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Low Prevalence but High Intensity of Infestation by Amblyceran Lice in Red-necked Grebes (*Podiceps grisegena*) in Eastern Poland

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Abstract.—Patterns of louse infestation in waterbirds deserve attention due to potential impacts on host populations. Red-necked Grebes (*Podiceps grisegena*) were visually examined for the presence of amblyceran chewing lice during the 2000-2015 breeding seasons in eastern Poland. Only one species of lice (*Pseudomenopon dolium*) was recorded. Amblyceran prevalence was low; four young birds (2.7%) were found infested out of 147 banded individuals (66 adults and 81 chicks). However, all infested Red-necked Grebes bore heavy parasite loads. Negative consequences of infestation for physiological condition were indicated by low hemoglobin and total protein values when compared with same-age non-infested birds. The low infestation rate can be related to the biology of the host (a diving species undergoing molt at sea, solitary breeder) and the apparent high pathogenicity of *P. dolium*, which left numerous holes in feathers and bleeding bites in all parasitized birds. *Received 29 May 2016, accepted 7 July 2016.*

Key words.—Amblycera, chewing lice, host-parasite relationships, infestation rate, *Podiceps grisegena*, Red-necked Grebe, virulence.

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Chewing lice (Phthiraptera: Amblycera and Ischnocera), once called Mallophaga, comprise the most widespread ectoparasites of birds. They exert a wide array of effects on parasitized individuals ranging from negligible harm to serious implications for fitness (Ash 1960; Clayton and Tompkins 1995; Møller and Rózsa 2005). There is a clear need for studies on infestation rates in free-living populations, especially employing large sample sizes, which increase the accuracy of estimates (Jovani and Tella 2006). With the exception of Anseriformes (e.g., Rékási *et al.* 1997; Hinojosa-Saez *et al.* 2009; Garbarino *et al.* 2013), patterns of louse parasitism have been particularly poorly explored in precocial aquatic species, most of which are notoriously difficult to sample using non-lethal techniques. Here, we investigated lice prevalence and intensity in Red-necked Grebes (*Podiceps grisegena*) in eastern Poland. The Red-necked Grebe is a migratory diving bird, distributed across the eastern and western Palearctic. The available information on the ectoparasite fauna of grebes (Podicipediformes), summarized by Storer (2000), has been based mainly on taxonomic investigations. We also compared the individual condition of parasitized and non-parasitized Red-necked Grebes using blood parameters proven to be indicators of physiological stress related to lice infestation in birds: hemoglobin concentration (Prelezov *et al.* 2002; Dudaniec *et al.* 2006) and total plasma protein levels (Quillfeldt *et al.* 2004).

Methods

Study Area

Adult and young Red-necked Grebes were captured on nests or by night-lighting and were metal- and color-banded during the 2000-2015 breeding seasons at fish ponds in eastern Poland (50° 55′ to 51° 27′ N, 21° 58′ to 22° 26′ E) (Kloskowski 2012) as part of a long-term research project on the species. All captured birds at least 2 weeks old were visually inspected for lice infestation. Their sex was determined using a standard molecular assay targeting differentially sized introns in the sex-linked CHD genes on the Z and W chromosomes (see Kloskowski *et al.* 2006 for more details on capture and sexing techniques). Hatching dates of clutches in the study area, and thus information on the approximate age of sampled chicks and data on resighting of marked individuals in subsequent years, were collected.

The method used for lice detection was similar to that described by Clayton and Drown (2001); for species determination a sample of specimens collected from various parts of the body was fixed in a 75% ethanol solution. At least two persons participated in each sampling event, helping each other with the immobilization of the bird and the search for parasites (Carrillo *et al.* 2007). The whole plumage and the skin at the base of the feathers were examined for lice and traces of lice (bite marks, damaged feathers). For birds sampled during the night, two million candlepower spotlights were used. Scanning of free-living birds is naturally time-limited, and we considered visual examination to be an unreliable method for quantifying occurrence of ischnocerans, which do not leave conspicuous traces (bites, feather holes) and are considered to exhibit low levels of pathogenicity (Clayton and Tompkins 1995; Møller and Rózsa 2005). However, we believe that the method provides a good estimate of amblyceran prevalence. The lice individuals collected were identified according to the key by Złotorzycka (1976). To verify the accuracy of the results obtained from the living birds, we examined, by ruffling feathers after storing carcasses in separate plastic bags at -20 °C, four birds found freshly dead at the nest.

To check whether lice infestation affected the health status of the birds, we compared the hematological indices of two parasitized young males with 11 non-infested young males. Since these indices are sensitive to age, time of sampling and capture and handling stress (Dawson and Bortolotti 1997; Dudaniec *et al.* 2006), birds sampled for condition-related indices were of similar age (46-47 days of age vs. 6-7 weeks post-hatching in controls) and captured with the same method (i.e., night-lighting) (Kloskowski *et al.* 2006) in July-August 2001-2006. All these young were reared on ponds stocked with common carp (*Cyprinus carpio*) fry, an abundant food supply for Red-necked Grebes; consequently, we assumed that the favorable food conditions overwhelmed the possible effects of between-year variation in habitat conditions (Kloskowski 2012). Blood samples were taken by venipuncture and processed within 6 hr of collection. Hemoglobin concentration was photometrically measured after using Drabkin's reagent (cyanohemoglobin method; Drabkin and Austin 1935). Total plasma protein levels, after the separation of plasma from blood cells by centrifugation, were determined spectrophotometrically (at wavelength λ = 545 nm) using the standard Biuret method (Gornall *et al.* 1949). The sampled birds were also weighed. We predicted that hemoglobin and plasma protein levels would be lower in infested than in healthy individuals.

RESULTS

A total of 66 adult and 81 young (3-7 weeks old) Red-necked Grebes were examined for lice; 14 of them were individuals repeatedly recaptured in different years. Lice were not detected on any of the adults, including the four carcasses. Four young approaching fledging from two broods (two males and two females, captured in August 2003 and July 2010, respectively) in the 5th-7th week post-hatching, were found to be infested (2.7% of all marked birds). The only louse species identified was the amblyceran *Pseudomenopon dolium* Rudow, 1869 (see Price 1974 for synonyms). Lice (both sexes and eggs) were observed on the whole body of the infested individuals, since they were highly motile during examination of the hosts. We only roughly estimated the numbers of lice visible on the wing undersurface (both wings were examined) all at one time to be more than 10 per wing in each infested grebe. All birds harboring lice bled from bite marks at the bases of feathers and had numerous holes (again both > 10 per wing), as large as 2-3 mm in diameter, mainly in the flight feathers and underwing coverts.

Infested young males had lower concentrations of hemoglobin and total plasma proteins than non-infested young males; Range = 12.0-13.0 g dL^{-1} vs. 14-19.9 g dL^{-1} for hemoglobin and 3.3-3.4 g dL^{-1} vs. 3.8-10.1 g dL^{-1}, respectively, for total plasma proteins; Mann-Whitney test, both $U = 0$, $P = 0.030$. The weights of infested young overlapped those of the control group (730-820 g vs. 620-857 g, respectively; $U = 9.0$, $P = 0.693$).

None of the parasitized young were resighted in the following study year; however, the resighting rates of individuals banded prior to fledging were very low overall, so the resighting probability did not differ between infested and non-infested young ($P = 0.874$, χ^2_1 test, Yates corrected).

DISCUSSION

The observed low number of louse species carried by Red-necked Grebes is not surprising, since the taxonomic richness of lice tends to be low in diving species (Felsõ and Rózsa 2006). Traditionally, parasitologists show little interest in populations with zero or low parasite prevalences, this trend being enhanced by an alleged publication bias;

nonetheless, very low infestation rates are ecologically and evolutionarily informative (Jovani and Tella 2006; Bensch *et al.* 2012). The low proportion of parasitized birds in the studied population can be explained by the species' typically solitary breeding regime (i.e., monogamy and strict territoriality) (Rékási *et al.* 1997; Storer 2000), which limits opportunities for the horizontal (between unrelated hosts) transmission of parasites. On the other hand, *P. dolium* is hosted by other grebe species sympatric with Red-necked Grebes (Price 1974). European Red-necked Grebes are reported to molt mainly in shallow sea areas in northern latitudes (Fjeldså 2004), where both water salinity and low temperatures may contribute to reducing lice abundance (Felsõ and Rózsa 2006). Although our sample of birds is the largest documented one in research on lice in the family Podicipedidae, methodological differences make comparisons with previous work problematic. Previous reports based on examinations of dead Red-necked Grebes from central and eastern Europe indicated a significant prevalence of ischnoceran lice (*Aquanirmus emersoni*) but not of amblycerans (Złotorzycka 1961; Rékási and Kiss 1980). Admittedly, under field conditions, we might have occasionally not detected lice on minimally infested birds. Visual examination is a reliable indicator of lice abundance; however, it usually allows only a minor fraction of the lice harbored by the bird to be detected (Clayton and Drown 2001). On the other hand, previous studies using birds collected for museums or hunter-killed might have occasionally overestimated lice prevalence, because they did not account for ectoparasite transmission between the collected birds often stored together prior to examination (Złotorzycka 1961; Storer 2000).

Our sample of parasitized individuals examined for hematology was extremely small; moreover, they came from the same brood and cannot be considered fully independent data points. Therefore, the hematological comparisons should be treated with great caution. Nonetheless, low total plasma protein levels (hypoproteinemia) indicate dysfunctions in the preservation of blood volume and of an optimal blood pH (Sturkie 1976). Depressed hemoglobin, presumably due to the destruction of mature erythrocytes by parasites may affect flight capacity (O'Brien *et al.* 2001; Dudaniec *et al.* 2006). As indicated by high feeding rates of the infested chicks reared in high-quality habitats (Kloskowski 2012) and given that their weights overlapped with those in the control group, the low condition indices and the heavy infestation of the chicks obviously did not result from malnutrition (in contrast to Ash 1960).

The fitness consequences of intense infestation by amblycerans, which feed on both feathers and skin, can be considerable. To obtain blood, amblycerans scratch the delicate skin around the feather base and chew the emerging tips of growing feathers (Ash 1960; Møller and Rózsa 2005). Ectoparasites transmitted horizontally are assumed to be more virulent, because their fitness is less dependent on the host's reproductive rate (Clayton and Tompkins 1994, 1995). The transmission of *P. dolium* presumably occurs both vertically, from parents to chicks (however, we failed to capture the parents of the infested broods), and horizontally, including spreading from species to species (Storer 2000). We argue that infestation with this species may have serious fitness consequences for Red-necked Grebes, especially since all the birds hosting lice were heavily parasitized and bled from bite wounds. Considering the high wing loading in grebes (Fjeldså 2004), the damage inflicted to flight feathers by *P. dolium* might affect flight capacities (Barbosa *et al.* 2002). Anemia following erythrocyte destruction may additionally compromise bird activity (see also Fairn *et al.* 2012 for possible immune investment). Blood ingestion adapts *P. dolium* to being a likely vector for the nematode parasite *Pelecitus fulicaeatrae* (Bartlett and Anderson 1987). The pattern of infestation by *P. dolium* in the studied population suggests that the Red-necked Grebes may lack protective defenses against this parasite and supports the general contention that highly pathogenic species typically exist at low levels of prevalence (May and Anderson 1979). However, further

research is required on endoparasites transmitted by amblyceran louse vectors and their aggregate virulence in waterbirds (Seegar *et al.* 1976; Bartlett and Anderson 1987).

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