Zur Homepage der Dissertation

CHAPTER 7

ISOSPORA (PROTISTA: COCCIDIIDA) INFECTION IN MIGRATING PASSERINE BIRDS

Isospora (Protista: Coccidiida) infection in migrating passerine birds

Introduction

The optimal way for a migrating bird to reach its destination within the appropriate time differs depending on the demands that act on the bird. Time, energy, and safety from predators are of main current concern (Alerstam & Lindström 1990, Alerstam & Hedenström 1998). A yet almost unidentified subject is the role of parasites and diseases in migrants, and the adaptations of the birds to cope with. Some studies reveal that migratory species indeed have more severe protozoan infections than residents (Dogiel 1962, Greiner *et al*. 1975). This may have implications for the fitness of the birds and for our understanding of the susceptibility of migratory birds to environmental perturbations. The immune system is probably one of the most efficient anti-parasite defence systems that hosts have evolved against parasites (Roitt *et al*. 1996, Wakelin 1996). It was shown, that migratory bird species have larger immune defence organs than closely related resident species, and this difference is suggested to be caused by exposure of migrants to a more diverse parasite fauna than experienced by residents (Møller & Erritzøe 1998). Migratory birds are thought to be very susceptible to the negative impact of parasites owing to a condition-dependent immune response (Chandra & Newberne 1977, Gershwin *et al*. 1985). The relationship between body condition, refuelling and parasite load in migratory and resident species has, however, hardly been studied, and it deserves more attention (Dawson & Bortolotti 2000, Yorinks & Atkinson 2000).

The effect of blood Haemosporidian parasites on migrating passerine birds was shown by many authors, the most complete overview was made by Valkiūnas (1993, 1997). He showed influence of *Haemoproteus fringillae* on growth, body mass, locomotor activity and behaviour of nestling Chaffinches. Hayworth *et al*. (1987) found that *Plasmodium relictum* infection reduces the ability of birds to keep thermoregulation and oxygen transport. However, there is very few data about intestinal coccidian infections in passerine birds in the wild (Mazgajski & Kędra 1998, Kruszewicz & Dyrcz 2000) and their consequences.

Most of intestinal coccidian species that infect passerine birds belong to the genus *Isospora* (Pellerdy 1974). They are abundant and widespread. In some populations of passerine birds in Europe prevalence of infection has been frequently recorded to be over 50 % (Scholtyseck & Przygodda 1956, Grulet *et al*. 1985, Dolnik 1998). *Isospora* spp.

are monoxenous parasites that require no intermediate transmitter for the spread of infection (see Long 1982 for a review). With a few exceptions (Barré & Troncy 1974, Doran 1978) bird intestinal coccidia are thought to be specific on the level of host genera (Box 1977, Levine 1982). In the host, mostly occasionally swallowed with food or water, *Isospora* oocysts pass several merogonies so that the amount of these parasites increases rapidly (Long, 1982). After gametogony and fertilisation new oocysts are released from the bird together with faeces. *Isospora* oocyst output from passerine birds has a clear diurnal pattern with one peak of oocyst release in the afternoon (Grulet *et al*. 1985, Dolnik 1999a, 1999b).

Coccidian infections are self-limiting and after a specific number of generations, schizogony terminates and merozoites develop into sexual stages (Hammond 1973). *Isospora* in passerine birds, however, seems to be an exception, as first noticed by Labbé (1893). Boughton (1937) showed chronic *Isospora* infection in birds for two months, despite he sterilised cages, food and water every 6 hours. Similar results for other *Isospora* species were recorded by Anwar (1966). Box (1977) showed that Canaries (*Serinus canarius*) experimentally infected with *Isospora serini* remain infected for months, whereas *I. canari* infection passes after 16-18 days. Some immunity develops as a result of infection but it does not prevent re-infection (Long 1982).

Intestinal coccidians are well-known to be pathogenic in poultry as well as in some wild birds in captivity (Gylstorff & Grimm 1998). Therefore, it is of a great interest to know more about their interactions with migratory birds in the wild.

The aim of this study was to explore *Isospora* infection during autumn migration in passerine bird species at two stopover sites, on the Courish Spit (Baltic Sea) and on the island of Helgoland (North Sea).

Migratory species are suggested to carry more severe protozoan parasite infections (Greiner *et al*. 1975) and to invest more in immune defence than resident ones (Møller & Erritzøe 1998). Therefore, we also compared prevalence and intensity of infection between long- and short distance migrants.

We checked whether intensity or prevalence of *Isospora* infection in the investigated migrating bird species were associated with body condition of the bird. The only data about the relationship between *Isospora* infection and body condition of passerine birds in the wild concern nestlings. They show no or even a slight positive correlation between intensity of infection and body mass of the host (Mazgajski & Kędra 1998, Kruszewicz & Dyrcz 2000).

It was recorded that Chaffinches infected with *Leucocytozoon* spp. are concentrated at the end of their bird migration flow (Valkiūnas 1997). Therefore, we investigated whether the prevalence and intensity of infection increases at the end of migration, which may indicate that infected birds are hindered in time of departure.

Material and methods

The research was carried out in the period 20 August − 16 October 1997-98 at two sites. The first study site is Biological Station Rybachy, that is located on the Courish Spit, SE Baltic coast (55°12′N, 20°46′E). The second site is on the island of Helgoland in the North Sea (54°11′N, 07°55′E), 53 km from the mainland (Fig. 1). Birds were trapped by mistnets in Rybachy and by funnel traps on Helgoland. On both sites birds were ringed and processed following the guidelines of the ESF-programme (Bairlein 1995). Recorded data include date and time of capture, species, age, sex, moult, body mass and fat score.

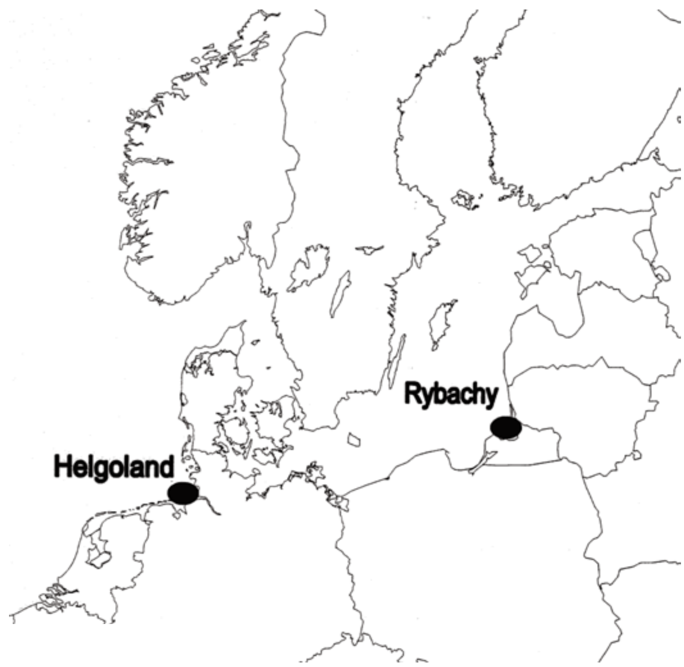

Fig. 1. Study sites.

We made our study on five target host species that are the most numerous at both sites during autumn migration. These were Blackcap (*Sylvia atricapilla*), Garden Warbler (*Sylvia borin*), Robin (*Erithacus rubecula*), Willow Warbler (*Phylloscopus trochilus*), and Chaffinch (*Fringilla coelebs*). Garden Warblers, Willow Warblers, and Blackcaps were selected as long distance migrants, whereas Robins and Chaffinches are regarded as medium to short distance migrants (Zink 1973-1975, Zink & Bairlein 1995).

To avoid possible influence of birds' age only juvenile birds were considered in the analysis. Moreover, because of a diurnal pattern in *Isospora* oocysts output (Dolnik 1999b), only birds caught between 4 p.m. and 6 p.m. were sampled.

In total, 105 Garden Warblers, 94 Robins, 81 Blackcaps, 52 Willow Warblers, and 32 Chaffinches were sampled.

The intensity of *Isospora* infection can be estimated without dissecting the host by using standard method of counting oocysts in faecal samples. At both sites the same protocol of sampling was used.

After ringing the birds were kept for 5-15 minutes in small individual cages with clean ground paper. After defecation one fresh dropping of each individual bird was put into an individually labelled tube with 5 ml 2% $K_2Cr_2O_7$ aqueous water solution.

In the lab, the samples were kept opened for a week at room temperature to allow the oocysts to sporulate. Then the samples were checked using a standardised method. For concentrating the oocyst flotation in saturated NaCl solution was used. Each sample was shaken well and put into 10 ml centrifuge-tube. Tap water was added up to 10 ml volume. The sample was centrifuged for 5 minutes at 1500 R.P.M., and the upper layer was removed, so that 2 ml of the lower layer were left. 8 ml saturated NaCl solution were added and centrifuged again for 5 minutes at 1500 R.P.M. A standard quantity of the surface layer (5 loops of 5 mm diameter) was placed on slides and immediately examined at 100× magnification to determine the occurrence and intensity of infection. The whole slide was checked to avoid mistakes that can be caused by oocyst clustering. As intensity of infection the number of oocysts on the slide was used. Parasites were identified under high magnification (1000×). The data were statistically analysed using SPSS 8.0 programme (SPSS Base System und Professional Statistics). Data are presented as means ± standard error (SE) or standard deviation (SD).

Results

Parasite species

No other coccidia genera except *Isospora* were found. The same host species on the Courish Spit and on Helgoland were infected by the same *Isospora* species. In Blackcaps two species of *Isospora* were found: *Isospora sylvianthina* Schwalbach 1959 and *Isospora sylviae* Schwalbach 1959. The first species occurred in 91% of infected birds in Rybachy and in 94% of infected Helgoland birds. The second species was presented in 22% of infected birds from Rybachy and in 21% of infected birds from Helgoland. 13% of infected Rybachy Blackcaps and 15% of infected Helgoland birds were infected by both parasite species. The same two species of parasites were found in Garden Warblers. On the contrary, in Garden Warblers *Isospora sylviae* predominated (74% of infected birds in Rybachy and 80% of infected birds on Helgoland), while *Isospora sylvianthina* occurred in 31% of infected birds in Rybachy and in 28% of infected birds on Helgoland. Mixed infection of both species of parasites was seen in 5% of infected Garden Warblers in Rybachy and in 8% on Helgoland. Willow warblers on both sites were infected by *Isospora* sp. that does not fit to any species description but which is identical to *Isospora* sp. type 21 mentioned by Svobodova (1994). In all infected Robins on both sites only *Isospora erithaci* Anwar 1972 oocysts were found. Chaffinches on both sites were exclusively infected by *Isospora fringillae* Yakimoff et Gousseff 1938.

Prevalence of infection

There was no significant difference in prevalence of infection between birds from the Courish Spit and from Helgoland (Fig. 2), nor between the five host species. Prevalence of infection did not show significant seasonal variation.

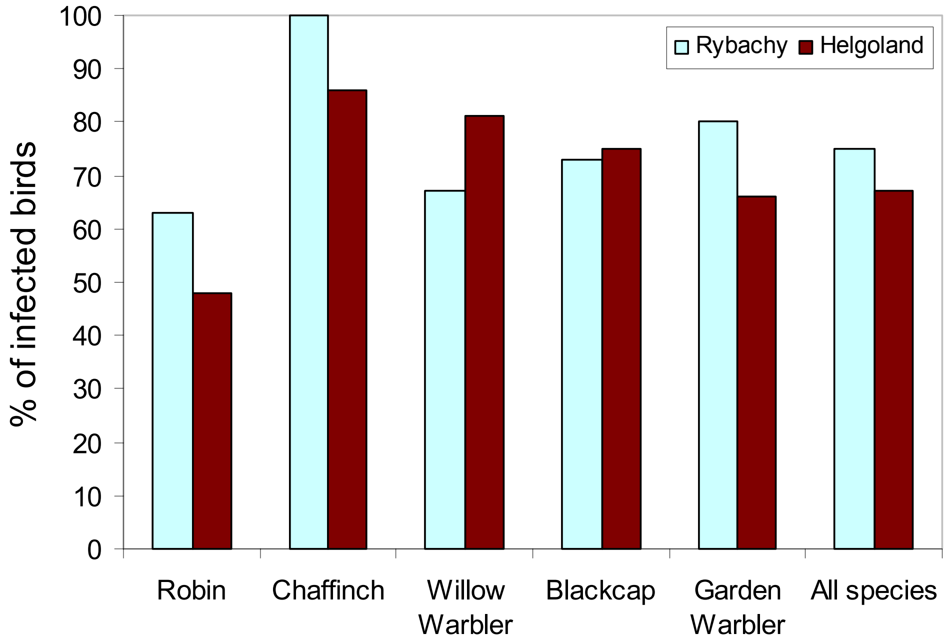

Fig. 2. Prevalence of *Isospora* infection in some birds' species at two sites in autumn.

Intensity of infection

Within-site the intensity of infection varied between hosts species, but significant only on Helgoland where Willow Warblers were heavier infected than Blackcaps (P=0.04) and Garden Warblers (P=0.008), respectively.

In all five species the intensity of infection was higher in birds from Rybachy (Fig. 3), although significant only in Garden Warblers (P=0.01), Willow Warblers (P=0.01) and in the combined sample (P=0.000). In total, the intensity of *Isospora* infection in birds of these two species caught on the Courish Spit was more than 10 times higher than in birds from Helgoland.

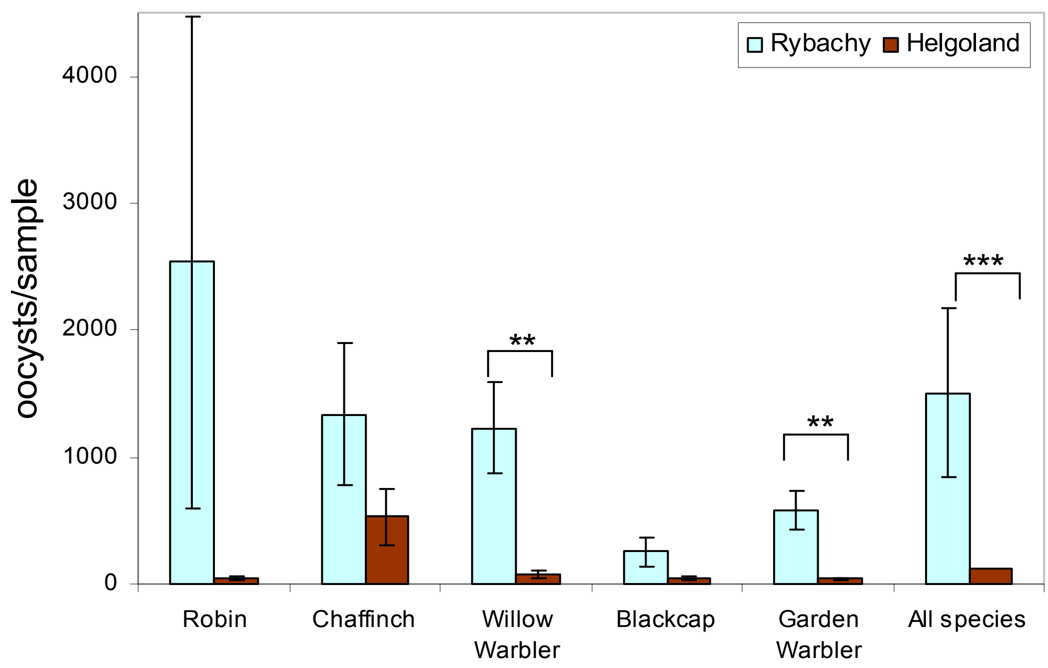

Fig. 3. Average intensity of *Isospora* infection (±SE) in some birds' species at two sites in autumn.

If we separate the three long-distance species and the two medium-distance migrants (Fig. 4), the intensity of infection in long-distant migrants within a site is lower than in medium- and short-distant migrants, though this difference is significant only on Helgoland (P=0.000). The difference in intensity of infection between the sites is highly significant in long-distance migrants (P=0.000) and not significant in short distance migrants because of a large standard error in Rybachy birds.

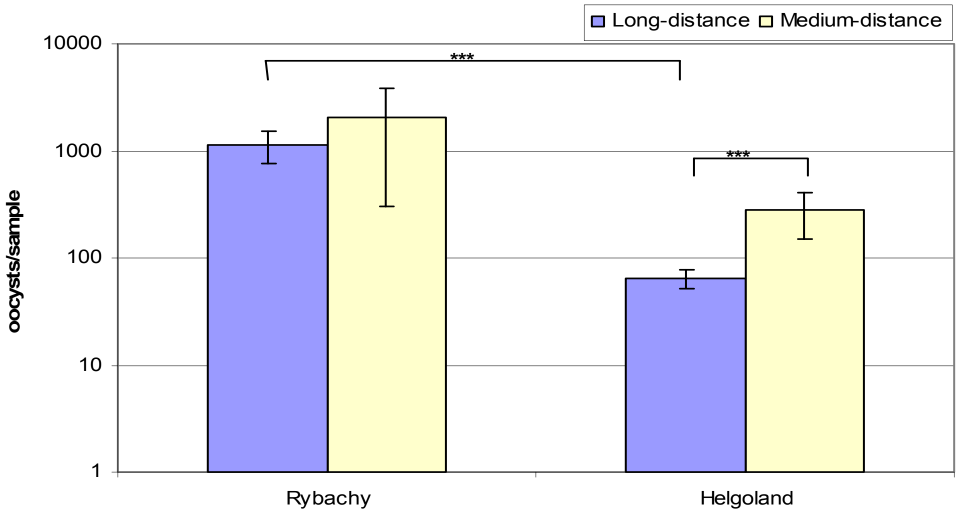

Fig. 4. Average intensity of infection (±SE) in long- and medium-distance migrants.

Fat score and body mass of the birds

With the exception of Garden Warblers fat scores and body mass did not differ significantly between the two sites (Table 1). We did not find any significant relationship between the intensity of *Isospora* infection and the bird's body mass or fat score neither in Rybachy nor on Helgoland.

Table. Average fat score (± SD) and body mass (± SD) and number (n) of investigated birds at two study sites.

Bird species	Measured parameter	Rybachy	Helgoland	U-test
Blackcap	fat	3.5±2.2	2.8±1.2	0.179
	mass	20.8±2.0	19.8±2.0	0.13
	n	17	64	
Garden warbler	fat	3.6±1.4	2.4±1.3	0.000
	mass	20.5±1.9	19.5±2.3	0.028
	n	30	75	
Robin	fat	1.9±1.0	1.6±0.7	0.182
	mass	16.1±1.3	16.1±1.1	0.790
	n	27	67	
Willow warbler	fat	3.0±1.1	2.6±0.9	0.112
	mass	9.3±1.0	8.8±0.9	0.077
	n	17	35	
Chaffinch	fat	1.2±1.4	2.7±1.0	0.060
	mass	22.7±2.1	22.5±2.0	0.966
	n	10	22	

There was no significant seasonal variation of intensity of infection neither on Helgoland nor in Rybachy (correlation analysis: P>0.3-0.5).

Discussion

Parasite fauna

Coccidia species carried by young birds during autumn migration can be those from the breeding sites or be picked up along the migration route. However, young birds trapped on Helgoland carried the same *Isospora* species in similar proportion as birds trapped on the Courish Spit. Therefore, we can suggest that the coccidia faunas did not differ much between the breeding and the migration areas of these birds. Infection of birds on both sites with the same parasite species and their similar prevalence allows us to analyse the data about *Isospora* spp. infection on the level of genera.

Fat score and body mass of the birds

There was no significant correlation between the intensity of *Isospora* infection and the birds' body mass or fat score neither in Rybachy, nor on Helgoland. Rather, the heavier and fatter birds in Rybachy carried higher intensity than the birds on Helgoland. This may relate to the observation by Dogiel (1962) who postulated that parasites with endogenous multiplication stages control their multiplication according to the "parasitic capacity of the host". Hosts in better physical condition are likely to offer more resources for parasites, thus being more infected than weaker hosts. A positive correlation between the intensity of infection and body mass was found in Starling nestlings (Mazgajski & Kędra 1998) and in adults and nestlings of several *Acrocephalus* species (Kruszewicz & Dyrcz 2000). Moreover, recent experiments with captive Blackcaps also show no linear relationship between body mass and intensity of *Isospora* infection (Dolnik & Bairlein, in prep.). These birds appear to cope with *Isospora* as long as the intensity of infection is not too high and enough food is available.

Prevalence and intensity of infection

In wild birds during breeding, prevalence of *Isospora* infection can be over 50% (Svobodová 1994, Dolnik 1998, Kruszewicz & Dyrcz 2000). For example on the Courish Spit the prevalence of infection in young Chaffinches in summer can be 55% (Dolnik, unpubl.). The current data revealed even much higher prevalence in passage migrants with some 70% in the combined sample of species and 100% in Chaffinches on the Courish Spit. Scholtyseck (1956) showed an increase of the proportion of Coccidia infected birds in autumn compared to summer with a peak in September – October. However, he combined data of 1381 birds from 146 species, even of different taxonomic orders. Similar increase of prevalence and intensity of *Isospora* infection in autumn was observed in pooled data of several passerine species on the Courish Spit (Dolnik 1998). The causes of higher prevalence of *Isospora* infection in migrating birds are not clear. It could be due to differences in feeding mode or flocking (in Chaffinches). In any case, however, these high levels may indicate that *Isospora* in migrating birds is less harmful for wild passerine birds as *Eimeria* is for poultry where such high rates of occurrence may reveal serious disease (Fernando 1982).

In contrast to prevalence, the intensity of *Isospora* infection was significantly higher in Rybachy than on the island of Helgoland. As in other parasites with endogenous multiplication stages in their life cycles, the intensity of *Isospora* infection does not depend much on the dose of the infective oocysts. Even an infection with one oocyst may cause heavy infection.

The reasons for lower infection in birds on Helgoland as compared to Rybachy are again unclear. There may be differences in the intensity of infection in the different areas of origin. While the Rybachy birds are mainly originating from the Baltic (Payevsky 1971), the Helgoland migrants are coming mainly from Norway and central Sweden (Zink 1973-1975, Zink & Bairlein 1995). Habitats in both areas of origin differ, thus the intensity of *Isospora* infection may also be different. A striking difference between both study sites is their location within the migration journey. While birds on passage at Rybachy are mainly passing over land, the birds on Helgoland must have crossed the open sea. Consequently, only those individuals may have done it to the island which were less infected, whereas such a selection may not play a role in Rybachy. This is also supported by data on Willow Warblers at the island Greifswalder Oie (Baltic Sea) where the intensity of *Isospora* spp. infection is considerably lower than on the Courish Spit. In racing pigeons, coccidia infected birds returned less and at lowed speed than non-infected birds (Bachmann *et al*. 1992). Moreover, recent studies on small migrants reveal some considerable reduction in organ size, including intestine, due to sustained flights across ecological barriers (Biebach 1998, Bauchinger & Biebach 2001). Piersma (1998) showed that wader species shrink their nutritional organs already before the long-distance flight. Digestive tract mass in Garden Warblers, on the contrary, changes during the flight. It can reduce by 39% of the pre-flight condition, with a shortening of the small intestine by 18% its length (Biebach 1998). Consequently, the host’s capacity for intestine parasites could be reduced, resulting in lower levels of parasite infection in those birds.

Finally, the different intensity levels of *Isospora* infection in birds in Rybachy and on Helgoland may be related to stopover duration. On Helgoland, stopovers are very short, and most migrants leave the island within a day (Dierschke, in prep.). In Rybachy, however, passage migrants stay much longer (Chernetsov 1998). Consequently, the risk of re-infection could be higher in Rybachy, as well as the possibility to catch it in our study, because it was shown, for example, on captive Blackcaps that oocyst release peaks 3-4 days after infection (Dolnik & Bairlein, in prep.)

Neither prevalence nor the intensity of infection showed any tendency to increase towards the end of migration time. Thus, *Isospora* infection is unlikely to cause delay in migration.