

# Metazoan parasite community as a potential biological indicator in juveniles of the starry smooth-hound *Mustelus asterias* Cloquet, 1819 (Carcharhiniformes Triakidae)

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**Abstract** – The starry smooth-hound *Mustelus asterias* is a near-threatened coastal shark in Europe, whose parasitofauna is largely unknown. We studied metazoan parasites of 20 immature sharks (13 males and seven females) from the English Channel and we examined their relationships with host condition and their use as host bioindicators. All the sharks were parasitized by one to six metazoan taxa among the twelve recorded in the whole sampling (one trematode, six cestodes and two nematodes trophically-transmitted; one monogenean, one copepod and one myxosporean on gills), with a mean abundance of  $30.5 \pm 21.4$  parasites per fish (myxosporeans not included). The three major taxa were in decreasing order: the nematode *Acanthocheilus rotundatus* (prevalence: 75%, Confidence Interval 53–89%), the cestode *Eutetrarhynchus* sp. (70%, CI 48–85%), and the monogenean *Erpocotyle laevis* (60%, CI 39–78%). The gill copepod *Kroyeria lineata* and the gut nematode *Proleptus obtusus* were identified as significant pathogens. Parasite community differed between males and females despite their immature stage, suggesting early spatial sex-segregation, with *E. laevis*, *Eutetrarhynchus* sp. and *Anthobothrium* sp. proposed as tags. We discuss results in terms of host fitness loss and information given by parasites on diet ecology and stock discrimination. We recommend incorporating parasitology in further research to improve shark conservation and management.

**Keywords:** Metazoan parasites / *Mustelus asterias* / immatures / host fitness loss / sex discrimination / biological tags

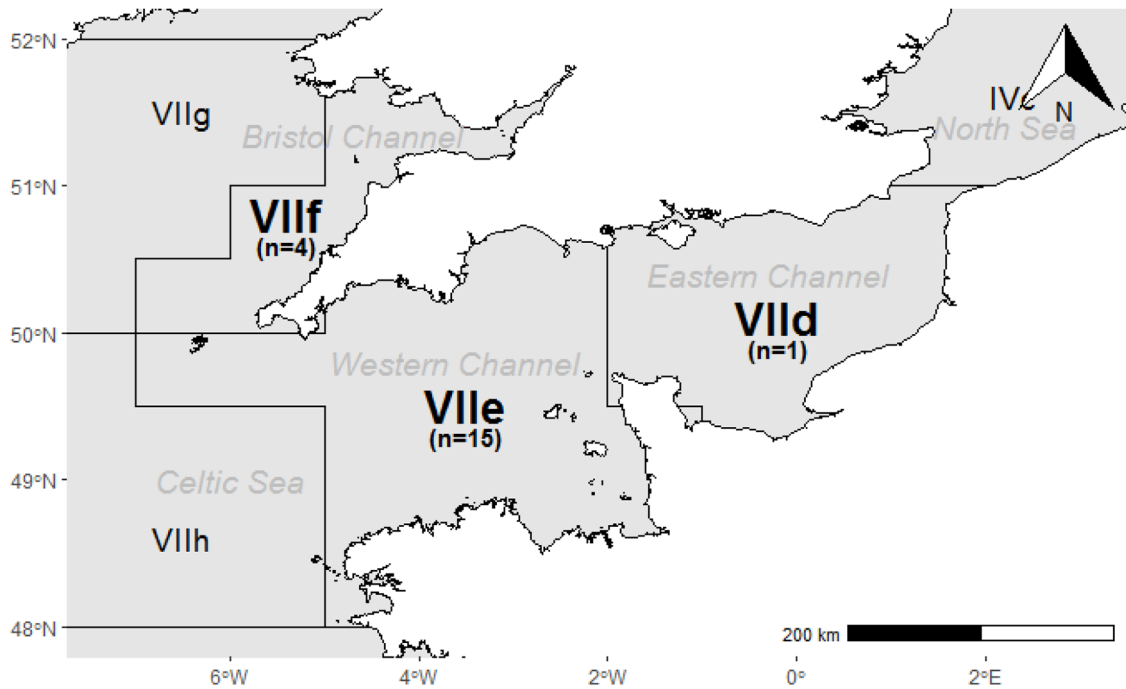
## 1 Introduction

The starry smooth-hound *Mustelus asterias* Cloquet, 1819 is a demersal coastal species of triakid shark, commercially exploited and distributed on the continental shelf to 200 m depth from Northern Europe to Northwest Africa, including the Mediterranean Sea (Jabado et al., 2021). ICES Working Group on Elasmobranch Fishes (ICES WGEF, 2019) considers there is a single biological stock unit in the continental shelf waters of Northeast Atlantic (ICES areas IV, VI–VIII), but with uncertain southern limits in the absence of relevant studies on stock identity. According to the IUCN's Red List

(Jabado et al., 2021), *M. asterias* is considered “Near Threatened”. This assessment is mainly due to its decline in the Mediterranean Sea recorded from 1994 onwards and its disappearance from most Mediterranean coastal areas, suggesting a high risk of regional extinction (Colloca et al., 2017; Jabado et al., 2021). Stock evaluation of *M. asterias* required for management and conservation measures remains difficult due to current taxonomical confusion between *M. asterias* and *Mustelus mustelus*, and sometimes between *Mustelus* spp. and early juveniles of *Galeorhinus galeus*, as demonstrated in the Northeast Atlantic (Farrell et al., 2009; ICES WGEF, 2019).

Although some recent data occurred on reproduction, growth and seasonal migrations of *M. asterias* (Farrell et al., 2010a,b;

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**Fig. 1.** Geographical position of the three ICES areas in the North-East Atlantic where *Mustelus asterias* was caught: VIId (Eastern Channel), VIIe (Western Channel) and VIIf (Bristol Channel).

McCully Phillips and Ellis, 2015; Brefeld and Meyer, 2018; ICES WGEF, 2019; Brevé et al., 2016, 2020; Griffiths et al., 2020), it remains a lack of knowledge on various biological and ecological aspects such as population structure, distribution, and behaviour. Surprisingly, the parasitofauna has received very little attention in previous works on *M. asterias*, despite parasites are ubiquitous natural stressors of great interest to understand population dynamics and to implement management strategies. Indeed, first, metazoan parasites decrease the host fitness (e.g., lower energy reserves and life traits such as growth and fecundity) and increase the host mortality risk (almost thrice higher for infected hosts compared to uninfected) (e.g., Thomas et al., 2007; Robar et al., 2010; McElroy and De Buron, 2014; Timi and Poulin, 2020 for reviews). Second, metazoan parasites influence biotic interactions (e.g., competition, predation) and host responses to other environmental stresses (e.g., overfishing, pollution, and degradation of marine habitat), reducing animal health in myriad ways (e.g., Price et al., 1986; Marcogliese and Pietrock, 2011; Frainer et al., 2018; Timi and Poulin, 2020 for reviews). Third, metazoan parasites such as helminths may provide perspective on the biology of their hosts (e.g., diet, habitat use, movements, stock discrimination), environmental pollution (e.g., heavy metals), and on free-living biodiversity, as shown by their increasingly use as bioindicators (e.g., MacKenzie, 2002; Marcogliese, 2005; De Buron et al., 2009; Catalano et al., 2014; Timi and Poulin, 2020 for reviews).

To date, the parasitofauna of *M. asterias* is poorly known, especially in case of immature sharks, and data have been scarcely and scattered published [e.g., a parasitological study on three females of *M. asterias* among other shark species in Ireland (Henderson et al., 2003); a phylogenetic study on cestodes of

triakids including 11 specimens of *M. asterias* from the North Sea (Bernot et al., 2016)].

In this context and to address this knowledge gap, we have investigated the metazoan parasites of *M. asterias* juveniles sampled in the English Channel, an important fishing ground of Northeast Atlantic. Our aims were: (i) to describe the community of metazoan parasites in *M. asterias*; (ii) to study the relationships between parasite occurrence and *M. asterias* health using two condition indices (hepato-somatic ratio, Fulton's K) as proxies of fitness (Bolger and Connolly, 1989; Lloret et al., 2012; Gérard et al., 2017; Silva et al., 2017; Ryberg et al., 2020); (iii) to examine the potential use of metazoan parasites as host bioindicators.

## 2 Material and methods

### 2.1 Study-sites and fish samplings

Twenty immature starry smooth-hounds were caught in 2018 by professional fishermen in the Northeast Atlantic in three ICES areas, respectively VIId (Eastern Channel = one individual in September 2018), VIIe (Western Channel = 15 in January 2018) and VIIf (Bristol Channel = four in March 2018) (Fig. 1).

### 2.2 Fish measurements

We measured total mass (TM in g) and total length (TL in cm) of each fish, then we determined the sex and assessed the immaturity stage (McCully Phillips and Ellis, 2015). Upon dissection, we recorded liver mass (LM in g) and eviscerated mass of each fish (EM in g). We calculated two indices of body condition as proxies of fitness: Hepato-Somatic Index (HSI)

and Fulton's K (K) (Bolger and Connolly, 1989; Lloret et al., 2012; Gérard et al., 2017; Silva et al., 2017; Ryberg et al., 2020). For both indices, we used the eviscerated mass to avoid bias due to the mass of parasites and gut contents (Lagrué and Poulin, 2015) as follows:  $HSI = (LM/EM) \times 100$  and  $K = 10^5 EM/TL^3$ . We chose HSI to evaluate host condition because the liver of elasmobranchs is the main lipid storage site and a major site of lipid synthesis (Speers-Roesch and Treberg, 2010 for review). We selected K among the primary condition factors based on length-mass relationships because of the quasi-isometric length-weight growth of *M. asterias* (Bolger and Connolly, 1989; Silva et al., 2013), and because K has previously been documented to be the best predictor of parasite load in *Lepomis macrochirus* (Centrarchidae) (Neff and Cargnelli, 2004).

### 2.3 Parasitological research

All the fish were frozen before the search for metazoan parasites. The following organs and tissues: eyes, skin, muscles, gills, heart, digestive tract, liver, and body cavity were meticulously dissected under a binocular stereomicroscope. Excepted for Myxozoa (myxosporean cysts), all the 609 metazoan parasites found were numbered per organ and per fish, and then morphologically identified. Morphological identification referred to Price (1942) for Monogenea; Curran and Overstreet (2000) and Gibson et al. (2001) for Digenea; Barker et al. (1984), Khalil et al. (1994), Ruhnke (2011), Palm and Walter (2000), Ruhnke and Caira (2009), Schaeffner (2014) and Bernot et al. (2016) for Cestoda; Petter et al. (1991), Moravec et al. (2002) and Anderson et al. (2009) for Nematoda; Lom and Dyková (2006) for Myxozoa; and Isawa (2008) for Copepoda.

Some nematode specimens (i.e., *Achanthocheilus rotundatus* and *Proleptus obtusus*) were preserved in 70% ethanol for molecular identification to validate morphological identification (see below).

The parasitological parameters used to describe the parasite community structure were: prevalence (P, number of hosts infected with a particular parasite taxon divided by the number of hosts examined), taxa richness (R, number of parasite taxa infecting a host species), abundance (A, number of individuals of a particular parasite taxon divided by the total number of hosts, infected plus uninfected), and intensity (I, number of individuals of a particular parasite taxon divided by the number of infected hosts) (Bush et al., 1997).

### 2.4 Molecular identification of nematodes via DNA sequencing

The DNA of each individual parasite was extracted using Wizard<sup>®</sup> Genomic DNA Purification Kit (Promega) following manufacturer's instructions after grinding of the parasite with a sterile piston pellet. DNA was kept at  $-20^{\circ}\text{C}$  until used. The 18S rDNA partial gene (18S) was amplified using the primers 18SF (5'-CGCGAATRGCTCATTACAACAGC-3') and 18SR (5'-GGGCGGTATCTGATCGCC-3') as described in Floyd et al. (2005), with an initial denaturation of 15 min at  $95^{\circ}\text{C}$ , 40 cycles of  $94^{\circ}\text{C}$  for 30 s,  $55^{\circ}\text{C}$  for 40 s and  $68^{\circ}\text{C}$  for 1 min 30 s and a final elongation step of 10 min at  $68^{\circ}\text{C}$ . The

amplification of the nuclear rDNA region comprising ITS-1, 5.8S, and ITS-2 sequences was carried out with the primers A (5'-GTCTGAATTCGTAGGTGAACCTGCGGAAGGAT-3') and B (5'-CCGATCCGAATCCTGGTGA-GTTTCTTTTCCCT-3') as described in D'Amelio et al. (2000). The amplification of the nuclear rDNA ITS-2 region was carried out with the primers XZ1F (5'-ATTGCGCCATCGGGTTCATTCC-3') and NC2 R (5'-TTAGTTTCTTTTCTCCGCT-3') as described in Zhu et al. (2000). Automated DNA sequencing was performed by Genoscreen (Lille, France), and then sequences were analyzed using BioEdit software to obtain consensus sequences from forward and reverse sequences. They were subsequently compared with available 18S or ITS sequences of nematode species by GenBank Blast software (Basic Local Alignment Search Tool) (Altschul et al., 1997).

### 2.5 Statistical analysis

Throughout the following sections, data are reported as means followed by 95% confidence interval (CI). For prevalence, 95% CI was calculated using the score method (Newcombe, 1998). All statistical analyses were performed with the R software v. 4.0.2 (R Core Team, 2020).

Prevalence was compared between parasite taxa using a likelihood ratio test (LRTTest) applied to a Generalized Linear Model (GLM, distribution: binomial, link function: logit) including the parasite taxon and the shark individual as independent variables. Pairwise comparisons of estimated marginal means (EMMeans) were subsequently performed using the 'emmeans' R package (Lenth, 2021), with *P*-values adjusted using the false discovery rate correction (Benjamini and Hochberg, 1995). The same approach was used to analyse abundance data (GLM distribution: negative binomial, link function: log) and intensity data (GLM with parasite taxon as only independent variable, distribution: negative binomial, link function: log).

Condition parameters were compared between host sexes using both a multivariate approach (redundancy analysis (RDA) on centred and scaled data, and associated permutation test with 9999 permutations; 'vegan' R package (Oksanen et al., 2020)) and a univariate approach (*t* test for each parameter). The same procedure was used to compare the parasite community (parasite taxa with prevalence  $>5\%$  only) between host sexes (presence-absence data: RDA on Hellinger-transformed data (Legendre and Gallagher, 2001) and univariate LRTTests on GLMs (distribution: binomial, link function: logit); abundance data: RDA on Hellinger-transformed data and univariate F tests on GLMs (distribution: quasi-Poisson, link function: log)). Total parasite abundance and taxa richness (both including all parasite taxa) were compared between sexes using LRTTests based on GLMs (distribution: negative binomial and Poisson respectively, link function: log in both models).

Relationships between the parasitofauna and host condition parameters were assessed using F tests on LMs. For each condition parameter, two models were built per parasite taxon (taxa with prevalence  $>5\%$  only): one relating the presence of the parasite (binary factor) to the condition parameter and

**Table 1.** Prevalence ( $P \pm CI$  %), mean abundance ( $A \pm CI$ ) and mean intensity ( $I \pm CI$ ) of metazoan parasites in 20 immatures of *Mustelus asterias*, and ecological parameters: ICES area, microhabitat (MH) and infection pathway according to Joyeux and Baer (1936), Dogiel et al. (1958), Diaz (1971), Gibson and Bray (1977), Caira and Ruhnke (1991), Pascual et al. (1996), Palm and Walter (2000), Lom and Dyková (2006), Moravec (2007), Isawa (2008), Jensen and Bullard (2010) and Tedesco et al. (2020a). Different letters (a, b, c, d) indicate statistically significant differences between parasite taxa for each parasitological descriptor (P, A and I). ES = esophagous-stomach, D = duodenum, G = gills, SV = spiral valve, HI = intermediate host. Myxosporeans were not numbered.

Parasite taxa	Area	MH	P $\pm$ CI %	A $\pm$ CI	I $\pm$ CI	Range	Infection pathway
Myxozoa							
Unidentified Myxosporea	VIIe	G	30.0 (14.5–51.9) <sup>bc</sup>	–	–	–	Random contact with spores
Monogenea							
<i>Erypcotyle laevis</i>	VIIdef	G	60.0 (38.7–78.1) <sup>ab</sup>	3.55 $\pm$ 2.83 <sup>ab</sup>	5.92 $\pm$ 4.41 <sup>b</sup>	1 – 26	Active attachment on gill lamellae
Digenea							
<i>Ptychogonimus megastomum</i>	VIIe	ES	20.0 (8.1–41.6) <sup>c</sup>	1.20 $\pm$ 1.33 <sup>bc</sup>	6.00 $\pm$ 5.52 <sup>b</sup>	3 – 11	HI Ingestion (crabs, e.g., <i>Portunus</i> )
Cestoda							
<i>Anthobothrium</i> sp.	VIIdef	SV	30.0 (14.5–51.9) <sup>bc</sup>	1.85 $\pm$ 2.23 <sup>bc</sup>	6.17 $\pm$ 7.54 <sup>b</sup>	1 – 20	HI Ingestion (teleosts and cephalopods)
<i>Eutetrarhynchus</i> sp.	VIIdef	SV	70.0 (48.1–85.5) <sup>a</sup>	3.35 $\pm$ 2.20 <sup>ab</sup>	4.79 $\pm$ 2.81 <sup>b</sup>	1 – 20	HI Ingestion (crabs, pagures) <sup>†</sup>
<i>Heteronybelinia robusta</i>	VIIe	SV	5.0 (0.9–23.6) <sup>c</sup>	0.10 $\pm$ 0.21 <sup>d</sup>	2.00 <sup>ab</sup>	2	HI Ingestion (various teleosts)
<i>Orymatobothrium</i> sp.	VIIe	SV	5.0 (0.9–23.6) <sup>c</sup>	0.05 $\pm$ 0.10 <sup>d</sup>	1.00 <sup>b</sup>	1	HI Ingestion (octopod <i>Eledone</i> )
<i>Phyllobothrium</i> sp.	VIIe	SV	5.0 (0.9–23.6) <sup>c</sup>	0.05 $\pm$ 0.10 <sup>d</sup>	1.00 <sup>b</sup>	1	HI Ingestion (various cephalopods)
<i>Symcallio leuckarti</i>	VIIe	SV	5.0 (0.9–23.6) <sup>c</sup>	0.05 $\pm$ 0.10 <sup>d</sup>	1.00 <sup>b</sup>	1	HI Ingestion (probably pagures and crabs)
Nematoda							
<i>Acanthocheilus rotundatus</i>	VIIdef	ES,D	75.0 (53.1–88.8) <sup>a</sup>	4.40 $\pm$ 2.12 <sup>a</sup>	5.87 $\pm$ 2.34 <sup>b</sup>	1 – 13	HI Ingestion ( <i>Pagurus prideauxi</i> )
<i>Proleptus obtusus</i>	VIIdef	ES	15.0 (5.2–36.0) <sup>c</sup>	1.45 $\pm$ 2.71 <sup>cd</sup>	9.67 $\pm$ 25.99 <sup>ab</sup>	1 – 26	HI ingestion (crabs, pagures) <sup>‡</sup>
Copepoda							
<i>Kroyeria lineata</i>	VIIe	G	35.0 (18.1–56.7) <sup>bc</sup>	14.40 $\pm$ 18.69 <sup>ab</sup>	41.14 $\pm$ 55.79 <sup>a</sup>	1 – 155	Active attachment on gill lamellae

<sup>†</sup>Cancer pagurus, Carcinus maenas, Liocarcinus depurator, Hyas araneus, Pachygrapsus marmoratus, Inachus dorsettensis, Macropodia longirostris, Eupagurus bernhardus (Joyeux and Baer, 1936; Dogiel et al., 1958).

<sup>‡</sup>Carcinus maenas, Eupagurus bernhardus, Hyas araneus, Pachygrapsus marmoratus (Moravec, 2007).

another one relating the abundance of the parasite to the condition parameter. The effect of the total parasite abundance was also assessed in the same way. The whole procedure was repeated for all host individuals, males only and females only.

Differences were considered statistically significant at  $P < 0.05$ .

### 3 Results

#### 3.1 Metazoan parasite community of *M. asterias* juveniles

Each immature specimen of *M. asterias* was infected (total prevalence of 100%; CI 84–100%) by one to six metazoan parasite taxa among the 12 identified in the whole sampling

(Tab. 1). The mean number of parasite taxa per fish was  $3.55 \pm 0.70$  (range: 1–6) and the mean total abundance (= mean total intensity) of parasites (Myxozoa excluded) was  $30.45 \pm 21.36$  parasites per fish (range: 1–170).

Parasites were mainly recorded in the digestive tract, i.e., nine gastro-intestinal helminth taxa in 19 fish, with a prevalence of 95% (CI 76–99%) and a mean intensity of  $13.16 \pm 5.09$  helminths per fish (Tab. 1). In a lesser extent, parasites occurred on the gills, i.e., three taxa in 13 fish, with a prevalence of 65% (CI 43–82%) (Tab. 1). The other organs examined (eyes, skin, muscles, heart, liver, and body cavity) were not found infected.

Among the 12 parasite taxa recorded, helminths were the best represented (10 taxa among them six cestodes), in contrast with crustaceans (one copepod species) and Myxozoa (one

myxosporean taxon) (Tab. 1). At least one helminth taxon was recorded in each specimen of *M. asterias* (helminth prevalence of 100%; CI 84–100%) with a mean of  $16.05 \pm 6.37$  helminths per fish, and in total, 80% (CI 58–92%) of the sharks harboured a mean of  $6.81 \pm 2.93$  cestodes in their spiral valve.

Prevalences, mean abundances and intensities significantly varied between parasite taxa ( $\chi^2 = 82.416, 98.795$  and  $53.309$  respectively,  $df = 11, 10$  and  $10$  respectively, all  $P < 0.001$ ) (Tab. 1). The three major parasite taxa were in decreasing order (prevalence value in parenthesis): the nematode *Acanthocheilus rotundatus* (75%, CI 53–89%), then the cestode *Eutetrarhynchus* sp. (70%, CI 48–85%), both in the digestive tract, and the monogenean *Erpocotyle laevis* (60%, CI 39–78%) on the gills. All these highly prevalent taxa were recorded in the three ICES areas (Tab. 1). Five other parasite taxa had intermediate prevalence (15–35%); among them, the copepod *Kroyeria lineata* (35%, CI 18–57%) on the gills, characterized by a significantly much higher mean intensity ( $41.14 \pm 55.79$ , up to 155 parasites per fish) compared to all other parasite taxa (Tab. 1). The four last taxa (cestodes in the spiral valve) were rare with only one specimen of *M. asterias* found infected in VIIe ICES area (Tab. 1).

Among the 97 isolated nematodes from 13 immature starry smooth-hounds, 27 individuals were molecularly identified, i.e., 20 *A. rotundatus* and seven *Proleptus* sp. (*P. obtusus* according to morphology, not confirmed by molecular analysis due to the absence of reference sequence of this species) (Tab. 2). Individual 18S sequences had an identity percentage with sequences present in National Center for Biological Information above 99.8% and 99.6% respectively for *A. rotundatus* and *Proleptus* sp. (Tab. 2). For three *A. rotundatus* individuals, identification was also confirmed by ITS2 sequences with identity percentage above 99.6%. Percentage of coverage was always at least 96% whatever sequences. All the sequences generated in this study were deposited in GenBank (accession numbers OM177246-OM177262, OM177185-OM177187 and OM200080-OM200086).

### 3.2 Parasites as potential biological indicators

All the 20 specimens of *M. asterias* were immature based on their size (Tab. 3) and comprised seven females and 13 males. Despite the relatively low and unbalanced sample size between females and males, no significant differences occurred between sexes in total mass, total length and body condition indices HSI and K (RDA:  $F = 1.250, P = 0.299$ ;  $t$  tests:  $t = 0.679, 0.277, 1.883$  and  $1.128$  respectively, all  $df = 18, P \geq 0.076$ ), as well as in total parasite abundance ( $\chi^2 = 0.814, df = 1, P = 0.367$ ) and number of parasite taxa ( $\chi^2 = 0.215, df = 1, P = 0.643$ ) (Tab. 3). Except the four rare taxa of cestodes found in a single host individual, females and males harboured the same taxa of metazoan parasites (eight in common) (Tab. 4). However, the parasitic assemblage significantly differed between sexes in terms of prevalences ( $F = 2.914, P = 0.007$ ) and abundances ( $F = 3.018, P = 0.011$ ) (Tab. 4, Fig. 2). In particular, the cestode *Eutetrarhynchus* sp. was clearly both more prevalent and more abundant in males than in females, whereas the gill monogenean

*E. laevis* and the cestode *Anthobothrium* sp. were significantly more abundant in females than in males (Tab. 4, Fig. 2, and Tab. S1). The prevalence of *E. laevis* tended also to be higher in females than in males (Fig. 2a).

### 3.3 Parasites and host condition

When considering the total number of metazoan parasites, no significant relationship was detected between the parasite abundance and the total length, total mass or condition indices (HSI and K) of *M. asterias*, whatever taking or not into account the sex (Fig. 3, Tabs. S2–S7). However, some relationships were significant when considering parasite taxa separately (Fig. 3, Tabs. S2–S7). Indeed, the abundance of the nematode *P. obtusus* was negatively related to K considering all individuals or males only (not females); and for males only, the prevalence of *P. obtusus* was negatively related to the total length and total mass (Figs. 3A and 3B). A negative relationship was also found for males only between the prevalence of the copepod *K. lineata* and K (Fig. 3B). On the contrary, positive relationships occurred between gill myxosporean prevalence and HSI considering all individuals (Fig. 3), and for females only, between the prevalence of the nematode *A. rotundatus* and total mass, and between its abundance and both total length and total mass (Fig. 3C). Finally, considering all individuals, contrasting relationships occurred for the monogenean *E. laevis*: its prevalence was negatively related to total length whereas its abundance was positively related to HSI (Fig. 3A).

## 4 Discussion

### 4.1 Metazoan parasite community of *M. asterias* juveniles

Our study demonstrates for the first time the importance to take into account the parasitofauna of *M. asterias* in further investigations since all the specimens were infected despite their immature stage (McCully Phillips and Ellis, 2015) and the relatively low sample size of 20 individuals. It is worthy to note that both parasitic load and diversity are expected to be greater for adults (vs immatures) of *M. asterias*, based on the increasing probability of meeting parasites with age (Polyanski, 1958) and the strong positive correlation between maximum parasite biomass and host mass (Poulin and George-Nascimento, 2007).

In total, we recorded 12 metazoan parasite taxa (one monogenean, one trematode, six cestodes, two nematodes, one copepod, and one myxosporean) in immature sharks (Tab. 1). Among them, seven taxa were already described in adults of *M. asterias*, i.e., *K. lineata*, *E. laevis*, *Anthobothrium* sp., *Proleptus* sp. (Henderson et al., 2003); *Heteronybelinia robusta* (Palm and Walter, 2000); *Phyllobothrium* sp. (Ruhnke, 2011); *Symcallio leuckarti* (Bernot et al., 2016). The other parasite taxa are a priori new records for *M. asterias*, previously registered in the closely related *M. mustelus* (*Acanthocheilus rotundatus*, *Eutetrarhynchus* sp., *Ptychogonimus megastomum*)

**Table 2.** Molecular identification of 27 nematodes from *Mustelus asterias* immatures: comparison of 18S and ITS2 sequences with previously published data in GenBank.

Sequence	Present study samples			BLAST results				
	GenBank Reference	Length	Target	Identification	GenBank reference	Identity percentage	Query coverage	References
MA-10-ES-04	OM177246	656	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-11-ES-13	OM177247	656	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-1-ES-02	OM177248	656	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-1-ES-03	OM177249	650	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-1-ES-04	OM177250	657	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	99.85	100	Li et al., 2018
MA-1-ES-05	OM177251	665	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-1-D-04	OM177252	656	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-2-ES-01	OM177253	664	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	99.85	100	Li et al., 2018
MA-3-ES-01	OM177254	660	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-11-ES-04	OM177255	655	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-11-ES-16	OM177256	667	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-11-ES-18	OM177257	656	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	99.85	100	Li et al., 2018
MA-11-ES-19	OM177258	663	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-11-ES-20	OM177259	656	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-11-ES-21	OM177260	663	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-11-D-02	OM177261	631	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-19-ES-03	OM177262	663	18S	<i>Acanthocheilus rotundatus</i>	MF072699.1	100	100	Li et al., 2018
MA-1-D-3	OM177185	311	ITS2	<i>Acanthocheilus rotundatus</i>	MT967229.1	99.67	96	Tedesco et al., 2020b
MA-3-ES-2	OM177186	311	ITS2	<i>Acanthocheilus rotundatus</i>	MT967229.1	100	96	Tedesco et al., 2020b
MA-19-ES-08	OM177187	311	ITS2	<i>Acanthocheilus rotundatus</i>	MT967229.1	100	96	Tedesco et al., 2020b
MA-1-ES-1	OM200080	650	18S	<i>Proleptus sp.</i>	JF934733.1	99.85	100	Laetsch et al., 2012
MA-5-ES-1	OM200081	637	18S	<i>Proleptus sp.</i>	JF934733.1	100	100	Laetsch et al., 2012
MA-5-ES-3	OM200082	671	18S	<i>Proleptus sp.</i>	JF934733.1	99.85	100	Laetsch et al., 2012
MA-11-ES-2	OM200083	637	18S	<i>Proleptus sp.</i>	JF934733.1	100	100	Laetsch et al., 2012
MA-11-ES-3	OM200084	637	18S	<i>Proleptus sp.</i>	JF934733.1	99.68	100	Laetsch et al., 2012
MA-11-ES-33	OM200085	674	18S	<i>Proleptus sp.</i>	JF934733.1	100	100	Laetsch et al., 2012
MA-12-ES-5	OM200086	670	18S	<i>Proleptus sp.</i>	JF934733.1	99.85	100	Laetsch et al., 2012

**Table 3.** Biometrics [total mass (TM, g) and length (TL, cm), body condition indices (Hepato-Somatic Index HSI and Fulton's K)] and parasitological parameters [total prevalence (P, %), mean abundance (A), mean taxa richness (R), and number of metazoan parasite taxa] ( $\pm$  CI) in 20 immatures of *M. asterias* (7 females and 13 males). Mean abundance of metazoan parasites equals mean intensity because prevalence equals to 100% whatever the sex. No significant differences between sexes occur in biometrics and parasitological parameters.

	Females (7)	Range	Males (13)	Range	All sharks
TM (g)	881.43 $\pm$ 134.90	690–1090	834.62 $\pm$ 87.29	580–1040	851.00 $\pm$ 67.79
TL (cm)	64.56 $\pm$ 3.56	59.3–70.5	64.06 $\pm$ 2.23	55.5–67.8	64.24 $\pm$ 1.74
HSI	8.61 $\pm$ 1.34	6.52–10.19	7.24 $\pm$ 0.94	5.21–11.51	7.72 $\pm$ 0.77
K	263.40 $\pm$ 10.74	245.10–282.94	257.44 $\pm$ 6.55	234.99–274.93	259.53 $\pm$ 5.31
P (%)	100.0 (64.6–100.0)		100.0 (77.2–100.0)		100.0 (83.9–100.0)
A <sup>†</sup>	21.57 $\pm$ 17.94	1–50	35.23 $\pm$ 33.01	1–170	30.45 $\pm$ 21.36
R	3.29 $\pm$ 1.43	1–6	3.69 $\pm$ 0.89	1–5	3.55 $\pm$ 0.70
Taxa number	9		11		12

<sup>†</sup>Myxozoa not included (not numbered).

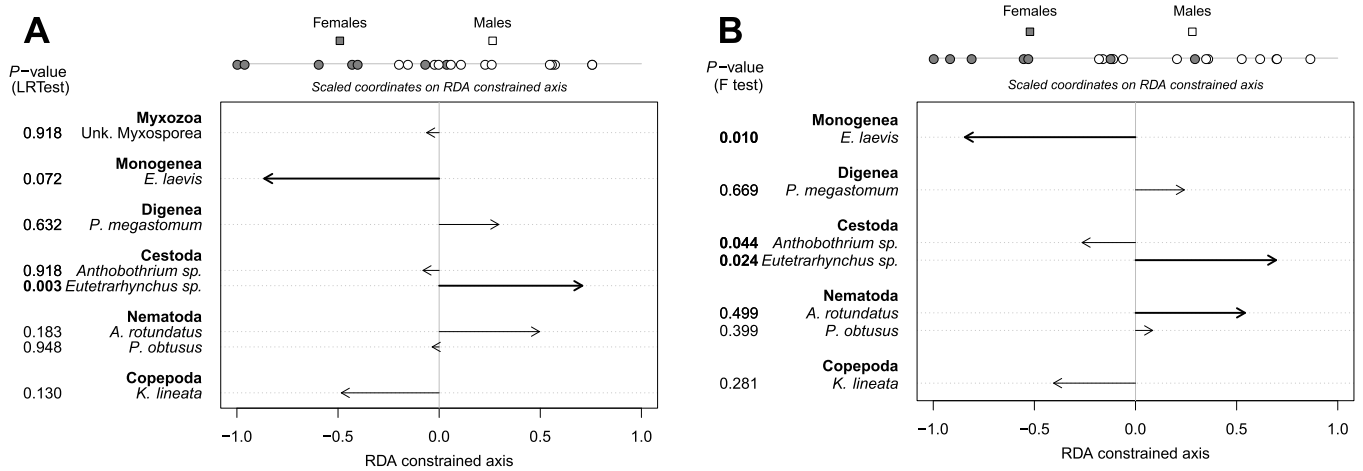
**Table 4.** Community of metazoan parasites in *Mustelus asterias* according to the sex: total prevalence (P  $\pm$  CI %), mean abundance (A  $\pm$  CI) and mean intensity (I  $\pm$  CI) of metazoan parasites in immature females (7) and immature males (13). Myxosporeans were not numbered.

Parasite taxa	Females (7)				Males (13)			
	P $\pm$ CI %	A $\pm$ CI	I $\pm$ CI	Range	P $\pm$ CI %	A $\pm$ CI	I $\pm$ CI	Range
Myxozoa								
Unidentified Myxosporea	28.6 (8.2–64.1)	–	–	–	30.8 (12.7–57.6)	–	–	–
Monogenea								
<i>Erpocotyle laevis</i>	85.7 (48.7–97.4)	7.43 $\pm$ 8.14	8.67 $\pm$ 9.30	1–26	46.2 (23.2–70.9)	1.46 $\pm$ 1.08	3.17 $\pm$ 1.19	2–5
Digenea								
<i>Ptychogonimus megastomum</i>	14.3 (2.6–51.3)	1.57 $\pm$ 3.72	11.00	11	23.1 (25.1–84.2)	1.00 $\pm$ 1.20	4.33 $\pm$ 2.81	3–6
Cestoda								
<i>Anthobothrium</i> sp.	28.6 (8.2–64.1)	4.14 $\pm$ 6.93	14.5 $\pm$ 23.67	9–20	30.8 (12.7–57.6)	0.62 $\pm$ 0.67	2.00 $\pm$ 1.60	1–3
<i>Eutetrarhynchus</i> sp.	28.6 (8.2–64.1)	0.71 $\pm$ 1.12	2.50 $\pm$ 2.15	2–3	92.3 (66.7–98.6)	4.77 $\pm$ 3.18	5.17 $\pm$ 3.36	1–20
<i>Heteronybelinia robusta</i>					7.7 (1.4–33.3)	0.15 $\pm$ 0.33	2.00	2
<i>Orygmatobothrium</i> sp.	14.3 (2.6–51.3)	0.14 $\pm$ 0.34	1.00	1				
<i>Phyllobothrium</i> sp.					7.7 (1.4–33.3)	0.08 $\pm$ 0.17	1.00	1
<i>Symcallio leuckarti</i>					7.7 (1.4–33.3)	0.08 $\pm$ 0.17	1.00	1
Nematoda								
<i>Acanthocheilus rotundatus</i>	57.1 (25.1–84.2)	3.43 $\pm$ 4.55	6.00 $\pm$ 7.77	1–13	84.6 (57.8–95.7)	4.92 $\pm$ 2.59	5.82 $\pm$ 2.72	1–13
<i>Proleptus obtusus</i>	14.3 (2.6–51.3)	0.29 $\pm$ 0.68	2.00	2	15.4 (4.3–42.2)	2.08 $\pm$ 4.31	13.50 $\pm$ 53.79	1–26
Copepoda								
<i>Kroyeria lineata</i>	57.1 (25.1–84.2)	3.86 $\pm$ 5.02	6.75 $\pm$ 8.43	1–12	23.1 (25.1–84.2)	20.08 $\pm$ 29.46	87.00 $\pm$ 139.57	5–155

(Petter et al., 1991; Gračan et al., 2014) and *Mustelus manazo* (*Orygmatobothrium* sp.) (Yamaguchi et al., 2003), apart from the unknown Myxosporea. To date, only 20 species of Myxosporea have been described in Carcharhiniformes, but not on the host gills, and a large number of Myxosporea species certainly remains to be discovered (Lom and Dyková, 2006; MacKenzie and Kalavati, 2014 for reviews). Moreover, we did not find *Calliobothrium wightmanorum*, a cestode described in each of the 11 adults of *M. asterias* (78–109 cm TL) from the North Sea by Bernot et al. (2016), possibly due to the age difference with our immature specimens (55–70 cm). Overall, further molecular systematic investigations are needed to specifically identify all metazoan parasites and assess new records, as demonstrated by

our DNA sequences for *A. rotundatus*, recorded for the first time in *M. asterias*.

The diversity of metazoan parasites we found in immature *M. asterias* is of the same order than for *M. manazo* (life stage and size not mentioned) in which Yamaguchi et al. (2003) recorded 13 taxa (eight cestodes, one nematode, two myxosporeans, and two copepods), but from 1038 host specimens examined. Moreover, prevalences of each parasite taxon recorded in *M. asterias* (from 5% to 75%) appear higher than for *M. manazo* (from 0 to 41%) (Yamaguchi et al., 2003). Some parasitological studies focused on helminths from the digestive tract of adult *Mustelus* spp. (Cisio and Caira, 1993; Gračan et al., 2014). According to these studies, *Mustelus*



**Fig. 2.** (A) Redundancy analysis (RDA) performed on the parasite community (Hellinger-transformed presence-absence data). Individual coordinates on the RDA constrained axis scaled to [-1;1] (circles) and sexes placed at the mean of the corresponding individuals (squares and names). Arrows show correlations of each parasite taxon with the RDA constrained axis (bold arrows for  $|r| > 0.5$ ). P-values on the left show results of univariate Likelihood ratio tests. (B) Same as A for abundance data (Hellinger-transformed data, univariate F tests).

*canis* (16 females and 28 males of 43–123 cm TL) harboured four species of cestodes (Cisio and Caira, 1993), whereas *M. mustelus* (six females and nine males of  $101.1 \pm 27.3$  cm TL, range 50.5–152.5 cm) hosted four helminth taxa (among them three recorded in our study): one nematode, two cestodes and one trematode (Gračan et al., 2014). In comparison, helminths found in the digestive tract of *M. asterias* immatures are more diverse with nine taxa recorded, despite the difference in host size/age. However, the total prevalence and the mean intensity of gastrointestinal helminths were of the same order for *M. asterias* (95% and  $13 \pm 5$  helminths per host) and *M. mustelus* (87% and 29 helminths per host) (Gračan et al., 2014).

The community of metazoan parasites of immature *M. asterias* includes three core (dominant) taxa, five secondary taxa and four satellite taxa (i.e., cestodes in the spiral valve).

The most prevalent species (75%) is the nematode *A. rotundatus*, which is a priori a first record for *M. asterias*. This dominant species is widely distributed (North Sea, Adriatic Sea, Mediterranean Sea, North Pacific Ocean) and occurred in closely related species such as *M. mustelus*, *Mustelus griseus*, *M. manazo* and *G. galeus* (Diaz, 1971; Petter et al., 1991; Moravec and Nagasawa, 2000; Yamaguchi et al., 2003). The two-host life cycle comprises the hermit crab *Pagurus prideauxi* as intermediate host ingested by the elasmobranch definitive host (Diaz, 1971). Both prevalence (50%) and intensity (1–15) of *P. prideauxi* reported on the French Mediterranean Coast (Sète) are relatively high, suggesting it is the main intermediate host of *A. rotundatus* (Diaz, 1971). *Pagurus* spp. such as *P. prideauxi* and *P. bernhardus* are common in the North-East Atlantic where they are the main prey species ingested by *M. asterias* at Plymouth, UK, i.e., found in 64.6% of 48 stomachs (a priori of male adults) and representing 24.6% of the diet (Ford, 1921). In the Irish Sea, *Pagurus* spp. constitute 7.1% of the preys ingested by *M. asterias* (46 individuals of  $72.2 \pm 18.7$  cm total length, range 43–100 cm) (Ellis et al., 1996).

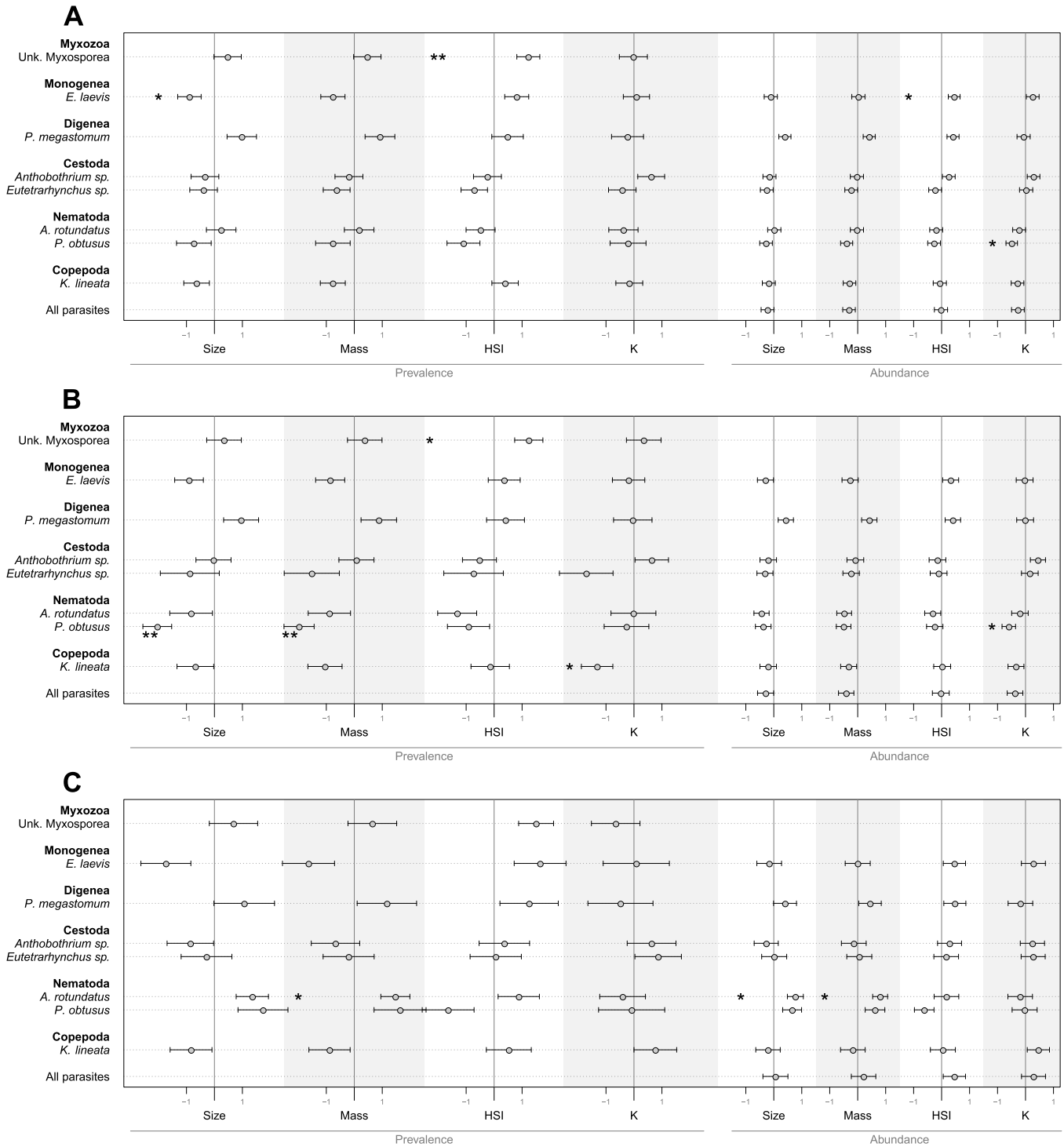
The second most prevalent parasite (70%) is the cestode *Eutetrarhynchus* sp., already found in *M. mustelus* (Gračan et al., 2014), and possibly belonging to the type species, *Eutetrarhynchus ruficollis*, described before modern taxonomy

in *M. asterias* on the French coast (Joyeux and Baer, 1936). *E. ruficollis* parasitizes decapods as second intermediate host, mostly crabs, i.e., *Cancer pagurus*, *Carcinus maenas*, *Liocarcinus depurator*, *Hyas araneus*, *Pachygrapsus marmoratus*, *Inachus dorsettensis*, *Macropodia longirostris*, and pagures, i.e., *Eupagurus bernhardus* (Joyeux and Baer, 1936; Ginetsinskaya, 1958). These prey species frequently occur in the stomach of *M. asterias* where crabs and pagures constitute respectively up to 70% and 25% of the diet in the Northeast Atlantic (Ford, 1921; Ellis et al., 1996).

The third most prevalent species (60%) is the blood-feeder gill monogenean *Erpocotyle laevis*, already recorded from *M. asterias* in the Northeast Atlantic (Henderson et al., 2003). The life cycle is direct and swimming larval oncomiracidia actively attach themselves on gill lamellae of fish host where sexual reproduction of the monogenean parasite occurs (Ginetsinskaya, 1958).

The five secondary parasite taxa whose prevalence varied from 15% to 35% correspond to two branchial parasites (the copepod *K. lineata* and the unidentified myxosporean) and three trophically-transmitted helminths (the cestode *Anthobothrium* sp., the digenean trematode *P. megastomum* and the nematode *P. obtusus*). These taxa are a priori first records for *M. asterias* except *K. lineata* and *Anthobothrium* sp. already recorded by Henderson et al. (2003) in the Irish Sea. These authors also found *Proleptus* sp. in *M. asterias* but without species identification. As for *E. laevis*, the direct life cycle of *K. lineata* comprises free-living infective larvae (copepodid stage) that actively attach themselves on gill lamellae of *M. asterias* (Raibaut et al., 1998). It is different for heteroxenous myxosporeans for which actinospores, discharged from an annelid definitive host into water, randomly encounter the fish intermediate host (Lom and Dyková, 2006). Regarding the gastro-intestinal helminths, the intermediate hosts preyed by the shark definitive host are mainly crabs (e.g., *Portunus* sp.) for *P. megastomum* (Gibson and Bray, 1977) and various crabs and pagures (i.e., *C. maenas*, *E. bernhardus*, *H. araneus* and *Pachygrapsus marmoratus*) for *P. obtusus* (Moravec, 2007), but teleosts and cephalopods for *Anthobothrium* sp. (Jensen and Bullard, 2010; Tedesco et al., 2020a).





**Fig. 3.** Relationships between host condition and parasite prevalence (left) or abundance (right), for all individuals (A), males only (B) and females only (C). Effect sizes are represented, with symbols depicting results of the corresponding F tests ( $\sim < 0.1$ ,  $* < 0.05$ ,  $** < 0.01$ ). Effect sizes for the impact of parasites' presence are regression coefficients of the Linear Model (LM) linking parasite presence (binary factor) with centered-scaled condition parameters. Effect sizes for the impact of parasites' abundance are standardized regression coefficients, i.e., regression coefficients of the LM linking centered-scaled parasite abundance with centered-scaled condition parameters.

The four satellite taxa (prevalence of 5%), i.e., the cestodes *H. robusta*, *Phyllobothrium* sp., *S. leuckarti* and *Orygmatobothrium* sp., were already described in *M. asterias* or in *M. manazo* for the latter (Ruhnke, 2011; Palm and Walter, 2000; Yamaguchi et al., 2003; Bernot et al., 2016). In contrast with the wide geographical and low host specificity of tentaculariid trypanorhynchs such as *H. robusta* (Palm and Walter, 2000), the three tetraphyllideans are specific of the *Mustelus* genus (Barker et al., 1984; Ruhnke, 2011; Bernot et al., 2016). The second intermediate host of *H. robusta* may be various teleosts (Palm and Walter, 2000), whereas a great variety of cephalopod species have been found infected by larval plerocercoids of *Phyllobothrium* sp. (Pascual et al., 1996), but only the octopod *Eledone moschata* for *Orygmatobothrium* sp. (Joyeux and Baer, 1936). To our knowledge, the life cycle is unknown for *S. leuckarti*. However, *Calliobothrium verticillatum*, a closely related species to *S. leuckarti* infecting *M. mustelus* and *M. canis* as definitive hosts, uses *Pagurus pollicaris*, and possibly *C. maenas*, as second intermediate hosts (Joyeux and Baer, 1936; Caira and Ruhnke, 1991).

#### 4.2 Parasites as potential biological indicators

The taxonomically diverse metazoan parasites hosted by *M. asterias* may provide a wide variety of information and be potentially used as biological tags, as shown for many teleost species but scarcely for elasmobranch species (MacKenzie, 2002; Marcogliese, 2005; Poulin and Kamiya, 2013; Catalano et al., 2014 for reviews). In that respect, Yamaguchi et al. (2003) demonstrated the feasibility of using helminth parasites, in particular cestodes, for the identification of different host stocks of *M. manazo*.

Interestingly, our study on immatures of *M. asterias* highlights differences in the distribution patterns of the parasitofauna according to the sex, even if the same parasite taxa occurred in males and females apart from rare cestodes. In particular, three helminths, i.e., the gill monogenean *E. laevis* and the gut cestodes *Eutetrarhynchus* sp. and *Anthobothrium* sp., were of interest due to significant inter-sex differences in their prevalence and/or abundance.

*E. laevis* was nearly thrice more abundant and tended to be twice more prevalent in females than in males. Several reasons may explain these differences. First, immune response and susceptibility to parasite infection may be different between sexes, possibly in relation with the more or less attractiveness of mucus covering the gills and with humoral immune factors and immune cells contained in gill tissues (Ilgová et al., 2021 for review). Second, immature females may be more exposed than immature males to infective oncomiracidia of *E. laevis*, possibly due to differences in their spatial distribution. Information suggested by *E. laevis* patterns is in accordance with spatial segregation by sex demonstrated for adults of *M. asterias* in the Northeast Atlantic, where females disperse across a wider geographic range than males (Brevé et al., 2016, 2020; Griffiths et al., 2020). Some gill monogeneans such as *Mazocraes alosae* and *Dactylogyrus* sp. have been already found to discriminate fish host species or hybrids with different spatial distribution (Dupont and Crivelli, 1988; Gérard et al., 2016). In the same way, *E. laevis* may constitute a valuable marker of spatial segregation between males and females of *M. asterias* at an immature stage.

In contrast to monoxenous *E. laevis*, *Eutetrarhynchus* sp. and *Anthobothrium* sp. are heteroxenous and trophically-transmitted, and therefore may be indicative of inter-sex differences in preys consumed by *M. asterias* immatures, and more generally in feeding ecology and habitat use.

*Eutetrarhynchus* sp., the dominant taxon of the parasite community in males, was both thrice more prevalent and twice more abundant than in females. These differences suggest that immature males ingest more frequently and in higher quantity the second intermediate host species of *Eutetrarhynchus* sp., and therefore are more exposed to parasite infection. Another explanation may be that male and female immatures forage in different habitats varying in food resource availability and parasite infection risk.

Contrarily to *Eutetrarhynchus* sp., *Anthobothrium* sp. was more abundant in immature females than in immature males, but similarly prevalent in both sexes. It suggests that the second intermediate hosts of *Anthobothrium* sp. preyed by immature females were more heavily infected than those ingested by immature males, and possibly originated from different areas. Immature females of *M. asterias* may be more pelagic vs immature males more benthic, since pelagic sharks eat more teleosts and cephalopods, and less crustaceans than benthic ones [database on the diet of 29 shark species (Capapé, 1975)].

The inter-sex differences in the infection patterns of *E. laevis*, *Eutetrarhynchus* sp. and *Anthobothrium* sp. strongly suggest different spatial distribution and habitat use of male and female immatures. Therefore, these three helminth taxa may constitute useful tags of spatial segregation between males and females at immature stage. Despite recent studies on *M. asterias* distribution and the demonstration of sex-biased dispersal (Brevé et al., 2016, 2020; Griffiths et al., 2020), knowledge on immatures is lacking. Predicted suitable habitats of *M. asterias* immatures generally coincide with those of adults but sex of immatures was not identified in available data (Brefeld and Meyer, 2018). Thus, potential differences in behaviour, migrations and diet ecology of *M. asterias* according to both developmental stage and sex should be further investigated for a better understanding and use of information provided by parasite taxa of *M. asterias*.

Our data on trophically-transmitted parasites (nine taxa) also underline high resemblances in the diet of immature males and females of *M. asterias*, such as the importance of preying crabs and pagures [already described by Ford (1921), Capapé (1975) and Ellis et al. (1996)] based on the occurrence in both sexes of *Eutetrarhynchus* sp., *A. rotundatus*, *P. obtusus* and *P. megastomum*. Moreover, the occurrence of *Orygmatobothrium* sp. in females and of *Phyllobothrium* sp. in males, even rare, proves the consumption of cephalopods, not mentioned among the preys of *M. asterias* in the Northeast Atlantic (Ford, 1921; Ellis et al., 1996), but recorded in the Tunisian coast, i.e., *Sepia officinalis* and *E. moschata* (Capapé, 1975). Concerning teleosts, the record of two specimens of *H. robusta* in one male of *M. asterias* proved that teleosts may be preyed by males (Palm and Walter, 2000). We cannot assess that females of *M. asterias* from our study have ingested teleosts based on the occurrence *Anthobothrium* sp. since second intermediate hosts may be both cephalopods and teleosts (Jensen and Bullard, 2010; Tedesco et al., 2020a). According to stomach analysis, teleosts are rarely consumed by *M. asterias* in the Northeast Atlantic where they constitute

only 2% of its diet (Ellis et al., 1996) or are not found (Ford, 1921), whereas 11 species of teleosts are distinguished in the stomach contents of *M. asterias* in the Tunisian coast (Capapé, 1975). Further studies on the relationships between parasitofauna and diet of *M. asterias* at different ages of both sexes and from different geographical areas are needed to increase knowledge and make the best use of parasites as host biological tags.

#### 4.3 Parasites and host condition

Whatever host-parasite combination, parasites and their hosts compete for resources in such way that host survival and fecundity could be affected, even if no pathology is obvious and even if this effect may be drowned in the background noise of all other factors that affect these life-traits (Thomas et al., 2007; Robar et al., 2010; McElroy and De Buron, 2014 for reviews). Metazoan parasites are ubiquitous natural stressors inducing a host fitness loss, with more or less pathogen effects depending, among several factors, on host life stage, infected organs/tissues and parasite load, most parasites being pathogen by accumulation (Bauer, 1958; Woo, 2006; Thomas et al., 2007; Robar et al., 2010; McElroy and De Buron, 2014 for reviews). The body condition can be used as a proxy of fitness to evaluate the severity of parasite infection (e.g., Neff and Cargnelli, 2004; Bean and Bonner, 2009; Gérard et al., 2013, 2017; Ryberg et al., 2020). Here, we obtained contrasting results depending on the parasite taxa considered, and we demonstrated a clearly negative relationship between parasites and host condition for the gill copepod *K. lineata* and the gut nematode *P. obtusus*, suggesting they induced a host fitness loss.

Gill parasites, including the three taxa found in *M. asterias*, can result in lesions that facilitate infections by various opportunist pathogens (virus, bacteria, and fungi), and, in case of blood-feeders such as copepods and polyopisthocotylid monogeneans, in potentially lethal anemia (Bauer, 1958; Woo, 2006 for reviews). Moreover, severe hyperplastic lesions of gills can also reduce or block the respiratory water flow over lamellae, and reduce gas and ion exchange across the lamellar epithelium, as demonstrated for sharks highly infected by the blood-feeder monogenean *Erpocotyle tiburonis* (about 100 parasites per fish) (Bullard et al., 2001). The death of two aquarium-held *M. asterias* adults was attributed to respectively 50 and 70–90 specimens of *Erpocotyle* sp. on the gills (MacKenzie and Smith, 2016). In our study, the parasitic load of *E. laevis* in immature *M. asterias* was lower (up to 26 monogeneans per host) and no negative impact was evident since contrasting correlations occurred, i.e., prevalence negatively related to total length but abundance positively related to HSI. By contrast, the negative relationship between the hematophagous *K. lineata* and host condition was clearly assessed, but only for males. Among them, two males harbored intense infections on their gills, with respectively 101 and 155 copepods, probably inducing respiration impairment and anemia. Regarding the unknown Myxosporea, the positive relation only between its prevalence and HSI is difficult to interpret since myxosporeans may cause necrosis and destruction of gill tissues (Bauer, 1958; Lom and Dyková, 2006). According to our results, multiple infections of gill parasites frequently

occur on the same host individual, i.e., 77% with two taxa, 15% with three taxa, and probably induce cumulative pathogen effects on *M. asterias*. This issue needs to be explored in further investigations.

Pathogen effects can also occur due to helminth parasites in the digestive tract, such as the nine taxa recorded in *M. asterias* (nematodes, cestodes and trematodes). Indeed, parasitic helminths may lead to obstruction of the lumen of the gut and damage to its wall, and have an impact on the host's physiology and nutrition (Bauer, 1958; Petkevičius, 2007 for reviews). Some of them such as spirurine nematodes are especially pathogenic and known as causative agents of serious fish diseases (Moravec, 2007 for review). Due to host-parasite competition for resources, gastro-intestinal helminths reduce the host reserves from their own gain and commonly induce weight loss and lower condition (e.g., Bean and Bonner, 2009; Gérard et al., 2017; Ryberg et al., 2020). In our study, *M. asterias* immatures hosted up to 43 helminths in their digestive tract with a mean of  $13 \pm 5$  worms per host, probably resulting in a decrease of energy available for the host with side-effects on its life traits. The spirurine nematode *P. obtusus* was the single helminth species negatively related with host condition (K), and in case of males, with host total mass, suggesting a negative impact on host health. *P. obtusus* is known to induce histopathology since repeated attachment to the gut wall, toxic secretions and external digestion of host tissues by the nematode result in lesions, ulcerations and hemorrhage (Schuurmans Stekhoven and Botman, 1932). No negative relationship was obvious for the other nematode *A. rotundatus* for which, in case of females only, prevalence and abundance were even positively related with host total mass. Here again, we need further parasitological studies to understand these results.

## 5 Conclusions

To conclude, our study describes for the first time the widely diverse and significant community of metazoan parasites of *M. asterias* at an immature stage. We demonstrate a negative relationship between host condition and parasitism by *K. lineata* and *P. obtusus* in immature sharks, suggesting a pathogeny that may compromise future reproduction and/or affect reproductive success. Even if host fitness loss was not obvious for the other taxa of metazoan parasites, one can expect a severe impact of gill and gut metazoan parasites on the health of the most heavily infected *M. asterias* individuals or those infected by especially harmful parasites, with potential consequences at the population level. Parasitism is ubiquitous and an additional stress that may regulate host populations (e.g., Esch et al., 1997; Knudsen et al., 2002; Fraimer et al., 2018). Therefore, it is crucial to understand complex host-parasite relationships and cumulative pathogen effects depending on parasite taxa, host characteristics (life stage, sex) and environmental factors, and to know how metazoan parasites may contribute to the decline of vulnerable host species. Moreover, we demonstrated the use of some parasite taxa to increase knowledge on host feeding ecology and spatial distribution. Since conflicting results occur about the existence of a single biological stock unit for *M. asterias* (McCully Phillips and Ellis, 2015; Brevé et al., 2016; ICES WGEF, 2019), parasites may help to discriminate sub-populations and to assess the

metapopulation-like stock structure. Another aspect to consider in further research is the bioindication by parasitic helminths of environmental pollutants such as heavy metals, which accumulate more in parasites than in host tissues (De Buron et al., 2009 for review), as demonstrated for *Anthobothrium* sp. infecting the shark *Carcharhinus dussumieri* (Malek et al., 2007). Parasites are ubiquitous but rarely incorporated into framework, despite they may constitute a confounding factor leading to biased interpretations if not accounted for (Frainer et al., 2018; Timi and Poulin, 2020 for reviews). Thus, future research must include parasitological studies in multidisciplinary research programs on *M. asterias* in order to improve the efficiency of sustainable conservation and management strategies.

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## Appendix A

Appendix comprises seven tables that detail statistical results. [Table A1](#) Effect of host sex on the prevalence and abundance of parasite taxa with total prevalence >5%. [Table A2](#) Effect of parasites' prevalence on host biometrics and body condition (both sexes confounded). [Table A3](#) Effect of parasites' abundance on host biometrics and body

condition (both sexes confounded). [Table A4](#) Effect of parasites' prevalence on host biometrics and body condition (males only). [Table A5](#) Effect of parasites' abundance on host biometrics and body condition (males only). [Table A6](#) Effect of parasites' prevalence on host biometrics and body condition (females only). [Table A7](#) Effect of parasites' abundance on host biometrics and body condition (females only).

**Table A1.** Effect of host sex on the prevalence and abundance of parasite taxa with total prevalence >5%. Prevalence: likelihood ratio test on generalized linear models (distribution: binomial, link function: logit). Abundance: F tests on generalized linear models (distribution: quasi-Poisson, link function: log).

	Prevalence			Abundance		
	$\chi^2$	df	P	F	df	P
Myxozoa						
Unidentified Myxosporea	0.011	1	0.918	–	–	–
Monogenea						
<i>Erpocotyle laevis</i>	3.234	1	0.072	8.261	1. 18	0.010
Digenea						
<i>Ptychogonimus megastomum</i>	0.229	1	0.632	0.188	1. 18	0.670
Cestoda						
<i>Anthobothrium</i> sp.	0.011	1	0.918	4.701	1. 18	0.044
<i>Eutetrarhynchus</i> sp.	9.008	1	0.003	6.064	1. 18	0.024
Nematoda						
<i>Acanthocheilus rotundatus</i>	1.770	1	0.183	0.475	1. 18	0.500
<i>Proleptus obtusus</i>	0.004	1	0.948	0.747	1. 18	0.399
Copepoda						
<i>Kroyeria lineata</i>	2.292	1	0.130	1.233	1. 18	0.281

**Table A2.** Effect of parasites' prevalence on host biometrics and body condition (both sexes confounded). F tests on linear models.

	Total length		Total mass		Hepato-Somatic Index		Fulton's K	
	$F_{1,18}$	P	$F_{1,18}$	P	$F_{1,18}$	P	$F_{1,18}$	P
Myxozoa								
Unidentified Myxosporea	0.931	0.347	0.908	0.353	8.887	0.008	0.002	0.966
Monogenea								
<i>Erpocotyle laevis</i>	4.564	0.047	3.114	0.095	3.552	0.076	0.035	0.853
Digenea								
<i>Ptychogonimus megastomum</i>	3.447	0.080	2.976	0.102	0.727	0.405	0.162	0.692
Cestoda								
<i>Anthobothrium</i> sp.	0.469	0.502	0.145	0.707	0.230	0.637	1.650	0.215
<i>Eutetrarhynchus</i> sp.	0.622	0.441	1.733	0.205	2.254	0.151	0.724	0.406
Nematoda								
<i>Acanthocheilus rotundatus</i>	0.209	0.653	0.109	0.745	0.874	0.362	0.520	0.480
<i>Proleptus obtusus</i>	1.413	0.250	1.535	0.231	3.459	0.079	0.108	0.746
Copepoda								
<i>Kroyeria lineata</i>	1.951	0.180	2.962	0.102	0.690	0.417	0.121	0.732
All parasites	–	–	–	–	–	–	–	–

**Table A3.** Effect of parasites' abundance on host biometrics and body condition (both sexes confounded). F tests on linear models.

	Total length		Total mass		Hepato-Somatic Index		Fulton's K	
	$F_{1,18}$	$P$	$F_{1,18}$	$P$	$F_{1,18}$	$P$	$F_{1,18}$	$P$
Myxozoa								
Unidentified Myxosporea	–	–	–	–	–	–	–	–
Monogenea								
<i>Erpocotyle laevis</i>	0.213	0.650	0.011	0.917	4.554	0.047	1.277	0.273
Digenea								
<i>Ptychogonimus megastomum</i>	3.365	0.083	3.657	0.072	3.606	0.074	0.085	0.774
Cestoda								
<i>Anthobothrium</i> sp.	0.443	0.514	0.016	0.902	1.273	0.274	1.635	0.217
<i>Eutetrarhynchus</i> sp.	1.215	0.285	1.011	0.328	1.017	0.327	0.009	0.927
Nematoda								
<i>Acanthocheilus rotundatus</i>	0.005	0.943	0.014	0.907	0.699	0.414	0.991	0.333
<i>Proleptus obtusus</i>	1.504	0.236	3.335	0.085	1.395	0.253	5.770	0.027
Copepoda								
<i>Kroyeria lineata</i>	0.594	0.451	1.691	0.210	0.074	0.788	1.601	0.222
All parasites	0.947	0.344	1.913	0.184	0.009	0.924	1.442	0.245

**Table A4.** Effect of parasites' prevalence on host biometrics and body condition (males only). F tests on linear models.

	Total length		Total mass		Hepato-Somatic Index		Fulton's K	
	$F_{1,18}$	$P$	$F_{1,18}$	$P$	$F_{1,18}$	$P$	$F_{1,18}$	$P$
Myxozoa								
Unidentified Myxosporea	0.308	0.590	0.367	0.557	6.043	0.032	0.318	0.584
Monogenea								
<i>Erpocotyle laevis</i>	3.145	0.104	2.752	0.125	0.399	0.541	0.109	0.747
Digenea								
<i>Ptychogonimus megastomum</i>	2.327	0.155	1.908	0.195	0.364	0.559	0.004	0.952
Cestoda								
<i>Anthobothrium</i> sp.	0.003	0.958	0.015	0.904	0.745	0.407	1.132	0.310
<i>Eutetrarhynchus</i> sp.	0.699	0.421	2.378	0.151	0.477	0.504	3.171	0.103
Nematoda								
<i>Acanthocheilus rotundatus</i>	1.198	0.297	1.388	0.264	3.587	0.085	0.001	0.982
<i>Proleptus obtusus</i>	15.772	0.002	13.448	0.004	1.474	0.250	0.112	0.745
Copepoda								
<i>Kroyeria lineata</i>	1.082	0.321	2.942	0.114	0.042	0.842	5.509	0.039
All parasites	–	–	–	–	–	–	–	–



**Table A5.** Effect of parasites' abundance on host biometrics and body condition (males only). F tests on linear models.

	Total length		Total mass		Hepato-Somatic Index		Fulton's K	
	$F_{1,18}$	$P$	$F_{1,18}$	$P$	$F_{1,18}$	$P$	$F_{1,18}$	$P$
Myxozoa								
Unidentified Myxosporea	–	–	–	–	–	–	–	–
Monogenea								
<i>Erpocotyle laevis</i>	1.064	0.325	0.865	0.372	1.290	0.280	0.010	0.921
Digenea								
<i>Ptychogonimus megastomum</i>	2.486	0.143	2.297	0.158	2.133	0.172	0.002	0.966
Cestoda								
<i>Anthobothrium</i> sp.	0.453	0.515	0.080	0.783	0.247	0.629	2.706	0.128
<i>Eutetrarhynchus</i> sp.	1.224	0.292	0.646	0.439	0.128	0.728	0.267	0.615
Nematoda								
<i>Acanthocheilus rotundatus</i>	2.620	0.134	3.352	0.094	1.231	0.291	0.455	0.514
<i>Proleptus obtusus</i>	1.836	0.203	3.724	0.080	0.703	0.420	6.102	0.031
Copepoda								
<i>Kroyeria lineata</i>	0.462	0.511	1.290	0.280	0.004	0.950	1.448	0.254
All parasites	1.025	0.333	2.252	0.162	0.009	0.927	1.801	0.207

**Table A6.** Effect of parasites' prevalence on host biometrics and body condition (females only). F tests on linear models.

	Total length		Total mass		Hepato-Somatic Index		Fulton's K	
	$F_{1,18}$	$P$	$F_{1,18}$	$P$	$F_{1,18}$	$P$	$F_{1,18}$	$P$
Myxozoa								
Unidentified Myxosporea	0.621	0.466	0.551	0.491	5.785	0.061	0.565	0.486
Monogenea								
<i>Erpocotyle laevis</i>	3.781	0.110	3.097	0.139	3.145	0.136	0.005	0.947
Digenea								
<i>Ptychogonimus megastomum</i>	0.965	0.371	1.204	0.323	1.439	0.284	0.172	0.695
Cestoda								
<i>Anthobothrium</i> sp.	1.073	0.648	0.603	0.473	0.164	0.702	0.521	0.503
<i>Eutetrarhynchus</i> sp.	0.097	0.768	0.049	0.834	0.004	0.952	1.084	0.346
Nematoda								
<i>Acanthocheilus rotundatus</i>	5.504	0.066	8.017	0.037	1.423	0.286	0.248	0.640
<i>Proleptus obtusus</i>	3.781	0.110	3.097	0.139	3.145	0.136	0.005	0.947
Copepoda								
<i>Kroyeria lineata</i>	1.250	0.314	1.443	0.283	0.429	0.542	1.002	0.363
All parasites	–	–	–	–	–	–	–	–

**Table A7.** Effect of parasites' abundance on host biometrics and body condition (females only). F tests on linear models.

	Total length		Total mass		Hepato-Somatic Index		Fulton's K	
	$F_{1,18}$	$P$	$F_{1,18}$	$P$	$F_{1,18}$	$P$	$F_{1,18}$	$P$
Myxozoa								
Unidentified Myxosporea	–	–	–	–	–	–	–	–
Monogenea								
<i>Erpocotyle laevis</i>	0.139	0.725	0.000	0.999	1.327	0.301	0.431	0.541
Digenea								
<i>Ptychogonimus megastomum</i>	0.965	0.371	1.204	0.323	1.439	0.284	0.172	0.695
Cestoda								
<i>Anthobothrium</i> sp.	0.396	0.557	0.101	0.764	0.439	0.537	0.323	0.594
<i>Eutetrarhynchus</i> sp.	0.001	0.977	0.016	0.905	0.139	0.725	0.404	0.553
Nematoda								
<i>Acanthocheilus rotundatus</i>	7.295	0.043	9.017	0.030	0.154	0.711	0.191	0.681
<i>Proleptus obtusus</i>	3.781	0.110	3.097	0.139	3.145	0.136	0.005	0.947
Copepoda								
<i>Kroyeria lineata</i>	0.224	0.656	0.155	0.710	0.011	0.922	1.344	0.299
All parasites	0.017	0.901	0.230	0.652	1.320	0.301	0.446	0.534