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Incidence of a *Haemoproteus lari* parasitemia in a threatened Gull: *Larus audouinii*

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Data on haematozoan parasitemias in Laridae are scarce and, since blood parasites may influence birds fitness, it is important to report their incidence in threatened species such as Audouin's Gull. Blood smears of 90 Audouin's Gulls caught during incubation at their two main breeding sites, the Ebro Delta and the Chafarinas Islands, were examined for *Haemoproteus lari* parasitemia. Overall prevalence of the parasite was 92.1% for the Chafarinas Is. and 28.9% for the Ebro Delta. To our knowledge, prevalences reported in this study are the highest ever recorded for Laridae. Differences in prevalence between localities were significant, but not differences between the sexes within each locality. Males were more intensely parasitized than females in Chafarinas, and gulls from Chafarinas were more intensely parasitized than those from the Ebro Delta. Differences in intensity were also checked using data from sympatric Yellow-legged Gulls, *L. cachinnans*, and only the factor locality was significant. There was no significant relationship between body condition and intensity of parasitemia in males or females in either locality. These data indicate higher susceptibility to *H. lari* for gulls breeding at the Chafarinas Islands.

1. Introduction

The interaction of haematozoa and their avian hosts has received increased attention since parasites have been recognized as agents influencing many host fitness components (reviews by Møller et al. 1990 and Clayton 1991). This influence may occur through sexual selection (Hamilton & Zuk 1982, Clayton 1991), through differential survival rates of parasitized vs. non-parasitized birds (Atkinson & van Riper 1991, Davidar & Morton 1993) or through lowered reproductive success (Johnson & Boyce 1991, Korpimäki et al. 1993, Rätti et al. 1993).

There are few data on haemotazoan parasitemias in Laridae, and studies reporting the incidence of infections in wild birds are needed to evaluate their potential effects (Bennett et al. 1992, May 1995), particularly in the case of rare or endangered species, as such Audouin's Gull, one of the few SPEC species (Species of European Conservation Concern) belonging to category 1 (species of global conservation concern) (Tucker & Heath 1995). The main potential threat for this species comes from the concentration of 84% of the world population in only two breeding colonies, those of the Ebro Delta and Chafarinas Islands (Pedrocchi & Ruiz 1995). This situation is particularly sensitive to the action of epizooties, thus deserving particular interest to the study of prevalence (the proportion of individuals infected in a population) and intensity (the number of infected red blood cells in a standard

sample from an infected individual) of any kind of parasitemias, especially those affecting the population during the breeding season.

In this contribution we report and analyze data on prevalence and intensity of the haematozoan *Haemoproteus lari* in Audouin's Gulls *Larus audouinii* while breeding at its two main world colonies and compare data of this species with those obtained for a sympatric gull species, the Yellow-legged Gull *L. cachinnans*.

2. Material and Methods

2.1. Study areas

The Chafarinas Islands (Melilla, Spain: 35°11′N, 3°46′35″E) are three small volcanic islands situated 4.5 Km offshore from the Moroccan Mediterranean coast. The only inhabited island is the central one (Isabel II) and gulls nest on the other two: Rey Francisco, placed very near Isabel II, which contains most of the Audouin's Gull nests (ca. 3.700 in 1994) and about 600 nests of Yellow-legged Gull (*Larus cachinnans*); and Congreso Is., where most of the nests correspond to Yellow-legged Gull (1.000), but also holding about 800 nests of Audouin's Gull in 1994. On the Rey Is. the dominant vegetation is formed by *Suaeda vera*, *Lycium intricatum* and *Atriplex halimus*, whereas on Congreso Is. *Salsola oppositifolia* and *Pancratium foetidum* are almost exclusive. In both cases the vegetation covers almost completely the islands surface (Blanco 1988).

The Ebro Delta (40°37′N, 0°21′E), is an alluvial plain of about 250 Km^2 presenting a sandy flat peninsula of 2500 Ha, where Audouin's Gull breeds (more than 10.000 pairs in 1994) together with other seabird species. The landscape is formed by a mosaic of saltmarshes and scattered halophytic vegetation (Oro & Martinez 1994).

2.2. Sampling parasites

Blood smears of 90 incubating Audouin's Gulls, caught under license using nest-traps on April 1994, were obtained by venipuncture both in the Ebro Delta (N = 44) and the Chafarinas Islands (N = 46). Animals were sexed using a bill biometry index (Witt et al. 1982). Smears were air dried and fixed in methanol within a few minutes of sampling. A numeric code identified each slide. In the laboratory the slides were stained with GIEMSA and blind scores were obtained by examining smears under oil at 1000X.

Haemoproteus lari was identified following Bennett et al. (1992). No other blood parasites were found (G.F. Bennett, pers. comm.). Smears were scored by one observer. Prevalence was established through inspection of 100 fields containing about 100 erythrocytes each, thus on the basis of 10.000 erythrocytes observed per sample. Intensity was established by counting the number of infected erythrocytes in 40 fields, thus on the basis of 4.000 red blood cells observed per sample. Additionally, some blood smears of Yellow-legged Gulls breeding sympatrically with the Audouin's Gulls were also collected both in the Ebro delta (N = 26) and the Chafarinas (N = 6), and processed in the same way.

2.3. Statistics

Two collections of 15 randomly choosen smears from each locality were scored to test intraobserver variations in prevalence values using the kappa index of concordance (Fleiss 1981), while variations in the degree of intensity recorded were tested using the intraclass correlation index, approached by means of the Model II of single factor ANOVA (Zar 1984). Intra-observer repeatibilities were high both for prevalence (Kappa Index of Concordance $k = 0.88$) and for intensity of parasitemias (intraclass correlation $r_i = 0.93$).

Independence among prevalence, sex, and locality was tested using chi-square statistics on a three-dimensional contingency table (Zar 1984). Independence of intensity and sex was tested using the Mann-Whitney U test, and association between body condition (body mass/tarsus length) and infection intensity, in each locality and sex, was tested using the Pearson correlation. Since, in the case of Yellow-legged Gull, some expected frequencies were lower than 1%, we used the Fisher's exact test to check for differences in prevalence between localities in this species. A two-factor ANOVA was used on log-transformed data (K-S Lilliefors = 0.07, d.f. = 74, n.s.) to check for significant differences in

intensity between localities using data from both species. All tests were two-tailed.

3. Results

Table 1 shows the number of Audouin's Gulls infected vs. non-infected for each locality and sex. Global prevalence was 92.1% for the Chafarinas Is. (94.1% for males and 90.5% for females) and 28.9% for the Ebro Delta (17.6% for males and 35.7% for females). Overall independence among prevalence, sex, and locality was rejected ($\chi_4^2 = 62.01$, $P < 0.001$) and tests of partial independence for each variable failed to reject the null hypothesis only for sex ($\chi_4^2 = 2.48$, n.s.). Thus, a 2×2 contingency table was used to test the independence between prevalence and locality. Results show that gulls from Chafarinas Is. presented significantly higher levels of prevalence ($\chi^2_1 = 39.11$, $P < 0.0001$) than those of the Ebro Delta.

The results obtained checking for differences by locality using the Yellow-legged Gull samples (Table 3) also indicated significantly higher prevalences for the Chafarinas colony (Fisher's exact test $P = 0.002$).

Parasitism intensity values are given in Table 2. Since prevalence for males at the Ebro Delta was much lower than in the Chafarinas Is., thus providing a largely unbalanced sample to test intensities (Table 2), the association of intensity with sex was tested only at the Chafarinas Is., where males had significantly higher intensities than females ($U = 96.0$, $P < 0.001$). The correlation analyses of body condition with intensity showed that the correlation was not significant for males or females at either locality (Ebro Delta males, $r_1 = 0.56$, $P = 0.62$; Ebro Delta females, $r_8 = -0.08$, $P = 0.83$; Chafarinas males, $r_{10} = 0.57$, $P = 0.052$; Chafarinas females, $r_{18} = -0.07$, $P = 0.75$).

Significant differences in intensity of parasitemia between localities were also checked using data from both species, and only locality was significant ($F_1 = 29.3$, $P = 0.0001$) (Table 4); while neither species ($F_1 = 2.9$, $P = 0.09$), nor interaction between species and locality ($F_1 = 1.1$, $P = 0.3$) was significant.

4. Discussion

The scant data available about haematozoans in Laridae show that the prevalences are usually very low (ca. 5% or less, see e.g. Peirce 1981, Bennet et

Table 1. Frequencies of parasitism by *Haemoproteus lari* on Audouin's Gull by sex and locality.

	EBRO DELTA		CHAFARINAS	
	♂	♀	♂	♀
PARASITIZED	3	10	20	23
NON PARASITIZED	14	17	1	2
TOTAL	17	27	21	25

Table 2. Intensity of *Haemoproteus lari* parasitism among infected individuals by sex and locality. Mean number of infected erythrocytes (standard deviation) and sample size are given.

	EBRO DELTA	CHAFARINAS
MALES	30.0 (40.9) N = 3	27.35 (16.6) N = 20
FEMALES	2.8 (3.36) N = 10	12.91 (8.1)N = 23

Table 3. Prevalence of parasitism by *Haemoproteus lari* on Yellow-legged Gulls by locality. Differences are statistically significant (Fisher's exact test P=0.002) indicating higher prevalences in the Chafarinas colony.

	EBRO DELTA	CHAFARINAS
PARASITIZED	7	6
NON PARASITIZED	19	0
TOTAL	26	6

Table 4. Intensity of *Haemoproteus lari* parasitism among infected individuals by species and locality. Mean number of infected erythrocytes (standard deviation) and sample size are given. A two factor ANOVA on long trans formed data has shown that only locality is responsible for significant differences ($F_1 = 29.3$, $P = 0.0001$), while neither species nor the interaction between species and locality were significant.

	EBRO DELTA	CHAFARINAS
L. cachinnans	13.5 (14.03) N = 7	22.7 (14.33) N = 6
L. audouinii	8.7 (19.97) N = 13	19.6 (14.26) N = 43

al. 1992). To our knowledge the present results show much larger prevalences than ever recorded for Laridae. Since large seasonal variation in prevalence occurs in other species (Weatherhead & Bennett 1991, 1992; Allander & Bennett 1994, Norris et al. 1994), these high values might be attributable to the fact that all our samples were taken during the breeding season, when chronic infections may relapse owing to either the immunosuppressive action of sexual hormones (Zuk 1991, Atkinson & van Riper 1991, Folstad & Karter 1992), increased exposure to parasite vectors (Møller 1994) or breeding effort (Norris et al. 1994).

At present three striking patterns in prevalence of blood parasites amongst birds have been recognized (Norris et al. 1994), one of which is that amongst nonraptorial altricial land birds prevalences tend to be larger at higher latitudes (Ricklefs 1992). According to our data northern populations of both gull species showed lower prevalence than more southern ones. Clearly further research is needed to ascertain whether this is an exceptional case or whether seabirds exhibit different trends to land birds (see Peirce 1981 for values in European countries). Ricklefs (1992) explains this trend at interspecific level through a possible link between the length of the embryonic period and the maturation of an effective immune system, since prevalence is inversely related to the length of the incubation period for a given egg-size. However, this should not be the case in an intraspecific comparison in which only slight differences in the incubation period are found (authors, unpubl.).

On the other hand, Norris et al. (1994) hypothesized that all three patterns observed for haematozoan prevalence in birds (Read 1991, Ricklefs 1992) can be explained through breeding effort. Thus, the fact that gulls from the Chafarinas Islands showed significantly higher prevalences than those of the Ebro Delta, would mean that gulls breeding in Chafarinas invest greater parental efforts than gulls in the Ebro Delta. This is consistent with data on reproductive parameters such as clutch size, since there was a larger proportion of two-egg clutches in Chafarinas (25%) than in the Ebro Delta (12%) (authors unpubl.). Since in gulls, clutch-size variability reflects mainly nutritional constraints during the egg-synthesis period (Reid 1987, Salzer & Larkin 1990, Bolton et al. 1992), these data could indicate that the Chafarinas population was under poorer feeding conditions than that of the Ebro Delta. However, since, in contrast, Yellow-legged Gulls showed larger proportions of two-egg clutches at the Ebro Delta (30%) than in Chafarinas (20%) (authors, unpublished), but higher incidence of parasites at Chafarinas, other variables non mutually exclusive with breeding effort, such as the degree of exposure to parasite vectors, Ceratopogonid flies of the genus *Culicoides* (Allander & Bennet 1994), or the incidence of other immunosuppressive agents, e.g. organochlorine pollutants such as PCBs (Peakall 1986), need to be considered.

According to Allander & Bennett (1994), and G. F. Bennett (pers. comm.), prevalence values in haematozoan parasitemias are mainly dependent on the abundance and activity of parasite vectors, i.e. exposure, so we can expect higher densities or activity of *Culicoides* at Chafarinas than at the Ebro Delta. We have no data on *Culicoides* density at the two colony sites, but, since temperature and wind may affect the feeding activity of dipteran parasite vectors (McCreadie et al. 1986, quoted in Allander & Bennett 1994), we can report that weather parameters are consistent with this: during the incubation stage (April month) the Chafarinas Is. were hotter (mean temperature 18°C) than the Ebro Delta (mean temperature 13°C), and, furthermore, the Ebro Delta is a windy area, and the vegetation cover of the colony site is scarcer than in the Chafarinas (see methods).

The fact that there were no significant differences in prevalence between the sexes indicates a similar degree of exposure to parasite vectors during incubation. This is consistent with our observations on the time spent by each sex at the colony site at this breeding stage (authors unpubl.). This is also the most usual trend found in the literature on blood parasites in birds (Stacey et al. 1990, Allander & Bennett 1994, O'Dell & Robbins 1994), although the possibility of finding differences may rely on sampling date (Weatherhead & Bennett 1992).

Because *Haemoproteus* infections have prepatent periods of about 14 days (G.F. Bennett, pers. comm.), in Audouin's Gull the significantly higher intensity of parasitemia shown by males at Chafarinas colony, should reflect greater susceptibility to *H. lari* during the pre-laying period. This coincides with the courtship feeding and egg-synthesis period, which lasts about 20 days in gulls (Salzer & Larkin 1990), and also with other costly

male activities such as sperm competition (Birkhead & Møller 1992, Norris et al. 1994). Moreover, during the pre-laying period drastic changes occur in hormone balance of the birds, which may interact in a complex way with the immunocompetence of the sexes (Korpimäki et al. 1993, Saino & Møller 1994). In any case, differences in intensity between localities are also indicative of gulls being more susceptible to parasites when reproducing in the Chafarinas Islands.

The absence of relationship between body condition and parasitemia intensity does not suggest any effect of these parasites on gulls' health. Moreover, the only relation approaching significance is that of males from Chafarinas, but it is in the reverse direction, i.e. males in best condition were those more intensely parasitized. This could be because *Haemoproteus* are relatively benign (Bennett 1993), and then males in better condition, could be older birds, i.e. those which have had longer exposure to the parasites. Values of relationship for females are too low to suggest any trend.

However, the effects of parasites on other fitness components, such as breeding success (including extra-pair paternities) or endurance to migratory effort, need to be thoroughly investigated before concluding that such parasitemias, at their present level, are irrelevant to gulls' fitness.

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Selostus: *Haemoproteus lari-* veriloisen esiintyminen Välimerenlokilla

Kirjoittajat tutkivat *Haemoproteus lari* -veriloisen esiintymistä Välimerenlokilla (*Larus audouinii*) ja keltajalkalokilla (*L. cachinnans*) kahdella tutkimusalueella, Chafarinas saarilla, jotka sijaitsevat lähellä Afrikan pohjoisrannikkoa Marokon edustalla, ja Ebro joen suistossa Espanjan itärannikolla. Linnut pyydystettiin haudonta-aikaan pesimäsaariltaan verinäytteiden ottoa varten. Loisittuja Välimerenlokkiyksilöitä esiintyi runsaammin Chafarinas saarilla (92% yksilöistä loisittu) kuin Ebron suistossa (29% yksilöistä loisittu). Näin korkeita loisintafrekvenssejä ei lokkilinnuilla ole aikaisemmin havaittu. Naaraita ja koiraita oli loisittu yhtä suurella frekvenssillä, mutta koirailla loisia esiintyi lukumääräisesti enemmän loisittuja yksilöä kohti. Tulokset Keltajalkalokilta olivat samansuuntaisia. Linnun kunnolla ja loistartunnan voimakkuudella ei näyttänyt olevan yhteyttä. Kirjoittajat pohtivat loisien esiintymiseen vaikuttavia tekijöitä ja loisten vaikutuksia isäntiinsä.

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