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INFLUENCE OF HOST AGE AND SEX ON THE HELMINTH FAUNA OF THE YELLOW-LEGGED GULL (*LARUS MICHAHELLIS*) IN GALICIA (NORTHWESTERN SPAIN)

M. F. Álvarez, J. A. Cordeiro, J. M. Leiro, and M. L. Sanmartín*

Laboratorio de Parasitología, Instituto de Investigación y Análisis Alimentarios, Universidad de Santiago de Compostela, C/ Constantino Candeira, s.n., 15782 Santiago de Compostela, Spain. *e-mail: mpduran@usc.es*

ABSTRACT: We studied the influence of host age and sex on the helminth fauna of 324 *Larus michahellis* captured in different locations in the region of Galicia (northwestern Spain). Gulls were grouped into prefledglings, first-year immature birds, second- and third-year immature birds, and adults. Second-year, third-year, and adult birds were grouped by sex. Thirty-six helminth species were recorded. Total species richness and mean infracommunity species richness were both significantly lower for prefledglings than for the other age groups. Prevalence increased significantly with age for *Brachylecithum microtesticulatum*, probably reflecting changing feeding habits. Likewise, 8 species (*Cardiocephaloides longicollis, Microphallus similis, Maritrema gratiosum, Gynaecotyla longiintestinata, Brachylecithum microtesticulatum, Himasthla elongata, Parorchis acanthus*, and *Renicola* sp.) were absent or had very low prevalence in prefledglings. At least 5 of these 8 species are transmitted to gulls through ingestion of molluscs or crustaceans, which suggests that these types of prey are seldom fed to prefledglings. In *Gymnophallus deliciosus, G. longiintestinata*, and *Cosmocephalus obvelatus*, mean intensity, and in the latter case prevalence, declined with age, suggesting that protective immunity against these species increase with age. Only *G. deliciosus, Microphallus similis*, and *G. longiintestinata* presented significant differences between the sexes.

Many gull species of the genus *Larus* show wide distributions and a marked capacity to thrive in humanized habitats, particularly in view of their ability to feed on waste material (Harris, 1970; Lloyd et al., 1991). As a result, populations of many *Larus* species have increased dramatically in many parts of the world.

Gulls are omnivorous and feed in both marine and terrestrial environments (Cramp and Simmons, 1983; Munilla, 1997). As a consequence, they are susceptible to infection by a wide range of parasites whose larval forms infect fishes, molluscs, or marine crustaceans. They also may be infected by parasites normally harbored by terrestrial animals.

In Galicia (northwestern Spain), the most abundant species is the yellow-legged gull (*Larus michahellis*). Recently (Sanmartín et al., 2005), we provided overall prevalence and intensity data for the helminth species found in this species in Galicia, but there is little information on the effect of such host factors as age and sex on the parasite fauna of *L. michahellis*. The only other study on the helminth fauna of the yellow-legged gull in Spain is that by Bosch et al. (2000), of gulls on the Medes Islands in the Mediterranean Sea, and it studies only the effect of host sex on these parasites. In view of much more diverse helminth fauna of this host species in the Atlantic versus the Mediterranean, we think there also may be differences in the influence of sex on this fauna. Also, an analysis of the variations between age groups may cast some light on the feeding patterns of the different age groups of this gull species, of which there are no studies. Here, we investigate effects of host age and sex on helminth species richness, prevalence, and intensity of *L. michahellis* in Galicia.

MATERIALS AND METHODS

We collected 324 specimens of yellow-legged gull from different locations in the region of Galicia, including the estuaries of Arousa, Pontevedra, and Vigo; various islands close to the coast (now Parque Natural de las Islas Atlánticas, containing very large breeding colonies); and the municipal waste dumps of the cities of A Coruña (on the coast) and Santiago de Compostela (inland), where gulls feed in large numbers on organic waste. Gull capture was performed between June 1994 and February 1996 with appropriate permission from the regional and local authorities, by using nets and traps. In addition, the Wild Bird Recovery Centre in Cotorredondo supplied us with several specimens that had been found dead or dying in different locations within the above-mentioned estuaries and that had been stored by deep freezing.

Live gulls were killed by an overdose of anesthetic and then measured, weighed, aged, and sexed. They were classified to species following Burger and Gochfeld (1996) in all cases as *Larus cachinnans*, more recently reclassified as *L. michahellis* (Liebers et al., 2001; Yésou, 2002). Most birds were frozen at −20 C until dissection. After thawing, the viscera, eyes, and air sacs were removed and examined. The digestive tract was divided into esophagus, proventriculus, gizzard, intestine, intestinal caeca, rectum, and cloaca. All helminths found were washed in physiological saline. Helminths from birds examined fresh were relaxed and killed in Berland's fluid, fixed and preserved in 70% alcohol, and then mounted for microscopic observation by using standard procedures (Cordeiro Paredes, 2004). Samples of muscle tissue were examined for *Trichinella* spp.

The gulls were categorized into 4 age groups on the basis of plumage: (1) prefledglings (not yet able to fly); (2) first-year immature birds (hereafter "Y1 birds"); (3) second- and third-year immature birds (hereafter "Y2/3 birds"); and (4) adults. Sex was recorded only for Y2/3 and adult birds, because it was difficult to determine in younger birds.

Prevalence, mean abundance, and mean intensity were calculated after Bush et al. (1997). Total species richness (S_T) for age and sex groups was determined as total number of helminth species detected considering all host individuals in that age or sex group. Mean infracommunity species richness (MS_i) within each gull age or sex group was calculated as $\Sigma S_i/n$, where S_i is the number of helminth species detected in each gull of that sex/age group, and n is the number of gulls in that group.

Prevalences were compared between groups by Fisher's exact test. Mean abundances, mean intensities and MS_i values were compared among age groups by the Kruskal–Wallis test with Dunn's post-test for subsequent one-to-one comparisons, and between the sexes by the Mann–Whitney *U*-test. Significance was assumed when $P \leq 0.05$. All statistical tests were performed with GraphPad InStat, version 3.05 (GraphPad Software Inc., San Diego, California).

Species with an overall prevalence under 1% (Sanmartín et al., 2005) were considered to be accidental in *L. michahellis* in Galicia, and no statistical analyses were performed on them.

RESULTS

Species richness

Thirty-six helminth species were recovered. S_T and MS_i were lowest in prefledglings (S_T = 19, MS_i = 2.3) and highest in Y1 birds (S_T = 29, MS_i = 4.0). Values for older birds were inter-

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* To whom correspondence should be addressed.

TABLE I. Prevalence and intensity statistics considering all helminth parasites as a group.

Bird	No. of birds examined	No. of birds infected	Prevalence (%)	Total intensity*	Mean total intensity*	Mean total abundance*
Prefledgling	29	22	75.9	467	21.2	16.1
Y1	87	84	96.6	5,199	61.9	59.8
Y2/3	47	47	100	1,970	41.9	41.9
Adult	161	160	99.4	10,845	67.8	67.4
Female	124	124	100	9,463	76.3	76.3
Male	84	83	98.8	3,352	40.4	39.9
Total	324	313	96.6	18,481	59.0	57.0

* Total intensity is the total number of helminths (regardless of species) found in all infected birds; mean total intensity is the mean number of helminths per infected host individual; and mean total abundance is the mean number per examined host individual.

mediate (Y2/3 birds S_T = 20, MS_i = 3.7; adults S_T = 28, MS_i = 3.4). The S_T and MS_i values for prefledglings were significantly lower than the corresponding values for the other age groups, but the differences between the other age groups were not significant. The difference between the sexes was not significant: S_T was 26 in both males and females, whereas MS_i was 3.7 in males versus 3.3 in females.

Prevalence and intensity statistics

Prevalence and intensity for all helminths as a group are shown in Table I. All three parameters were significantly lower in prefledglings than in the older groups. Helminth prevalence scarcely differed between the sexes.

Prevalence and mean intensity for individual parasite species (>1% overall prevalence) are summarized by age group in Table II and by sex in Table III.

Of the species with overall prevalence over 1%, *Diplostomum spathaceum* and *Echinostephilla virgula* were present in only 1 age group (Table II), so no age-related comparisons could be performed on them.

Diplostomum spathaceum was absent from Y2/3 and adult birds, so that no sex-related comparison could be performed;

TABLE II. Prevalence and mean intensity statistics for the individual helminth species, showing values for each host age group.

	Prevalence				Mean intensity			
	PF* (n = 29)	Y1 (n = 87)	Y2/3 (n = 47)	AD* (n = 161)	PF (n = 29)	Y1 (n = 87)	Y2/3 (n = 47)	AD* (n = 161)
Trematoda								
Diplostomum spathaceum		4.6				45.5		
C. longicollis		6.9	8.5	1.9		1.8	5.0	5.3
Brachylaima sp.	3.4	2.3	8.5	5.0	58.0	5.5	5.7	6.5
G. deliciosus	17.2	43.7	25.5	28.0	23.4	23.1	7.2	5.0
C. lingua	37.9	51.7	40.4	28.6	5.6	23.6	8.3	12
M. similis		10.3	12.8	9.3		29.9	20.8	367.7
M. gratiosum		6.9	2.1	2.5		24.8	29.0	7.0
M. linguilla	10.3			1.9	8.0			12
G. longiintestinata		23.0	40.4	21.7		43.6	21.4	13.6
B. microtesticulatum		3.4	17.0	28.6		5.0	18.4	37.6
Renicola sp.		5.8	14.9	6.2		12.6	2.1	7.0
H. elongata		14.9	2.1	4.4		23.9	3	21.4
P. acanthus		3.4	4.3	1.9		2.0	1.0	2.0
E. virgula				3.1				13.2
Cestoda								
A. micracantha	3.4	1.2		18.0	12.0	1.0		4.6
Tetrabothrius erostris	48.3	67.8	100	85.7	3.1	3.8	15.8	6.6
Nematoda								
Anisakis simplex	3.4	6.9	8.5	6.8	1.0	3.7	2.2	2.0
C. obvelatus	62.1	66.7	38.3	37.9	4.3	4.5	3.3	2.6
P. adunca	6.9	39.1	17.0	16.8	1.5	4.4	2.0	1.9
Tetrameres skrjabini	6.9	2.3	6.4	1.2	4.0	1.5	1.7	61.5
E. contortus	6.9	10.3	12.8	18.6	9.0	12.9	4.2	2.5
Acanthocephala								
Arhythmorhynchus longicollis	6.9	2.3		7.5	5.0	13.5		5.0

* PF, prefledglings; AD, adults.

TABLE III. Prevalence and intensity statistics for the individual helminth species, showing values for each host sex. All values are for Y2/3 immature birds and adults.

	Prevalence		Mean intensity	
	Females (n = 124)	Males (n = 84)	Females (n = 124)	Males (n = 84)
Trematoda				
C. longicollis	1.6	6.0	3.5	5.8
Brachylaima sp.	8.1	2.4	7.3	1.0
G. deliciosus	33.9	17.9	5.4	5.6
C. lingua	27.4	36.9	9.0	13.0
M. similis	13.7	4.8	328.0	16.0
M. gratiosum	3.2	1.2	9.0	21.0
M. linguilla	2.4		12.0	
G. longiintestinata	19.4	35.7	10.3	21.2
B. microtesticulatum	29.0	21.4	28.5	47.4
Renicola sp.	6.4	10.7	2.1	7.6
H. elongata	5.6	1.2	21.3	4.0
P. acanthus	3.2	1.2	1.8	1.0
E. virgula	4.0		13.2	
Cestoda				
A. micracantha	16.9	9.5	4.4	5.0
T. erostris	87.1	91.7	10.0	7.4
Nematoda				
A. simplex	8.1	6.0	2.0	2.2
C. obvelatus	38.7	36.9	2.5	3.1
P. adunca	19.4	13.1	1.9	2.0
T. skrjabini	3.2	1.2	31.8	1.0
E. contortus	19.4	14.3	2.8	2.6
Acanthocephala				
A. longicollis	5.6	6.0	5.3	4.6

like the accidental species, it is therefore not included in Table III.

Many parasite species showed statistically significant differences in prevalence, mean intensity among age groups, or both, but only 3 species (*G. deliciosus*, *G. longiintestinata*, and *M. similis*) showed significant differences between the sexes.

Of the species present in several age groups, 8 (*C. longicollis*, *M. similis*, *M. gratiosum*, *G. longiintestinata*, *B. microtesticulatum*, *H. elongata*, *P. acanthus*, and *Renicola* sp.) were absent from prefledglings.

The only species in which all three parameters increased with age was *B. microtesticulatum*. Prevalence was significantly lower in Y1 birds than in Y2/3 birds and adults. Mean abundance was significantly higher in Y1 birds than in adults. Mean intensity of *C. longicollis* also increased with age, whereas prevalence and mean abundance peaked in Y2/3 birds. Prevalence differed significantly between Y2/3 birds and adults.

Maritrema linguilla was found only in prefledglings and adults. Prevalence and mean abundance were both significantly higher in prefledglings, whereas mean intensity was not significantly higher in adults.

Gymnophallus deliciosus showed significantly higher prevalence in Y1 birds than in the other age groups. Mean infection intensity by *G. deliciosus* was highest in prefledglings and declined with age, with significant differences between prefledglings and adults and between Y1 birds and adults. Similarly, in *G. longiintestinata*, mean intensity was significantly higher in Y1 birds than in Y2/3 birds and in Y2/3 birds than in adults. Prevalence was significantly higher in Y2/3 birds than in the other age groups.

In *C. lingua*, all 3 parameters were highest in Y1 birds, with significant differences with respect to adult birds. Prevalence and mean abundance of *H. elongata* were significantly higher in Y1 birds than in Y2/3 birds and adults. Mean intensity did not differ significantly between groups.

The cestode *Alcataenia micracantha* was not detected in Y2/3 birds and was present in only 1 prefledgling and 1 Y1 bird. By contrast, it was found in 29 adults (18%). The difference was statistically significant between Y1 and adult birds.

Tetrabothrius erostris showed significant differences in all 3 parameters between almost all the age groups. Prevalence increased with age until the second/third year, and then dropped; all between-group differences were significant except those between prefledglings and Y1. This species had the highest prevalence of any helminth species in all age groups except prefledglings, in which the nematode *C. obvelatus* showed the highest value. Mean intensity and mean abundance showed similar patterns: all differences were significant except those between prefledglings and Y1; and for mean intensity, those between prefledglings and adults.

Cosmocephalus obvelatus showed markedly higher prevalence in prefledglings and Y1 birds, with significant differences between prefledglings and adults, between Y1 birds and Y2/3 birds, and between Y1 birds and adults. Mean intensity was slightly higher in prefledglings and Y1 birds than in Y2/3 birds, with the lowest value being obtained in adults; the difference between Y1 birds and adults was statistically significant. Mean abundance differed significantly between prefledglings and adults, between Y1 birds and adults, and between Y1 birds and Y2/3 birds.

Paracuaria adunca showed significantly higher prevalence in Y1 birds than in prefledglings and older birds. Mean intensity and mean abundance showed similar patterns, with a higher value for Y1 birds than for the other age groups. For mean intensity, only the difference between Y1 and adults was statistically significant; for mean abundance, the Y1 value was significantly different from all other values.

DISCUSSION

Species richness in *L. michahellis* from Galicia is very high, with 36 species (Sanmartín et al., 2005), in comparison with the 10 species found by Bosch et al. (2000), of which 9 also were found in our gulls. As mentioned above, prefledglings showed the lowest S_T value and Y1 birds the highest values. Nevertheless, this parameter can be considered to be more or less stable in the three fledged gull groups, because the difference between Y1 birds and adults is of only 1 species. In contrast, Bakke (1972) found that S_T increased with age. As this author suggested, the difference in species richness between prefledglings and older gulls is probably due to diet, because prefledglings are limited to the food their parents bring and whatever prey they can catch by themselves on the breeding ground. In contrast, older birds are able to move about freely and capture more varied prey, and they are therefore exposed to more parasite species. Similar trends occur with total helminth prevalence, which is lower in prefledglings and practically stable in older birds.

Only 3 species presented significant differences between sexes: *G. deliciosus*, which was more prevalent in females and had higher mean abundance in males; *G. longiintestinata*, in which both parameters were significantly higher in males; and *M. similis*, with a higher prevalence in females. In contrast, Threlfall (1967) found the prevalence of *G. deliciosus* to be significantly higher in male *Larus argentatus* from the U.K. Threlfall (1967) did not find any significant between-sex difference in *M. similis*, although Bakke (1972) obtained mixed results, with higher prevalence and mean intensity in adult males than in adult females, but the reverse pattern in immature birds. All three species are transmitted by molluscs or crustaceans and have relatively high overall prevalences (Sanmartín et al., 2005). In yellow-legged gulls from the Medes Islands (Mediterranean Sea), the only species showing a significant difference between sexes was *Eucoleus contortus*, with a higher prevalence in females (Bosch et al., 2000). In the present work, *E. contortus* also showed a higher prevalence in females, but the difference was not statistically significant. *Eucoleus contortus* has a direct life cycle but uses earthworms as paratenic host (Anderson, 2000), so differences in prevalence may reflect a more frequent ingestion of earthworms by females, which would be more noticeable in the Medes Islands than in Galicia. These results might be related with the rather low prevalence of this species in *L. michahellis* from Galicia (Sanmartín et al., 2005), resulting from the low frequency of ingestion of this prey observed by Munilla (1997).

Of the 8 species not found in prefledglings, at least 5 species are transmitted to gulls through ingestion of molluscs or crustaceans. *Microphallus similis* uses crabs as second intermediate host (Galaktionov and Bustnes, 1996; Granovitch and Johanesson, 2000; Skirnisson and Galaktionov, 2002). *Himasthla elongata* uses mussels and other molluscs (Montaudouin et al., 2000). The cercariae of *P. acanthus* encyst on hard submerged objects, such as mollusc or crab shells (James, 1973). The second intermediate hosts of *M. gratiosum* are cirripeds (Deblock and Tran Van Ky, 1966; Ching, 1978; Irwin et al., 1990), frequently consumed by *L. michahellis* in Galicia because of their presence on mussels, a key component of this gull's diet in this region (Munilla, 1997). Threlfall (1967, 1968b) likewise did not find *P. acanthus* in juveniles of *L. argentatus*, whereas Bakke (1972) found it with low prevalence in prefledglings of *Larus canus* and higher prevalence in older birds, with a significant difference between prefledglings and adults. *Gynaecotyla longiintestinata* (Prévot, 1974) is transmitted by crustaceans. Another species, *G. deliciosus*, was not detected in chicks aged less than 1 mo (9 of the 29 birds in the prefledglings age group), suggesting that these small chicks were not fed the mollusc species (mussels, *Mytilus* sp. and limpets, *Nucella* sp.) that are thought to be the second intermediate hosts of this parasite (Threlfall, 1968a; Hoberg, 1984). Molluscs and large crustaceans are intertidal prey species, whose presence in *L. michahellis* pellets in Galicia declines during the first few weeks of the reproductive period and then generally increases (although not in all areas) from the second week after hatching onward (Munilla, 1997). This, too, suggests that, at least in the first 2 wk of their lives, chicks are not fed crabs and molluscs.

Nevertheless, a small number of mollusc- and crab-transmitted species were present in prefledglings, such as *Larus propinquus*, *M. linguilla*, and *Brachylaima* sp., although the first species was found in only 1 bird. The presence of *Brachylaima* sp. in *L. michahellis* is of interest, because the species of Brachylaimidae are among the few trematodes using terrestrial gastropods as both first and second intermediate hosts (Cribb, 1992; Guisantes et al., 1994). *Gymnophallus deliciosus* also was present in prefledglings over 1 mo of age. That mean intensity of this species was highest in prefledglings and declined with age suggests that younger birds are more susceptible to this parasite, with older birds perhaps showing protective immunity. Similar findings are noted with *G. longiintestinata*.

In *C. lingua*, all 3 parameters were highest in Y1 birds. Both Threlfall (1967) in *L. argentatus* from the U.K. and Bakke (1972) in *L. canus* found higher prevalence of *C. lingua* in immature gulls, and similar values in juveniles and adults, with mean intensities lowest in juveniles. In contrast, Pemberton (1963) in *Larus ridibundus* and Threlfall (1968a) in *L. argentatus* found a higher prevalence of this species in adults. *Paracuaria adunca* also showed higher values in Y1 birds, in accordance with Bakke and Barus (1976) who found higher prevalence in immature birds than in adults. By contrast, Threlfall (1968b) found this species (= *Paracuaria macdonaldi* = *Cosmocephalus aduncus* = *Streptocara tridentata*) with higher prevalence and mean intensity in adults than in juveniles of *L. argentatus* in Canada. *Cosmocephalus obvelatus* showed higher prevalence and mean intensity in prefledglings and Y1 birds. This is in line with the findings of Threlfall (1967) and Bakke and Barus (1976), who found this parasite with higher prevalence in first-year birds than in adults. Nevertheless, Threlfall (1968b) found this parasite with higher prevalence in adult birds than in first-year birds. Threlfall (1967) also found a higher intensity of infection in first-year birds than in adults. Other species with significantly higher prevalence in Y1 birds are *G. deliciosus* and *H. elongata*. The first 3 species are transmitted by fish (Marcogliese, 1995; Anderson, 2000; Zander, 2003), and the last 2 species by crustaceans and molluscs (Threlfall, 1968a; Hoberg, 1984; Montaudouin et al., 2000), so diet does not seem to be the factor influencing their age distribution pattern. Y2/3 birds also have a mixed helminth fauna with fish- and shellfish-transmitted species.

The very low prevalence of *A. micracantha* in immature gulls contrasts with the results of Pemberton (1963) in *L. ridibundus* and Threlfall (1968b) in *L. argentatus*.

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