Some aspects of the biology and host-parasite interactions of *Isospora* spp. (Protozoa: Coccidiida) of passerine birds

vom Fachbereich Biologie,
Geo- und Umweltwissenschaften
der Carl von Ossietzky Universität Oldenburg

angenommene Dissertation
zur Erlangung des Grades
eines Doktors der Naturwissenschaften

Olga Dolnik

geb. am 16.09.1973 in St. Petersburg

Angefertigt im Institut für Vogelforschung
„Vogelwarte Helgoland"
Wilhelmshaven

in Kooperation mit dem Zoologischen Institut
der Russischen Akademie der Wissenschaft
St. Petersburg, Russland

Erstreferent: Prof. Dr. Franz Bairlein

Zweitreferent: Prof. Dr. Georg Klump

Tag der Disputation: 16.12.2002

CONTENTS

CHAPTER 1

GENERAL   INTRODUCTION

General introduction

In natural animal populations parasites can be considered as important as predators (Hudson 1997). Parasites comprise a great selection pressure on host, simply because of their abundance and diversity (Price 1980). Since 1990, more than a dozen books have been published on animal host-parasite evolution, ecology and behaviour (see Clayton & Moore 1997 for a review). Birds, and especially songbirds, are regarded as excellent model systems for testing many of major questions of host-parasite interactions.

The great majority of bird endoparasites are protozoa. During the last years much attention is paid in parasitological and ornithological literature to blood Haemosporidia of passerine birds (see Valkiūnas 1997 for a review). However, enormous gaps remain in our knowledge of intestinal protozoan parasites, in particular Coccidia, in wild songbirds. The most successful genus of intestinal coccidia in passerine birds is *Isospora*. It represents about 90-95 % of known intestinal coccidia fauna of passerine birds (Pellerdy 1974) and in some bird populations 50% to over 90% of individuals can be infected by *Isospora* spp. (Scholtyseck & Przygodda 1956, Grulet *et al*. 1985, Dolnik 1998). Despite the high prevalence of *Isospora* spp. in wild passerine birds, our knowledge about consequences of an *Isospora* infection is very fragmentary. On contrary, the genus *Eimeria* (Eimeriidae) from poultry that is closely related to *Isospora* is very well studied, because of the high economical importance of the disease. Probably our knowledge of *Eimeria* spp. from poultry and lack of information about *Isospora* spp. from passerine birds is the reason why biology of *Isospora* genera is supposed to be similar to *Eimeria* spp., unless the opposite is proved.

There are several points of view on the systematic position of *Isospora* coccidia, though the differences concern the position and the name of the class. I do not discuss this problem here, but present the systematic position of *Isospora* genera according to the system of Krylov & Dobrovolsky (1980):

Phylum **Sporozoa** Leucart 1897 emend. Krylov, Mylnikov 1986

Class **Coccidiomorpha** Doflein 1901 emend. Krylov 1980

Subclass **Coccidiomorphina** Doflein 1901 emend. Krylov 1980

Order **Coccidiida** Leucart 1879 emend. Krylov 1980

Family **Eimeriidae** Minchin 1903

Genera *Isospora* Schneider 1881

Life cycle of *Isospora* spp.

Coccidia of *Isospora* genera have a monoxenous life cycle (Fig. 1) and require no intermediate transmitter for the spread of infection (Long 1982). Immunity that develops as a result of *Isospora* spp. infection does not suffice to prevent re-infection (Long 1982). Infections are transmitted by faecal contamination. Sporulated oocysts get into the bird occasionally swallowed, mostly with food or water, and after ingestion the sporozoits emerge from the oocysts and enter the wall of the intestine (Fig. 1, *1*). In epithelial cells of villi in the ileum and duodenum (Anwar 1972, Box 1977) several merogonies take place (Fig. 1, *2-12*) so that the number of parasites increases rapidly (Long 1982, Grulet *et al*. 1985). After gametogonie (Fig. 1, *13-22*), fertilisation (Fig. 2, *23*), and development of oocysts (Fig. 1, *24-25*) unsporulated oocysts are released from the bird together with faeces.
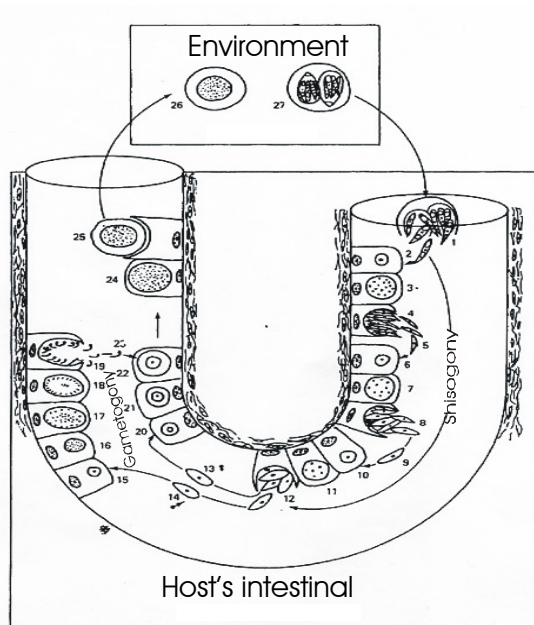
Fig. 1. Scheme of life cycle of *Isospora canaria* (Grulet *et al*. 1985).
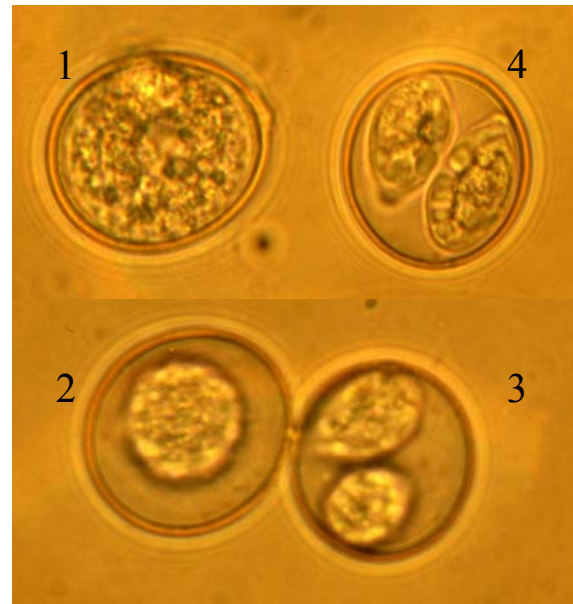
Fig. 2. Sporulation of *Isospora fringillae* oocyst from Chaffinch, *Fringilla coelebs*. 1 – unsporulated oocyst, 2-3 – stages of sporulation, 4 – sporulated

To complete their development oocysts have to sporulate (Fig. 1, *26-27*; Fig. 2). Only oocysts that completed sporulation are able to infect a new host. This process takes from 48 hours to 7 days in different *Isospora* species (Pellerdy 1974). With the only known exception of *Isospora xerophyla* (Barré & Troncy 1974), *Isospora* oocysts from passerine birds, and especially unsporulated ones, can easily be damaged by drying out or by being exposed to direct sunlight (Long 1982).

Sporulated oocyst of *Isospora* genus contains two sporocysts, each with four sporozoits. Thickness of oocyst wall, presence or absence of micropile, form and size of oocyst,

sporocysts and sporozoits, oocyst and sporocyst residuum, form and number of polar granulas, form of Stieda and substieda body, and other morphological characteristics of sporulated oocysts are used to identify the species of *Isospora*.

Fauna of *Isospora* spp. in passerine birds

Until now, about 100 species were described from birds all around the world. The latest overview of coccidia fauna was made by Pellerdy (1974) in his book "Coccidia and Coccidiosis". Since that time, some new coccidia species were described. Because of the difficulty in finding and isolating tissue stages, and because the study of these endogenous stages would require killing the host animal, it is the description of the structures of the sporulated oocysts upon which the taxonomy of most eimeriid coccidia is based (Duszynski *et al*. 1999). Unfortunately, as it was already mentioned by Pellerdy (1974) many of the descriptions are not complete or synonymous that makes it very difficult to determine the species. Further collecting from additional host taxa proved that nearly every species of bird is a potential host for one or several *Isospora* species, most of which have yet to be collected, even more to be described taxonomically or studied ecologically. However, for further investigations of *Isospora* fauna in passerine birds, more data on host specificity of these parasites would be necessary.

Host specificity of *Isospora* spp.

In general, coccidia of Eimeriidae family are thought to be narrow host specific. Nevertheless, partly because of structural resemblance between the oocysts of the forms from the various hosts, partly because of tradition, for a long time many authors reported most of the *Isospora* spp. oocysts found in more than a hundred passerine bird species as *Isospora lacazei* (see Levine 1982 for a review). Studies on *Isospora* fauna in passerine birds, description of new parasite species and revision of those already described requires better knowledge on *Isospora* host specificity. These data can be obtained only from cross-transmission studies, but up to now only three such experiments were carried out (Černá 1973, Barré & Troncy 1974, Box 1980). All three showed that *Isospora* sp. from passerine bird does not infect a host from another taxonomic family, and only Barré & Troncy (1974) showed successful transmission of the parasite between hosts of different genus within one family. Therefore, other experiments on cross-transmission are necessary to be carried out. If a parasite is indeed narrow host specific, an investigation of new bird species can lead to describing new parasite species, as it was suggested by Svobodova (1994).

Periodicity of oocysts output

The high metabolic rate of birds requires strict habits of feeding, activity, and rest, which produce a marked regularity in certain fundamental physiological functions. This seems a desirable condition for studies on the host-environment of parasites. The physiological processes within the host body are controlled indirectly by the regular alternation of the light and dark periods, which, in this case, may be the principal factors in the formation of various habits involving nutrition, muscular and nervous activity, rest, and sleep. The complex animal body, then, when considered in relation to its parasites becomes an intricate environment, which, nevertheless, often exhibits a regularity in activity ruled by external factors (Boughton 1933). Hence, in studying the parasite species living within it, one must consider the influence of physiological processes of the host organism on the parasite.

Eimeria spp. from poultry do not demonstrate any diurnal rhythmicity neither in their endogenous stages nor in oocyst output (Long 1982). As long as nothing was known about *Isospora* genus itself, most of ecological studies were based on the knowledge about biology of this closely related and well-studied genus. Therefore, the samples from passerine birds were collected at different time of the day, and as far as most of the birds can be caught in the early morning, the great majority of data belong to this time of day (e.g. Scholtyseck 1956). Only few people independent from each other found that in House Sparrow (Boughton 1933, Schwalbach 1959, Milde 1979, Grulet *et al.* 1985) and two other Ploceidae species (Barre & Troncy 1974) in captivity the oocyst output shows a daily rhythm, and nearly no oocysts can be found in morning faeces of infected birds. Moreover, it was shown in House Sparrows that all endogenous stages of *Isospora* life cycle have 24 - hours rhythms (Grulet *et al.* 1985). All these authors suggested that this knowledge should be considered when collecting samples from birds in the wild. However, this work concerned only *Isospora* from one taxonomic family of birds and remained unnoticed by many investigators. It remains open if the diurnal periodicity of oocyst output that was noticed by several authors for *Isospora* spp. from three bird species of Ploceidae family is the rule or an exception. This knowledge is important to understand more about problems of periodicity in host-parasite relationships. Moreover, knowing more about oocyst output models of different *Isospora* spp. from different host will allow to collect more exact data about prevalence and intensity of infection.

Estimation of prevalence and intensity of infection

Prevalence of infection is the proportion of the host population showing infection, often expressed as percentage. Analysis of faecal samples collected in the afternoon is sufficient to conclude whether an individual is infected by *Isospora* spp. or not. Concentration of oocysts from faecal samples using flotation centrifuging allows to determine the infection in low infected individuals, and to estimate the prevalence of *Isospora* spp. infection in bird populations.

On contrary, estimation of intensity of coccidial infection is often based on the damages of the intestine. Due to ethical rules, however, it is not appropriate to dissect wild birds, therefore sometimes only data about prevalence but not intensity of infection in wild bird population is collected. Alternatively, the intensity can be estimated from counting the oocysts in faecal samples, though not all the investigators agree (e.g. Kruszewicz & Dyrcz 2000). Some investigators estimated intensity of infection in passerine birds in captivity (e.g. Schwalbach 1959), using different methods. However, up to now, it was not proved if any of these methods gives repeatable and comparable results. An appropriate standard method is not described in literature and still needs to be established.

Effects of birds feeding style on *Isospora* spp. infection

As a consequence of the life cycle of *Isospora* species, ground feeding birds are likely to be more exposed to infection than those feeding in the air. This should be reflected in the prevalence and probably also in the intensity of infection of these species, because immunity does not suffice to prevent re-infection (Long 1982). Nevertheless, no attempts to prove it were done up to now. The only work on this subject concerns the prevalence but not the intensity of *Isospora* infection in birds of different diets (Scholtyseck 1956). He suggested that in passerine birds prevalence of *Isospora* spp. infection in insectivores birds is lower than in omnivores, but unfortunately the data were collected without taking the daily rhythm of oocyst output into account. The question if the feeding style of the birds influences prevalence and intensity of *Isospora* infection remains, therefore, unsolved.

Effects of birds age on *Isospora* spp. infection

Younger animals are generally assumed to be more susceptible to coccidial disease than older ones (Long 1982, Gylstorff & Grimm 1998). It is supposed that different susceptibility to *Eimeria* spp. infection in young and adult birds is connected with some acquired immunity against coccidia with age (Long 1982). *Isospora* infection in birds of different age has rarely been studied. It was shown that prevalence of infection with

Isospora spp. is higher in older nestlings than in younger ones (Scholtyseck & Przygodda 1959, Svobodova & Cibulkova 1995), and higher in adult Icterine Warblers than in their nestlings (Svobodova & Cibulkova 1995). On the other hand, it was shown that prevalence of *Isospora* infection does not differ between adult and juvenile Chaffinches (Gryczynska *et al*. 1999) and *Acrocephalus* spp. (Kruszewicz & Dyrcz 2000). Nevertheless, there is no literature that compares the intensity of *Isospora* infection in young and adult passerine birds, though the probable development of immunity with age can cause differences in infection intensity.

Reaction of birds on re-infection and dose-dependent response

Re-infection of chronically infected birds happens very often in the wild, and the immunity system can not prevent it (Long 1982) but probably can help the bird to stabilise the infection. Therefore, re-infection with coccidia may increase or reduce the severity of disease, but the number of reports discussing it is limited and concerns only *Eimeria* spp., which makes generalisation difficult. There is no data on the reaction of chronically infected passerine birds on re-infection.

Experiments in chicken showed that an increase in the number of *Eimeria* oocysts ingested by the host is usually accompanied by an increase in severity of disease (Hein 1968, 1969, 1971, 1974, Long 1973). On contrary, Leathem and Burns (1968) noted that very heavy doses of oocysts produced lower mortality in cecal coccidiosis of chickens. It is possible that the invasion of very large numbers of sporozoites and/or the development of the early stages produce a host reaction resulting in loss of some invasive stages (Rose *et al*. 1975). Nothing is known about the reaction of wild passerine birds on infection with different amounts of *Isospora* oocysts.

Body mass changes and infection

It is known that pathogenesis of *Eimeria* spp. leads to disturbances of absorption and permeability, and thus results in reduced food and water consumption (Yvoré & Mainguy 1972). Correspondingly, one can expect effects of intensive *Isospora* infection on bird's body mass and food intake. Nevertheless all attempts to find any correlation between *Isospora* infection and body mass of the host failed, for example, in studies on Starling nestlings (Mazgajski & Kędra 1998), or on adults and nestlings of several *Acrocephalus* species (Kruszewicz & Dyrcz 2000) in the wild. What really is going on with birds body mass can be shown only in experiments.

Bird migration and coccidia infection

Many species of passerine birds migrate to considerable distances and this migration can influence the parasites, as well as the parasites can influence the success of migration. For example, it was recorded that in Chaffinches, the individuals infected with *Leucocytozoon* spp. (Haemosporidia) are concentrated at the end of migration flow (Valkiūnas 1997), which indicates that infected birds are hindered in time of departure. However, there is no investigation on the possible influence of *Isospora* spp. on migration performance of passerine birds.

Prior to or as a consequence of sustained flights migratory birds can reduce their intestines (Biebach 1998, Piersma 1998, Bauchinger & Biebach 2001). Thus, the host's capacity for intestine parasites may be affected, and this can result in lower levels of parasite infection in those birds. Therefore, we can expect that short and long distance migrants may have different intensity or prevalence of infection as well as the intensity and prevalence of infection during migration can differ if birds migrate over land or over sea, because of possibly different selection pressures. We can also expect that the intensity of *Isospora* infection is associated with body condition of the migrating bird.

This thesis

The aim of the research described in this thesis is two-fold. On one hand, establishing a reliable method of estimating of *Isospora* infection intensity and using it in the field as well as in laboratory experiments allowed us to fill some gaps in understanding of interactions between wild passerine birds and their *Isospora* parasites. On the other hand, the research should contribute to our knowledge of *Isospora* fauna of passerine birds, and intensity and prevalence of *Isospora* infection in different host species.

Chapter 2 presents a standardised method of sampling and estimating intensity of infection. I tested our method under controlled laboratory conditions and proved that it gives repeatable and comparable results.

Chapter 3 deals with diurnal periodicity in *Isospora* oocyst output. I showed that afternoon release of *Isospora* spp. oocysts is likely to be a general rule for passerine birds, since it was shown for six bird species (**Chapter 3.1** and **3.2**). Knowledge of diurnal pattern of oocyst output in combination with using the standardised method of counting the oocyst in samples gives excellent opportunities for a study on the ecology of these parasites.

Chapter 4 deals with fauna of *Isospora* coccidia in passerine birds on the Courish Spit, where most of our field work took place. The problem of *Isospora* specificity is discussed in **Chapter 4.1**. **Chapter 4.2** gives an overview of the fauna of *Isospora* spp. in passerine birds on the Courish Spit. In **Chapters 4.3** and **4.4** new *Isospora* species from Sedge Warbler and Tree Creeper are described.

Chapter 5 results from analysing the data from the field study on the effect of feeding style of the host species on intensity and prevalence of *Isospora* infection. Prevalence and intensity of infection in birds catching insects in the air and ground feeders are compared.

The effect of age of the host and dose of infective oocysts on intensity of *Isospora* spp. infection and its consequences were studied in field and in laboratory experiments and the results are presented and discussed in **Chapter 6**. Following artificial infection of the birds I checked changes of oocyst output, body mass, and food intake of the birds. Possible reasons why many authors found no correlation between body mass of the birds and their intensity of infection in the wild are also discussed in Chapter 6.

In **Chapter 7** I compare prevalence and intensity of *Isospora* infection in birds at two stopover sites during autumn migration. Possible reasons of differences in intensity of infection between birds migrating over land and over sea, as well as between long- and medium distance migrants are discussed.

METHOD OF ESTIMATING
ISOSPORA INFECTION INTENSITY
IN PASSERINE BIRDS

Method of estimating *Isospora* infection intensity in passerine birds

Introduction

Investigation of the *Isospora* infections of wild passerine birds requires estimation not only of the prevalence of infection in the population, but also of the intensity of infection in individual birds. A typical approach to estimate prevalence is to examine the intestines of dead birds for the presence of oocysts (see for example Scholtyseck & Przygoda 1956) but such an approach requires the bird be killed and a more simple and harmless method is needed to obtain good estimates. Previous workers have suggested that "faecal analysis do not allow for clear assessment of parasite load" (Kruszewicz & Dyrcz 2000) although other workers have used faecal samples to estimate prevalence and relative intensity. For example, Boughton (1933) estimated oocysts intensity in fresh faecal smears using a subjective scale of intensity that ranged from "0" to "5". Schwalbach (1959) used a second method but this required the assessment of fresh samples within one or two days of collection, an approach that is rarely possible in field conditions. Barré & Troncy (1974) counted the number of oocysts in 1 g of native smear while the absolute number of oocysts in daily faeces was counted using flotation techniques by Milde (1979). Arnastauskene (1985) argued that comparable results required the same amount of faeces and the same amount of sediment in every estimate, but did not propose a workable method. The central issue is that the absence of any standard method of intensity estimation in the literature has meant that studies are not comparable. In this paper we propose a simple method that allows the collection of data in laboratory and field conditions and to estimate intensity of *Isospora* infection in small passerine birds. This method was tested under controlled laboratory conditions, and provides repeatable and comparable results.

Methods

Standardised method of sampling

Fresh faecal samples should be collected at the same time of each day, ideally 2 to 6 hours before sunset. If faecal samples are collected in the field, birds should be kept in a clean cotton bag or better still in a small washable cage with plastic walls and samples collected on clean floor paper. Fresh droppings need to be removed within 10 minutes of production and care should be taken to ensure the samples do not dry out. Samples from each bird should be placed in individual vials with 2% water solution of potassium dichromate ($K_2Cr_2O_7$).

To distinguish between species of *Isospora*, samples should be kept in room temperature for several days to allow sporulation. In this case it is important that the vial is not completely full and there is sufficient air present. After sporulation, the samples should be stored in a fridge (2-8 °C), although our experience is that samples can also be stored at room temperature at least for a year.

Oocysts should be concentrated by flotation in saturated NaCl solution. Each sample should then be fully mixed by shaking and put into a 10 ml centrifuge-tube topped up to 10 ml volume with water. Samples should be centrifuged for 5 minutes at 1500 R.P.M., and the supernatant removed, so that 2 ml of the lower layer remain. Add 8 ml saturated NaCl solution and centrifuge again for 5 minutes, at 1500 R.P.M. A standard quantity of the surface layer (5 loops of 5 mm diameter) is placed on slides and immediately examined under 100× magnification to determine the presence and the number of oocysts. The whole slide should be checked to avoid mistakes that can be caused by oocyst clustering.

*Evaluating standardised method*

To determine if the method developed for oocyst counting can give repeatable results, two tests were undertaken using samples collected from chronically infected young Blackcaps.

A group of chronically infected Blackcaps trapped as juveniles from a natural population were kept in the Institute of Avian Research in Wilhelmshaven (Germany). For the experiments we chose birds that were naturally infected with just one coccidia species, *Isospora sylvianthina* Schwalbach 1959. Body mass of each bird was recorded daily. Birds were maintained individually under controlled laboratory conditions (LD 14:10, 20±1 °C, 50-60% R.H.) and fed ad libitum on a standard diet prepared from dried insects, casein, saccharose, vegetable oil, minerals and cellulose, containing 15% crude protein, 10% crude fat and 5% digestible carbohydrates (Bairlein 1986). Water was also available ad libitum. For the experiments we selected 10 individuals that had not shown changes in body mass within the last 20 days.

Fresh faecal samples were collected onto clean paper from each of 10 individual birds over a period of one hour. Subsequently, we collected faecal samples from each of 8 birds, each day for 12 days at the same time of day time, 3 hours before the light was off.

Results

Variation in oocyst production in consecutive samples collected within 1 hour was greater between than within individuals (ANOVA, P=0.000) (Fig. 1).
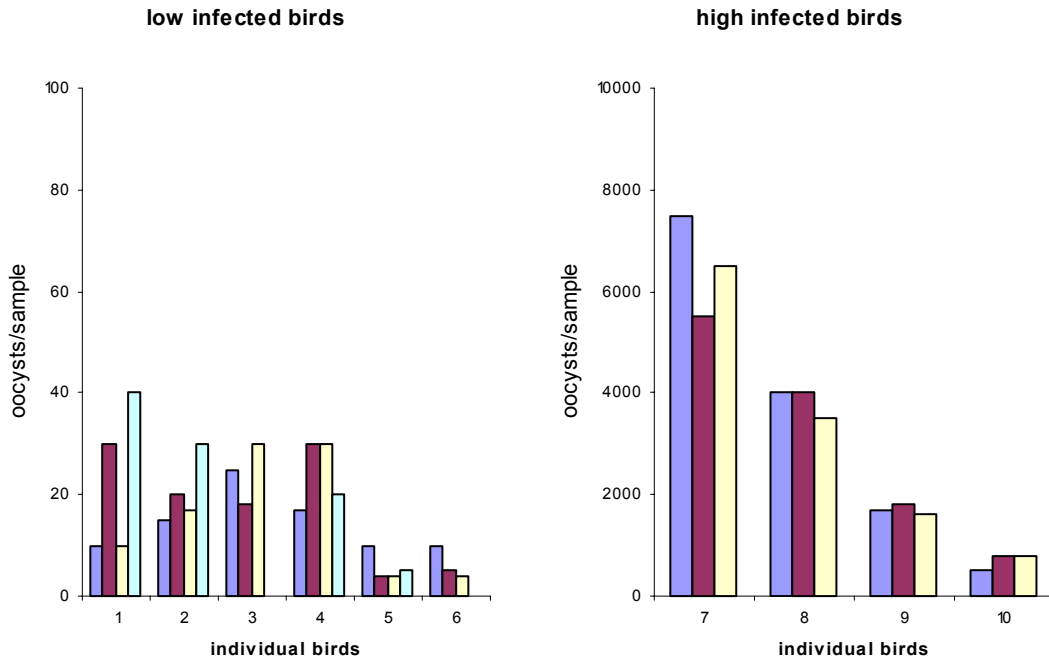
Fig. 1. The number of *Isospora* oocysts counted from consecutive samples from 10 Blackcaps.

The variation in the intensity of *Isospora* infection in chronically infected birds under controlled conditions during 12 consecutive days exhibited greater variation between than within individuals (ANOVA, P=0.000) (Fig. 2).
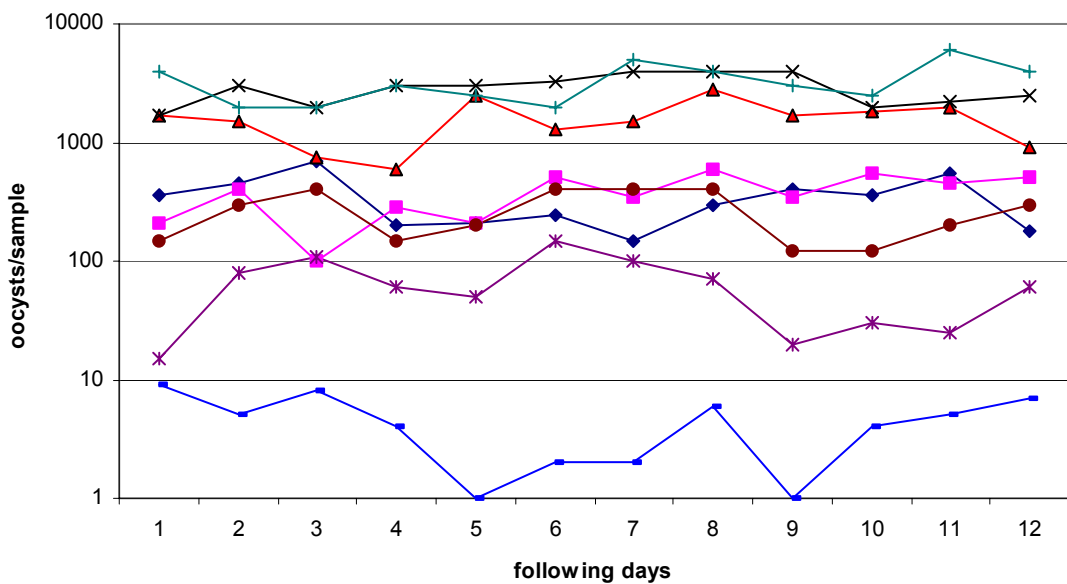
Fig. 2. Variation in the production of *Isospora* oocysts in 8 Blackcaps over a period of 12 consecutive days of sampling.

Discussion

We have identified and described a standardised flotation method for estimating the intensity and prevalence of *Isospora* species in the faecal samples of passerine birds. Testing of the methods demonstrated that *Isospora* counts were consistent within individuals, both over a range of consecutive droppings within one hour and over a period of 12 days. The flotation method is important because it first allows the oocysts to separate from the faeces and be easily visible, and secondly, allows the oocysts to be concentrated so that makes it possible to distinguish the infection at low intensity.

As a flotation solution we propose NaCl solution because in comparison to glycerin it does not deform the oocysts, it is also easy to handle, cheap and available. We suggest that the differences in the size of droppings in the evening when the second peak of birds feeding activity takes place is not that high to be recalculated. Our experimental data also prove it (Figs. 1, 2).

All workers that have reported daily periodicity in *Isospora* oocyst output from passerine birds recorded a maximum production in the afternoon (e.g. Boughton 1933, Schwalbach 1959, Barré & Troncy 1974, Grulet *et al*. 1985, Kruszewicz 1995, Dolnik 1999). Therefore we stress that it is very important to collect samples at the same time of day, and if the pattern of diurnal oocyst production for that species is not known then samples should be collected in the afternoon, 2 to 6 hours before sunset.

Our experiments showed that there is relatively little variation in oocyst abundance in consecutive droppings taken over a period of one hour. Furthermore, under controlled laboratory conditions, the oocyst output of chronically infected birds varied more between individuals than within individuals indicating that the method is repeatable and representative over a period of time. We suggest that all the possible variations in oocysts output are caused by some endogenous factors, such as re-infection (Chapter 6). Thus, we conclude, that this method allows us to get comparative data on intensity of infection. The proposed method has been successfully applied to wild populations of different bird species (Dolnik 1998, Dolnik 1999a, 1999b, 1999c, Dolnik 2000).

CHAPTER 3

DIURNAL PERIODICITY
IN *ISOSPORA* OOCYST OUTPUT

CHAPTER 3.1.

Diurnal periodicity of oocysts release of *Isospora dilatata* (Sporozoa: Eimeriidae) from the Common Starling (*Sturnus vulgaris*) in nature

СУТОЧНАЯ ПЕРИОДИЧНОСТЬ ВЫДЕЛЕНИЯ ООЦИСТ ISOSPORA DILATATA (SPOROZOA; EIMERIIDAE) ИЗ ОБЫКНОВЕННОГО СКВОРЦА (STURNUS VULGARIS) В ПРИРОДЕ

© О. В. Дольник

Сто молодых скорцов (*Sturnus vulgaris*) было поймано и обследовано в разное время суток на Куршской косе Балтийского моря (55°12′N, 20°46′E).

У птиц, начинающих постювенильную линьку, интенсивность заражения изоспорами была ниже, чем у птиц, заканчивающих линьку.

При этом ооцисты рода *Isospora* были обнаружены только в помете птиц, обследованных во второй половине дня. Максимальное количество ооцист было отмечено в пробах помета, взятых в промежутке времени между 16 и 19 ч.

Скворец, сидевший в отдельной клетке в течение 4 дней, также продемонстрировал выделение ооцист преимущественно во второй половине дня.

Возможно, такое массовое выделение ооцист в вечернее время имеет адаптивное значение для паразита. Оно может повышать концентрацию инвазионных ооцист в местах кормежки хозяев или предохранять ооцисты от немедленного высыхания под воздействием прямых солнечных лучей и малой влажности.

———

Циркадные ритмы у птиц известны уже очень давно. Установлено, что они контролируются эпифизом (пинеальным органом) головного мозга (Menaker, Oksche, 1974). Эпифиз секретирует разные по химической природе вещества, среди них гормон мелатонин. Свет подавляет, а темнота активирует его синтез. Химический механизм влияния света на мелатонин известен (Чернышева, 1995). Показано, что ритм выделения мелатонина in vitro сходен с ритмом локомоторной активности птиц при том же освещении и корректируется по фотопериоду (Takahaschi, 1982). Реверсия освещенности приводит к реверсии ритма выделения мелатонина изолированным эпифизом (Deguchi, 1982). Инъекция мелатонина живой птице модифицирует ритм ее локомоторной активности, а также задает его птице с удаленным эпифизом (Gwinner, 1978). Из всего выше сказанного можно сделать вывод, что мелатонин принимает важное участие в регуляции циркадных ритмов активности птиц.

74

Явление периодичности выделения ооцист кокцидий впервые обнаружил Боутон в 1933 г. на примере рода *Isospora* из домового воробья *Passer domesticus* (Boughton, 1933). Он также показал, что реверсия освещенности приводит к реверсии ритма выделения ооцист. Французские исследователи (Grulet e. a., 1986), работая с воробьями, содержавшимися в неволе, показали, что все стадии эндогенного развития изоспор также строго синхронизированы. Проф. Гвиннер (Gwinner) летом 1995 г. изучал суточный ритм выделения ооцист изоспор зараженными скворцами (*Sturnus vulgaris*). В неволе при естественном освещении пик выделения ооцист приходился на вторую половину дня. После удаления птицам эпифиза циркадный ритм выделения ооцист рассыпался, а после инъекции таким птицам мелатонина — восстанавливался (Гвиннер, лич. сообщ.). Однако наличие вечернего пика выделения ооцист изоспор у воробьиных птиц в природе, насколько нам известно, пока не исследовано.

Мы попытались проследить наличие циркадного ритма выделения ооцист у многих видов воробьиных птиц в природе. К настоящему времени у нас набралось достаточно материала по одному из самых массовых видов на Куршской косе — обыкновенному скворцу (*Sturnus vulgaris*). Эти данные и легли в основу нашей статьи.

МАТЕРИАЛ И МЕТОДЫ

Работа проводилась в летние сезоны 1995—1997 гг. на Куршской косе Балтийского моря (55°12′N, 20°46′E). Сбор материала производился на базе Биологической станции Зоологического института РАН в пос. Рыбачий. Восход в период отлова птиц приходился на 5.00—5.30 утра, заход солнца — на 22.30—23.00 ч. Птиц отлавливали паутинными сетями в рамках международной программы кольцевания «MRI». За три года было поймано и обследовано 100 молодых скворцов.

Птиц вынимали из путанок каждый час, кольцевали и записывали дату и время поимки, номер кольца, вид птицы, ее пол, возраст, балл жира, мускулатуры, стадию линьки, вес и др. Стадию линьки скворцов определяли по системе Ньютона (Newton, 1968).

Оценить интенсивность заражения птицы непосредственно путем вскрытия не представлялось возможным, поскольку сбор материала осуществлялся на территории национального парка. Поэтому оценку интенсивности заражения проводили по результатам анализа проб помета птиц, используя стандартизированный метод Фюллеборна. Для этого каждого окольцованного скворца сажали в отдельный садок, дно которого было покрыто чистой бумагой. Получив одну пробу помета, птицу выпускали, а пробу заливали 2%-ным водным раствором бихромата калия и выдерживали 6 сут для споруляции. Затем пробы центрифугировали со скоростью 1000 об/мин в насыщенном растворе NaCl 5 мин, после чего стандартное количество поверхностной пленки (5 петель диаметром 5 мм) переносили на предметное стекло. Изучение препаратов проводили с помощью светового микроскопа. Подсчет ооцист производили при просмотре всего препарата. За интенсивность заражения птицы условно принимали количество ооцист изоспор в препарате.

Собранный нами материал содержал сотни тысяч ооцист, и, вполне естественно, что измерить и описать их все не представлялось возможным. Поэтому мы измеряли по 30 выбранных наугад ооцист в каждой пробе и, кроме того, просматривали весь препарат в поисках ооцист, отличающихся от типичных.

Один зараженный скворец был отсажен в индивидуальную клетку. Он находился там с 14 ч 21.07 до 20 ч 24.07.1995. Клетка находилась в большом помещении со множеством окон, на естественном освещении. В клетке постоянно присутствовали корм и вода. Дно клетки было застелено белой бумагой, которую заменяли на чистую каждые 2 ч круглосуточно в течение всего эксперимента.

Весь помет с каждого листа собирали в пробирку, на которой была обозначена дата и временной интервал. Пробы выдерживали для споруляции в 2%-ном растворе бихромата калия и просматривали так же, как пробы от птиц из природы.

РЕЗУЛЬТАТЫ

Все измеренные нами ооцисты по своим морфологическим характеристикам совпадали с описанным от скворца видом *Isospora dilatata* Schwalbach, 1959. Кроме ооцист *I. dilatata* у скворца описаны ооцисты *Eimeria balozeti* Yakimoff and Gousseff, 1938, но представителей рода *Eimeria* нам обнаружить не удалось. У родственного обыкновенному скворцу вида *Sturnus contra* в Индии были описаны ооцисты *Isospora lonchurae* Mandal and Chakravarty, 1964. В отличие от *I. dilatata* ооцисты этого вида имеют остаточное тело и обладают более крупными размерами и овальной формой. Такие ооцисты у скворцов нами обнаружены не были.

Количество ооцист в пробах от птиц, находящихся на более ранних стадиях постювенильной линьки, было меньшим, чем в пробах птиц с более поздними стадиями линьки. Таким образом, интенсивность заражения молодых скворцов изоспорами возрастала с продвижением линьки (рис. 1, *а*). Однако интересно отметить, что экстенсивность заражения птиц на всех стадиях линьки оставалась практически постоянной (рис. 1, *б*).

Распределение количества выделенных птицами ооцист по часам суток выглядит следующим образом (рис. 2).

До 14 ч количество ооцист, выделяемых скворцами, незначительно. К 16 ч наблюдается достоверное увеличение этого количества, в 17 ч. опять спад. К
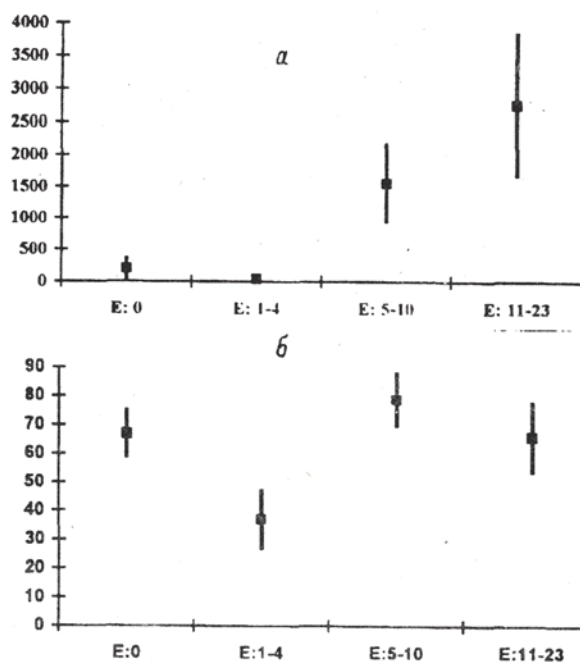

Рис. 1. Зависимость зараженности молодых скворцов от стадии их линьки, по данным 1995—1997 гг.

а — интенсивность заражения; по оси абсцисс — баллы линьки (от 0 до 23); по оси ординат — количество ооцист, шт.; *б* — экстенсивность заражения; по оси ординат — процент зараженных птиц.

Fig. 1. Dependence of *Isospora* infection of young starlings on the stage of their moult, according to data 1995—1997 years.
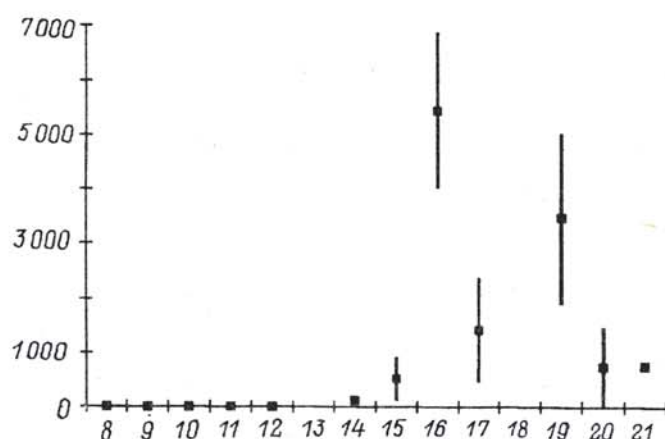
Рис. 2. Распределение количества ооцист изоспор, выделенных пойманными в природе скворцами по часам суток.

По оси абсцисс — часы суток.
Остальные обозначения такие же, как на рис. 1.

Fig. 2. The distribution of the number of *Isospora* oocysts from the birds caught in nature by the time of day.

сожалению, у нас отсутствуют данные за 18 ч, так как в это время не было поймано ни одной птицы. После еще одного подъема в 19 ч, в 20 и 21 ч число выделенных ооцист опять уменьшается. Несмотря на возможное наличие двух пиков выделения ооцист между 15 и 21 ч, общая тенденция выделения ооцист в этот период времени очевидна.

В результате просмотра проб от скворца, сидевшего в индивидуальной клетке, мы наблюдаем пик выделения ооцист между 16 и 19 ч (рис. 3) и почти полное отсутствие выделяемых ооцист с полуночи до 2 ч дня. В разные дни интенсивность выделения ооцист во время вечернего пика также значительно варьировала (рис. 4), причем каждый последующий день количество ооцист, выделяемых птицей во время вечернего пика, значительно уменьшалось.
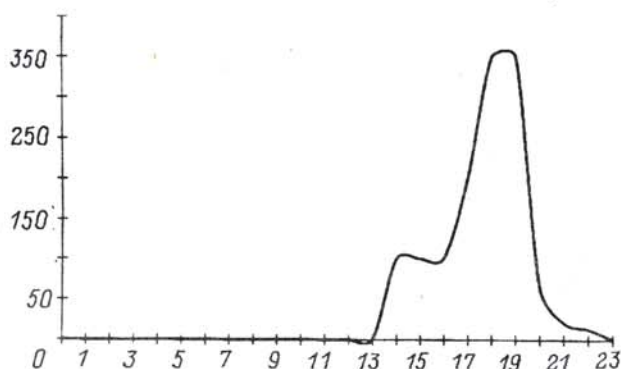

Рис. 3. Распределение количества ооцист изоспор, выделенных скворцом в неволе по часам суток.

По оси абсцисс — часы суток.
Остальные обозначения такие же, как на рис. 1.

Fig. 3. The distribution of the number of *Isospora* oocysts from the starling in captivity by the time of day.
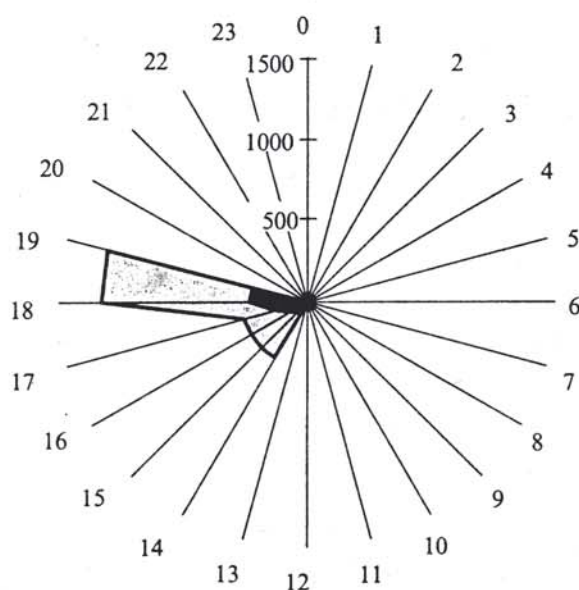
Рис. 4. Распределение количества ооцист, выделенных скворцом в неволе по часам суток в разные дни.

Fig. 4. The distribution of the number of *Isospora* oocysts frim the starling in captivity by the time of day in different days.

ОБСУЖДЕНИЕ

Зависимость интенсивности заражения молодых скворцов от стадии их линьки можно рассматривать как зависимость зараженности от возраста. Известно, что спустя 20—25 дней после вылета из гнезд молодые скворцы начинают постювенильную линьку. Начало линьки у скворцов приходится на возраст 42—53 сут и продолжается 110—130 дней (Носков, 1990). Таким образом, скворцы со стадией линьки Е:0 не старше 53 сут, а возраст скворцов со стадией линьки Е:50 составляет от 150 до 180 сут. Возраст скворцов с самой продвинутой стадией линьки из тех, что попались нам в период эксперимента (Е:23) составлял соответственно около 120 сут.

Наблюдаемое нами возрастание интенсивности заражения молодых скорцов в ходе продвижения их линьки может объясняться несколькими причинами. Возможно, что линька как энергоемкий процесс понижает сопротивляемость организма птицы, что и приводит к увеличению интенсивности ее заражения изоспорами. Однако более вероятным представляется, что повышение интенсивности заражения птиц связано с их возрастом. Становясь старше, молодые скворцы объединяются в стаи сперва по 10—20, затем по 100—200, а позже по несколько тысяч особей (Feare, 1984). Такие стаи на Куршской косе совершают ярко выраженные кочевки, называемые промежуточными миграциями (Schüz, 1932). Следует отметить, что род *Isospora* не нуждается в промежуточном хозяине и заражение происходит пассивно при заглатывании зрелых ооцист с пищей или с водой. Таким образом, стайное поведение и массовые кочевки скворцов могут способствовать их первичному и повторному заражениям.

При этом интересным фактом оказывается практически не изменяющаяся в ходе продвижения линьки доля зараженных молодых скворцов (рис. 1, б). В связи с этим можно предположить наличие определенного процента птиц, генетически или по какой-то другой причине более устойчивых к заражению изоспорами. В зараженных птицах, по-видимому, происходит очень интенсивная эндогенная агломерация паразита.

Теперь обратимся к суточной динамике выделения ооцист. Из представленных данных ясно видно наличие вечернего пика их выделения. К сожалению, из-за отсутствия данных за 18 ч мы не можем сказать определенно, является ли уменьшение интенсивности выделения ооцист птицами в 17 ч случайным или же имеется два вечерних пика. В отличие от данных за другие часы данные за 17 ч получены от птиц, принадлежавших к одной стае, и следовательно, небольшое количество выделяемых ими ооцист может быть лишь следствием низкой зараженности всей стаи. Кроме того, все птицы из этой стаи имели начальные стадии линьки, т. е. были недавно вылетевшими из гнезд, что может также быть причиной низкой интенсивности их заражения, как было показано выше (рис. 1). Следует отметить, что основная масса ооцист у всех птиц относилась к одному и тому же виду, и следовательно, если предположить наличие двух вечерних пиков, то их нельзя объяснять простым присутствием разных видов изоспор в каждом из них.

Можно было бы предположить, что выделение птицами ооцист во второй половине дня связано с более частой поимкой зараженных особей в это время. Действительно, не исключено, что поведение зараженных и незараженных птиц отличается, и зараженные птицы более активны в вечерние часы, потому чаще попадаются именно в это время. Однако пробы от скворца, сидевшего в клетке, демонстрируют пик выделения ооцист вечером и полное отсутствие ооцист в помете в первой половине дня. То же подтверждают опыты Гвиннера со скворцами и работы с воробьями (Boughton, 1933; Grulet e. a., 1986).

Таким образом, просматривая утреннюю пробу помета зараженного скворца, можно сделать ошибочный вывод о его незараженности изоспорами. Для сравнения интенсивности заражения изоспорами скворцов (и, по-видимому, многих других видов воробьиных птиц), а также при сравнении экстенсивности заражения стай по количеству ооцист в пробах помета, следует учитывать наличие циркадного ритма выделения ооцист.

В настоящее время мы не можем объяснить наличие вечернего пика выделения ооцист. Из работ Гвиннера следует, что этот пик совпадает с повышением концентрации мелатонина в крови хозяина. По-видимому, массовое выделение ооцист во второй половине дня может иметь адаптивное значение для паразита.

В вечернее время кормовая активность птиц очень высока. Можно предположить, что ооцисты изоспор, выделившиеся в это время, имеют более высокий шанс быть проглоченными птицей того же вида на том же месте несколько дней спустя. Во всяком случае, такой механизм позволяет паразитам повысить концентрацию инвазионных ооцист на местах кормежки хозяев. Кроме того, ооцисты изоспор гибнут при высыхании. Поэтому возможно также, что выделение неспорулировавших ооцист в вечерние, а не в утренние часы предохраняет их от высыхания из-за немедленного и длительного воздействия солнечных лучей и малой влажности.

Если принять одну из этих гипотез, то мелатонин служит лишь условным сигналом для выделения ооцист изоспор, так как повышение его концентрации в крови птицы связано с наступлением определенного времени суток. Кроме того, можно предположить, что мелатонин сам является причиной выведения ооцист из организма хозяина. Поскольку мелатонин активизирует иммунную систему животного (Чернышева, 1995), возможно, что массовое выделение ооцист при увеличении концентрации мелатонина в крови хозяина происходит в ответ на изменение активности его иммунной системы.

Все эти гипотезы не исключают друг друга. Возможно, что выделение ооцист изоспор в вечерние часы является сложной адаптацией к физиологическим ритмам и поведению хозяев. Как бы то ни было, взаимосвязь циркадных ритмов активности воробьиных птиц и ритмов выделения ооцист изоспор является интересной темой и нуждается в дальнейшем исследовании.

Список литературы

Носков Г. А. Скворец — Sturnus vulgaris // Линька воробьиных птиц Северо-Запада СССР. Л.:
    Изд-во ЛГУ, 1990. С. 264—268.
Чернышева М. П. Гормоны животных // Введение в физиологическую эндокринологию.
    СПб.: Глагол, 1995. 269 с.
Boughton D. S. Diurnal gametic periodicity in avian Isospora // Am. J. Hyg. 1933. Vol. 18, N 1.
    P. 161—184.
Deguchi T. Endogenous oscillator and photoreception for serotonin N-acetyltransferase rhythm in
    chicken pineal gland // Vertebrate cyrcadian systems. Structure and physiology. Berlin, 1982.
    P. 146—172.
Feare C. The Starling. Oxford: Oxford University Press, 1984. 386 p.
Grulet O., Landau I., Millet P., Baccam D. Les Isospora du moineau. 2 — Etudes sur la bio-
    logie // Ann. Parasitol. Hum. Comp. 1986. T. 61, N 2. P. 161—192.
Gwinner E. Effects of pinealectomy on cyrcadian locomotor activity rhythms in European starling,
    Sturnus vulgaris // J. Comp. Physiol. 1978. Vol. 126. P. 123—129.
Menaker M., Oksche A. The avian pineal organ // Avian biology. 1974. Vol. 4. P. 79—118.
Newton J. The moult of the Bullfinch (Pyrrhula pyrrhula L.) // Ibis. 1968. Vol. 108. P. 41—67.
Schüz E. Frühesommerzug bei Star und Kiebitz // Vogelzug. 1932. N 3. S. 49—57.
Takahaschi J. S. Cyrcadian rhythms of the isolated chicken pineal in vitro // Vertebrate circadian
    systems. Structure and physiology. Berlin, 1982. P. 158—163.

DIURNAL OOCYST PERIODICITY IN ISOSPORA DILATATA (SPOROZOA,
EIMERIIDAE) FROM THE COMMON STARLING (STURNUS VULGARIS) IN NATURE

O. V. Dolnik

SUMMARY

One hundred of young starlings were caught in the different time of day on the Courish spit of the Baltic Sea (55°12′N, 20°46′E). The feces of these birds were collected at the time of capture and then examined to find *Isospora* oocysts.

The intensity of infection was higher among the starlings at an early stage of the postjuvinal moult that among those who were at the last stages of the moult.

The *Isospora* oocysts were observed only in feces that were collected in the afternoon. The most number of oocysts were observed in feces collected between 4 p. m. and 7 p. m.

In the droppings of one starling, who was sitting in a cage for four days, the oocysts were also observed only in the afternoon.

The appearance of *Isospora* oocysts in the droppings of birds only in the afternoon may have adaptive meaning. For example, it can increase the concentration of invasion oocysts at forage places of the host. It can also preserve the oocysts from drying up immediately because of the straight sunlight and low humidity.

CHAPTER 3.1. (Translation)

Diurnal periodicity of oocysts release of *Isospora dilatata* (Sporozoa: Eimeriidae) from the Common Starling (*Sturnus vulgaris*) in nature

Parasitologiya 1999, 33 (1): 74-80 (in Russian, English summary).

Introduction

Birds have circadian rhythms that are controlled by the pineal organ in the brain (Menaker & Oksche 1974). Its predominant cell types are modified photoreceptors that produce the hormone melatonin. Synthesis of melatonin depends on the light condition, it is inhibited by light and initiated by darkness. Chemical mechanisms of the influence of light on melatonin synthesis are known (Chernisheva 1995). It is shown that the rhythm of melatonin production coincides with locomotion activity rhythm of the bird and is corrected according to photoperiod (Takahaschi 1982). The reverse of the light (light at p.m. hours and darkness at a.m. hours) leads to reverse in melatonin production by isolated pineal organ. Injection of melatonin modifies the locomotor activity rhythm of a bird and introduces a rhythm to a bird with removed pineal organ (Gwinner 1978). Thus melatonin plays an important role in regulation of circadian rhythms in birds.

Periodicity in coccidian oocyst appearance was first shown by Boughton (1933) on *Isospora* oocysts from a House Sparrow (*Passer domesticus*). He also showed that reverse of light leads to reverse of oocyst output rhythm (Boughton 1933). It was shown on captive House Sparrows that all the endogenous stages of *Isospora* development are strictly synchronised by time with a 24-hours period (Grulet *et al*. 1985). The existence of oocyst output rhythm in birds in the wild was not shown up to now.

We collected material to follow the diurnal pattern in *Isospora* oocyst output in many bird species in the wild. Here we present the data from Starling, one of the most numerous bird species at the site of our investigation.

Material and methods

The study was carried out in summer 1995-97 on the Courish Spit Baltic Sea (55°12´N, 20°46´E). At the time of our study the sunrise was between 5.00-5.30 a.m. and the sunset at 10.30-11 p.m. The material was collected at the Biological Station of the Zoological Institute RAS in Rybachy where birds were trapped by mistnets and ringed in frames of international bird ringing programme MRI.

For this study 100 of young Starlings were trapped. Birds were taken out from the nets every full hour, and ringed following routine protocol. Recorded data included date and time of capture, ring number, bird species, sex, age, fat and muscle rate, stage of moult and body mass. Stage of moult of starlings was described by the standard 50 gradation scale (Newton 1968).

It was impossible to estimate the intensity of *Isospora* infection directly by dissecting the host because we carried out our work on the territory of a national park. Therefore, we estimated the intensity of infection using faeces samples analysed by a modified standardised method of Fülleborn. Every ringed Starling was put into a small separate cage with clean ground paper. From every individual bird we collected one dropping of faeces. The bird was released and the dropping was put into an individually marked tin with 2% kalium dichromate solution The samples were kept at room temperature for 6 days to allow sporulation. Then the samples were centrifuged for 5 minutes (1500 RPM) in saturated NaCl solution. Standard quantity of surface level (5 loops of 5 mm diameter) was taken. Light microscope ($\times100$, $\times400$, $\times1000$) was used. To avoid mistakes in counting the oocysts that can be caused by oocyst clustering, we looked through the whole preparate.

Some of the collected samples contained thousands of oocysts, and in that case it was impossible to measure and determine every oocyst. We measured 30 oocysts per sample and were searching for untypical oocysts while looking through the whole preparate.

One infected starling was kept in an individual cage from 2 p.m. 21 July until 8 p.m. 24 July. The cage was exposed to natural light conditions. Food and water were offered *ad libitum*. The bottom of the cage was covered by paper that was changed every two hours during days and nights along the whole experiment. All the faeces from the paper were immediately collected into a tin with kalium dichromate to avoid drying. The tins were marked with date and time. The samples were kept in kalium dichromate for the sporulation and then checked the same way as the samples from wild birds.

Results

All the oocysts that we observed in the Starling samples belonged to *Isospora* genera. According to their morphological characteristics all the oocysts that we measured belonged to *Isospora dilatata* Schwalbach 1959.

The amount of oocysts in the samples from birds with earlier stages of moult was lower than from birds with later stages. Thus, the intensity of infection in young starlings increased during moult (Fig. 1a). The prevalence of infection, however, was not significantly different in birds of different moult stages (Fig. 1b).

Oocyst production before 2 p.m. was very low. More than 90 % of the oocysts were found in the samples that were collected between 4 p.m. and 8 p.m. (Fig. 2).

The peak of oocyst production from the captive Starling was between 4 p.m. and 7 p.m. (Fig. 3). There were no oocysts in the samples taken at the time between midnight and 2 p.m. The oocysts output was different in different days (Fig. 4) so that on the second and third day the intensity of infection decreased.

Discussion

We did not observe any oocysts of *Eimeria* in the Starlings' samples, though *Eimeria balozeti* Yakimoff et Gousseff 1938 is known for Starlings. We also found no oocysts of *Isospora lonchurae* Mandal & Chakravarty 1964, that was described from *Sturnus contra* in India. These oocysts are larger than *I. dilatata*, have oval form, and residuum body. Presence of only one *Isospora* species in samples is an important fact that excludes the possibility of overlapping the oocyst output patterns of different species.

The increase of intensity of infection with moult can be caused by several factors. We can suppose that moult as an energy consuming process reduces the immune defence of the organism that leads to the increase in *Isospora* infection. But dependency of infection intensity in young starlings on their stage of moult can be also interpreted as dependency from age. Young starlings in NW Russia start postjuvenile moult at an age of 42-53 days and the moult continues for 110-130 days (Noskov 1990). Thus, Starlings that did not start their moult yet (stage E:0) are not older than 53 days, and the age of Starlings with the highest moult stage among those we sampled (E:23) should be about 120 days. After fledging the young starlings gather first in small flocks of 10-20, then 100-200, and later of several thousands of birds (Feare 1984). On the Courish Spit these flocks show summer migrations, so-called "Zwischenzug" (Schüz 1932). *Isospora* parasites do not include intermediate host and the infective oocysts enters a new host passively, swallowed with food or water. Therefore, gathering in flocks and groups can rise birds' risk to become infected or re-infected with *Isospora*.

It is interesting to notice that the prevalence of infection does not change significantly with age. We can suggest that there is a certain part of birds in the population that by some reasons are resistant to *Isospora* infection. On the other hand, in infected birds a very intensive multiplication of the parasites takes place.

Now about the diurnal pattern of oocyst output. We can see that oocyst output occurred in the late afternoon, mostly between 4 p.m. and 8 p.m. Unfortunately we did not trap any Starlings at 6 p.m. and therefore we can not say if the lower oocyst output at 5 p.m. was just occasional, or if there were two peaks of oocyst output. Five o'clock was the only hour when all the samples were taken from birds of one flock, that means, lower intensity at this hour can be caused by lower intensity of that particular flock. Moreover, all the birds from that flock were recently fledged, at the very beginning of their moult stages, which can also explain their lower oocyst output level. As there was only one species of *Isospora* found in the samples, the probable two peaks can not be caused by the presence of two different parasite species.

One could suppose that the higher number of oocysts in evening samples is due to the effect that more infected birds are trapped in the afternoon. It is probable that the behaviour of highly infected birds differs from the behaviour of uninfected ones. But our experimental Starling, as well as the experiments of Boughton (1933) and Grulet *et al.* (1986) with Sparrows prove that there are diurnal changes of *Isospora* oocyst output within an individual.

To compare intensity of *Isospora* infection of Starlings (and probably many other species of passerine birds), as well as to estimate the prevalence of *Isospora* infection in passerine birds population based on counting of oocysts in the faeces samples one should always take into account the diurnal periodicity of *Isospora* appearance.

We can not explain why the oocysts appear in the afternoon. From the work of Gwinner (unpubl.) it seems that the peak of oocyst production coincides with increase of melatonin concentration in the blood of the host. It is possible that mass oocyst appearance in the afternoon may have some adaptive reasons for the parasite.

The feeding activity of birds in the evening is high. We can suppose that the oocysts that are released at that time have higher probability to be swallowed by the next host at the same place some days later. Releasing at the feeding places of the birds allows to increase the concentration of invasious oocysts at the feeding places of hosts. Drying out kills the oocysts, therefore we can also suggest that afternoon output protects the unsporulated oocysts from immediate drying out from direct sunlight and low humidity.

If to accept one of these hypothesis, then melatonin is just a signal to oocyst output and it's increase in blood indicates the time of day for the parasite. Another hypothesis is that melatonin by itself is the reason for the oocyst output. Melatonin activates the immune system, and oocyst output during melatonin concentration increase in blood can be the outcome of changes of the activity of the immune system.

The oocyst output in the afternoon is likely to be a complicated adaptation to physiological rhythms and behaviour of the host. The interaction of circadian rhythms of birds and rhythms of parasite development may be the result of long-lasting co-evolution and this interesting subject deserves further investigations.

CHAPTER 3.2.

Olga V. Dolnik

Zoological Institute, Russian Academy of Sciences, Universitetskaya nab. 1,
St. Petersburg, 199034, Russia

DIURNAL PERIODICITY IN APPEARANCE OF *ISOSPORA* (PROTOZOA: COCCIDEA) OOCYSTS FROM SOME PASSERINE BIRDS

Coccidia are wide spread one-cell parasites of invertebrates and vertebrates. Diurnal periodicity in appearance of some stages of Coccidea development is well known for *Plasmodium* sp. (ord. Haemosporida), but it is not described for *Eimeria* sp. (ord. Coccidiida). At the same time diurnal periodicity of *Isospora* sp. (ord. Coccidiida) oocysts has been recorded from sparrows (*Passer domesticus*) in captivity (Boughton, 1933; Schwalbach 1959, Grulet *et al.* 1986). We checked the existence of this diurnal rhythm of *Isospora* oocysts from several other species of passerine birds in the wild, and compared the results with data from caged birds.

MATERIAL AND METHODS

Studies were undertaken on Biological Station on the Courish Spit of the Baltic Sea. During the years 1995-1998 we checked more than 950 passerine birds of 50 species in the wild, and more than 40 birds of 9 species in captivity. Some of the material is presented in this paper. 112 young Starlings (*Sturnus vulgaris*), 66 Scarlet grosbeaks (*Carpodacus erythrinus*), 60 Garden warblers (*Sylvia borin*), 45 Reed warblers (*Acrocephalus scirpaceus*), 42 Willow warblers (*Phylloscopus trochilus*), and 37 Chaffinches (*Fringilla coelebs*) were trapped by mistnets at different time of day during June and July, 1996-98. Fresh feces from the birds were collected at the moment of capture, the birds were released after ringing them. When the sporulation of the oocysts was completed, the samples were checked using the standard method.

Flotation method of oocysts concentration was used. Each sample was centrifuged in saturated NaCl solution (5 minutes, 1500 R.P.M.). The standard quantity of surface layer (5 loops, diameter of the loop is 5 mm) was taken. The number of oocysts was counted while checking the whole sample.

Fourteen birds (6 White wagtails, *Motacilla alba*, 5 Chaffinches, *Fringilla coelebs*, 2 Starlings, *Sturnus vulgaris*, and one Blackcap, *Sylvia atricapilla*) were kept in cages under natural light conditions. Feces samples from the caged birds

were collected every 2 hours during several days and were checked using the same method.
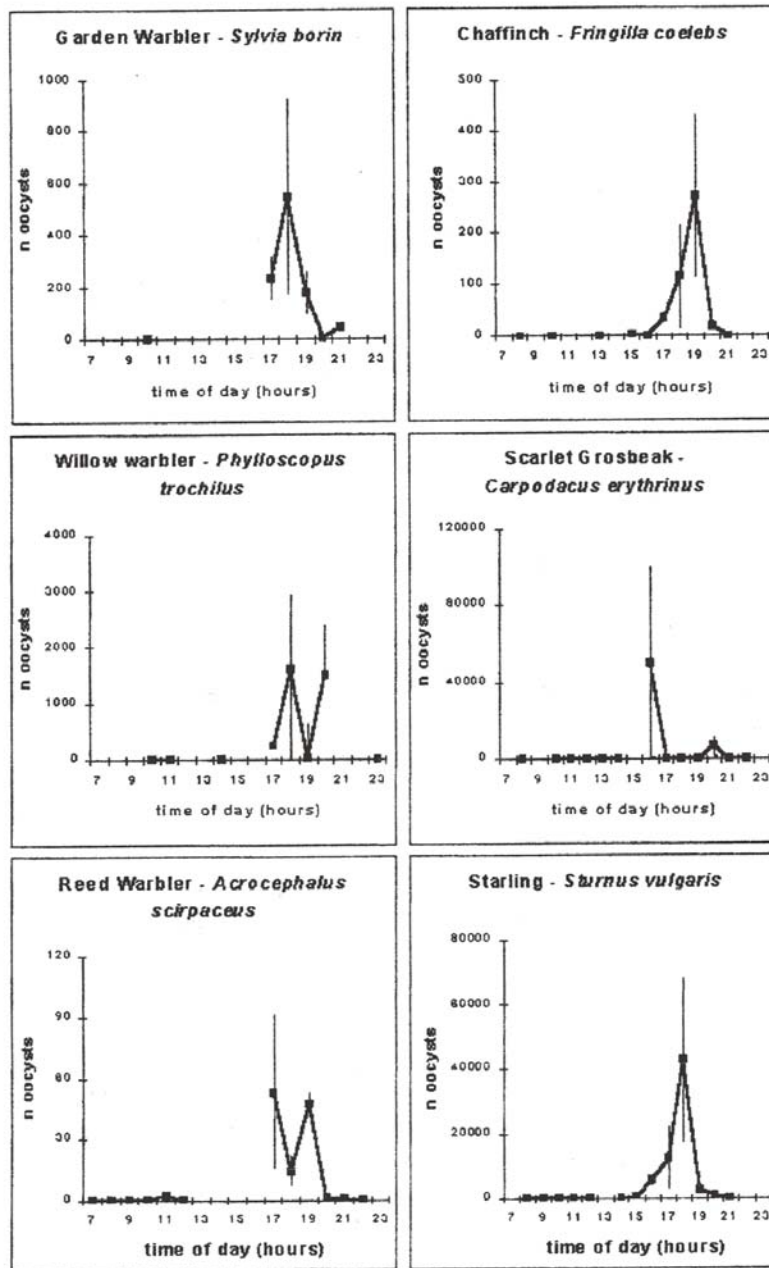
Fig. 1. *Isospora* oocysts' output from birds in the wild (summer)

RESULTS

In all six selected species of free-living passerine birds oocysts of *Isospora* were found. The number of oocysts in the samples occurred to be different in different time of day (Fig. 1). All data concern only the light time of day, because these species of birds can't be caught by mistnets in the darkness. The six species belong to four families of Passeriformes, and they had different species of *Isospora*. We also can see a great difference in the intensity of *Isospora* infection between the host species as well as between the individuals. Nevertheless, all of them clearly show one peak of oocysts' output in the afternoon. There are no or nearly no oocysts in the morning samples. The peaks vary in forms, they can start at earlier or later time, but they are always between 4 and 8 p.m.

In captivity we can see the similar model (Fig. 2). The four species of birds belong to four different families. Under the experimental conditions it was possible to collect samples from both light and dark time of day. On Fig. 2 the hours of darkness are marked by black bars. There was nearly no oocysts output not only during morning time, but also at night. The oocysts output was only in the afternoon and had one peak. Compared to the free living birds, birds in captivity can start oocysts output earlier, and finish it later; so the time when the peak appears is between 1 and 9 p.m. It is also interesting to notice that in most of the caged birds during the first day of captivity the number of oocysts in afternoon samples was significantly higher, than during the next days.

DISCUSSION

The existence of a diurnal rhythm in *Isospora* oocysts output was described for the first time by Boughton (1933). He collected feces from caged House sparrows (*Passer domesticus*) at different time of the day, and he showed that the oocysts appear mostly in the afternoon, their output has one peak, and that the time of their appearance doesn't depend on the time of birds feeding. He also showed in the experiments, that the rhythm of oocysts output is connected with the photoperiodical conditions: after a reversion of light and darkness, there was a reversion of the oocysts output time. His paper attracted no attention and later on it was forgotten, because at that time there was no theory of birds' circadian rhythms yet. Schwalbach (1959) also revealed such a rhythm of oocysts' output in the caged house sparrows, and showed that there can be a slight difference in the form of peak and in its time between individual birds. In 1986 it was shown that in caged sparrows not only the oocysts' appearance, but also all the other stages of *Isospora* development are well synchronised. All the three papers were made on one species of birds and under experimental conditions. In our previous research we showed the existence of evening *Isospora* oocysts' output from free-living Starlings on the Courish Spit (Dolnik, 1999) and from free-living Chaffinches in north-eastern

Poland (Gryczynska *et al.*, 1999). We also showed the later time of maximum *Isospora* oocysts' output in autumn compared to summer (Dolnik, 1998).
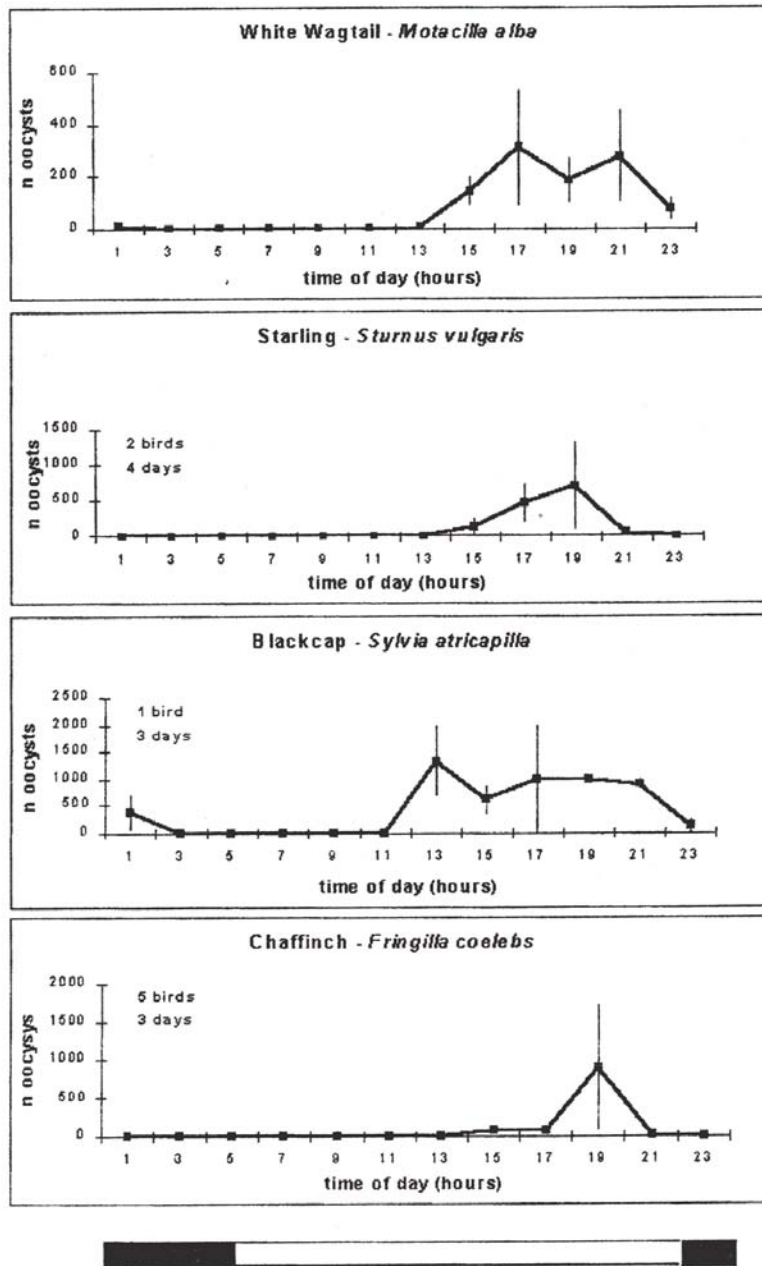

Fig. 2. *Isospora* oocysts' output from birds in captivity (July;black bars -darkness, white bar - daylight)

116

In this paper we present the diurnal models of *Isospora* oocysts' appearance in the wild for six species of hosts. Unfortunately sometimes it was impossible to cover every next hour of a day by trapping the birds of the same species, but despite some missing data, the general model of oocysts' output within the period between 4 and 8 p. m. is obvious (Fig. 1). Up to now nobody knows the effect of *Isospora* on host's behaviour. That is why one can suppose, that the increase of oocysts in the samples from birds caught in the evening can be connected with higher activity of infected birds at this time of day. But the experiments with caged Sparrows that were described above as well as our data (Fig. 2) show that individual birds have rhythms of oocysts output. So the rhythm exists in different species of *Isospora* and their hosts. What kind of biological meaning can it have for the parasite?

Isospora from passerine birds are homoxenous parasites, so such a rhythm cannot be an adaptation to the time of intermediate host's activity, as it is in case with *Plasmodium*.

It is known that in nature passerine birds have two peaks of feeding activity, one in the morning, and the other one in the evening. During this time birds visit the same territories for feeding. In breeding season they collect food at the same territory every day, during the migration time many species gather in flocks and feed all together. If parasites appear in feces during this time, the concentration of oocysts at such places increases. This gives better chances to oocysts to infect a new host of the same species. But then why don't oocysts appear at both feeding peaks of the host? May be the appearance in the afternoon prevents the oocysts from straight sunlight and from getting dry during the first hours. There can be also another explanation. It is known, that in some passerine species with the start of so-called "migration state" the morning peak of feeding activity disappears, but never the evening one (Dolnik, 1974).

What can be a signal for the oocysts to get out from cells into intestines and then outside? As it was said before, in experimental conditions they don't react on the time of host's feeding, but on photoperiod. Circadian rhythms of birds are controlled by pineal organ and by its hormone melatonin. Pinealectomy destroys not only the circadian rhythms of pinealectomized starlings but also the rhythm of their *Isospora* oocysts' output (E.Gwinner, personal communication). Nevertheless, up to now we can't say anything about how in blood melatonin can influence intracellular intestinal parasites. But even irrespective of a mechanism, the diurnal *Isospora* oocysts' output rhythms, that exist in nature as well as in caged birds of different species and families, should be an important adaptation of these parasites to their hosts, and it is an important special feature of *Isospora* genus. The existence of such a rhythm must be taken into account while collecting material about *Isospora* infection of birds. For receiving comparable data sampling should be done in the afternoon, during the peak of oocysts' output.

O.V. DOLNIK

REFERENCES

Boughton, D.S. 1933. Diurnal gametic periodicity in avian *Isospora. The American Journal of Hygiene* 18 (1): 161-184.

Dolnik, O.V. 1998. *Isospora* coccidia (Protozoa, Eimeriidae) of passerine birds on the Courish Spit. *Bulletin of the Scandinavian Society for Parasitology* 8 (2): 58.

Dolnik, O.V. 1999. [Diurnal oocyst periodicity in *Isospora dilatata* (Sporozoa ; Eimeriidae) from the Common starling (*Sturnus vulgaris*) in nature]. *Parasitologiya* 33 (1): 74-80.

Dolnik, V. R. 1974. [Diurnal feeding and locomotor patterns in migratory birds]. In: *Birds' biology researches.* (R.L.Potapov. Ed.). pp. 3-13. Leningrad, Nauka.

Grulet, O., Landau, I., Millet, P.& D.Baccam. 1986. Les *Isospora* du moineau. 2 - Etudes sur la biologie. *Ann. Parasitol. Hum. Comp.* 61 (2): 161-192.

Gryczynska, A., Dolnik, O., & T.Mazgajski. 1999. Parasites of Chaffinch (*Fringilla coelebs*) population. Part I. Coccidia (Protozoa, Apicomplexa). *Wiadomosci Parasitologiczne*. In press.

Schwalbach, G. 1959. Die Coccidiose der Singvoegel. I. Der Ausscheidungsrhythmus der *Isospora*-Oocysten beim Haussperling (*Passer domesticus*). *Zentralblatt fuer Bakteriologie, Parasitenkunde, Infectionskrankenheiten und Hygiene. I. Orig.* 178: 263-276.

CHAPTER 4

SPECIES AND SPECIFICITY

CHAPTER 4.1.

Isospora sylvianthina (Protozoa: Coccidiida), parasite of Blackcap, does not infect Reed Warbler

O.V. Dolnik

Dolnik, O.V. 2002. *Isospora sylvianthina* (Protozoa: Coccidiida), parasite of Blackcap, does not infect Reed Warbler. *Zoosystematica Rossica*, **10**(2), 2001: 240.

Sporulated oocysts of *Isospora sylvianthina* were extracted from faeces of Blackcaps. Reed Warblers and control group of Blackcaps (both species belong to the family Sylviidae) received a similar dose of the oocysts. *Isospora sylvianthina* did not infect Reed Warblers. This experiment provides one more evidence that at least some *Isospora* coccidia are narrow host specific.

O.V. Dolnik, Zoological Institute, Russian Academy of Sciences, Universitetskaya nab. 1, St.Petersburg 199034, Russia, and Institut für Vogelforschung "Vogelwarte Helgoland", An der Vogelwarte 21, D-26386 Germany.

Most of intestinal coccidian species that infect passerine birds belong to the genus *Isospora* (Pellerdy, 1974). Many authors studied isosporans from over 100 bird species and called them all *Isospora lacazei* (see Levine, 1982 for a review). However, this is not based on cross-transmission studies, which have very rarely been carried out, but on a structural resemblance between the oocysts of the forms from the various hosts, and on tradition (Levine, 1982). To orientate oneself in the descriptions that already exist in literature and to describe new *Isospora* species from passerine birds, the question of specificity of these parasites is very important.

Levine (1982) assumed that "a coccidian species may be transmissible from one species to another in the same genus, but not from one genus to another in the same family until otherwise demonstrated". Unfortunately, up to now there are only three cross-transmission experiments that are published (Černá, 1973; Barré & Troncy, 1974; Box, 1980). Therefore it is clear that other experiments that will support or disprove this suggestion are necessary.

Young Blackcaps (*Sylvia atricapilla*) and Reed Warblers (*Acrocephalus scirpaceus*) were kept in the Institute of Bird Research, Wilhelmshaven (Germany) under controlled laboratory conditions. Seven Blackcaps and seven Reed Warblers were chosen for the experiment. The Blackcaps were naturally chronically infected by *Isospora sylvianthina* Schwalbach, 1959. The Reed Warblers were naturally infected by another *Isospora* species. There is no described *Isospora* species from Reed Warblers but the oocysts observed fit the *Isospora* sp. type 14 mentioned by Svobodová (1994).

To prepare oocysts for infection, we used one highly infected Blackcap that was infected with *Isospora sylvianthina* only. We extracted the oocysts from the faeces and concentrated them in tap water. The birds were orally infected by a standard dose of ca. 1×10^4 oocysts per bird. Faeces were sampled daily at the same time (3 hours before the light was off) because this time is the peak of

Isospora oocyst output (Dolnik, 1999). Oocysts in samples were counted by the improved method (Dolnik & Bairlein, in prep.).

Oocyst output in Reed Warblers did not show any changes. On the contrary, in all the Blackcaps the oocyst output increased more than 1000 times on the third post-infection day, and this peak continued for two days.

Blackcap and Reed Warbler belong to the same family Sylviidae but to different genera. We showed that *Isospora sylvianthina*, parasite of Blackcap, does not infect Reed Warbler. This experiment provides one more evidence that at least some *Isospora* coccidia are stringent host specific. This fact is important for describing new species of these parasites.

References

Barré, N. & Troncy, P.M. 1974. Note sur une coccidie rencontrée chez quelques Ploceidae du Tchad: *Isospora xerophila* n. sp. *Z. Parasitenk.*, **44**: 139-147.
Box, E. 1980. *Isospora* as an extra-intestinal parasite of passerine birds. *Prog. Abstr. Amer. Soc. Parasitol.*, **55**: 39-40.
Černá, Ž. 1973. Interesting coccidians from wild birds. *J. Protozool.*, **20**: 535-536.
Dolnik, O.V. 1999. Diurnal periodicity in appearance of *Isospora* (Protozoa: Coccidea) oocysts from some passerine birds. *Proc. Zool. Inst. RAS,* **281**: 113-118.
Levine, N.D. 1982. *Isospora passeris* n. sp. from the house sparrow *Passer domesticus, I. lacazei*, and related apicomplexan Protozoa. *Trans. Amer. Microscop. Soc.*, **101**: 66-74.
Pellerdy, L. 1974. *Coccidia and coccidiosis.* 2nd ed. 959 p. Akadémiai Kiadó, Budapest.
Svobodová, M. 1994. *Isospora, Caryospora* and *Eimeria* (Apicomplexa: Eimeriidae) in passeriform birds from Czech Republic. *Acta Protozool.*, **33**: 101-108.

CHAPTER 4.2.

Isospora fauna of passerine birds on the Courish Spit, Baltic Sea

Introduction

Coccidia are ubiquitous intracellular parasites of vertebrates and invertebrates and represent some of the most prevalent and abundant parasites known (Duszynski & Upton 2000). Most of the Eimeriidae species are within two genera, *Eimeria* and *Isospora*, and the most numerous genera in passerine birds is *Isospora* (Pellerdy 1974). The great majority of *Isospora* species are known only from the structure of their sporulated oocysts. The morphology of sporulated oocysts of different species is structurally different from each other. Cross-transmission experiments with *Isospora* from passerine birds have shown that species from one host generally do not infect hosts from other genus (Černá 1973, Box 1980, Dolnik 2002), though some species cross generic boundaries (Barré & Troncy 1974). Therefore we can expect that every species of passerine birds is a potential host for some *Isospora* parasites. Investigation of many bird species will contribute to our knowledge on *Isospora* fauna.

Courish Spit of the Baltic Sea is situated on an excellent place of migration route of passerine birds and gives outstanding opportunities to study bird migration. This was noticed already over a century ago when Johannes Thienemann founded the world's first ornithological station there. Apart from local birds, short-distant migrants on their journey from Scandinavia to Central and Western Europe and back as well as long-distance trans-sahara migrants can be trapped there, and it is a stopover site for many bird species. This site can be also taken as an excellent opportunity to study parasite fauna of passerine birds. The aim of our study was to explore which species of *Isospora* occur in local and migrating passerine birds on the Courish Spit.

Material and methods

Birds were trapped at the Courish Spit, Baltic Sea ($55^o12'$N, $20^o46'$E) in the afternoon in spring, summer and autumn 1995 - 2000. In total, 1038 birds of 55 species that belong to 17 taxonomic families were checked for *Isospora* oocysts. Faeces were collected and stored individually in 2% aqueous solution of $K_2Cr_2O_7$ at room temperature to allow the sporulation. Each sample was examined immediately after flotation centrifuging in saturated NaCl solution. Preparates were examined under 100× magnification to determine the presence and the number of oocysts. For the species determination 1000× magnification with immersion oil was used.

Results

We found *Isospora* oocysts in the samples of 40 investigated bird species. Out of 41 types of *Isospora* oocysts that were found, 17 types were identified as previously described species, and for 1 type the identification was uncertain. A further 14 types we determined as previously mentioned by some authors as "*Isospora* sp.", and the rest 9 types found are probably new species (Table). The infection in 25 bird species was single with only one type of oocysts, 15 species had double infections and in one species also triple infection appeared.

Table. *Isospora* coccidia found in passerine birds. n – number of examined individuals, +– number of infected individuals.

Host species	Host family	n	+	Parasite species
Acrocephalus arundinaceus	Sylviidae	11	5	***Isospora schoenobaeni*** Dolnik 1999 (syn. *Isospora* sp. type 12 Svobodova 1994)
Acrocephalus dumetorum	Sylviidae	1	0	—
Acrocephalus palustris	Sylviidae	15	6	***Isospora* sp. type 13** Svobodova 1994
Acrosephalus schoenobaenus	Sylviidae	20	13	***Isospora schoenobaeni*** Dolnik 1999 (syn. *Isospora* sp. type 12 Svobodova 1994), ***Isospora* sp. type 13** Svobodova 1994
Acrosephalus scirpaceus	Sylviidae	61	29	***Isospora schoenobaeni*** Dolnik 1999 (syn. *Isospora* sp. type 12 Svobodova 1994) ***Isospora* sp. type 13** Svobodova 1994
Aegithalos caudatus	Paradoxonithidae	7	4	***Isospora* sp. type 24** Svobodova 1994
Cannabina cannabina	Fringillidae	1	1	***Isospora arrui*** Quesada et Cringoli 1990
Carpodacus erythrinus	Fringillidae	81	51	***Isospora* sp. (new species 1)**
Certhia familiaris	Certhiidae	6	3	***Isospora certhiae*** Dolnik 1999
Chloris chloris	Fringillidae	1	1	***Isospora chloridis*** Anwar 1966
Coccothraustes coccothraustes	Fringillidae	7	5	***Isospora* sp. (new species 2)**
Corvus monedula	Corvidae	1	1	***Isospora monedulae*** Yakimoff et Matschoulsky 1936
Delichon urbica	Hirunididae	3	0	—
Emberiza schoeniclus	Emberizidae	5	3	***Isospora* sp.** Matschoulsky 1941 (syn. *Isospora* sp. type 37 Svobodova 1994)
Erithacus rubecula	Turdidae	96	71	***Isospora erithaci*** Anwar 1972 (syn. *Isospora* sp. type 6 Svobodova 1994)
Fringilla coelebs	Fringillidae	88	75	***Isospora fringillae*** Yakimoff et Gousseff 1938 (syn. *Isospora* sp. type 32 Svobodova 1994)
Fringilla montifringilla	Fringillidae	1	1	***Isospora* sp. type 34** Svobodova 1994
Hippolais icterina	Sylviidae	10	3	***Isospora* sp. type 16** Svobodova 1994
Hirundo rustica	Hirunidiae	4	1	***Isospora hirundinis*** Schwalbach 1959
Locustella fluviatilis	Sylviidae	1	0	—
Locustella luscinioides	Sylviidae	1	0	—
Loxia curvirostra	Fringillidae	1	0	—
Luscinia luscinia	Turdidae	10	8	***Isospora* sp. (new species 3)** ***Isospora* sp. (new species 4)**
Motacilla alba	Motacillidae	20	19	***Isospora* sp. type 2** Svobodova 1994, ***Isospora* sp**. Misra 1947
Motacilla flava	Motacillidae	2	1	***Isospora* sp. type 2** Svobodova 1994, ***Isospora* sp**. Misra 1947

Host species	Host family	n	+	Parasite species
Muscicapa hypoleuca	Muscicapidae	10	4	***Isospora ficedulae*** Schwalbach 1959, ***Isospora* sp. type 23** Svobodova 1994
Muscicapa parva	Muscicapidae	2	0	—
Muscicapa striata	Muscicapidae	6	0	—
Oriolus oriolus	Oriolidae	1	0	—
Parus caeruleus	Paridae	22	5	***Isospora pari*** Dolnik 1998 (syn. *Isospora* sp. type 26 Svobodova 1994) ***Isospora caerulei*** Dolnik 1998 (syn. *Isospora* sp. type 25 Svobodova 1994)
Parus major	Paridae	17	9	***Isospora pari*** Dolnik 1998 (syn. *Isospora* sp. type 26 Svobodova 1994) ***Isospora caerulei*** Dolnik 1998 (syn. *Isospora* sp. type 25 Svobodova 1994)
Parus palustris	Paridae	1	0	—
Passer domesticus	Ploceidae	3	3	***Isospora* sp. type 30** Svobodova 1994, ***Isospora* sp. type 31** Svobodova 1994
Phoenicurus ochruros	Turdidae	1	1	***Isospora* sp. type 8** Svobodova 1994
Phoenicurus phoenicurus	Turdidae	9	3	***Isospora* sp (new species 5)**
Phylloscopus collybitus	Sylviidae	16	8	***Isospora* sp. type 20** Svobodova 1994, ***Isospora* sp. type 21** Svobodova 1994
Phylloscopus sibilatrix	Sylviidae	2	0	—
Phylloscopus trochiloides	Sylviidae	2	0	—
Phylloscopus trochilus	Sylviidae	57	38	***Isospora* sp. type 21** Svobodova 1994, ***Isospora* sp. type 20** Svobodova 1994
Prunella modularis	Prunellidae	10	9	***Isospora* sp. type 4** Svobodova 1994
Pyrrhula pyrrhula	Fringillidae	14	10	***Isospora perroncitoi*** Carpano 1937 (syn. *Isospora* sp. type 35 Svobodova 1994)
Regulus regulus	Regulidae	43	24	***Isospora* sp. (new species 6)**
Remiz pendulinus	Paridae	23	2	***Isospora* sp. (new species 7)**
Saxicola rubetra	Turdidae	1	0	—
Sitta europaea	Paridae	1	0	—
Spinus spinus	Fringillidae	9	5	***Isospora* sp. (new species 8)**
Sturnus vulgaris	Sturnidae	116	70	***Isospora dilatata*** Schwalbach 1959
Sylvia atricapilla	Sylviidae	60	44	***Isospora. sylviae*** Schwalbach 1959 (syn. *Isospora* sp. type 18 Svobodova 1994) ***Isospora sylvianthina*** Schwalbach 1959 (syn. *Isospora* sp. type 17 Svobodova 1994)
Sylvia borin	Sylviidae	68	54	***Isospora sylvianthina*** Schwalbach 1959 (syn. *Isospora* sp. type 17 Svobodova 1994) ***Isospora sylviae*** Schwalbach 1959 (syn. *Isospora* sp. type 18 Svobodova 1994)
Sylvia communis	Sylviidae	38	23	***Isospora wurmbachii*** Schwalbach 1959, ***Isospora sylvianthina*** Schwalbach 1959
Sylvia curruca	Sylviidae	30	19	***Isospora sylviae*** Schwalbach 1959 (syn. *Isospora* sp. type 18 Svobodova 1994) ***Isospora sylvianthina*** Schwalbach 1959 (syn. *Isospora* sp. type 17 Svobodova 1994), ***Isospora ampullacea***(?) Schwalbach 1959
Troglodytes troglodytes	Troglodytidae	8	5	***Isospora* sp. (new species 9)**
Turdus iliacus	Turdidae	1	0	—
Turdus merula	Turdidae	13	11	***Isospora turdi*** Schwalbach 1959 *(syn. *Isospora* sp. type 9 Svobodova 1994)
Turdus philomelos	Turdidae	4	0	—

Discussion

Despite many descriptions of new *Isospora* species from passerine birds, species determination is still problematic. For example, for long time practically all findings of *Isospora* species in over 100 passerine bird species were recorded as *Isospora lacazei* Labbe 1893. Now, most of the authors agree that *Isospora* can be counted as genus-specific parasite until otherwise demonstrated. Another problem one is faced with identification of *Isospora* species in passerine birds is that the literature is full of uncomplete and synonimical descriptions. Though good reviews are made in coccidia of mammalians (e.g. Duszynski & Upton 2000, Duszynski et al. 1999) there is no such review on bird coccidia since the book of Pellerdy appeared in 1974. As it was already mentioned by Pellerdy (1974) in many species descriptions, especially in old ones, some precise information on oocyst size in combination with some morphological characteristics is lacking. As soon as no standardised method has been developed yet to preserve sporulated oocysts long term (Duszynski & Gardner 1991), line drawings are required to such descriptions, and they also lack in many works. Therefore, such descriptions are very difficult to apply for determination of the oocysts. On the other hand, some authors provided nearly all data that are necessary to determine the species (e.g. Svobodova 1994), but did not give any name to the species (it is called "*Isospora* sp.") and gave no comparative analysis of the species, nor did line drawing, therefore the species can not be counted as a valid one. In our work we present these species under the name, for example, "*Isospora* sp. type 34 Svobodova 1994". However, while this is not a valid taxonomic species name, the complete descriptions of these species with photos and line drawings, as well as descriptions of the new species we found in passerine birds on the Courish Spit are prepared for publication (Dolnik, in prep.).

We did not find any *Isospora* in 17 bird species likely due to a low number of investigated individuals, and in some of the species probably due to low intensity of infection. For some of these species oocysts of *Isospora* are known, as, for example, *Turdus philomelos* and *Delichon urbica* (Svobodova 1994). We believe that probably every bird species contains at least one *Isospora* species.

CHAPTER 4.3.

Isospora schoenobaeni sp. n. (Protozoa: Eimeriidae) from the Sedge Warbler (*Acrocephalus schoenobaenus*)

O.V. Dolnik

Dolnik, O.V. 1999. *Isospora schoenobaeni* sp. n. (Protozoa: Eimeriidae) from the Sedge Warbler (*Acrocephalus schoenobaenus*). *Zoosystematica Rossica*, **8**(1): 6.

Faeces from *Acrocephalus schoenobaenus* caught on the Courish spit (Baltic Sea) were examined for coccidia. 9 of 15 birds (60%) had undescribed isosporan oocysts in their faeces. Sporulation took 72 hours at 20 °C. Sporulated oocysts of *Isospora schoenobaeni* sp. n. are spherical, 27.0 (24.3-29.0) µm, with oocyst wall ca. 1.5 µm thick; a polar granule is present, but no oocyst residuum or micropile occured. Sporocysts are ovoid, 20.7 (18.2-22.4) × 12.8 (12.0-13.8) µm, with a nipple-like Stieda body and a medium substieda body. A sporocyst residuum was present in the form of numerous minute globules, and 4 sporozoites 6.7 × 4.6 µm in average were lying in the sporocysts. This is the first description of *Isospora* from the genus *Acrocephalus*.

O.V. Dolnik, Zoological Institute Russian Academy of Sciences, Universitetskaya nab. 1, St.Petersburg 199034, Russia.

In July and August 1997 15 Sedge Warblers (*Acrocephalus schoenobaenus*) were trapped by mistnets in the time between 5 p.m. and 7 p.m. on the Courish spit of the Baltic Sea. Faecal samples were collected immediately, put in 2.5% $K_2Cr_2O_7$ solution and left at 20 ± 2 °C for oocyst sporulation. Sporulation took 72 hours at 20 °C. Samples were examined by flotation using NaCl. Sporocysts were found in 9 (60%) of 15 birds examined. The new species of *Isospora* revealed is described below. In all measurements size ranges in parentheses follow the means. Photographs are kept in the Zoological Institute, St.Petersburg.

Isospora schoenobaeni sp. n.

(Figs 1-2)

Host type: *Acrocephalus schoenobaenus* (Passeriformes: Sylviidae).
Location in host: unknown; oocysts were found in faeces.
Type locality: Courish spit of the Baltic Sea, Russia.
Description. Sporulated oocysts spherical, 27.0 (24.3-29.0) µm. Oocyst wall ca. 1.5 µm thick, without a micropile. One polar granula is present, but no oocyst residuum occurs. Sporocysts ovoid, 20.7 (18.2-

22.4) × 12.8 (12.0-13.8) µm, with a nipple-like Stieda body and a medium substieda body. Sporocyst residuum present in the form of numerous minute globules; 4 sporozoites 6.7 × 4.6 µm in average are lying in the sporocysts.
Discussion. No *Isospora* species have been described previously from *Acrocephalus*. As all species of *Isospora* are genus-specific parasites, it is obvious that the species described above is new.

Acknowledgements

Fig. 1. *Isospora schoenobaeni* sp. n. from *Acrocephalus schoenobaenus*. Magnification 1000×.

Fig. 2. Line drawing of sporulated oocyst of *Isospora schoenobaeni* sp. n. Scale: 10 µm.

CHAPTER 4.4.

Isospora certhiae sp. n. (Protozoa: Eimeriidae) from the Tree Creeper (*Certhia familiaris*)

ISOSPORA CERTHIAE SP. N. (PROTOZOA : EIMERIIDAE) ИЗ ОБЫКНОВЕННОЙ ПИЩУХИ (CERTHIA FAMILIARIS)

© О. В. Дольник

Впервые описаны ооцисты изоспор из обыкновенной пищухи (*Certhia familiaris*). Ооцисты *Isospora certhiae* sp. n. круглые, 21.6—29.7 (в среднем 27) мкм в диаметре. Оболочка ооцисты однослойная, двухконтурная, не имеет микропиле. В ооцисте отсутствует остаточное тело, но имеется одна полярная гранула треугольной формы. Спороцисты 12.2—14.9 × 14.9—17.6 мкм (в среднем 13.5 × 16.2 мкм), штидовское тельце имеет колпачок. Спороциста содержит компактное остаточное тело и клиновидные спорозоиты 8 мкм длины и 3 мкм ширины в основании. Вид обнаружен у пищух, пойманных осенью 1996 г. на Куршской косе Балтийского моря (55° 12′ N, 20° 46′ E).

———

Обыкновенная пищуха (*Certhia familiaris*) относится к птицам семейства пищуховых (Certhiidae) отряда воробьиных. У представителей этого семейства к настоящему времени кокцидии сем. Eimeriidae не описаны. Данная работа является первым описанием кокцидий рода *Isospora* из пищуховых.

Материал и методика. Работа проводилась в сентябре и октябре 1996 г. на базе Биологической станции Зоологического института РАН на Куршской косе Балтийского моря (55° 12′ N, 20° 46′ E). Отлов птиц производили паутинными сетями. Среди пойманных птиц было 6 обыкновенных пищух. Пойманных птиц помещали в индивидуальные садки, дно которых было застелено чистой бумагой. Спустя 1 ч птицу выпускали, а свежую пробу помета помещали в 2 %-ный раствор бихромата калия и выдерживали 5—6 сут при 20 ± 2°. Для концентрации ооцист применяли метод Дарлинга, однако в качестве флотационного раствора использовали насыщенный раствор поваренной соли. Подсчет ооцист производили стандартным методом. Для этого после центрифугирования с каждой пробы одинаковое количество поверхностной пленки (5 петель 5 мм в диаметре) переносили на предметное стекло и производили подсчет ооцист, просматривая весь препарат в световом микроскопе (окуляр × 7, объектив × 10). Таким образом, удавалось косвенно оценить интенсивность заражения птицы. Морфологию ооцист изучали, используя объективы × 40 и × 90. Измерения ооцист проводили с помощью окуляр-микрометра с ценой деления 2.7 мкм.

Результаты и обсуждение. У всех обследованных нами пищух были обнаружены ооцисты, относящиеся к роду *Isospora*. У двух молодых пищух, пойманных 22 сентября в 18 ч, было обнаружено по 50 ооцист в каждой пробе помета. У двух других молодых птиц, пойманных 16 октября в 10 ч, было обнаружено 2 и 5 ооцист в пробе. У каждой из двух взрослых птиц, пойманных 16 октября в 14 и 15 ч, было обнаружено по 500 ооцист в пробе помета.

Все ооцисты морфологически были схожи между собой и отличались только диаметром, который варьировал в широких пределах — от 21.6 до 29.7 мкм. Однако большинство ооцист имело диаметр 27 мкм. Ооцисты не имели микропиле, и каждая ооциста содержала одну светопреломляющую гранулу треугольной формы. Остаточное тело в ооцисте отсутствовало. Спороцисты на заостренном конце имели штидовское тельце с колпачком. Размеры спороцист колебались от 12.2 × 14.9 до 14.9 × 17.6 мкм, составляя в среднем 13.5 × 16.2 мкм. Внутри каждой спороцисты находились компактное остаточное тело и клиновидные спорозоиты, длина которых составляла 8, а ширина в основании — 3 мкм.

Предполагают, что изоспоры обладают довольно узкой видоспецифичностью. К настоящему времени имеется очень мало опытов по перекрестному заражению изоспорами разных видов хозяев. Черна (Černa, 1973) описал неудавшуюся попытку заражения изоспорами домового воробья (*Passer domesticus*) от канарейки. В работе

Бокс (Box, 1980) эти данные были подтверждены. Отметим, что домовой воробей и канарейка относятся к разным семействам отряда воробьиных (Ploceidae и Fringillidae соответственно). Барре и Тронси (Barre, Troncy, 1974), заражавшим красноклювых ткачиков (*Quelea quelea*) и больших масковых ткачей (*Ploceus cucullatus*), относящихся к семейству Ploceidae, подсем. Ploceinae, изоспорами *I. xerophila* в Африке, удалось заразить этими изоспорами также виды *Ploceus capuitalis*, *Euplectes oryx*, *E. afra* и *Sporopipes frontalis* (все из сем. Ploceidae, подсем. Ploceinae), но не *Poliospiza leucipygia* (Fringillidae) и не *Lonchura cucullatus* (сем. Ploceidae, подсем. Estrildinae). Ливайн (Levine, 1982) считает, что предположение о том, что изоспоры могут заражать представителей любого рода внутри одного семейства хозяев, безосновательно.

Исключение составляет вид *Isospora lacazei* Labbe, 1893, описанный разными авторами у представителей различных родов и даже различных семейств отряда воробьиных. Описания ооцист *Isospora lacazei* из разных видов хозяев, приводимые разными авторами, часто неполные и сильно отличаются друг от друга. Так, согласно Пеллерди (Pellerdy, 1974), Лаббе описал у этого вида ооцисты 23—25 мкм в диаметре, Боутон относил к тому же виду ооцист 21 мкм в диаметре, Генри указывал диаметр 22—32 × 16—26 мкм, а некоторые другие авторы описывали ооцисты *I. lacazei* диаметром 17—29 мкм. Большинство авторов не наблюдали микропиле, однако, как сообщает Пеллерди (Pellerdy, 1974), Чакраварти и Карр, а также Генри, утверждают, что иногда оно имеется.

Исходя из различий в описании одного и того же вида разными авторами, многие в настоящее время полагают, что *Isospora lacazei* является сборной группой. Некоторые авторы склонны разбивать этот вид на несколько самостоятельных видов (Grulet e.a., 1986; Levine, 1982).

Итак, если исключить сборный (?) вид *Isospora lacazei*, в настоящее время нет данных о возможности заражения представителей различных семейств воробьиных птиц одним и тем же видом изоспор.

Исходя из того, что у представителей сем. Certhiidae кокцидии рода *Isospora*, как и другие кокцидии сем. Eimeriidae, ранее обнаружены не были, нам представляется возможным признать встреченные нами ооцисты самостоятельным видом. Для найденного нами вида мы предлагаем название *Isospora certhiae* sp.n.

Isospora certhiae Dolnik sp.n (рисунок).

Х о з я и н: *Certhia familiaris* (обыкновенная пищуха).

Р а с п р о с т р а н е н и е: вид обнаружен в Калининградской обл. (55° 12′ N, 20° 46′ E).

М а т е р и а л: гапантотип, препарат № 3, хранится в коллекции лаборатории протозоологии Зоологического института РАН, Санкт-Петербург.

Д и а г н о з. Круглые ооцисты 21.6—29.7 мкм в диаметре (в среднем 27 мкм). Оболочка ооцисты однослойная, двухконтурная, не имеет микропиле. В ооцисте отсутствует остаточное тело, но имеется одна полярная гранула треугольной формы. Спороцисты 12.2—14.9 × 14.9—17.6 мкм, в среднем 13.5 на 16.2 мкм, штидовское тельце имеет колпачок. Спороциста содержит компактное остаточное тело и клиновидные спорозоиты 8 мкм длины и 3 мкм ширины в основании.

Д и ф ф е р е н ц и а л ь н ы й   д и а г н о з. Описания изоспор у представителей сем. Certhiidae в литературе к настоящему времени отсутствуют. Сравнение найденного нами вида с *Isospora lacazei* сильно затруднено из-за отсутствия четкого диагноза этого вида. Однако отметим, что обнаруженные нами ооцисты содержат более крупные спороцисты и спорозоиты, чем *Isospora lacazei* в описании, приводимом Пеллерди (Pellerdy, 1974).

Ближайшим к Certhiidae семейством воробьиных птиц, у которого описаны изоспоры, является сем. Paridae. У представителей этого семейства известно два вида

Isospora certhiae sp. n. из обыкновенной пищухи.

Isospora certhiae sp. n. from the Tree creper.

изоспор. От *I. parusae* обнаруженный нами вид отличается отсутствием микропиле и значительно более крупными спороцистами. От *I. sylvianthina* ооцисты изоспор пищухи отличаются наличием только одной полярной гранулы, округлой формой спороцист и более мелкими спорозоитами.

Статья выполнена в рамках ГНТП «Биологическое разнообразие».

10 мкм
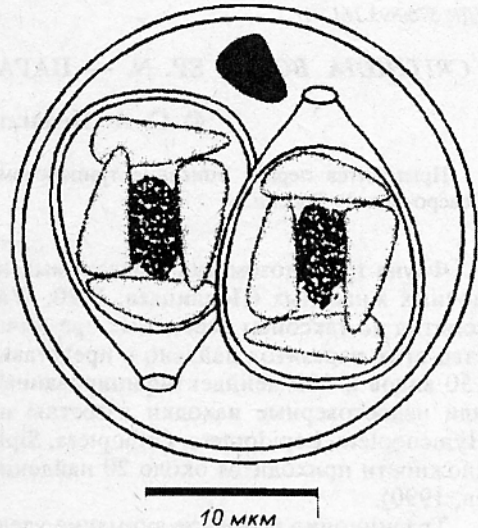
Список литературы

Barre N., Troncy P.-M. Note sur une coccidie recontres chez quelques Ploceidae du Tchad: Isospora xerophila n.sp. // Z. Parasitenk. 1974. Bd 44. S. 139—147.

Box E. Isospora as an extra-intestinal parasite of passerine birds // Prog. Abstr. Am. Soc. Parasitol. 1980. Vol. 55. P. 39—40.

Cerna Z. Interesting coccidians from wild birts // J. Protozool. 1973. Vol. 20. P. 535—536.

Grulet O., Landau I., Millet P., Baccam D. Les Isospora du moineau II — Etudes sur la biologie // Ann. Parasitol. Hum. Comp. 1986. T. 61, № 2. P. 161—192.

Levine N. Isospora passeris n.sp. from the house sparrow Passer domesticus, I. lacazei, and related Apicomplexan Protozoa // Trans. Am. Microsc. Soc. 1982. Vol. 101, N 1. P. 66—74.

Pellerdy P. L. Coccidia and coccidiosis. Budapest: Akademiai Kiado, 1974. 959 p.

ISOSPORA CERTHIAE SP. N. (PROTOZOA: EIMERIIDAE) FROM THE TREE CREEPER (CERTHIA FAMILIARIS)

O. V. Dolnik

SUMMARY

The first description of *Isospora* oocysts from *Certhia familiaris*. *Isospora certhiae* sp.n. has round oocysts 21.6—29.7 µm in diameter (27.7 µm in average). The cyst wall lacks a micropile. A triangular polar granule appears, but no oocyst residium. The sporocysts 12.2—14.9 × 14.9—17.6 µm size (average 13.5 × 16.2 µm), bear a knob-like Stieda body and contain pyramid-like sporozoites 8 µm length and 3 µm width. The species was found in Tree creepers on the Courish spit of the Baltic sea (55° 12′ N, 20° 46′ E) during the autumn 1996.

CHAPTER 4.4. (Translation)

Isospora certhiae sp. n. (Protozoa, Eimeriidae) from the Tree Creeper (*Certhia familiaris*)

Parasitologiya 1999 (2): 149-151 (in Russian, English summary).

The first description of *Isospora* oocysts from *Certhia familiaris*. *Isospora certhiae* sp. n. has round oocysts 21.6-29.7 μm in diameter (27.7 μm in average). The cyst wall lacks a micropyle. A triangular polar granule appears, but no oocyst residuum. The sporocysts 12.2-14.9 × 14.9-17.6 μm size (average 13.5 × 16.2 μm), bear a knob-like Stieda body and contain pyramid-like sporozoites 8 μm length and 3 μm width. The species was found in Tree creepers on the Courish spit of the Baltic sea (55° 12′ N, 20° 46′ E) during the autumn 1996.

Tree Creeper (*Certhia familiaris)* belongs to fam. Certhiidae of passerine birds. Up to now there is no description of Eimeriidae coccidia from this family. Therefore, our study is the first description of *Isospora* species from Certhiidae birds.

Material and methods

The samples were collected in September-October 1996 at Biological station of the Zoological Institute RAS on the Courish Spit, Baltic sea (55°12′N, 20°46′E). The birds were trapped by mistnets and ringed. Among the trapped birds there were 6 Tree Creepers. These birds were kept for several minutes to one hour in small individual cages with fresh ground paper. As soon as a fresh dropping appeared, the bird was released and the fresh fecal sample was kept in 2% aqueous solution of $K_2Cr_2O_7$ at 20±2 °C for 5-6 days. For oocysts concentration flotation centrifuging in saturated NaCl solution was used. The same amount of surface layer (5 loops of 5 mm diameter) was placed on slides and immediately examined. The whole slide was examined under 100× magnification to determine the presence and the number of oocysts. For the species determination 400× and 1000× magnification with immersion oil was used.

Results and discussion

In the investigated birds we found oocysts that belong to *Isospora* genus. In two juvenile birds caught at 22 September at 6 p.m. we found 50 oocysts in each. In two other juvenile birds caught in October at 10 a.m. were 4 and 5 oocysts, and in adult birds caught in October at 2 p.m. and 3 p.m. there were 500 oocysts in each sample.

All the oocysts were morphologically similar and differed only in diameter that showed great variety from 21.6 μm to 29.7 μm, 27.0 μm in average. Micropyle and oocyst residuum were absent but there was a triangular polar granule. Sporocysts had a Stieda

body with a cap. The size of sporocysts varied from 12.2 × 14.9 μm to 14.9 × 17.6 μm, in average 13.5 × 16.2 μm. In every sporocyst there was a compact sporocyst residuum and wedge-shaped sporozoits 8 × 3 μm.

It is supposed that *Isospora* spp. are narrow host specific. Up to now there are very few experiments of cross-transmission of *Isospora* infection between different host species. Černa (1973) failed to infect a House Sparrow with *Isospora* sp. from the Canary, and experiments of Box (1980) proved this data. We should note that the House Sparrow and Canary belong to different families of Passerine birds (Ploceidae and Fringillidae, respectively). Barré and Troncy (1974) infected *Quela quela* and *Ploceus cucullatus* (fam. Ploceidae, subfam. Ploceinae) with *Isospora xerophila* as well as *Ploceus capuitalis, Euplectes oryx, E. afra* and *Sporopipes frontalis* (all Ploceidae: Ploceinae) but not *Poliospiza leucipiga* (Fringillidae) and *Lonchura cucullatus* (Ploceidae: Estrildinae). Levine (1982) supposed that there is no ground to believe that *Isospora* spp. may infect all the genera within one host family.

The only exception is the species *Isospora lacazei* Labbe 1893 that was described by many authors from different genera and even families of Passeriformes. The descriptions of *Isospora lacazei* from different host species that are shown by different authors are often uncomplete and differ from each other. So, according to Pellerdy (1974), Labbe described the oocysts 22-25 μm in diameter, Boughton mentioned diameter of 21 μm, Henry wrote that diameter is 22-32 × 16-26 μm, and some other authors mentioned 17-29 μm. Most of the authors observed no micropyle, but, according to Pellerdy (1974), Chakravarty and Kar as well as Henry note, that sometimes it occurs.

Because of such great variation in descriptions of the same species by different authors, it is suggested now that a group of species was understood under the name of *Isospora lacazei*. Some authors offer to split the species to several ones (Grulet *et al*., 1986, Levine 1982).

Therefore, if to exclude the combined (?) species *Isospora lacazei*, there is no data that host species from different taxonomic families can be infected by the same *Isospora* species.

We suggest that the species of *Isospora* we found in Tree Creeper is a new species, because no Eimeriidae coccidia were described from birds of Certiidae family up to now. For the new species we suggest a name *Isospora certhiae*.

Isospora certhiae Dolnik sp. n. (Fig.)

Type host: *Certhia familiaris* (Tree Creeper)

Type locality: Russia, Kaliningrad reg. (55°12′N, 20°46′E).

Material deposited: Preparate No 3 in collection of Lab. Protozoology, Zoological Institute St.Petersburg, Russia.

Diagnosis: Round oocysts 21.6-29.7 μm in diameter (27 μm in average). Oocyst wall single layer, two-contour, lacks a micropyle. There is no oocyst residuum, but a triangular polar granule presents. Sporocysts 12.2-14.9 × 14.9-17.6 μm, in average 13.5 × 16.2 μm, Stieda body bears a cap. Sporocyst contains a compact residuum and wedge-shaped sporozoits 3 × 8 μm.

Differential diagnosis: Up to now there is no description of *Isospora* species from birds of Eimeriidae family. Comparison with *Isospora lacazei* is difficult because there is no concrete diagnosis of this species. We can only note that *Isospora* from Tree Creeper has larger sporocysts and sporozoits than *Isospora lacazei* in original description reproduced by Pellerdy (1974).

Among the passerine bird families from which *Isospora* oocysts were described, the closest family to Certhiidae is Paridae. There are two *Isospora* species known for Paridae birds: *Isospora parusae* and *Isospora sylvianthina*. The species from Tree Creeper differs from *I. parusae* by larger sporocysts and absence of micropyle. From *I. sylvianthina* it differs by the round form of oocysts, the smaller sporozoits and the presence of only one polar granule.

CHAPTER 5

ISOSPORA (PROTOZOA, SPOROZOA) INFECTION
IN PASSERINE BIRDS OF VARIOUS FEEDING STYLES

Isospora (Protozoa, Sporozoa) infection in passerine birds of various feeding styles

Introduction

Coccidia of *Isospora* genus are intracellular parasites of intestines that are very common for wild passerine birds (Pellerdy 1974). They are monoxenous parasites that require no intermediate transmitter for the spread of infection (Long 1982). With a few exceptions (Barré & Troncy 1974, Doran 1978) bird intestinal coccidia are thought to be genera-specific (Box 1977, Levine 1982).

After ingestion of sporulated oocysts sporozoits emerge from them and enter the wall of the intestine (Long 1982), where several merogonies, gametogony and fertilisation take place (Long 1982, Grulet *et al.* 1985). This increases the amount of parasites within the host. Unsporulated oocysts are released from the bird together with faeces. Sporulation takes several days and only sporulated oocysts are able to infect a new host. Infection occurs if the oocysts are swallowed by appropriate host with food or water.

From the life cycle of *Isospora* spp. we can suppose that the feeding style of bird influences its probability to become infected by these parasites. To check if there are differences in infection rate between birds with different feeding style we screened different species of passerine birds in the wild and compared the prevalence and intensity of infection in species of birds with different feeding style.

Materials and methods

Studies were carried out in late summer and autumn at Biological Station Rybachy on the Baltic Sea coast (Curonian spit) and on the island of Helgoland (North Sea). 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, and age of the bird.

To avoid possible influence of birds' age (Chapter 6) only juvenile birds were considered in the analysis. Moreover, because of a diurnal pattern in *Isospora* oocysts output (Dolnik 1999b, Chapter 3), only birds caught between 4 p.m. and 6 p.m. were sampled. In total 1226 juvenile birds of 38 passerine species were sampled.

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. One fresh dropping of

each individual bird was put into an individually labelled tube with 5 ml 2 % $K_2Cr_2O_7$ aqueous solution.

In the lab the samples were kept opened for a week at room temperature to allow the oocysts to sporulate. The intensity of *Isospora* infection can be estimated by using the standardised method of counting oocysts in faecal samples (Chapter 2). 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 errors owing to oocyst clustering. As intensity of infection the number of oocysts on the slide was used. Parasites were identified under high magnification (1000×). For analysing the results the birds species were arranged into 4 groups according to their feeding style (Glutz von Blotzheim & Bauer 1985-97). The first group are birds species that catch insects in the air. Birds that collect insects from leaves and twigs are in the second group. The third group are species with vegetarian diet and species who include

Table. Examined bird species.

Aerial feeders	Foliage gleaners that include fruits into diet
Delichon urbica	*Carpodacus erythrinus*
Hirundo rustica	*Coccothraustes coccothraustes*
Muscicapa hypoleuca	*Pyrrula pyrrula*
Muscicapa parva	*Sylvia atricapilla*
Muscicapa striata	*Sylvia borin*
$\Sigma = 72$	*Turdus philomelos*
	$\Sigma = 395$
Foliage gleaners	**Ground feeders**
Acrocephalus palustris	*Erithacus rubecula*
Acrocephalus schoenobaenus	*Fringilla coelebs*
Acrocephalus scirpaceus	*Luscinia luscinia*
Aegithalos caudatus	*Motacilla alba*
Certhia familiaris	*Oenanthe oenanthe*
Emberiza schoeniclus	*Passer domesticus*
Hippolais icterina	*Sturnus vulgaris*
Parus caeruleus	*Troglodytes troglodytes*
Parus major	*Turdus merula*
Phoenicurus phoenicurus	$\Sigma = 317$
Phylloscopus collibitus	
Phylloscopus trochilus	
Prunella modularis	
Regulus regulus	
Remiz pendulinus	
Spinus spinus	
Sylvia communis	
Sylvia curruca	
$\Sigma = 442$	
	TOTAL: 1226 birds

berries into their autumn diet. The fourth group are ground feeding birds (Table).

The data were statistically analysed using SPSS 8.0 programme (SPSS Base System und Professional Statistics). Data are presented as means ± standard error (s.e.).
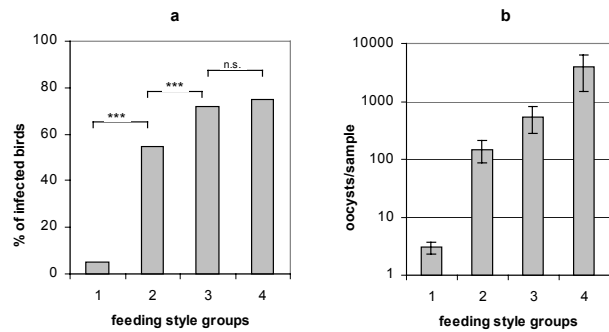
Results

Prevalence as well as intensity of infection were the lowest in aerial feeders, the highest in ground-feeding species, and intermediate in the two other groups (Fig. 1). The differences in prevalence of infection between the groups of aerial feeders, foliage gleaners, and foliage gleaners that include fruits into diet are significant. Differences in the intensity of the infection between aerial feeders and the three other groups, as well as between foliage gleaners and ground feeders are significant.

There is a positive correlation (r=0.6, P<0.01) between prevalence and average intensity of infection: bird species with lower prevalence of infection have lower average intensity of infection (Fig. 2).
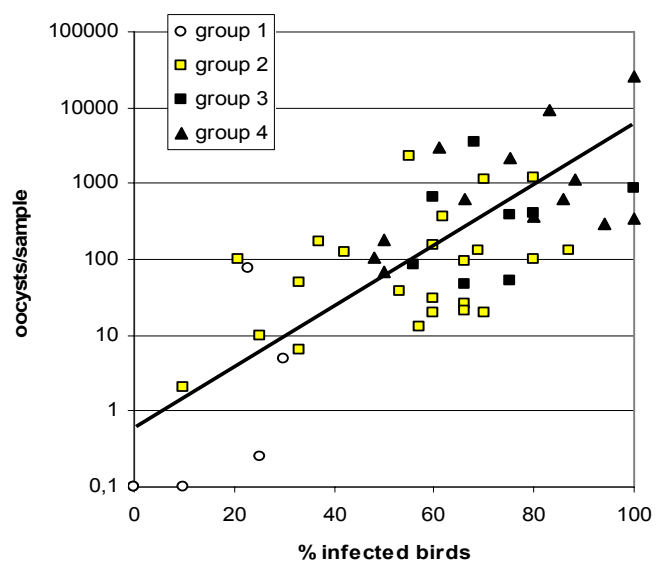
Discussion

Immunity that develops as a result of *Isospora* spp. infection does not suffice to prevent re-infection (Long 1982, Chapter 6). Therefore prevalence of infection reflects the

Fig. 1. Prevalence (**a**) and intensity (**b**) of *Isospora* spp. infection in aerial feeders (1), foliage gleaners (2), foliage gleaners that include fruits into diet (3) and in ground feeding birds (4).

Fig. 2. Correlation between prevalence and average intensity of infection in bird species of different feeding style (see text), trapped at Rybachy and Helgoland.

probability for the bird to become infected with *Isospora* spp., in other words, it shows the percent of the individuals that swallowed sporulated oocysts. For birds that catch insects in

the air this probability is low, that is reflected in lower prevalence of infection in these species. Collecting insects from leaves and twigs increases the probability to become infected and therefore prevalence of infection in these species is higher. Feeding on seeds and berries as well as collecting food from the ground is connected with the highest risk to swallow sporulated oocysts. The amount of trees and shrubs with ripe seeds and berries is in most cases limited and they attract many birds, whose droppings may cover the fruits. *Isospora* spp. oocysts from birds die quickly if they become dry or if exposed to direct sunlight. Therefore humid ground is the best place for accumulation and preservation of infectious oocysts. Those bird species that collect food on humid ground have the highest probability to swallow *Isospora* spp. oocysts, because the bird's droppings accumulate there. We suggest that feeding in flocks, as, for example, young Starlings in late summer do, also increases the risk to become infected by *Isospora* species.

Prevalence of infection

Scholtyseck (1956) arranged passerine bird species into three groups according not to their feeding style but to their diet. His results suggested that in passerine birds prevalence of *Isospora* spp. infection in insectivores birds is lower than in omnivores. Differences between omnivores and granivores were not significant. However, while collecting the material the author did not take into account diurnal rhythms of oocysts output that could influence the results of the investigation. Arranging bird species into groups according their feeding style and not the diet seems to us more logical. For example, in our investigations Wagtails were among the most infected birds, but on contrary, other insectivores as Flycatchers had the lowest prevalence of infection. We suggest the differences between these two strictly insectivores species are due to the way they collect their food, on the ground and in the air respectively. Therefore we suppose that the feeding style of the host species and not the diet determines the prevalence of infection with *Isospora* spp.

Intensity of infection

On contrary to prevalence, intensity of infection by parasites with endogenous multiplication can be regulated by the host and depends on the physiological state of the host, its immune system and how it can cope with the parasite. Thus, for *Isospora* spp., we state that intensity of infection gives us more information about the host's condition than prevalence of infection. We suggest that at least three factors may have influence on intensity of infection.

One of the possible explanations of higher infection intensity in ground feeders is that these birds may become more often re-infected, including self re-infection. Re-infection of a chronically infected individual causes an increase in oocyst output that can remain for some time (Chapter 6).

Infection by different species may increase the severity of infection (Long 1982) and lead to higher infection intensity. Another reason for higher intensity of infection in ground feeding species compared to those feeding in the air, are exposed to more endoparasites.

The nutritional status of the host is known both to increase and decrease the severity of coccidiosis. It has been shown that chicks infected with *Eimeria tenella* and fed 24% crude protein had a higher mortality rate than those fed 16 or 20% crude protein (Scharma *et al*. 1973). However, in *E. acervulina* infections, the higher crude protein diet was protective against weight loss. Britton *et al*. (1964) also found that birds on a high protein diet were more susceptible to infection.

In our case differences in intensity of infection between aerial feeders and foliage gleaners and non-significant difference between foliage gleaners and foliage gleaners that include fruits into diet as well as between the latter group and ground feeders suggest that average intensity of *Isospora* spp. infection in different bird species does not depend much on the diet of the host species. However, our investigation is made on different host species that are infected with different parasite species. In this case we can not separate the influence of the diet itself from the taxonomic factor. All the investigated birds species with different diet belong to various taxonomic groups. Moreover, they are infected by different species of *Isospora* that may have different pathogenisity. In general we do not exclude the possibility that diet may play some role in the severity of *Isospora* infection, but this has to be proved under experimental condition on one bird species infected with a single *Isospora* species.

The positive correlation between prevalence and average intensity of infection in bird species is a very interesting fact. This means that in those passerine bird species for which *Isospora* spp. is a common parasite the intensity of infection is higher. However, in this case it is difficult to distinguish a reason from the consequence and to decide if these species are stronger infected because of often re-infection due to the high prevalence of infected birds in the population is high, or vice versa. It is also interesting, how the parasite species from aerial feeders succeed to infect a new host of the same bird species. These parasites are very rare, the amount of oocysts produced and appearing in faeces of infected birds is very small, and the probability that an aerial feeder swallows sporulated oocysts is

also low. The probability of coinciding of these factors is extremely low, one would rather expect that parasites of aerial feeders should produce many oocysts and have high infection intensity. Probably there are some additional mechanisms we do not know that help to transfer the infection between the individuals, such as probable transmission from parents to nestlings (Svobodová & Cibulková 1995), for example.

We can conclude that the feeding style of passerine species influences both the intensity and prevalence of *Isospora* spp. infection. Probable influence of the diet on intensity of *Isospora* infection, nevertheless, has to be studied.

CHAPTER 6

EFFECTS OF AGE
ON THE INFECTION OF WILD AND CAPTIVE BIRDS
WITH *ISOSPORA* (PROTOZOA: EIMERIIDAE) PARASITES

Effects of age on the infection
of wild and captive birds with *Isospora* (Protozoa: Eimeriidae) parasites

Introduction

More than 90% of the Coccidia species that infect passerine birds in the wild belong to *Isospora* (Pellerdy 1974). However, prevalence and intensity of *Isospora* infection in wild bird populations and their consequences had rarely been studied.

The relationship between the age of wild passerine birds and prevalence and intensity of coccidia infection deserves more attention. Younger animals are generally assumed to be more susceptible to coccidial disease than their older counterparts (Long 1982, Gylstorff & Grimm 1998). However, it has also been shown, both in chicken and mammals, that older animals raised coccidia-free are as susceptible or even more susceptible than very young ones to similar doses of oocysts (see Long 1973, for review). Thus, not only the age of the host but also the immunity that develops as a result of coccidia infection is responsible for susceptibility of the host to coccidia. This immunity, nevertheless, may not be strong enough to prevent re-infection (Long 1982).

Most of the studies on the relationship between the age of birds and *Isospora* infection concern prevalence of infection in nestlings, and comparisons between nestlings and their parents. Because of the monoxenous oral-faecal life cycle of *Isospora* species, the nestlings are confronted to infections already in the nests if the feeding parents are infected with these parasites (Svobodová & Cibulková 1995) . The risk of infection increases with increasing age of the nestlings due to higher feeding frequencies by the parents. Scholtyseck & Przygodda (1959) found that the prevalence of infection with *Isospora* spp. was higher in older nestlings of passerine birds. Svobodová & Cibulková (1995) showed that 10-12 day old nestlings of Icterine Warblers (*Hippolais icterina*) were more frequently infected than 7-9 days old birds, and adult birds more frequently than nestlings. After the birds fledge and start feeding by their own, they are equally exposed to coccidia infection. Field data (Dolnik 1999) showed that in young Starlings prevalence of *Isospora* infection remained unchanged after fledging during summer, while the intensity of infection increased significantly with age during the first two months. In autumn, both, the prevalence and the intensity of infection in migrating passerine birds were high (Dolnik 1998), which may be due to the high proportion of young birds in autumn.

The aims of the present study were (1) to study intensity and prevalence of *Isospora* spp. infection in wild passerine birds of different age, and (2) to study the effects of age in captive birds held under controlled conditions.

Different intensities of infection in juvenile and adult birds could be due to the fact that young, highly infected birds may not survive the post-fledging period. Moreover, young birds may develop some kind of immunity against coccidiosis during early infection so that older birds show less severe chronic infections. In order to explore this, we kept infected birds under controlled conditions and screened their intensity of infection every three months during the first year of life. Different levels of intensity of infection in adult and young birds in the wild could also be due to different responses to re-infections. Re-infection of chronically infected birds happens very often in the wild. The immune system cannot prevent it but it could develop some reactions to avoid severe infections which may be dose-related. In this case, the response of young and adult birds to re-infections should be different. Therefore, we infected birds of different age artificially and recorded their responses.

As coccidia infection leads to disturbances of absorption and permeability in the intestine, and thus results in reduced food and water consumption (Yvoré & Mainguy 1972), we also monitored the birds' food intake and body mass following oocyst inoculations.

Material and methods

1. Field study

Birds were sampled in late summer and autumn at Biological Station Rybachy on the Courish Spit, SE Baltic coast ($55^o12'$N, $20^o46'$E).

Birds were trapped by mistnets, ringed and processed following the guidelines of the ESF-programme (Bairlein 1995). Recorded data include, among the others, date and time of capture, species, age, and body mass of the bird. Because of the diurnal pattern in *Isospora* oocysts output (Dolnik 1998), only birds caught between 4 p.m. and 7 p.m. were investigated.

In total, 315 birds of 5 passerine species were sampled. These were 73 Scarlet Grosbeaks *Carpodacus erythrinus*, 88 Chaffinches *Fringilla coelebs*, 54 Blackcaps *Sylvia atricapilla*, 63 Garden Warblers *Sylvia borin* and 37 Lesser Whitethroat *Sylvia curruca*.

Fresh faeces of each bird were examined for coccidia oocysts. The number of oocysts in the sample was counted after flotation centrifugation by standard method (see below).

2. *Laboratory trials*

A group of 58 Blackcaps was trapped as juveniles from a natural population and kept at the Institute of Avian Research in Wilhelmshaven (Germany). Birds were maintained individually under controlled laboratory conditions (LD 14:10, 20±1 °C, 50-60% R.H.). All birds were fed *ad libitum* a standard diet prepared from dried insects, casein, saccharose, vegetable oil, minerals and cellulose, containing 15% crude protein, 10% crude fat and 5% digestible carbohydrates (Bairlein 1986). Water was also available *ad libitum*. Body mass of the birds was recorded daily in the morning at the onset of light. For recording daily food intake, food for each bird was weighed in the morning, and remaining food was re-weighed 24 hours later. Loss of food mass due to evaporation was considered, and food intake for each individual bird was calculated as dry mass per day (Bairlein 1985).

Sampling and counting of oocysts

The fresh faecal samples were collected every day at the same time (3 hours before the light was set off). For sampling, fresh ground paper in the cage was used, and after about 10 minutes, a fresh dropping from the paper from each bird was collected into an individually labelled vial with 2% water solution of potassium dichromate ($K_2Cr_2O_7$).

In the lab, the samples were kept open 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 shacked well and put into a 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 presence and the number of parasites. The whole slide was checked to avoid errors owing to oocyst clustering. For species determination a 1000x magnification with immersion oil was used.

Screening of chronic infection

To screen the chronic intensity of infection, each caged bird was sampled repeatedly during the first year of life at the age of 1, 4, 7, 10, and 13 months.

Infection experiments

During 20 days prior to the infection experiments, we recorded daily body mass and oocyst release of each bird. For the experiments, we chose only birds that did not show changes in body mass during the last 20 days, and that were infected only by one coccidia species *Isospora sylvianthina* Schwalbach 1959, at low levels, on average below 200 oocysts per sample. These birds were arranged into groups of seven birds each: (group 1) 2 months old; (2 and 3) 7 months old; and (4 and 5) 20 months old. Group 2 was also used at an age of 8 month, group 3 at 14 months, and group 4 was used again at an age of 26 months, respectively.

To prepare oocysts for artificial infection we used one highly infected bird that was infected with only *Isospora sylvianthina*. Fresh faeces from this donor bird were collected over several consecutive days, and kept open in potassium dichromate to allow sporulation. Sporulated oocysts were concentrated in saturated NaCl solution, thereafter washed and the concentrated oocysts were kept in 2% water solution of potassium dichromate in darkness. Half an hour before infection, the oocysts were washed off potassium dichromate with tap water by repeated centrifugation. The final supernatant contained the oocysts in tap water and looked like a white suspension. The amount of oocysts was counted in 2 μl of suspension. By repeated washing and concentrating a final concentration of 1×10^4 oocysts in 50 μl of suspension was achieved. This was used as standard dose. In the morning of an experimental day, immediately after onset of light, the birds were orally infected with 1×10^4 oocysts. In one experiment, a dose of ca 1×10^5 oocysts per 50 μl was used.

Oocyst output, body mass and food intake of each experimental bird were recorded daily for at least four days prior and at least 18 days after experimental infection.

Three different experiments were conducted. (1) In order to explore the effect of age, birds were infected with the same dose of oocysts at ages of 2, 14, and 26 months. (2) As recent infection can weaken the bird, or, in contrary, refresh the immune system, we infected one experimental group twice with the same dose of oocysts at an age of seven months and 37 days later. (3) As the dosage of infection may also influence the bird's response (Long 1982), one group of 20 months old birds was infected with standard dose, while another group of the same age was infected with the 10 times higher dose.

The data were analysed using SPSS 8.0 programme (SPSS Base System und Professional Statistics).

In some cases there were big differences in individual reaction on infection, so that the individuals showed clear but opposite reaction to the infection. Therefore, for better illustration of this variety in the graphs we split the birds into two groups, "fit" and "weak", according to their reaction on the infection. "Fit" group are birds that managed to cope with the infection by their own. "Weak" are the birds who either died, or had to be medicated after the experiment because of tremendous body mass loss. "Fit" and "weak" birds did not differ by body mass before the experiments, so it was impossible to predict the individual bird response.

Results

1. Field data

In the five investigated bird species the prevalence of infection did not differ significantly between young and adult birds (Fig. 1a). The intensity of infection, however, was consistently higher in young birds than in adult birds (Fig. 1b), though significantly only in Scarlet Grosbeaks (P=0.047), Chaffinches (P=0.019) and Blackcaps (P=0.024) and in the combined sample (P=0.013). Intensity of *Isospora* infection in birds in the wild did not correlate with body mass of the host.

Fig. 1. Prevalence (**a**) and intensity ±SE (**b**) of *Isospora* spp. infection in young (white bars) and adult (black bars) birds in the wild.
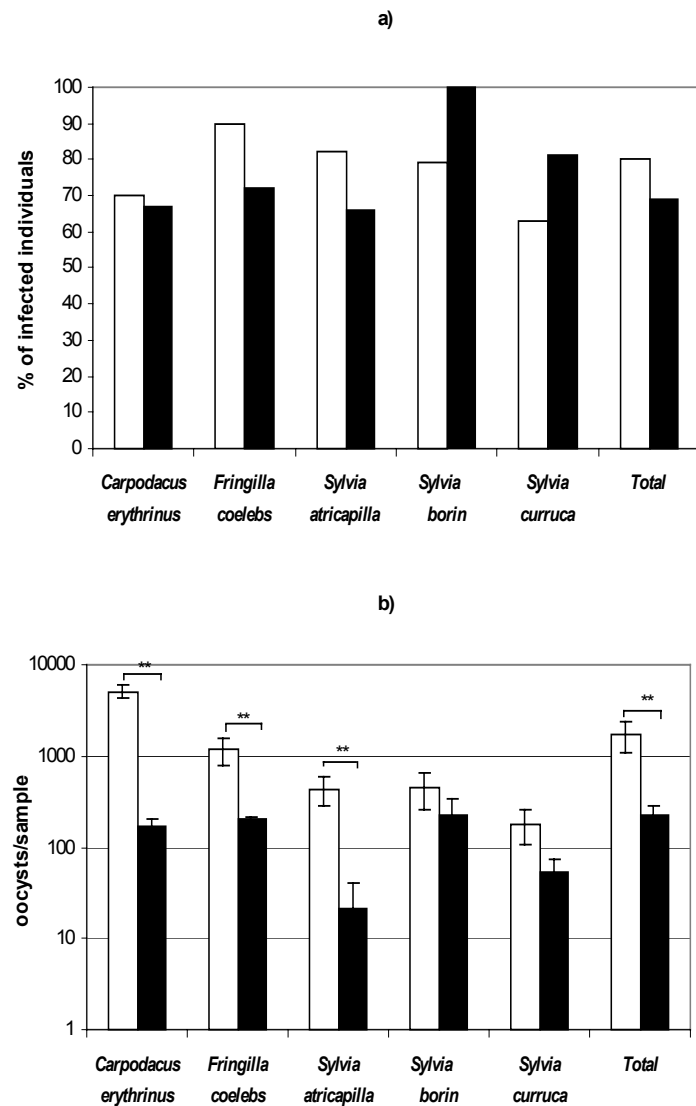
2. *Experimental data*

The intensity of chronic infection in captive Blackcaps was continuously decreasing during the first year of life (Fig. 2).
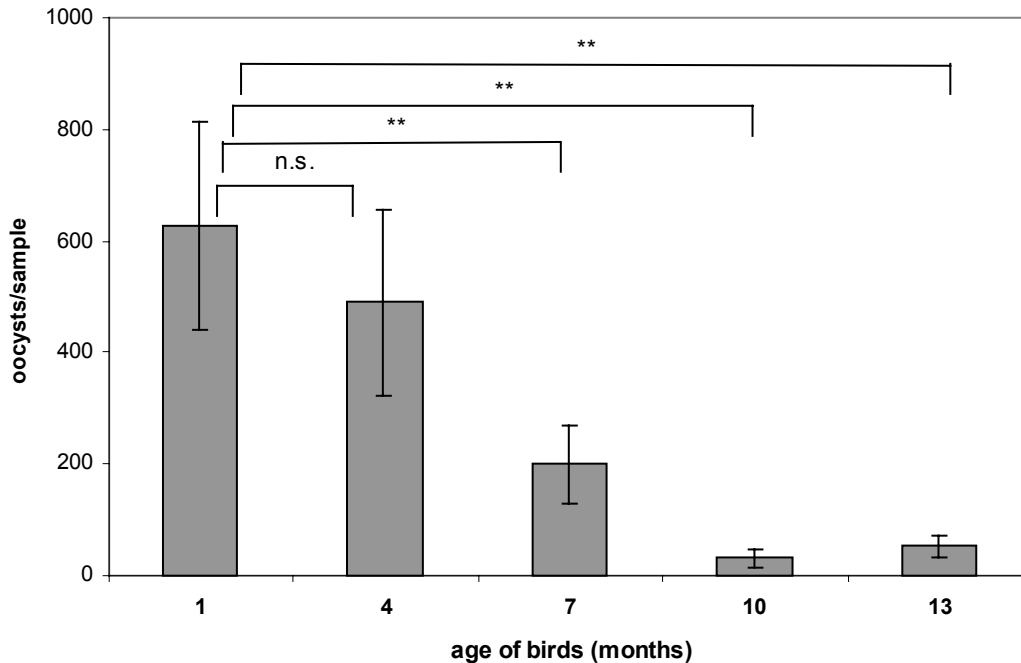

Fig. 2. Average intensity (±S.D.) of chronic *Isospora* infection in captive Blackcaps of different age during the first year of life.

Infection experiments

Experiment 1 (Fig. 3)

After infection, the oocyst releases decreased for 2 days and increased on the third day. Then it decreased in all birds except 2 months old ones (Fig. 3a). The group of 2 months old birds splitted into two subgroups. Two of the seven birds decreased oocyst output after the fourth postinfection day similarly to the older birds. In five other ("weak") 2 months old birds, however, the number of oocysts increased up to 100 times above pre-injection level during the first two weeks. On the 13th postinfection day the oocyst output of these 5 birds was still much higher, than in the other birds (Mann-Whitney U-test, P=0.01). Thereafter, the oocyst output in the five birds decreased but remained higher than the pre-infection level.

In all the groups during the first two weeks body mass did not decrease, rather showed a non-significant tendency to increase to the end of the second week (Fig. 3b). In the third post-
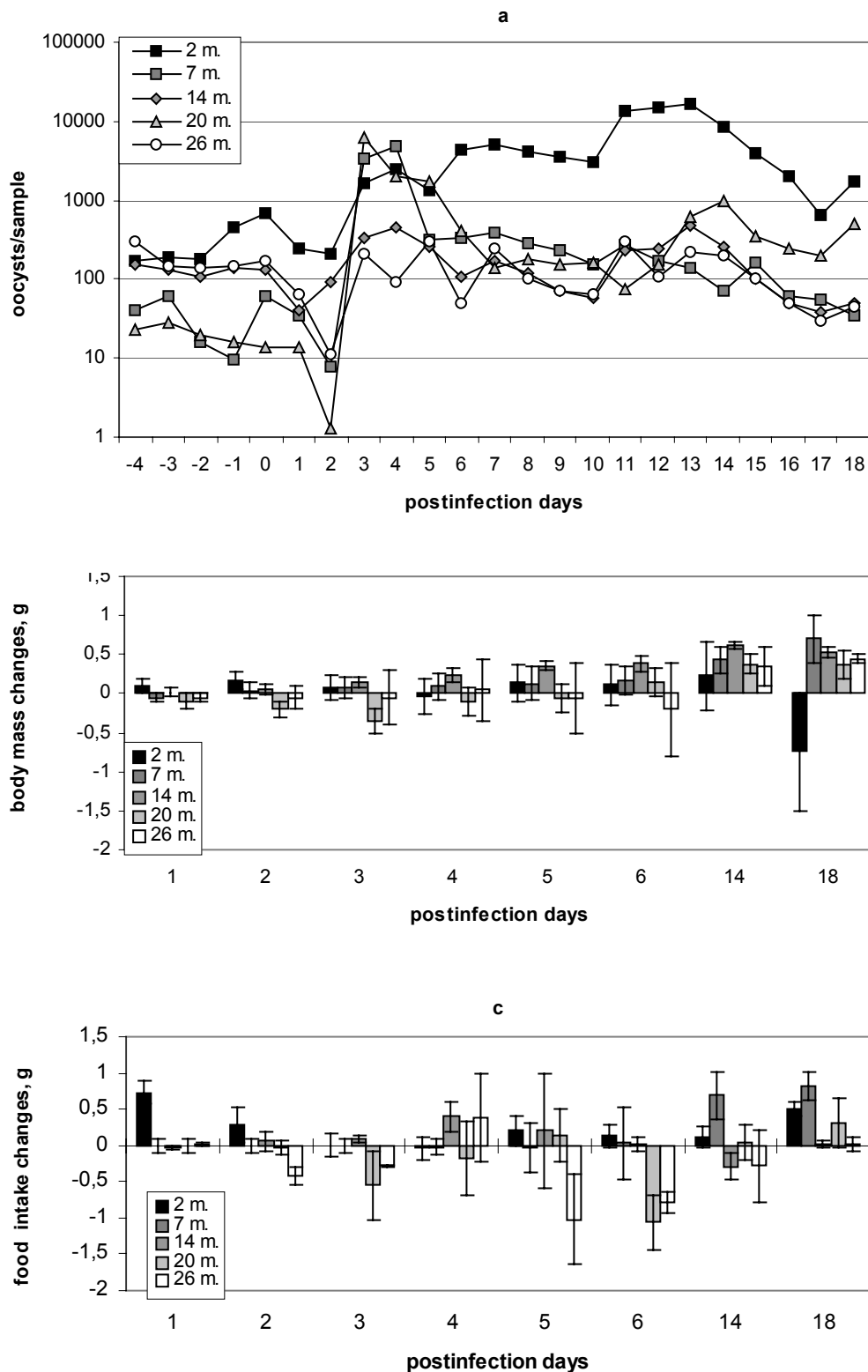
Fig. 3. Average oocyst release **(a)**, average body mass changes ±SE **(b)**, and average food intake changes ±SE **(c)** in birds of different age after artificial infection with 1×10^4 *Isospora sylvianthina* oocysts. For clarity error bars in figure **(a)** are omitted.
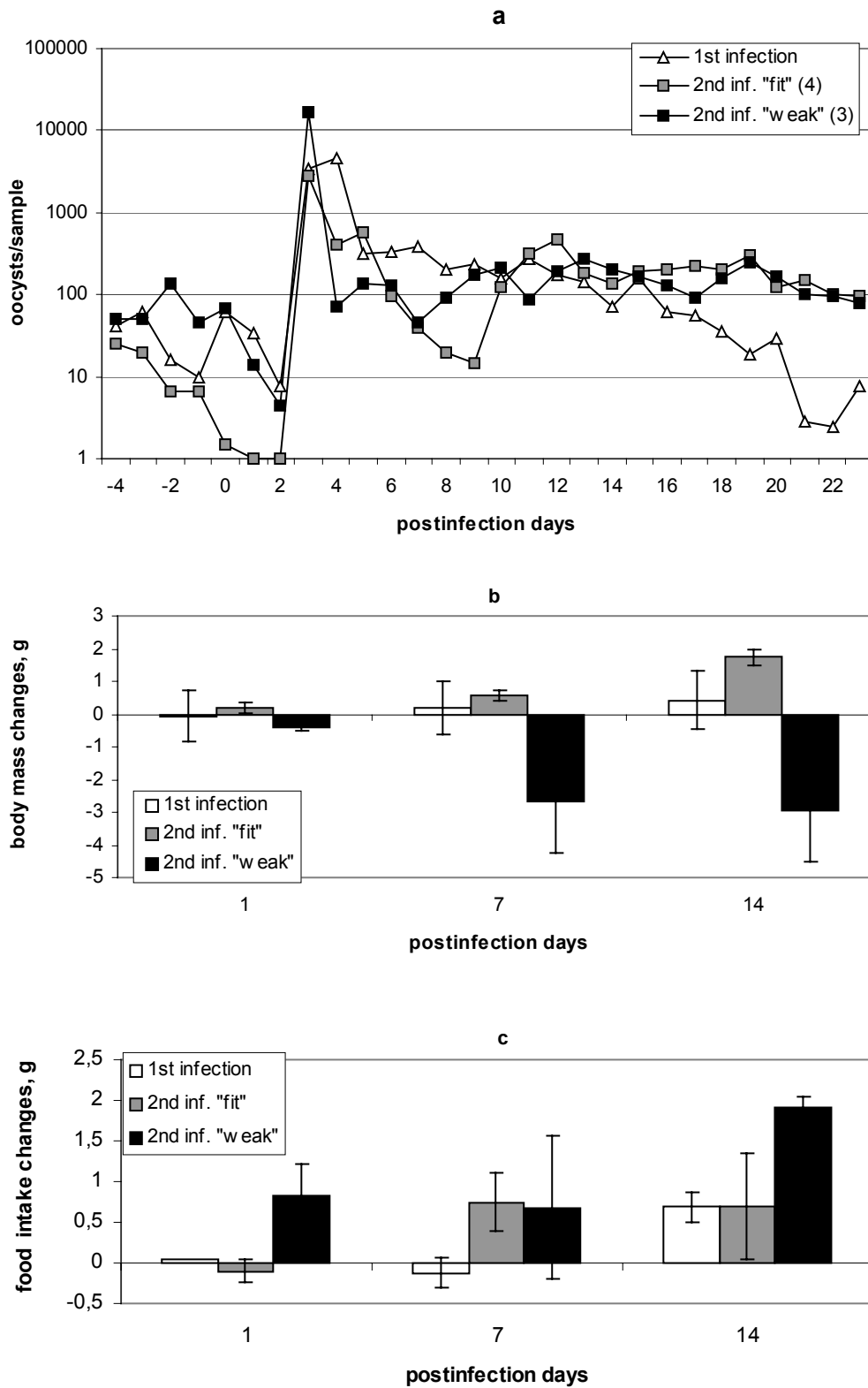
Fig. 4. Average oocyst release **(a)**, average body mass changes ±SE **(b)**, and average food intake changes ±SE **(c)** of 7 months old birds after first (white) and second (grey, black) artificial infection with 1×10^4 *Isospora sylvianthina* oocysts. For clarity error bars in figure **(a)** are omitted.

infection week the two months old birds showed some drop of body mass, though not significant because of one bird that increased body mass at that time.

Significant changes in food intake were observed only on the 1st postinfection day in 2 months old birds (Fig. 3c). On this day the food intake in all 2 months old birds was higher than at the day prior to infection (Wilcoxon test, P=0.018), and in the five "weak" 2 months old birds it increased significantly more than in the older ones (Mann-Whitney U-test, P=0.001).

Experiment 2 (Fig. 4)

Oocyst releases dropped the first two days after infection, both at first and at second infection. In all cases, the number of oocysts increased tremendously at day 3 after infection. After first infection all the birds returned to previous levels of infection within 3 weeks whereas the same birds maintained a higher level of oocysts output after the second infection (Fig. 4a). At 21st day after the second infection the birds still had significantly (P=0.003) higher oocyst output than at 21st day after the first infection, on average about 100 times higher.

Body mass did not change much in first infected birds (Fig. 4b). After the second infection the group splitted into 4 "fit" birds that increased body mass at about 2 g on average, and in three "weak" birds that decreased body mass at about 3 g on average. On day 14 after the second infection the difference in body mass change between the "weak" birds and the "fit" birds was significant (P=0.026), as well as the difference between the "weak" birds and the same birds at the 14th day after the first infection (P=0.005).

Daily food intake (Fig. 4c) did not change much in first infected birds, and the increases in food intake in birds following the second infection was more pronounced in the "weak" individuals.

Experiment 3 (Fig. 5)

All the 20 months old birds that were infected with a dose of 1x10^{4} oocysts showed similar reaction on the infection, but those birds who were infected with the 10 times higher dose splitted, according to their reaction, to 4 "fit" and 3 "weak" birds. As in the two previously described experiments, oocyst output in all the birds dropped on the first two days and increased on the third postinfection day. Thereafter, the birds at low dose as well as the "fit" birds of the high dose group returned to the chronic infection intensity level. On the 30th postinfection day the oocyst output in the birds infected with usual dose was lower
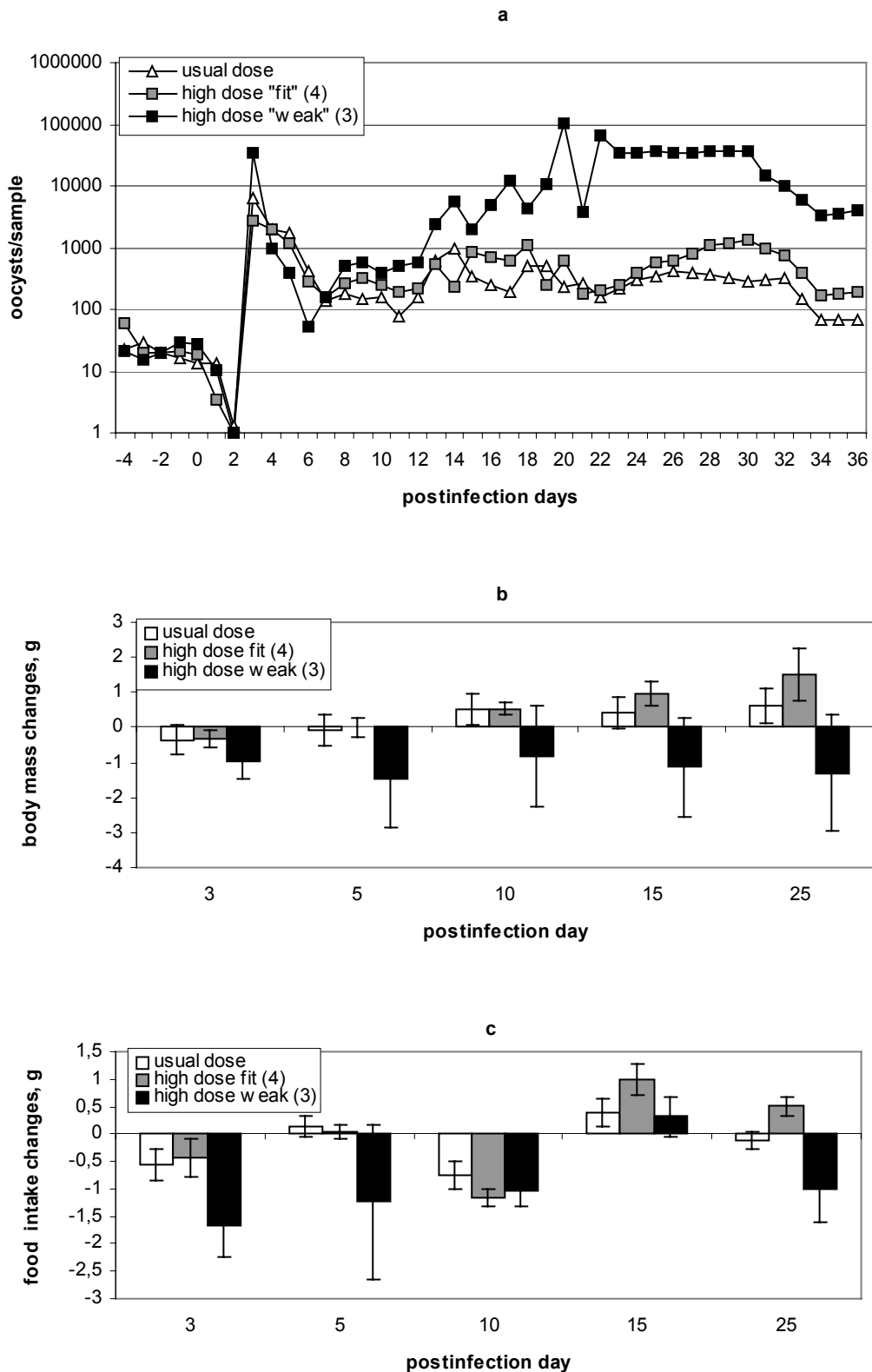
Fig. 5. Average oocyst release **(a)**, average body mass changes ±SE **(b)**, and average food intake changes ±SE **(c)** of 20 months old birds after artificial infection with 1×10^4 (white) and 1×10^5 (grey, black) *Isospora sylvianthina* oocysts. For clarity error bars in figure **(a)** are omitted.

than in "fit" birds infected with high dose (P=0.011) and much lower than in "weak" birds infected with high dose (P=0.000) (Fig. 5a).

Body mass of birds infected with usual dose, as well as of "fit" birds infected with high dose had a tendency to continuous increase during the first postinfection month, while the body mass of "weak" birds following infection with high dose decreased (Fig. 5b). On 25th day differences in body mass changes were not significant, but at 30th day the body mass increase in birds infected with usual dose was smaller than in "fit" birds infected with higher dose (P=0.046) and higher than in "weak" birds infected with higher dose (P=0.011). The "weak" birds infected with high dose differed in their body mass changes from the combined sample of the other birds (P=0.019), but the high dose infected birds calculated together did not differ from the ones infected with usual dose because of the opposite tendencies in "fit" and "weak" high dose infected individuals.

Food intake in birds infected with usual dose did not show any significant changes (Fig. 5c). In the "weak" high dose infected birds food intake decreased on the third postinfection day, and these changes were significantly different from the "fit" high dose infected birds (P=0.041) and from changes in birds infected with usual dose and in "fit" birds infected with high dose, calculated together (P=0.011).

Discussion

Immunity to *Isospora* can not prevent re-infection, therefore, similar prevalence of infection between adult and young birds of the investigated species in the wild reflects similar probabilities for young and adult birds to swallow infectious oocysts.

On contrary to prevalence, the intensity of infection by parasites that multiply inside the host can be regulated by the host. In this case the amount of parasites that survive and successfully develop in the host reflects how the host can cope with the parasite. Thus, intensity of *Isospora* spp. infection gives more information about the host's condition than prevalence of infection.

Screening of 5 bird species on the Courish Spit showed that in the wild the intensity of *Isospora* infection in young birds is higher than in adult ones. This concurs with the data that young animals are more susceptible to coccidial infection (Long 1982, Gylstorff & Grimm 1998), which is supposed to be due to some immunity against coccidia acquired with age (Long 1982).

The intensity of chronic infection in young captive Blackcaps decreased during the first year of life. This may serve as an indirect evidence of the development of immunity against *Isospora* spp.. This different level of chronic infection intensity may also be one of the possible reasons why young birds in the wild have higher intensity of *Isospora* spp. infection.

The second reason for a higher intensity of infection in young birds in the wild could be due to different reactions of adult and young birds to re-infection. The data from artificial infection experiment are supporting this suggestion.

Infection experiments

To understand the results of the experiments it is important to remember that in contrast to classical experiments of artificial infection in young chicken and other coccidia-free birds, all the birds that were used in our experiments were already chronically infected with the same *Isospora* species.

In all infection experiments a drop of oocyst output for the first two days following infection was observed. This drop shows that the inoculated oocysts indeed passed endogenous stages and the peak of oocyst production that we observe on the third day is a result of the parasite's multiplication within the host. If the oocysts would have been not able to infect the new host they would have passed through, and we would have observed many sporulated oocysts in the faeces at the day of infection. The peak of oocyst output appeared already on the third day that is the shortest known prepatent period of *Isospora* species. We can not yet explain why the prepatent period was this short.

The individual reaction of birds on the infection was very different, and in terms of body mass sometimes even opposite. This illustrates the natural variety in the population. We suggest that the "fit" groups are those individuals that survive the infection, and that surviving of "weak" individuals in nature will depend on many conditions, such as food availability, climatic factors, predator pressure, other diseases etc.

The first experiment showed that a high percentage of 2 months old birds (5 from 7) indeed suffered from the infection more than older birds. Only two of these birds managed to cope with the parasites by their own. On contrary, birds of 14 months age, and especially of 26 months age could easily cope with received infection. In these birds there was even a tendency to increase the body mass. The experiment also

showed that younger chronically infected birds in case of artificial infection react with higher oocyst output and keep it for a longer time than adult ones. This may also cause the observed differences in the infection intensity level between adult and young birds in the wild.

The differences in reaction of chronically infected birds on 1st and 2nd re-infection (experiment 2) can be taken as differences between healthy birds and birds that are already weakened because of some other diseases. The same dose of infective oocysts can be tolerated by healthy birds, but it can be more difficult to tolerate it for the birds who already have some health problems. The "weak" group after the second re-infection increased their food intake, but their body mass nevertheless decreased despite food was available *ad libitum*. In the wild when the birds have to search for the food actively, under pressure of abiotic factors, predators etc., this body mass changes may become more dramatic. In other words, for many individuals *Isospora* coccidia may become more harmful if the infection repeats after a short time. On the other hand, some individuals can cope with the infection and even accumulate body mass without increasing food intake, as shown by the "fit" group.

Experiments in poultry showed that an increase in the number of oocysts ingested by the host is usually accompanied by an increase in severity of disease (Hein, 1968, 1969, 1971, 1974, Long, 1973). However, Leathem and Burns (1968) noted that very heavy doses of oocysts produced lower mortality in cecal coccidiosis of chickens. It is possible that the invasion of very large numbers of sporozoites and/or the development of the early stages produce a host reaction resulting in loss of some invasive stages (Rose *et al.* 1975). Dogiel (1962) postulated that parasites with endogenous multiplication stages control their multiplication according to the "parasitic capacity" of the host. He suggests that not the amount of oocysts ingested, but the capacity of the host restricts the amount of parasites that will develop. In this case the amount of parasites developed will not depend on the dose of the infective oocysts. In our experiment (experiment 3) the amplitude of the peak did not depend on the dose of the infective oocysts. Increasing the infective dose did not influence much the amount of oocysts produced per day at the third postinfection day peak, but the number of days with high oocyst output. In other words, higher oocyst dose caused more severe infection, not in terms of higher oocyst output per day, but in the number of such days. As a result the birds that were infected with higher oocyst dose need more time to recover from the infection. As in previous experiments, there were fit" individuals that

could cope with the high dose infection (in terms of oocyst output) nearly as well as the birds which got 10 times lower dose. These birds even increased their body mass as a result of infection. On contrary, three of 7 birds dropped their body mass and reduced food intake, and at the end had to be medicated.

The absence of correlation or even a slight positive correlation between the intensity of *Isospora* infection and body mass of the bird in the wild was noticed by Mazgajski & Kędra (1998) in Starling nestlings and by Kruszewicz & Dyrcz (2000) in adults and nestlings of several *Acrocephalus* species. Our screening data from two different trapping sites also show no correlation between these two parameters (Chapter 7). In the present work we also did not find any correlation between the intensity of infection and body mass of the host in the wild. The absence of correlation between intensity of *Isospora* infection in birds in the wild and body mass of the host can be also explained by our experimental data. In some experimental birds there is a drop of body mass within the first two days after the infection. However, at this time there are nearly no oocyst in the faeces. This does not mean low intensity of infection, but that endogenous stages that preside appearance of oocysts prevail. If we sample the bird at this period of infection, we will observe positive correlation between oocyst output and body mass of the bird. After that in most cases there is no immediate influence of the infection on body mass, or sometimes there is even a slight positive effect, if enough food is available (Figs. 3, 4, 5). In cases of heavy infection, only 14-20 days after infection the body mass of some individuals started to decrease (Figs. 3, 4). Then it may even decrease rapidly and tremendously even when enough food is available, as we observed in some of 2 months old birds. At this time, however, the peak of oocyst output has already passed and the oocyst output decreases, and in birds sampled at this period of infection we will also observe positive correlation between oocyst output and body mass of the bird. In birds sampled between these two periods of infection no correlation between body mass and infection intensity will be found. Hence, we suggest that the fact that one can not find any correlation between oocyst output level and body mass of the host in the wild (Mazgajski & Kędra 1998), or even a slight positive correlation (Kruszewicz & Dyrcz 2000), is due to the sampling design. A single sampling of a bird does not allow to find the effect of intensity of oocysts output on the body mass of the bird.

We can conclude that the intensity of *Isospora* infection of wild passerine birds depends on age. Younger birds show higher intensity of infection than adult ones

because of higher intensity of chronic infection and more pronounced and prolonged oocyst output after re-infection. Nevertheless, even high infection intensity can be tolerated by some birds as long as the bird is not weakened and enough food is available. Any attempts of searching of a direct correlation between bird's body mass and its oocyst output should be done carefully because of the postponed effect of the parasite on host's body mass. The effect of parasite on a bird in a cage with food and water *ad libitum* will also be weaker than its effect in the wild.

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 ($55^{o}12'$N, $20^{o}46'$E). The second site is on the island of Helgoland in the North Sea ($54^{o}11'$N, $07^{o}55'$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.
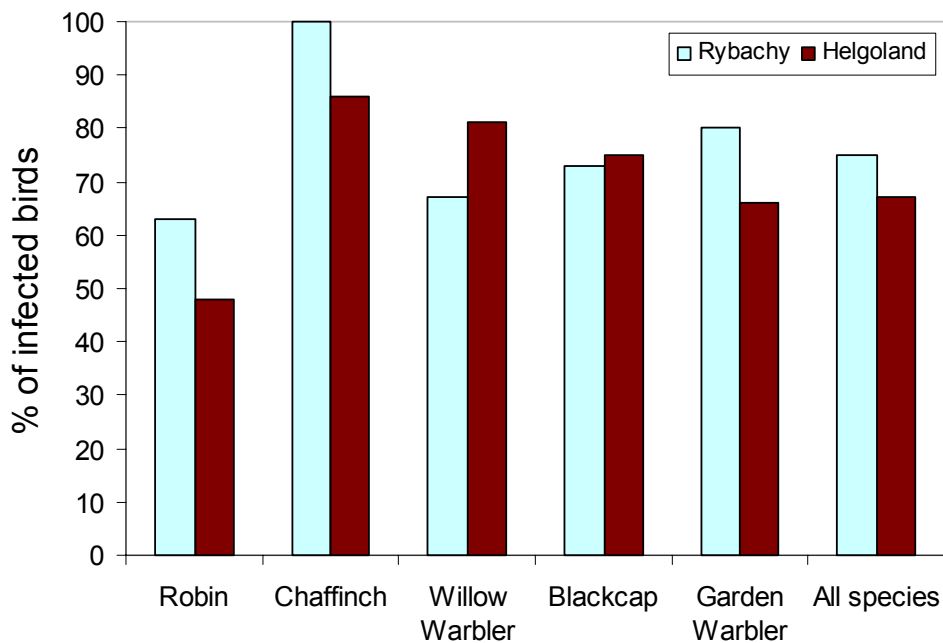
Fig. 2. Prevalence of *Isospora* infection in some birds' species at two sites in autumn.

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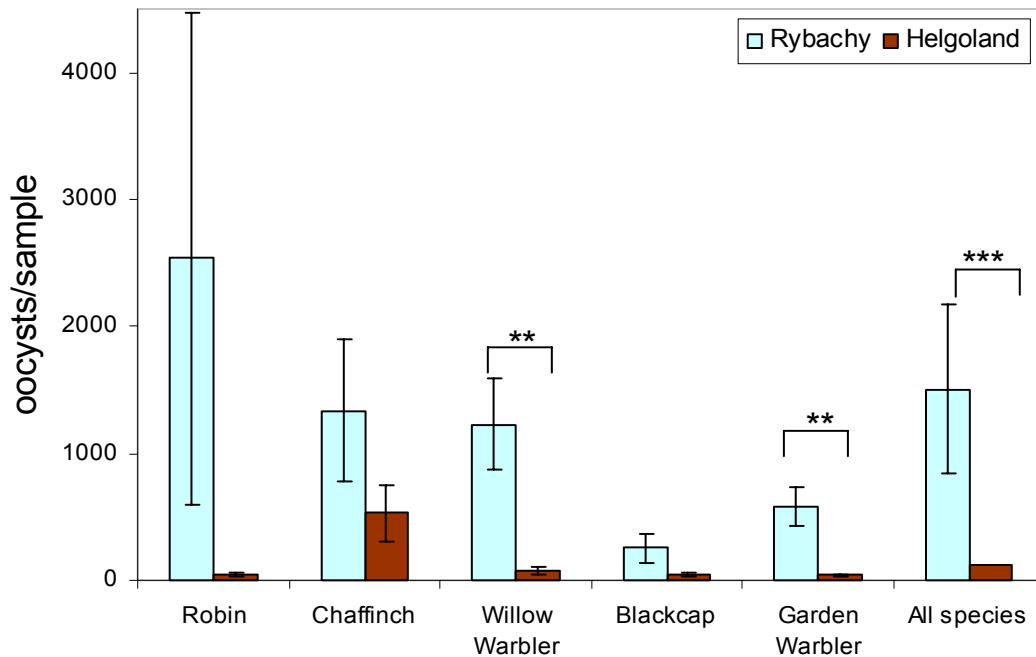
Fig. 3. Average intensity of *Isospora* infection (±SE) in some birds' species at two sites in autumn.

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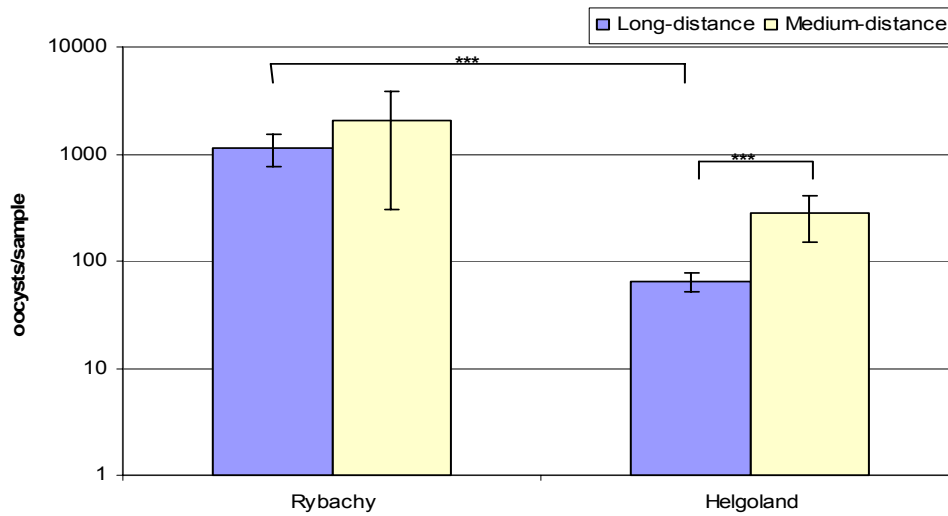
Fig. 4. Average intensity of infection (±SE) in long- and medium-distance migrants.

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.

References:

Alerstam, T. and Lindström, Ắ. (1990) Optimal Bird Migration: The Relative Importance of Time, Energy and Safety. In *Bird Migration* (E. Gwinner, ed.), pp.331-351. Springer-Verlag, Berlin, Germany.

Alerstam, T. and Hedenström, A. (1998) The development of bird migration theory. *J. Avian Biol*. **29,** 343-369.

Anwar, M. (1966) *Isospora lacazei* (Labbé 1893) and *I. chloridis* sp. n., (Protozoa,: Eimeriidae) from the English Sparrow (*Passer domesticus*), Green Finch (*Chloris chloris*) and Chaffinch (*Fringilla coelebs*). *J. Protozool*. **13**, 84-90.

Anwar, M. (1972) *Isospora erithaci* sp.n. (Protozoa, Eimeriidae) from the redbreast (*Erithacus rubecula*). *Acta protozoologica* **10**, 263-267.

Arnastauskene, T.V. (1985) *Coccidia and coccidiosis in domestic and wild animals of Lithuania*. Mokslas, Vilnius, Lithuania.

Bachmann, N., Daugschies, A., and Rommel, M. (1992) The effect of coccidial infections on the flight performance of racing Pigeons. *Tierärtzliche Umschau* **47**, 383-386.

Bairlein, F. (1985) Efficiency of food utilization during fat deposition in the long-distance migratory garden warbler, *Sylvia borin*. *Oecologia*, **68**, 118-125.

Bairlein, F. (1986) Ein standardisiertes Futter für Ernährungsuntersuchungen an omnivoren Kleinvögeln. *J. Ornithol*. **127**, 338-340.

Bairlein, F. (1995) *European-African Songbird Migration Network. Manual of field methods*. Institut für Vogelforschung, Wilhelmshaven, Germany.

Barré, N. and Troncy, P.M. (1974) Note on a coccidia of some Ploceidae in Chad: *Isospora xerophila* n. sp. *Z. f. Parasitenk*. **44,** 139-147.

Bauchinger, U. and Biebach, H. (2001) Differential catabolism of muscle protein in Garden Warblers (*Sylvia borin*): flight and led muscle act as a protein source during long-distance migration. *J. Comp. Physiol. B* **171**. 293-301.

Biebach, H. (1998) Phenotypic organ flexibility in Garden Warblers *Sylvia borin* during long-distance migration. *J. Avian Biology* **29**, 529-535.

Boughton, D.C. (1933) Diurnal gametic periodicity in avian *Isospora*. *Am.J.Hyg*. **33**, 161-184.

Boughton, D.C. (1937) Studies on oocyst production in avian coccidiosis. II. Chronic isosporan infections in the sparrow. *Am. J. Hyg*. **25**, 203-211.

Box, E.D. (1977) Life cycles of two *Isospora* species in the Canary, *Serinus canarius* Linnaeus. *J. Protozool*. **24**, 57-67.

Box E. D. (1980) *Isospora* as an extra-intestinal parasite of passerine birds. *Prog. Abstr. Am. Soc. Parasitol*. **55,** 39-40.

Britton, W.M., Hill, C.H., and Barber, C.W. (1964) A mechanism of interaction between dietary protein levels and coccidiosis in chicks. *J.Nutr*. **82**, 306-310.

Černá, Ž. (1973) Interesting coccidians from wild birds. *J.Protozool*. **20,** 535-536.

Chandra, R.K. and Newberne, P. M. (1977) *Nutrition, Immunity and Infection*. Plenum Press, New York, USA.

Chernetsov, N. (1998) Stopover length and weight change in juvenile Reed Warblers *Acrocephalus scirpaceus* in autumn in the Eastern Baltic. *Avian Ecology and Behaviour* **1**, 68-75.

Chernisheva, M.P. (1995) *Hormones of animals: Introduction into physiological endocrinology*. Glagol, St.Petersburg, Russia (in Russian).

Clayton, D.H. and Moor, J. (ed.) (1997) *Host-parasite evolution: general principles and avian models*. Oxford University Press, Oxford, UK.

Dawson, R.D. and Bortolotti, G.R. (2000) Effect of hematozoan parasites on condition and return rates of American Kestrels. *Auk* **117**, 373-380.

Deguchi, T. (1982) Endogenous oscillator and photoreception for serotonin N-acetyltransferase rhythm in chicken pineal gland. In: *Vertebrate cyrcadian systems. Structure and physiology*. (J. Aschoff), pp.146-172. Springer, Berlin, Germany.

Dogiel, V.A. (1962) *General Parasitology*. Leningrad University Press, Leningrad, USSR (in Russian).

Dolnik, O.V. (1998a) *Isospora* coccidia (Protozoa : Eimeriidae) of passerine birds on the Courish spit. Proceedings of the Symposium of Bird-Parasite Interactions, *Bulletin of the Scandinavian Society for Parasitology*. **8**, 58-59.

Dolnik, O.V. (1998b) *Isospora pari* sp. n. and *Isospora caerulei* sp. n. (Protozoa, Eimeriidae) from the Blue Tit (*Parus caeruleus*). *Parasitologiya* **32**, 277-281 (in Russian, English summary).

Dolnik, O.V. (1999a) Diurnal oocyst periodicity in *Isospora dilatata* (Sporozoa: Eimeriidae) from the Common Starling (*Sturnus vulgaris*) in nature. *Parasitologiya* **33**, 74-80. (in Russian).

Dolnik, O.V. (1999b) Diurnal periodicity in appearance of *Isospora* (Protozoa: Coccidea) oocysts from some passerine birds. *Proceedings of the Zoological Institute RAS* **281**,113-118.

Dolnik, O.V. **(1999c)** *Isospora certhiae* sp. n. (Protozoa, Eimeriidae) from a Tree Creeper (*Certhia familiaris*). *Parasitologiya* **33**, 149-151 (in Russian, English summary).

Dolnik, O.V. (2000) *Isospora* (Protozoa, Sporozoa) infection in passerine birds of various feeding habits. *Proceedings of the Symposium on Ecological parasitology on the turn of millenium, Bulletin of the Scandinavian Society for Parasitology* **10**, 69-70.

Dolnik, O.V. (2002) *Isospora sylvianthina* (Protozoa: Coccidiida), parasite of Blackcap, does not infect Reed Warbler. *Zoosystematica Rossic*a, **10**, 2001: 240.

Dolnik, V.R. (1974) Diurnal feeding and locomotor patterns in migratory birds. In: *Birds' biology researches*. (R.L. Potapov, ed.), pp. 3-13. Nauka, Leningrad, USSR.

Doran, D.J. (1978) The life cycle of *Eimeria dispersa* Tyzzer 1929 from the turkey in gallinaceous birds. *J. Parasitol*. **64**, 882-885.

Duszynski, D.W. and Gardner, S.L. (1991) Fixing coccidian oocysts is not an adequate solution to the problem of preserving protozoan type material. *J. Parasitol*. **77**, 52-57.

Duszynski, D.W. and Upton, S.J. (2000) Coccidia (Apicomplexa: Eimeriidae) of the mammalian Order Insectivora. *Special publication of the Museum of Southwestern Biology* **4**, 1-67.

Duszynski, D.W., Wilson, W.D., Upton, S.J., and Levine, N.D. (1999) Coccidia (Apicomplexa: Eimeriidae) in the Primates and the Scandentia. *Int. J. Primatol*. **20**, 761-797.

Feare, C. (1984) *The Starling*. Oxford University Press, Oxford, UK.

Fernando, M.A. (1982) Pathology and Pathogenicity. In *The biology of the Coccidia* (P.L. Long, ed.), pp. 287-327. Edward Arnold, London, UK.

Gershwin, M.E., Beach, R.S. and Hurley, L. S. (1985) *Nutrition and immunity*. Academic Press, Orlando, USA.

Glutz von Blotzheim, U.N. and Bauer, K.M. (1985) Handbuch der Vögel Mitteleuropas. Bd. 10 Wiesbaden.

Glutz von Blotzheim, U.N. and Bauer, K.M. (1997) Handbuch der Vögel Mitteleuropas. Bd. 14 Wiesbaden.

Greiner, E.C., Bennett, G.F., White, E.M. and Coombs, R.F. (1975) Distribution of the avian haemotozoa of North America. *Can. J. Zool*. **53**, 1762-1787.

Grulet, O., Landau, I., Millet, P. and Baccam, D. (1986) Les *Isospora* du moineau II - Etudes sur la biologie. *Ann. Parasitol. Hum. Comp*. **61,** 161-192.

Gryczyńska A., Dolnik, O., Pawełczyk, A., Mazgajski T. D. and Siemiątkovski, M. (2000) Parasites and pathogenes in population of Chaffinch (*Fringilla coelebs*) from Masurian Lakeland, NE Poland. *Acta Ornithologica*, **35**, 79-83.

Gwinner, E. (1978) Effects of pinealectomy on cyrcadian locomotor activity rhythms in European starling, *Sturnus vulgaris*. *J. Comp. Physiol*. **126**, 123-129.

Gylstorff, I. and Grimm, F. (1998) *Vogelkrankheiten*. Ulmer, Stuttgart, Germany.

Hammond, D.M. (1973) Life cycles and development of coccidia. *In The Coccidia, Eimeria, Isospora, Toxoplasma and related genera* (D.M. Hammond and P.L. Long eds.), pp.45-79. University Park Press, Baltimore, USA.

Hayworth, A.M., van Riper III, C., and Weathers, W.W. (1987) Effects of *Plasmodium relictum* on the metabolic rate and body temperature in Canaries (*Serinus canarius*). *J. Parasitol*. **73**, 850-853.

Hein, H. (1968) The pathogenic effects of *Eimeria acervulina* in young chicks. *Exp. Parasitol*. **22**, 1-11.

Hein, H. (1969) *Eimeria adenoides* and *E. meleagrimitis*: Pathogenic effect in turkey poults. *Exp.Parasitol*. **24**, 163-170.

Hein, H. (1971) Pathogenic effects of *Eimeria necatrix* in young chickens. *Exp. Parasitol*. **30**, 321-330.

Hein, H. (1974) *Eimeria brunetti*: pathogenic effects in young chickens. *Exp. Parasitol*. **36**, 333-341.

Hudson, P.J. (ed.) (1997) Wildlife diseases: the epidemiology of infectious diseases in wild animals and how they relate to mankind. Ecological issue **8**. British Ecological society, Liverpool, UK.

Kruszewicz, A. G. (1995) Occurrence of *Isospora lacazei* (Coccidia: Eimeridae) and its influence on nestlings growth in House Sparrows (*Passer domesticus*) and Tree Sparrow (*Passer montanus*). In: *Nestling mortality of granivorous birds due to microorganisms and toxic substances: synthesis*. (Pinowski J., Kavanagh B.P. and Pinovska B. eds.), pp. 291-305. PWN, Warszawa, Poland.

Kruszewicz, A. and Dyrcz, A. (2000) Intestinal parasites in five species of the genus *Acrocephalus*. *Acta Ornithologica* **35**, 153-158.

Krylov, M.V. and Dobrovolsky, A.A. (1980) Macrosystem and phylogeny of Sporozoa. Proceedings of Zoological Institute AS, **94**, 62-74.

Labbé, A. (1893) Sur les Coccidies des oiseaux. *C. R. Acad. Sci. Paris* **116**, 1300-1303.

Leathem, W.D and Burns, W.C. (1968) Duration of acquired immunity of the chicken to *Eimeria tenella* infection. *J. Parasitol*. **54**, 227-232.

Levine, N.D. (1982) *Isospora passeris* n. sp. from the house sparrow *Passer domesticus, I. lacazei*, and related apicomplexan Protozoa. *Trans. Am. Microsc. Soc*. **101**, 66-74.

Long, P.L. (1973) Pathology and pathogenicity of coccidial infections. In: D.M. Hammond and P.L. Long (eds.), The Coccidia: *Eimeria, Isospora, Toxoplasma*, and related Genera, pp. 253-294. University Park Press, Baltimore, USA.

Long, P.L. (ed.) (1982) *The biology of the Coccidia*. Edward Arnold, London, UK.

Mazgajski, T.D. and Kędra, A.H. (1998) Endoparasite *Isospora* sp. (Coccidia, Eimeriidae) affects the growth of starling *Sturnus vulgaris* nestling. *Acta Parasitologica* **43**, 214-216.

Menaker, M. and Oksche A. (1974) The avian pineal organ. *Avian biology* **4**, 79-118.

Milde, K. (1979) Light and electron microscopic studies on isosporan parasites (Sporozoa) in Sparrows (*Passer domesticus*). *Protistologica* **15**, 607-627.

Møller, A.P. and Erritzøe, J. (1998) Host immune defence and migration in birds. *Evolutionary Ecology* **12**, 945-953.

Newton, J. (1968) The moult of the Bullfinch (*Pyrrhula pyrrhula* L.). *Ibis* **108**, 41-67.

Noskov, G.A. (1990) Starling – *Sturnus vulgaris* in: *Moult of passerine birds of North-West USSR*. (T.A. Rymkevich, ed.), pp. 264-268. Leningrad State University, Leningrad, USSR (in Russian).

Payevsky, V.A. (1971) Atlas of bird migrations according to ringing data at the Kurische Nehrung. In *Ecological and physiological aspects of bird migration* (R.L. Potapov, ed.), pp. 3-110. Proceedings of Zoological Institute **50.** Nauka, Leningrad, USSR. (in Russian).

Pellerdy, P.L. (1974) *Coccidia and coccidiosis*. Akademiai Kiado, Budapest, Hungary.

Piersma, T. (1997) Do global patterns of habitat use and migration strategies co-evolve with relative investments in immunocompetence due to spatial variation in parasite pressure? *Oikos* **80**, 623-631.

Piersma, T. (1998) Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fueling and flight? *J. Avian Biol*. **29**, 511-520.

Price, P. W. (1980) Evolutionary Biology of Parasites. Princenton University Press, Princenton, NJ, USA.

Roitt, I.M., Brostoff, J. and Male, D.K. (1996) *Immunology*. Mosby, London, UK.

Rose, M.E., Long, P.L., and Bradley, J.W.A. (1975) Immune responses to infections with coccidia in chickens: Gut hypersensitivity. *Parasitology* **71**, 357-368.

Scharma, V.D., Fernando M.A., and Summers, J.D. (1975) The effect of dietary crude protein level on intestinal and caecal coccidiosis in chickens. *Can.J.Comp.Med*. **37**, 195-199.

Scholtyseck, E. (1956) Untersuchungen über die Coccidieninfektion bei Vögeln. *Zbl. Bakteriolog. Parasitenkde.Infectionskrankh. Hygiene*, Abt. 1 Orig. **165**, 275-289.

Scholtyseck, E. and Przygodda, W. (1956) Die coccidiose der Vögel. *Die Vogelwelt* **77**, 161-174.

Scholtyseck, E. and Przygodda, W. (1959) Untersuchungen über die Coccidien-infektion bei Nestlingen. *Z. f. Parasitenk*. **19**, 353-357.

Schuz, E. (1932) Fruhesommerzug bei Star und Kiebitz. *Vogelzug* **3**, 49-57.

Schwalbach, G. (1959) Die Coccidiose der Singvoegel. I. Der Ausscheidungsrhythmus der *Isospora*-Oocysten beim Haussperling (*Passer domesticus*). *Zentralblatt fuer Bakteriologie, Parasitenkunde, Infectionskrankenheiten und Hygiene. I. Orig.* **178**, 263-276.

Schwalbach, G. (1959) Untersuchungen und Beobachtungen an Coccidien der Gattungen *Eimeria*, *Isospora* und *Caryospora* bei Vögeln mit einer Beschreibung von sechzehn neuen Arten. *Arch.Protistenkd.* **104**, 431-491.

Svobodová, M. (1994) *Isospora*, *Caryospora* and *Eimeria* (Apicomplexa: Eimeriidae) in Passeriform Birds from Czech Republic. *Acta Protozoologica* **33**, 101-108.

Svobodová, M. and Cibulková, M. (1995*)* Isospora* sp. (Apicomplexa: Eimeriidae) in Icterine Warbler (*Hippolais icterina*, Passeriformes: Sylviidae): the possibility of parents to nestlings transmission . *Acta Protozoologica*, **34**, 233-235.

Takahaschi, J.S. (1982) Cyrcadian rhythms of the isolated chicken pineal in vitro In: *Vertebrate circadian systems. Structure and physiology*. (J. Aschoff), pp. 158-163. Springer, Berlin, Germany.

Valkiūnas, G. **(1993)** Pathogenic influence of haemosporidians and trypanosomes on wild birds in the field conditions: facts and hypotheses. *Ekologija* **1**, 47-60.

Valkiūnas, G. (1997) *Bird Haemosporidia*. Acta Zoologica Lituanica **3-5**: a monograph. Institute of Ecology, Vilnius, Lithuania. (in Russian).

Wakelin, D. (1996) *Immunity to parasites*. Cambridge University Press, Cambridge, UK.

Yorinks, N. and Atkinson, C.T. (2000) Effects of malaria on activity budgets of experimentally infected juvenile Apapane (*Himatione sanguinea*). *Auk* **117**, 731-738.

Yvoré, P. and Mainguy, P. (1972) Influence de la coccidiose duodénale sur la tenseur en carotenoides du serum chez le poulet. *Ann. Rech. Vet.* **3**, 381-387.

Zink, G. (1973-1975) Der Zug europäischer Singvögel: Ein Atlas der Wiederfunde beringter Vögel Bd.1. AULA-Verlag, Wiesbaden, Germany.

Zink, G. and Bairlein, F. (1995) Der Zug europäischer Singvögel: Ein Atlas der Wiederfunde beringter Vögel Bd.3. AULA-Verlag, Wiesbaden, Germany.

Summary

In wild bird populations parasites can be considered at least as important as predators, simply because of their abundance and diversity. The subject of interest of this work was *Isospora*, the most numerous genus among intestinal coccidia of wild passerine birds that has, nevertheless, been rarely studied.

These parasites require no intermediate transmitter for the spread of infection. Oocysts are released from the bird together with faeces, and the new host becomes infected through ingestion of sporulated oocysts. We established and proved a method that enables repeatable and comparable results on estimating the intensity of *Isospora* infection by counting oocysts in faecal samples.

Presence and the number of the oocysts in faeces of an infected host show a 24-hour rhythm. In 6 bird species we investigated in the wild and in captivity, maximum release of oocysts was found in late afternoon. This periodicity has to be taken into account when sampling birds.

Isospora species are considered to be narrow host specific. This hypothesis was supported by experimental transmission of *Isospora sylvianthina* from Blackcaps to Reed Warblers that did not lead to infection. In 41 from 56 investigated species of passerine birds on the Courish Spit (SE Baltic) 40 *Isospora* species were recorded, 2 of which are new species. The prevalence and average intensity of infection varied in different bird species. Ground feeders were more frequently and intensively infected than species feeding in the air. Hence, feeding style of birds influences their chances to become infected.

In the wild, the young birds appeared to be more intensively infected than adult birds. Under controlled laboratory condition the intensity of chronic infection of Blackcaps decreased continuously during the first year of life. Re-infection of chronically infected birds caused an increase in oocyst output for some days. In most young birds it took much longer to return to the low chronic infection level than in at least one year old birds. Subsequent re-infections weakened the birds so that the second re-infection led to pronounced body mass loss in some individuals. The decrease in body mass, however, occurred some days after the maximum of oocyst output. This may be the reason why many investigators did not find a correlation between body mass and infection intensity of birds in the wild.

During autumn migration the prevalence of infection in wild passerine birds was very high. Comparison of intensity and prevalence of infection in several bird species on the Courish Spit and on the island of Helgoland showed that there was no difference in prevalence of infection between both sites. In contrast, the intensity of infection was lower on the island than on land, and this difference was more pronounced in long-distance migrants.

Under natural circumstances most birds are likely to be able to tolerate *Isospora* infection. However, in very young birds, in case of high doses, repeated re-infection, or particular environmental constraints these parasites may have profound effects on fitness and survival.

Zusammenfassung

Schon auf Grund ihrer Häufigkeit und Vielfalt spielen Parasiten für Wildvögel mindestens eine genauso große Rolle wie Predatoren. Gegenstand dieser Untersuchung ist die Gattung *Isospora*. Obwohl sie die verbreitetste Kokzidien Gattung unter den Darmparasiten der Singvögel ist, wurde sie bisher kaum untersucht.

Diese Parasiten benötigen keine Überträger für ihre Ausbreitung, da die Oozysten mit dem Kot ausgeschieden und nach der Sporulation direkt vom neuen Wirt aufgenommen werden. Ich habe eine Methode entwickelt und getestet, um den Grad des *Isospora*-Befalls durch Auszählen der Oozysten in Kotproben abzuschätzen. Diese Methode ermöglicht wiederholbare und vergleichbare Ergebnisse.

Vorkommen und Menge der Oozysten in Kotproben zeigen einen 24-Stunden-Rhythmus. Für 6 Vogelarten, die wir unter Laborbedingungen und im Freiland untersucht haben, wurden maximale Werte für Oozysten am Spätnachmittag festgestellt. Dieser Tagesrhythmus muss bei der Kotprobennahme von Vögeln berücksichtigt werden.

Isospora werden als eng wirtsspezifisch angesehen. Diese Hypothese konnten wir experimentell unterstützen. Eine Übertragung von *Isospora sylvianthina* von Mönchsgrasmücken auf Teichrohrsänger führte zu keiner Infektion. In 40 von 55 untersuchten Singvogelarten der Kurischen Nehrung (Baltikum), wurden 41 *Isospora* Arten gefunden, von denen zwei Arten erstmalig beschrieben wurden. Vögel, die ihre Nahrung vom Boden aufnehmen, sind häufiger und stärker befallen als Vögel, die ihre Nahrung im Flug erbeuten. Die Art der Nahrungsaufnahme beeinflusst also die Wahrscheinlichkeit, infiziert zu werden.

Im Freiland waren Jungvögel häufiger stark befallen als Altvögel. Unter kontrollierten Laborbedingungen nahm die Befallsstärke chronisch infizierter Mönchsgrasmücken im ersten Lebensjahr kontinuierlich ab. Wiederholte experimentelle Infektion chronisch infizierter Mönchsgrasmücken führte zu einem mehrtägigen erhöhten Oozystenausstoß. Jungvögel brauchten in der Regel länger, um zu den anfänglichen chronischen Befallswerten zurückzukehren als einjährige und ältere Vögel. Wiederholte experimentelle Infektion schwächte die Vögel deutlich und führte bei einigen Individuen zu deutlichem Körpermasseverlust. Allein die Abnahme der Körpermasse zeigte sich erst einige Tage nach dem maximalen Oozystenausstoß. Vermutlich wurde deshalb von vielen Forschern kein Zusammenhang zwischen Körpermasse und Infektionsgrad an Freilandvögeln festgestellt.

Während des Herbstzuges ist der Kokzidienbefall von Singvögeln besonderes ausgeprägt. Ein Vergleich von Befallsgrad und -häufigkeit mehrerer Vogelarten auf der Kurischen Nehrung und auf Helgoland zeigte keinen Unterschied in der Häufigkeit des Befalls. Die Befallsstärke war jedoch niedriger bei Vögeln von der Hochseeinsel Helgoland als bei Vögeln vom Festland und dieser Unterschied war bei Langstreckenziehern deutlicher ausgeprägt.

Unter natürlichen Bedingungen scheinen die meisten Vögel einen *Isospora*-Befall ertragen zu können. Bei Jungvögeln jedoch können diese Parasiten bei hohem Infektionsgrad, wiederholtem Befall oder ungünstigen Umweltbedingungen einen gravierenden Effekt auf Kondition und Überleben haben.

Резюме

Паразиты, в силу своего обилия и разнообразия, оказывают не менее важное влияние на популяции диких птиц, чем хищники. Объектом данного исследования являются представители рода *Isospora*, самого распространенного и многочисленного рода кишечных кокцидий диких воробьиных птиц, который, тем не менее, слабо изучен.

Заражение изоспорами осуществляется без участия промежуточного хозяина. Ооцисты выделяются в окружающую среду с пометом и заражение птиц происходит пассивно при заглатывании спорулированных ооцист. Нами разработан и опробирован метод подсчета ооцист в пробах помета, позволяющий получать сравнимые данные по оценке интенсивности заражения изоспорами. У шести исследованных нами видов птиц как в природе, так и в эксперементе, максимум выделения ооцист приходился на вторую половину дня. Неравномерность выделения ооцист в течение суток следует учитывать при сборе проб.

Виды рода *Isospora* считаются узко видоспецифичными паразитами, что было подтвержденно нашим экспериментом по трансмиссии вида *Isospora sylvianthina* от славки-черноголовки тростниковой камышевке, который не привел к заражению последней. У 40 видов воробьиных птиц, из 55 исследованных на Куршской косе Балтийского моря, был обнаружен 41 вид изоспор, из которых 2 описаны впервые. Экстенсивность и средняя интенсивность заражения варьировала у разных видов птиц. Птицы, собирающие корм на земле, чаще и интенсивнее заражены, чем виды, кормящиеся в воздухе. Таким образом, установлено, что тип питания птицы оказывает влияние на риск заражения.

В природе молодые птицы оказываются более интенсивно зараженными, чем взрослые. В лабораторных условиях интенсивность хронического заражения славок-черноголовок постоянно снижалась в течение первого года жизни. Повторное заражение хронически зараженных птиц вызывало повышенное выделение ооцист в течение нескольких дней. Возврат к хроническому уровню выделения ооцист у молодых птиц наступал значительно позже, чем у взрослых. Повторное заражение ослабляло птиц и приводило к значительным потерям массы тела у некоторых особей. Потеря массы тела наблюдалась спустя несколько дней после массового выделения ооцист. Этот факт, возможно, является причиной того, что многим исследователям не удавалось обнаружить зависимости между массой тела и интенсивностью заражения птиц в природе.

Экстенсивность заражения изоспорами диких воробьиных птиц в течение осенней миграции регистрировалась очень высокой. Сравнение экстенсивности заражения изоспорами у нескольких видов птиц на Куршской косе и на острове Гельголанд (Северное море) не выявило различий, тогда как интенсивность заражения на острове была ниже, чем на косе, и эти различия были ярче выражены у дальних мигрантов.

Высокая доза изоспоридиозной инфекции, реинфекции и неблагоприятные условия окружающей среды оказывают сильное воздействие на состояние и выживаемость молодых птиц, тогда как большинство взрослых птиц, по-видимому, способно справится с инфекцией *Isospora*.

Acknowledgements

Curriculum Vitae

Persönliche Daten:

Name: Olga Dolnik

Geburtsdatum: 16. September 1973

Geburtsort: Leningrad (heute St. Petersburg), Rußland

Eltern: Victor Dolnik, Professor für Ornithologie, Zoologisches Institut, St.Petersburg
                           Tatjana Dolnik, Dipl. Biologin, Zoologisches Institut, St.Petersburg

Staatsangehörigkeit: russisch

Familienstand: verheiratet, ein Sohn, 9 Jahre alt

Schulausbildung:

1980-1983   allgemeinbildende Schule N 518 (Leningrad)
1983-1988   allgemeinbildende Schule N 483 (Leningrad)
1988-1990   allgemeinbildende Schule N 91, Biologische Klasse (St. Petersburg)

Studium:

1990-1997 Staatliche Universität St. Petersburg: Biologische Fakultät; Fachrichtung: Zoologie der Wirbellosen

07/1997   Studienabschluss mit Diplom, Diplomarbeit: "Kokzidien der Gattung *Isospora* in Singvögeln der Kurischen Nehrung" bei Prof. Dr. M.V. Krylov

07/1997-05/1999   Wissenschaftliche Angestellte am Zoologischen Institut der Russischen Akademie der Wissenschaft, Lehrstuhl für Protozoologie/Parasitologie

10/2000-07/2002   Promotions-Studium (DAAD-Stipendium) an der Carl von Ossietzky Universität Oldenburg bzw. am Institut für Vogelforschung in Wilhelmshaven über Kokzidien der Gattung *Isospora* in Zugvögeln.

List of publications

Dolnik, O.V. and Gracheva, T.I. (1990) Time and energy budgets of the White Wagtail (*Motacilla alba*) during feeding the nestlings. *Ornitologiya* **24**, 179-182 (in Russian, English summary).

Dolnik, O.V. **(1998)** *Isospora pari* sp. n. and *Isospora caerulei* sp. n. (Protozoa, Eimeriidae) from the Blue Tit (*Parus caeruleus*). *Parasitologiya* **32**, 277-281 (in Russian, English summary).

Dolnik, O.V. (1998) *Isospora* coccidia (Protozoa: Eimeriidae) of passerine birds on the Courish spit. *Proceedings of the Symposium of Bird-Parasite Interactions; Bulletin of the Scandinavian Society for Parasitology* **8**, 58-59.

Dolnik, O.V. (1998) Comparison of *Isospora* (Protozoa: Eimeriidae) infection intensity in passerine birds during autumnal migration at two geographical sites in two warbler species (*Sylvia atricapilla* and *Sylvia borin*). *Proceedings of the winners of "Grant 1998 for students, PhD-students and young scientists of St. Petersburg". Direction "Biology".* St. Petersburg, 76-77 (in Russian).

Dolnik, O.V. (1999) *Isospora certhiae* sp. n. (Protozoa, Eimeriidae) from a Tree Creeper (*Certhia familiaris*). *Parasitologiya* **33,** 149-151 (in Russian, English summary).

Dolnik, O.V. (1999) Diurnal oocyst output of *Isospora* (Eimeriidae) from Common Starling. *17th International Conference of the World Association for the Advancement of Veterinary Parasitology*, Copenhagen, Denmark, 37.

Dolnik, O.V. (1999) Diurnal oocyst periodicity in *Isospora dilatata* (Sporozoa ; Eimeriidae) from the Common Starling (*Sturnus vulgaris*) in nature. *Parasitologiya* **33**, 74-80 (in Russian, English summary).

Dolnik, O.V. (1999) Diurnal periodicity in appearance of *Isospora* (Protozoa: Coccidea) oocysts from some passerine birds. *Proceedings of the Zoological Institute RAS*, **281**, 113-118.

Dolnik, O.V. (1999) *Isospora schoenobaeni* sp. n. (Protozoa: Eimeriidae) from the Sedge Warbler (*Acrocephalus schoenobaenus*). *Zoosystematica Rossica* **8**, 6.

Dolnik, O.V. and Bairlein, F. **(1999)** Different level of *Isospora* (Protozoa: Sporozoa) infection in some Sylviidae in autumnal migration at Curonian Spit and Helgoland. *Proceedings of the 2nd Meeting of the European Ornithologists Union, The Ring*, **21,** 193.

Gryczynska, A., Dolnik, O., Pawelczyk, A., and Mazgajski, T. (1999) Parasites and pathogens in Masurian population of Chaffinch (*Fringilla coelebs*). *Proceedings of the 2nd Meeting of the European Ornithologists Union, The Ring*, **21,** 193.

Gryczynska, A., Dolnik, O., and Mazgajski, T. (1999) Parasites of Chaffinch (*Fringilla coelebs*) population. Part 1. Coccidia (Protozoa, Apicomplexa). *Wiadomosci Parasitologiczne*, **45**, 495-500.

Gryczynska, A., Dolnik, O., Pawelczyk, A., Mazgajski, T.D., and Siemiatkovski, M. (2000) Parasites and pathogenes in population of Chaffinch (*Fringilla coelebs*) from Masurian Lakeland, NE Poland. *Acta Ornithologica*, **35,** 79–83.

Dolnik, O.V. **(2000)** *Isospora* (Protozoa, Sporozoa) infection in passerine birds of various feeding habits. *Proceedings of the Symposium on Ecological parasitology on the turn of millenium, Bulletin of the Scandinavian Society for Parasitology*, **10**, 69-70.

Dolnik, O.V. and Bairlein, F. (2001) *Isospora* (Protozoa, Sporozoa) infection in wild passerine birds: the effect of age and diet. *Third Conference of the European Ornithologists' Union*, Groningen, The Netherlands, 39-40.

Dolnik, O.V. (2002) *Isospora sylvianthina* (Protozoa: Coccidiida), parasite of Blackcap, does not infect Reed Warbler. *Zoosystematica Rossica*, **10**, 2001, 240.

Submitted:

Dolnik, O.V., Bairlein, F. *Isospora* (Protista: Coccidiida) infection in migrating passerine birds. *Evolutionary ecology.*

Erklärung:

Hiermit erkläre ich, dass ich die Dissertation selbständig verfasst und nur die angegebenen Hilfsmittel benutzt habe.

Wilhelmshaven, im Oktober 2002

Olga Dolnik