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Macroparasites of five species of ray (genus *Raja*) on the northwest coast of Spain

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ABSTRACT: A parasitological study of rays captured on the Atlantic continental shelf off the estuary Muros-Noia in NW Spain (42° 35′ to 42° 41′ N, 9° 2′ to 9° 10′ W; mean capture depth 11.6 ± 4.1 m) was performed. A total of 128 rays were examined: 52 specimens of *Raja microocellata*, 60 of *R. brachyura*, 6 of *R. montagui*, 3 of *R. undulata* and 7 of an unidentified *Raja* species, known locally as 'fancheca'. A total of 23 macroparasite species were detected: 5 monogeneans (*Acanthocotyle* sp., *Calicotyle kroyeri*, *Empruthotrema raiae*, *Merizocotyle undulata*, *Rajonchocotyle emarginata*), 11 cestodes (*Acanthobothrium* sp., *Crossobothrium* sp., *Echeneibothrium* sp., *Echinobothrium brachysoma*, *Grillotia erinaceus*, *Grillotia* sp., *Lecanicephalum* sp., *Nybelinia lingualis*, *Onchobothrium uncinatum*, *Phyllobothrium lactuca*, *Tritaphros retzii*), 6 nematodes (*Anisakis simplex*, *Hysterothylacium* sp., *Histodytes microocellatus*, *Piscicapillaria freemani*, *Proleptus* sp., *Pseudanisakis baylisi*) and a copepod (*Holobomolochus* sp.). All parasite species were present in several ray species, except for *Acanthocotyle* sp. and *G. erinaceus* (detected only in *R. brachyura*), *H. microocellatus* (detected only in *R. microocellata*) and *T. retzii* (detected only in *R. montagui*). Three species (*C. kroyeri*, *M. undulata*, *E. brachysoma*) have not been reported previously from Spain. The host with the highest parasite species richness was *R. brachyura* (18 species), followed by *R. microocellata* (17) and the unidentified *Raja* species (14). The parasite with the highest prevalence in *R. microocellata* was *M. undulata*, followed by *R. emarginata*, *Acanthobothrium* sp. and *Echeneibothrium* sp. The species with the highest prevalence in *R. brachyura* was *R. emarginata*, followed by *C. kroyeri* and *P. baylisi*. Some differences in parasite prevalence were detected between sexes and among size classes in both *R. brachyura* and *R. microocellata*.

KEY WORDS: *Raja* spp. · Monogenea · Cestoda · Nematoda · Copepoda · Galicia (NW Spain)

INTRODUCTION

The genus *Raja* (Chondrichthyes: Rajidae) comprises numerous species that feed mainly on benthic invertebrates and vertebrates (Stehmann & Bürkel 1989). The rays act as definitive hosts for diverse parasites that use ray prey species as intermediate hosts. As in other elasmobranchs, the high concentration of urea in tissues and body fluids means that the parasite fauna is highly specialized (Williams 1964, Williams et al. 1970, Cislo & Caira 1993). Detailed knowledge of ray parasite faunas is desirable, since several ray species are currently being evaluated for aquaculture potential.

Most studies published to date on the parasite fauna of elasmobranch hosts have been species descriptions and listings without epidemiological data. The species that has been most intensively studied is *Raja radiata* (Lyaĭman & Borovkova 1926, Threlfall 1969, Zubchenko & Karasev 1986, Rokicki et al. 2001). There have also been studies of the parasite faunas of *R. hyperborea* (Rokicki et al. 2001), *R. naevus* (McVicar 1977) and *R. undulata* (Sanmartín et al. 2000b). We are not aware of any comprehensive studies of the parasite fauna of other species of this genus. The present study considered macroparasites of various species of *Raja* captured off the Atlantic coast of Galicia in NW Spain.

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MATERIALS AND METHODS

We examined 52 specimens of *Raja microocellata*, 60 of *R. brachyura*, 6 of *R. montagui*, 3 of *R. undulata* and 7 of an unidentified *Raja* species called 'fancheca' by local fishermen. At least 10 specimens were captured and examined per month between March 1999 and March 2000. All captures were from the Atlantic continental shelf off the mouth of the estuary of Muros-Noia, in an area situated at 42° 35′ to 42° 41′ N, 9° 2′ to 9° 10′ W. Mean capture depth was 11.6 ± 4.1 m. All specimens were captured with a traditional local fishing apparatus called a 'trasmallo' or 'miño' (Rivas Lago 1996). Fish were transferred to the laboratory in refrigerated containers, and examined after storage at 4°C for 2 nights or less.

Before dissection, rays were measured and weighed. The following organs and tissues were examined under a stereomicroscope for helminths and crustacean parasites: skin, nasal fossae, gills, heart, digestive apparatus, liver, spleen, gonads, kidneys and urinary ducts. The digestive apparatus was divided into esophagus, stomach, spiral intestine and cloaca; the spiral intestine was divided into successive loops, while the remaining parts of the digestive tract were opened longitudinally.

All helminths found were washed in physiological saline, then killed and relaxed in Berland's fluid. Both helminths and crustaceans were fixed and conserved in 70° alcohol. Specimens were then processed for microscopy by standard procedures.

Prevalence, mean abundance and mean intensity were calculated as described by Bush et al. (1997). These parameters were calculated only for *Raja microocellata* and *R. brachyura*, since host sample size for the remaining host species was insufficient to ensure reliable results. Calculations were performed using Microsoft Excel 2000. With the aim of assessing whether parasite faunas differ with host age, the rays of these 2 species were divided into classes on the basis of body length: <45, 46 to 50, 51 to 55 and >55 cm for *R. microocellata* and <45, 46 to 50, 51 to 55, 56 to 60 and >60 cm for *R. brachyura*. We then calculated prevalence, mean abundance and mean intensity in each size class of each species. These parameters were also calculated individually for each sex of these 2 species. The statistical significance of between-group differences was evaluated by Fisher exact tests (prevalence), by Mann-Whitney tests (mean abundance and mean intensity, comparisons between species and between sexes), or by Dunn tests after Kruskal-Wallis analysis (mean abundance and mean intensity, comparisons among size classes). Statistical analyses were done with GraphPad Instat Version 3.00; p-values of <0.05 were taken to indicate statistical significance.

Bootstrap means for plotting the cumulative species richness graph for *Raja montagui*, *R. undulata* and *Raja* sp. were calculated using the informatics application EstimateS Version 7.5 (Colwell 2005).

RESULTS

A total of 23 parasite species were detected: 22 helminths and 1 copepod. Table 1 summarizes prevalence, intensity and abundance data for parasites found in *Raja microocellata* and *R. brachyura*. Table 2 displays the presence/absence of the parasite species found in all the host species considered.

Considering all ray species together, in the gills we found the monogenean *Rajonchocotyle emarginata*. In the nasal fossae, we found *R. emarginata* and the monogeneans *Empruthotrema raiae*, *Merizocotyle undulata* and *Acanthocotyle* sp., as well as the copepod *Holobomolochus* sp. In the stomach, we found the nematodes *Anisakis simplex* (L_3 larvae) and *Pseudanisakis baylisi*. In both the stomach and intestine, we found the cestode *Nybelinia lingualis*; in the intestines only, we found the cestodes *Echinobothrium brachysoma*, *Grillotia erinaceus*, *Onchobothrium uncinatum*, *Phyllobothrium lactuca*, *Tritaphros retzii* and unidentified species of the genera *Grillotia*, *Acanthobothrium*, *Echeneibothrium*, *Lecanicephalum* and *Crossobothrium*, as well as L_3 larvae of the nematode *Hysterothylacium* sp. and adults of the nematodes *Piscicapillaria freemani* and *Proleptus* sp. Finally, we found *Calicotyle kroyeri* in the cloaca and *Histodytes microocellatus* in diverse organs and tissues. In the case of *M. undulata*, *C. kroyeri* and *E. brachysoma*, these are the first reports from Spain. All parasite species were present in several host species, except for *Acanthocotyle* sp. and *G. erinaceus*, which were present only in *Raja brachyura*; *H. microocellatus*, which was present only in *Raja microocellata*; and *T. retzii*, which was present only in *Raja montagui*.

The host with the largest number of parasite species was *Raja brachyura* (18 parasites), followed by *R. microocellata* (17) and the unidentified *Raja* sp. (14). Of particular note is the large number of parasite species found in the unidentified *Raja* sp., given that only 7 specimens of this species were examined. Host sample sizes were likewise small for *R. montagui* (6 hosts, 7 parasite species) and *R. undulata* (3 hosts, 10 parasite species). A cumulative species richness graph is given for more accurate estimation of the species richness in these 3 species (Fig. 1).

In *Raja microocellata*, the parasite with the highest prevalence was *Merizocotyle undulata*, followed by *Rajonchocotyle emarginata*, *Acanthobothrium* sp. and *Echeneibothrium* sp. (Table 1). The species showing

Table 1. *Raja microocellata* and *R. brachyura*. Prevalence, mean intensity and mean abundance of helminths and copepod parasites in *R. microocellata* (Rmi) and *R. brachyura* (Rbr) from the estuary Muros-Noia (Galicia, NW Spain). *Significant difference between both species at p ≤ 0.05. –: no parasites of this species found in this host

	Prevalence (%)		Mean intensity ± SD (range)		Mean abundance ± SD		Dispersion index	
	Rmi	Rbr	Rmi	Rbr	Rmi	Rbr	Rmi	Rbr
Monogenea								
Acanthocotyle sp.	–	5.0	–	13.7 ± 17.0 (1–33)	–	0.7 ± 4.3	–	27.6
Calicotyle kroyeri	19.2	46.7*	2.0 ± 1.6 (1–6)	1.9 ± 1.3 (1–6)	0.4 ± 1.0	0.9 ± 1.3*	2.8	1.8
Emprutohtrema raiae	–	11.7	–	1.0 (1)	–	0.1 ± 0.3	–	0.9
Merizocotyle undulata	96.2	–	64.0 ± 38.1 (2–181)	–	61.5 ± 39.4	–	25.2	–
Rajonchocotyle emarginata	94.2	91.7	47.6 ± 51.7 (1–213)	7.0 ± 5.6 (1–24)*	44.8 ± 51.4	6.4 ± 5.7*	59.0	5.1
Eucestoda								
Acanthobothrium sp.	71.2	33.3*	85.0 ± 173.6 (1–945)	142.0 ± 147.2 (12–631)*	60.5 ± 150.9	47.3 ± 107.4*	376.6	243.7
Crossobothrium sp.	19.2	6.7	3.6 ± 3.6 (1–13)	9.3 ± 13.9 (1–30)	0.7 ± 2.1	0.6 ± 3.9	6.3	24.7
Echeneibothrium sp.	61.5	3.3*	7.7 ± 10.6 (1–57)	1.5 ± 0.7 (1–2)	4.7 ± 9.1	0.1 ± 0.3*	17.5	1.6
Echinobothrium brachysoma	7.7	8.3	5.8 ± 4.9 (1–109)	3.0 ± 1.0 (2–4)	0.4 ± 2.0	0.3 ± 0.9	8.6	3.1
Grillotia erinaceus	–	25.0	–	1.5 ± 0.7 (1–3)	–	0.4 ± 0.8	–	1.5
Grillotia sp.	59.6	11.7*	22.5 ± 26.9 (1–112)	6.0 ± 6.5 (2–20)*	13.4 ± 23.5	0.7 ± 2.8*	41.0	11.5
Nybelinia lingualis	17.3	5.0	1.3 ± 0.5 (1–2)	1.3 ± 0.6 (1–2)	0.2 ± 0.5	0.1 ± 0.3	1.3	1.5
Onchobothrium uncinatum	15.4	–	2.1 ± 1.6 (1–5)	–	0.3 ± 1.0	–	2.8	–
Phyllobothrium lactuca	51.9	33.3	4.9 ± 3.6 (1–12)	3.4 ± 2.5 (1–8)	2.5 ± 3.6	1.1 ± 2.2*	5.0	4.1
Nematoda								
Anisakis simplex	5.8	8.3	1.3 ± 0.6 (1–2)	1.6 ± 0.5 (1–2)	0.1 ± 0.3	0.1 ± 0.5	1.5	1.6
Hysterothylacium sp.	1.9	1.7	1.0 (1)	1.0 (1)	0.02 ± 0.1	0.02 ± 0.1	1.0	1.0
Histodytes microocellatus	21.2	–	2.1 ± 1.6 (1–6)	–	0.4 ± 1.1	–	2.9	–
Piscicapillaria freemani	1.9	3.3	1.0 (1)	2.5 ± 0.7 (2–3)	0.02 ± 0.1	0.1 ± 0.5	1.0	2.6
Proleptus sp.	9.6	1.7	3.4 ± 3.7 (1–10)	1.0 (1)	0.3 ± 1.5	0.02 ± 0.1	6.4	1.0
Pseudanisakis baylisi	17.3	38.3*	2.9 ± 2.2 (1–8)	3.4 ± 1.7 (1–7)	0.5 ± 1.4	1.3 ± 2.0*	4.0	3.0
Copepoda								
Holobomolochus sp.	84.6	46.7*	31.4 ± 41.3 (1–163)	8.5 ± 15.0 (1–61)*	26.6 ± 39.6	4.0 ± 11.0*	59.1	30.5

Table 2. *Raja* spp. Presence (+)/absence (–) of helminths and copepod parasites in the 5 different *Raja* species from the estuary Muros-Noia (Galicia, NW Spain)

	R. microocellata	*R. brachyura*	*R. montagui*	*R. undulata*	*Raja* sp.
Monogenea					
Acanthocotyle sp.	–	+	–	–	–
Calicotyle kroyeri	+	+	–	–	+
Empruthotrema raiae	–	+	+	–	+
Merizocotyle undulata	+	–	–	+	+
Rajonchocotyle emarginata	+	+	–	+	+
Eucestoda					
Acanthobothrium sp.	+	+	+	+	+
Crossobothrium sp.	+	+	–	+	+
Echeneibothrium sp.	+	+	+	+	+
Echinobothrium brachysoma	+	+	+	–	–
Grillotia erinaceus	–	+	–	–	–
Grillotia sp.	+	+	–	+	+
Lecanicephalum sp.	–	–	+	–	+
Nybelinia lingualis	+	+	–	–	+
Onchobothrium uncinatum	+	–	–	+	+
Phyllobothrium lactuca	+	+	–	+	+
Tritaphros retzii	–	–	+	–	–
Nematoda					
Anisakis simplex	+	+	–	–	–
Hysterothylacium sp.	+	+	–	–	+
Histodytes microocellatus	+	–	–	–	–
Piscicapillaria freemani	+	+	–	–	+
Proleptus sp.	+	+	+	+	–
Pseudanisakis baylisi	+	+	–	–	–
Copepoda					
Holobomolochus sp.	+	+	–	+	–

the highest mean intensity and mean abundance was *Acanthobothrium* sp., followed by *M. undulata*, *R. emarginata* and *Grillotia* sp. In *Raja brachyura*, the parasite with the highest prevalence was *R. emarginata*, followed by *Calicotyle kroyeri* and *Pseudanisakis baylisi*. The species showing the highest mean intensity and mean abundance was again *Acanthobothrium* sp.

Acanthobothrium sp., *Echeneibothrium* sp., *Grillotia* sp. and *Holobomolochus* sp. showed significantly higher prevalence in *Raja microocellata* (Table 1). In contrast, *Calicotyle kroyeri* and *Pseudanisakis baylisi* showed significantly higher prevalence in *Raja brachyura*. Species with a significantly higher mean abundance in *R. microocellata* than in *R. brachyura* were *Rajonchocotyle emarginata*, *Acanthobothrium* sp., *Echeneibothrium* sp., *Grillotia* sp., *Phyllobothrium lactuca* and *Holobomolochus* sp., while species with a significantly higher mean abundance in *R. brachyura* than in *R. microocellata* were *P. baylisi* and *C. kroyeri*. (Note that both mean abundance and mean intensity of this latter species were very similar in the

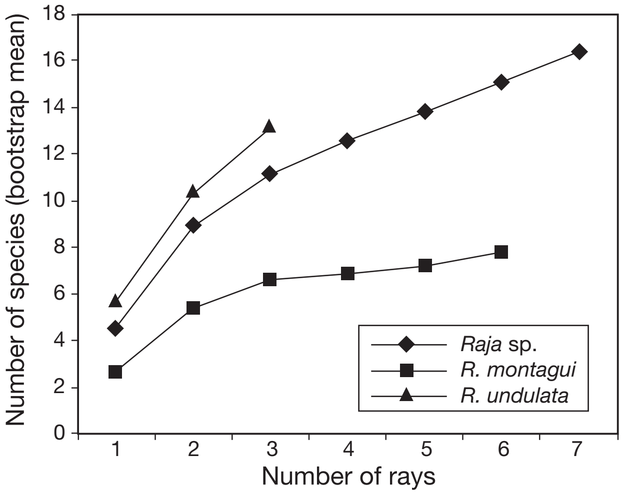

Fig. 1. *Raja montagui*, *Raja undulata* and *Raja* sp. Cumulative species richness curves for parasites in rays

2 host species.) Species with significantly higher mean infection intensities in *R. microocellata* than in *R. brachyura* were *R. emarginata*, *Holobomolochus* sp. and *Grillotia* sp., while *Acanthobothrium* sp. showed significantly higher mean infection intensity in *R. brachyura*. (Note that in the case of *Grillotia* sp., which had a mean intensity markedly higher in *R. microocellata*, see Tables 1 & 2, the Mann-Whitney test gave a marginally significant p-value of 0.08, but Kolmogorov-Smirnov tests indicated that intensity showed a normal distribution in both hosts, and a subsequent *t*-test with Welsh's correction gave a clearly significant p-value of 0.004.)

The number of species present increased with size in both *Raja brachyura* (Table 3) and *R. microocellata* (Table 4), although in *R. brachyura* species richness in the >60 cm group was slightly lower than in the 56 to 60 cm group.

In *Raja brachyura* (Table 3), parasites showing increasing prevalence with increasing host size were *Acanthocotyle* sp. and *Piscicapillaria freemani* (both present only in the largest size class), *Empruthotrema raiae* (present only in the 3 largest size classes), *Grillotia erinaceus* and *Holobomolochus* sp. (though this latter species showed higher prevalence in the <45 cm class rather than in the 46 to 50 cm size class). Statistically significant differences were observed only for *Holobomolochus* sp., between the >60 cm group and the <45, 46 to 50 and 51 to 55 cm groups. The same parasite species, and also *Rajonchocotyle emarginata*, showed increasing mean abundance with increasing size; again, statistically significant differences were observed only for *Holobomolochus* sp. between the >60 cm group and the 46 to 50 and 51 to 55 cm groups. Finally, parasites showing increasing mean intensity with increasing host size were *Acanthocotyle* sp.,

Table 3. *Raja brachyura*. Prevalence, mean abundance and mean intensity of helminths and copepod parasites in *R. brachyura* of different size classes (measurements in cm). Number of hosts examined given in **bold**. Superscripts: within each species and parameter, values sharing at least 1 letter are not significantly different (p > 0.05); '0' values have not been considered for statistical analysis and thus have no superscripts

	Prevalence (%)					Mean abundance					Mean intensity				
	<45	46–50	51–55	56–60	>60	<45	46–50	51–55	56–60	>60	<45	46–50	51–55	56–60	>60
	6	**10**	**20**	**16**	**8**	**6**	**10**	**20**	**16**	**8**	**6**	**10**	**20**	**16**	**8**
Monogenea															
Acanthocotyle sp.	0	0	0	6.3[a]	25.0[a]	0	0	0	0.1[a]	5.0[a]	0	0	0	1.0[a]	20.0[a]
Calicotyle kroyeri	16.7[a]	60.0[a]	55.0[a]	43.8[a]	37.5[a]	0.2[a]	1.2[a]	0.9[a]	0.3[a]	1.6[a]	1.0[a]	2.0[a]	1.5[a]	1.4[a]	4.3[a]
Empruthotrema raiae	0	0	15.0[a]	12.5[a]	25.0[a]	0	0	0.2[a]	0.1[a]	0.3[a]	0	0	1.0[a]	1.0[a]	1.0[a]
Rajonchocotyle emarginata	100[a]	80.0[a]	90.0[a]	100[a]	87.5[a]	4.2[a]	3.5[a]	6.8[a]	7.4[a]	8.6[a]	4.2[a]	4.4[a]	7.6[a]	7.4[a]	9.9[a]
Eucestoda															
Acanthobothrium sp.	100[a]	10.0[b]	15.0[b]	37.5[b]	50.0[b]	176.2[a]	63.1[a]	14.6[a]	14.1[a]	79.4[a]	176.2[a]	631.0[a]	97.3[a]	37.5[a]	158.8[a]
Crossobothrium sp.	0	10.0[a]	10.0[a]	0	12.5[a]	0	0.4[a]	0.2[a]	0	3.8[a]	0	4[a]	1.5[a]	0	30[a]
Echeneibothrium sp.	33.3	0	0	0	0	0.5	0	0	0	0	1.5	0	0	0	0
Echinobothrium brachysoma	50.0[a]	10.0[ab]	5.0[b]	0	0	1.5[a]	0.4[a]	0.1[a]	0	0	3.0[a]	4.0[a]	2.0[a]	0	0
Grillotia erinaceus	0	20.0[a]	25.0[a]	31.3[a]	37.5[a]	0	0.2[a]	0.3[a]	0.6[a]	0.8[a]	0	1.0[a]	1.2[a]	1.8[a]	2.0[a]
Grillotia sp.	66.7[a]	10.0[bc]	0	6.3[bc]	12.5[abc]	5.2[a]	0.2[a]	0	0.4[a]	0.3[a]	7.8[a]	2.0[b]	0	7.0[b]	2.0[b]
Nybelinia lingualis	0	0	5.0[a]	12.5[a]	0	0	0	0.1[a]	0.2[a]	0	0	0	1.0[a]	1.5[a]	0
Phyllobothrium lactuca	33.3[a]	40.0[a]	35.0[a]	31.3[a]	25.0[a]	0.5[a]	0.7[a]	1.5[a]	1.2[a]	1.1[a]	1.5[a]	1.8[a]	4.3[a]	3.8[a]	4.5[a]
Nematoda															
Anisakis simplex	0	10.0	5.0	12.5	12.5	0	0.2	0.1	0.1	0.3	0	2.0	2.0	1.0	2.0
Hysterothylacium sp.	0	0	0	6.3	0	0	0	0	0.1	0	0	0	0	1.0	0
Piscicapillaria freemani	0	0	0	6.3[a]	12.5[a]	0	0	0	0.2[a]	0.3[a]	0	0	0	3.0[a]	2.0[a]
Proleptus sp.	0	0	0	6.3	0	0	0	0	0.1	0	0	0	0	1.0	0
Pseudanisakisbaylisi	83.3[a]	40.0[ab]	40.0[ab]	25.0[b]	25.0[ab]	2.5[a]	1.6[a]	1.5[a]	0.8[a]	0.5[a]	3.0[abc]	4.0[ab]	3.8[ab]	3.3[abc]	2.0[c]
Copepoda															
Holobomolochus sp.	33.3[ab]	20.0[a]	40.0[a]	56.3[ab]	87.5[b]	1.5[ab]	0.9[a]	3.6[a]	3.3[ab]	12.1[b]	4.5[a]	4.5[a]	8.9[a]	5.9[a]	13.9[a]

Table 4. *Raja microocellata*. Prevalence, mean abundance and mean intensity of helminths and copepod parasites in *R. microocellata* of different size classes (measurements in cm). Number of hosts examined given in **bold**. Superscripts: within each species and parameter, values sharing at least 1 letter are not significantly different (p > 0.05); '0' values have not been considered for statistical analysis and thus have no superscripts

	Prevalence (%)				Mean abundance				Mean intensity			
	<45	46–50	51–55	>55	<45	46–50	51–55	>55	<45	46–50	51–55	>55
	7	**14**	**18**	**13**	**7**	**14**	**18**	**13**	**6**	**10**	**20**	**16**
Monogenea												
Calicotyle kroyeri	0	42.9^{a}	5.6^{b}	23.1^{ab}	0	0.6	0.3	0.5	0	1.3	6.0	2.0
Merizocotyle undulata	85.7^{a}	100^{a}	94.4^{a}	100^{a}	48^{a}	71.5^{a}	62.2^{a}	57.2^{a}	56^{a}	71.5^{a}	65.8^{a}	57.2^{a}
Rajonchocotyle emarginata	71.4^{a}	92.9^{a}	100^{a}	100^{a}	16.9^{a}	60.0^{a}	32.5^{a}	60.6^{a}	23.6^{a}	64.6^{a}	32.5^{a}	60.6^{a}
Eucestoda												
Acanthobothrium sp.	57.1^{a}	78.6^{a}	66.7^{a}	76.9^{a}	68.9^{a}	27.7^{a}	70.5^{a}	77.3^{a}	120.5^{a}	35.3^{a}	105.8^{a}	100.5^{a}
Crossobothrium sp.	14.3^{a}	21.4^{a}	22.2^{a}	15.4^{a}	0.1^{a}	0.6^{a}	1.0^{a}	0.7^{a}	1.0^{a}	2.7^{a}	4.5^{a}	4.5^{a}
Echeneibothrium sp.	85.7^{a}	71.4^{a}	66.7^{a}	30.8^{a}	14.9^{a}	5.6^{ab}	2.8^{ab}	1.1^{b}	17.3^{a}	7.8^{a}	4.2^{a}	3.5^{a}
Echinobothriumbrachysoma	28.6^{a}	0	5.6^{a}	7.7^{a}	1.6^{a}	0	0.6^{a}	0.2^{a}	5.5^{a}	0	10^{a}	2.0^{a}
Grillotia sp.	42.9^{a}	71.4^{a}	61.1^{a}	53.8^{a}	24.7^{a}	16.9^{a}	7.9^{a}	11.2^{a}	57.7^{a}	23.7^{a}	12.9^{a}	20.9^{a}
Nybelinia lingualis	0	21.4^{a}	27.8^{a}	7.7^{a}	0	0.2^{a}	0.4^{a}	0.2^{a}	0	1.0^{a}	1.4^{a}	2.0^{a}
Onchobothrium uncinatum	28.6^{a}	0	27.8^{a}	7.7^{a}	0.7^{a}	0	0.4^{a}	0.4^{a}	2.5^{a}	0	1.4^{a}	5.0^{a}
Phyllobothrium lactuca	28.6^{a}	50^{a}	55.6^{a}	61.5^{a}	1.3^{a}	2.4^{a}	2.4^{a}	3.6^{a}	4.5^{a}	4.7^{a}	4.3^{a}	5.9^{a}
Nematoda												
Anisakis simplex	0	7.1^{a}	0	15.4^{a}	0	0.1^{a}	0	0.2^{a}	0	2.0^{a}	0	1.0^{a}
Hysterothylacium sp.	0	0	5.6	0	0	0	0.1	0	0	0	1.0	0
Histodytes microocellatus	0	14.3^{a}	33.3^{a}	23.1^{a}	0	0.5^{a}	0.7^{a}	0.3^{a}	0	3.5^{a}	2.0^{a}	1.3^{a}
Piscicapillaria freemani	0	0	5.6	0	0	0	0.1	0	0	0	1.0	0
Proleptus sp.	14.3^{a}	7.1^{a}	0	23.1^{a}	0.1^{a}	0.1^{a}	0	1.1^{a}	1.0^{a}	2.0^{a}	0	4.7^{a}
Pseudanisakisbaylisi	0	35.7^{a}	11.1^{a}	15.4^{a}	0	1.3^{a}	0.3^{a}	0.2^{a}	0	3.6^{a}	2.5^{a}	1.5^{a}
Copepoda												
Holobomolochus sp.	100^{a}	92.9^{a}	77.8^{a}	76.9^{a}	9.7^{a}	24.7^{a}	31.8^{a}	30.5^{a}	9.7^{a}	26.6^{a}	40.9^{a}	39.6^{a}

R. emarginata and *Holobomolochus* sp., though in this case there were no statistically significant between-class differences.

Parasites showing declining prevalence with increasing host size in *Raja brachyura* (Table 3) were *Calicotyle kroyeri* (though prevalence was lower in the <45 cm class), *Echeneibothrium* sp. (only present in the <45 cm class), *Echinobothrium brachysoma* (absent from the >60 cm class; significantly higher prevalence in the <45 cm class than in the 51 to 55, 56 to 60 and >60 cm classes), *Phyllobothrium lactuca* (though prevalence was lower in the <45 cm class) and *Pseudanisakis baylisi* (significantly higher prevalence in the <45 cm class than in the 56 to 60 cm class).

In *Raja microocellata* (Table 4), *Rajonchocotyle emarginata* and *Phyllobothrium lactuca* showed increasing prevalence with increasing host size; *P. lactuca* showed increasing mean abundance with increasing host size; and *Crossobothrium* sp. and *Nybelinia lingualis* showed increasing mean intensity with increasing host size. No significant between-group differences were detected. In contrast with the pattern seen in *R. brachyura*, the prevalence of *Holobomolochus* sp. declined with increasing host size, while mean abundance and mean intensity of this species did not show any clear trend; again, no significant between-group differences were observed. The only parasite species showing the same trend in both *R. microocellata* and *R. brachyura* was *Echeneibothrium* sp., which showed declining prevalence with increasing size in both hosts: as noted, in *R. brachyura* it was present only in the <45 cm class. The between-group differences were statistically significant only in *R. brachyura* (between the <45 cm class and the 51 to 55 and 56 to 60 cm classes). *Echeneibothrium* sp. likewise showed declining mean abundance and mean intensity with increasing size in both hosts: mean abundance differed significantly between the <45 and >55 cm classes in *R. microocellata* and between the <45 and 51 to 55, 56 to 60 and >60 cm classes in *R. brachyura*.

We did not observe statistically significant differences between sexes in either host species, except for *Rajonchocotyle emarginata*, which showed significantly higher mean abundance in males than females of *Raja microocellata*, and *Grillotia* sp., which showed significantly lower mean intensity in males than in females, again of *R. microocellata* (Table 5). Despite the lack of statistically significant between-sex differences in prevalence, several parasite species showed differences of >10%.

Table 5. *Raja microocellata* and *R. brachyura*. Prevalence, mean intensity and mean abundance of helminths and copepod parasites in males and females of *R. microocellata* and *R. brachyura*. Number of examined hosts given in **bold**. *Significant difference between both sexes at p ≤ 0.05. –: no parasites of this species found in this host

	R. microocellata						*R. brachyura*					
	Prevalence (%)		Mean abundance		Mean intensity		Prevalence (%)		Mean abundance		Mean intensity	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
	24	**26**	**24**	**26**	**24**	**26**	**27**	**23**	**27**	**23**	**27**	**23**
Monogenea												
Acanthocotyle sp.	–	–	–	–	–	–	3.7	8.7	1.2	0.3	33.0	4.0
Calicotyle kroyeri	20.8	15.4	0.5	0.3	2.2	2.0	51.9	52.2	1.0	1.0	1.9	1.9
Empruthotrema raiae	–	–	–	–	–	–	18.5	8.7	0.2	0.1	1.0	1.0
Merizocotyle undulata	100	96.2	61.3	64.6	61.3	67.2	–	–	–	–	–	–
Rajonchocotyle emarginata	100	88.5	56.0	29.7*	56.0	33.6	96.3	95.7	6.4	7.1	6.6	7.5
Eucestoda												
Acanthobothrium sp.	75.0	73.1	90.7	37.2	120.9	50.9	40.7	30.4	70.4	39.4	172.9	129.6
Crossobothrium sp.	16.7	23.1	0.5	0.9	3.0	4.0	–	8.7	–	1.4	–	16.0
Echeneibothrium sp.	54.2	73.1	2.9	6.8	5.4	9.3	3.7	4.3	0.1	4.3×10^{-2}	2.0	1.0
Echinobothrium brachysoma	4.2	11.5	0.4	0.5	10	4.3	14.8	4.3	0.5	0.1	3.3	2.0
Grillotia erinaceus	–	–	–	–	–	–	25.9	17.4	0.5	0.3	1.9	1.5
Grillotia sp.	75.0	50.0	10.0	17.6	13.4	35.2*	18.5	8.7	0.7	1.0	4.0	11.0
Nybelinia lingualis	25.0	7.7	0.4	0.1	1.5	1.0	3.7	4.3	3.7×10^{-2}	0.1	1.0	2.0
Onchobothrium uncinatum	16.7	15.4	0.3	0.4	1.5	2.8	–	–	–	–	–	–
Phyllobothrium lactuca	62.5	46.2	3.3	2.0	5.3	4.3	29.6	34.8	0.9	1.3	3.0	3.8
Nematoda												
Anisakis simplex	4.2	7.7	4.2×10^{-2}	0.1	1.0	1.5	–	13.0	–	0.2	–	1.7
Hysterothylacium sp.	4.2	–	4.2×10^{-2}	–	1.0	–	–	4.3	–	4.3×10^{-2}	–	1.0
Histodytes microocellatus	29.2	15.4	0.5	0.4	1.9	2.5	–	–	–	–	–	–
Piscicapillaria freemani	4.2	–	4.2×10^{-2}	–	1.0	–	3.7	4.3	0.1	0.1	2.0	3.0
Proleptus sp.	12.5	7.7	0.6	0.1	4.7	1.5	–	–	–	–	–	–
Pseudanisakis baylisi	16.7	15.4	0.6	0.4	3.8	2.5	40.7	39.1	1.5	14.3	3.7	3.4
Copepoda												
Holobomolochus sp.	83.3	92.3	36.8	19.2	44.2	20.8	63.0	39.1	5.1	4.1	8.2	10.6

DISCUSSION

Most of the helminth species detected in the present study have been described previously in various elasmobranch species. It should be noted that we have not found digenean trematodes, as in the study by Sanmartín et al. (2000b) of *Raja undulata* in Galicia. Previous studies of other European species of *Raja* have found trematodes, including *Otodistomum* species, *Gonocerca phycidis*, *Derogenes varicus* and *Steganoderma formosum* (Threlfall 1969, McVicar 1977, Rokicki et al. 2001), although in the case of *D. varicus* the infection detected was probably accidental, due to ingestion by the ray of a fish with adult *D. varicus* in its intestinal tract (McVicar 1977).

We found a total of 5 monogenean species, of which *Merizocotyle undulata* and *Rajonchocotyle emarginata* were, respectively, the most prevalent species in *Raja microocellata* and *R. brachyura*. We also found *Calicotyle kroyeri*, an endoparasite that is located in the rectum and rectal gland of elasmobranchs. This species has been reported from >20 elasmobranch species (Lawler 1981, Llewellyn et al. 1984, Chisholm et al. 1997), including several species of the genus *Raja*, such as *R. radiata*, *R. batis*, *R. brachyura*, *R. garmani* and *R. olseni* (Chisholm et al. 1997); it is thus generally considered to show low host specificity. However, Chisholm et al. (2001) reported molecular studies which indicated that *C. kroyeri* is probably a species complex. These authors suggest that this may also be true of other monogeneans that are frequently reported from diverse elasmobranchs, such as *Acanthocotyle lobianchi*, *Empruthotrema raiae* and *R. emarginata* (the latter 2 species being among those detected in the present study).

Most of the cestodes detected in the present study belong to the order Tetraphyllidea, including *Lecanicephalum* sp., since it has been shown on both morphological and molecular grounds that the order Lecanicephalidea should be included within the Tetraphyllidea (Butler 1987, Caira et al. 1999). In Galicia, plerocercoids of the genera *Acanthobothrium* and *Onchobothrium* (generally referred to in previous studies as '*Scolex pleuronectis*') have been found in the digestive tract of diverse teleost species, including *Conger conger* (Sanmartín et al. 2000a), *Anguilla anguilla* (Outeiral et al. 2002), *Ciliata mustela*, *Callionymus lyra*, *Symphodus cinereus* (Peris Caminero 2000) and diverse species of flatfish (Álvarez et al. 2002).

Of the cestodes of the order Trypanorhyncha detected in the present study, *Grillotia* spp. use fishes as their second intermediate host. In Galicia, specifically in the estuaries of estuary Muros-Noia and Arousa, *Grillotia* plerocercoids have been found in species including various members of the genus *Gobius* (Sanmartín et al. 2001), *Conger conger* (Sanmartín et al. 2000a) and flatfish (Álvarez et al. 2002). *Nybelinia lingualis*, in contrast, has not been found to date within Galician estuaries, only offshore. This species uses intermediate host species like *C. conger*, *Hyperoplus lanceolatus* and *Microchirius varieatus* (Quinteiro et al. 1987), as well as cephalopods like *Todaropsis eblanae* (Pascual et al. 1996a,b).

Most authors only recognize *Hysterothylacium aduncum* as valid in European fishes, although Hartwich (1975) distinguishes 3 species (*H. gadi*, *H. aduncum* and *H. auctum*) and Balbuena et al. (1998) suggest that this is probably a species complex, similar to that of *Anisakis simplex*. *H. aduncum* sensu lato uses crustaceans as its first intermediate host, diverse aquatic invertebrates and fishes as its second intermediate and transport hosts and piscivorous fishes as definitive hosts (Køie 1993, Balbuena et al. 1998). Although its cycle is predominantly marine, this parasite has also been detected in freshwater fishes, and indeed its cycle has been completed in the laboratory in freshwater (Yoshinaga et al. 1987). In the present study, we found only 2 L_3 larvae, one in the intestine of a specimen of *Raja brachyura*, the other in the intestine of a specimen of *R. microocellata*. We know of only one previous report of this species in a ray, specifically *R. naevus* (McVicar 1977). In Galicia, adults of *H. aduncum* have been found in *Conger conger*, *Lophius piscatorius*, *Pollachius pollachius*, *Boops boops*, *Scomber scombrus* and *Scophthalmus maximus* (Quinteiro Alonso 1990), while larval forms have been found in diverse fish species (Quinteiro et al. 1987, Quinteiro Alonso 1990, Peris Caminero 2000, Sanmartín et al. 2001).

Anisakis simplex sensu lato uses crustaceans as its first intermediate host, notably euphausiids (Hays et al. 1998), although decapods may also be used (George-Nascimento et al. 1994). The second intermediate and transport hosts are fishes or cephalopods (Abollo et al. 2001), while the definitive hosts are marine mammals. Occasionally it may accidentally infect man, causing anisakiosis. In rays, larval forms of *A. simplex* have been found in *Raja radiata* (Threlfall 1969, Rokicki et al. 2001), *R. hyperborea* (Rokicki et al. 2001) and *R. naevus* (McVicar 1977). In Galicia, larval forms of *A. simplex* have been found in diverse fish species (Quinteiro et al. 1987, Sanmartín et al. 1989, Quinteiro Alonso 1990, Peris Caminero 2000, Abollo et al. 2001, Aguilar et al. 2005) and cephalopods (Pascual et al. 1996a,b, Abollo et al. 2001), as well as in gulls (*Larus cachinnans*; Sanmartín et al. 2005).

We do not know of any previous studies of the parasite fauna of *Raja microocellata*, *R. brachyura* and *R. montagui*. We have previously carried out a study (Sanmartín et al. 2000b) of the helminth fauna of *R. undulata* from an area very close to the capture area of the present study; however, these *R. undulata* specimens came from inside the estuary Muros-Noia (brackish water), while the rays examined in the present study were captured on the continental shelf off the mouth of this estuary. In line with this, there are evident differences between the helminth faunas of *R. undulata* in both localities, despite the fact that in the present study we were able to examine only very few specimens of this species; notably, 2 monogenean species, *Merizocotyle undulata* and *Rajonchocotyle emarginata*, were found in the present study, but not in our previous study of fish captured inside the estuary. Likewise, the only cestodes present in both capture areas were *Phyllobothrium lactuca* and *Onchobothrium uncinatum*; the *Acanthobothrium* and *Echeneibothrium* taxa detected in the present study could not be determined to species level, but are not the same species as the *Acanthobothrium benedeni* and *Echeneibothrium beauchampi* detected in our previous study, while an unidentified *Crossobothrium* species was detected in the present study but not in our previous study. The only nematode found in *R. undulata* in the present study was *Proleptus* sp., which was not detected in our previous study of fish captured within the estuary. In our previous study we did detect a *Schulmanella* (*Piscicapillaria*) species, probably *Piscicapillaria freemani*, as well as *Pseudanisakis baylisi* (=*P. rotundata*); in the present study these species were detected in other ray species, but not in *R. undulata*. This may be due to our small *R. undulata* sample size.

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