


Avian schistosome species in Danish freshwater lakes: relation to biotic and abiotic factors

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Research Paper

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Abstract

Due to the increased prevalence of human infections with bird schistosome larvae (cercarial dermatitis) associated with bathing in Danish lakes, a nationwide survey of infected intermediate host snails was conducted in 2018–2020. Pulmonate snails (10,225 specimens) were collected from 39 freshwater lakes (in the four major geographic regions in Denmark) and subjected to shedding. Released schistosome cercariae were isolated and identified by polymerase chain reaction and sequencing whereby *Trichobilharzia regenti*, *Trichobilharzia franki*, *Trichobilharzia szidati* and *Trichobilharzia anseri* were recorded. Infections were primarily determined by biotic factors such as the presence of final host birds and intermediate host snails and water temperature was noted as an important abiotic parameter associated with the infection. No clear connection with other abiotic factors (conductivity, alkalinity, pH, nitrogen, phosphorous) was seen. The widespread occurrence of infected snails, when compared to previous investigations, suggests that climate changes at northern latitudes could be responsible for the increased risk of contracting cercarial dermatitis.

Introduction

Avian schistosomes of the genus *Trichobilharzia* are digenean parasites occurring worldwide, with an increasing presence at northern latitudes – for example, Denmark, Norway and Sweden (Thors & Linder, 2001; Larsen *et al.*, 2004; Soleng & Mehl, 2011; Tracz *et al.*, 2019; Al-Jubury *et al.*, 2020). Several species within the genus are of medical importance as their cercariae cause cercarial dermatitis (swimmer's itch) in humans (Horák *et al.*, 2015; Christiansen *et al.*, 2016; Zbikowska & Marszewska, 2018; Tracz *et al.*, 2019; Al-Jubury *et al.*, 2020). The first connection between bird schistosomes and swimmer's itch was described from Michigan, USA, almost a century ago (Cort, 1928). In contrast, Denmark's first case was reported three decades later (Berg & Reiter, 1960).

The adult bird schistosomes occupy the visceral veins (visceral species) or the nasal mucosa (nasal species) of birds, where they release eggs that subsequently hatch and release ciliated larvae (miracidia) to the aquatic environment. They penetrate the intermediate pulmonate snail host and transform to mother sporocysts, which subsequently produce daughter sporocysts releasing furcocercariae able to penetrate the skin of the definitive host (waterfowl) for completion of the life cycle (Horák *et al.*, 2002). Bird schistosomes are zoonotic trematodes as their cercariae may not only seek birds but also penetrate the skin of humans in the freshwater lake due to the similarity in skin lipid composition between aquatic birds and humans (Haas & Van de Roemer, 1998). The resulting disease is termed cercarial dermatitis due to marked skin reactions with an itchy maculopapular eruption induced by the dying or dead parasite larvae (Haas & Pietsch, 1991; Horák & Kolářová, 2000, 2001; Bayssade-Dufour *et al.*, 2002). Re-infections of humans are generally more pronounced, possibly due to sensitization and allergic responses, whereby swelling of larger skin areas, fever and nausea can be seen (Kolářová *et al.*, 2013; Tracz *et al.*, 2019).

Recently, the occurrence of bird schistosomes and swimmer's itch has become a more frequent problem in recreational areas (Buchmann *et al.*, 2004; Soldánová *et al.*, 2013; Jouet *et al.*, 2015; Marszewska *et al.*, 2016; Al-Jubury *et al.*, 2020). Of special concern is the finding that larvae of certain visceral avian schistosome species have been detected in the lungs of experimentally infected murine hosts (Horák *et al.*, 2002), and feet paralysis has been seen in mice infected with the neurotropic species *Trichobilharzia regenti* (Horák *et al.*, 1998; Hrádková & Horák, 2002). Therefore, it is important to survey the occurrence of parasites at the species level in lakes attended by the public for recreational purposes. For further risk analysis, it is

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relevant to correlate the parasite occurrence with physicochemical environmental factors in the lakes, as the parameters such as pH, alkalinity, nitrogen, phosphorous, chlorophyll and conductivity were previously claimed to affect the survival of the intermediate host snails and cercariae (Hunter & Wigington, 1972; Madsen *et al.*, 1988; Marcogliese, 2001). The present study presents the results from an extensive survey, covering a representative part of the Danish lake territory, elucidating the occurrence of avian schistosome species in the lakes with different physicochemical conditions.

Materials and methods

Lake sampling and snails examined

From August 2018 to October 2020, a total of 10,225 pulmonate freshwater snails (*Lymnaea stagnalis*, *Radix* (syn. *Ampullaceana*) *balthica*, *Radix auricularia*, *Stagnicola palustris*, *Omphiscola glabra*, *Planorbis corneus*, *Planorbis planorbis*, *Anisus vortex*, *Bithynia tentaculata*, *Bithynia leachii*) were collected from 39 (natural and artificial) freshwater lakes distributed all over Denmark (table 1).

Snails were collected randomly by hand or forceps from stones and aquatic plants along several parts of the shore in shallow water (minimum three localities of each individual lake). Snails were subsequently transported to the Laboratory of Aquatic Pathobiology, University of Copenhagen, for further examination. The sampled snails were rinsed by dechlorinated water for removal of surface adhering organisms (preventing interference of false positive results), after which each snail was identified to species level based on morphometric features (Glöer, 2002), and subsequently placed separately in a 200 ml plastic beaker containing 100 ml dechlorinated water. All beakers with snails were placed in natural light and room temperature overnight. The following morning all *Trichobilharzia*-positive snails were identified by examining the water in the beaker using a dissecting microscope (Leica CLS 150X, Leica Microsystems, Wetzlar, Germany, magnification $\times 4$ –40).

Cercariae

The cercarial type and genus were determined morphometrically based on standard morphometric characters (Wesenberg-Lund, 1934; Schell, 1970; Horák *et al.*, 2002; Marszewska *et al.*, 2016). Specimens identified to genus level as *Trichobilharzia* sp. were isolated and preserved in 70–96% ethanol and kept at 4°C for subsequent molecular species identification.

Bird collection

A total of 12 specimens of waterfowl were subjected to examination for the presence of bird schistosomes. Nine mallards (*Anas platyrhynchos*, three males and six females, mean body weight 1200 g) were shot by a hunter in Roskilde municipality, Denmark, in 2019 and were within one day transported to the Laboratory of Aquatic Pathobiology at the University of Copenhagen for parasitological examination. In addition, three specimens of mute swan *Cygnus olor* were found dead at three different localities (Vanløse and Roskilde in Zealand and Norre Alslev in Lolland). They were collected and treated likewise. All birds were frozen at -18°C until autopsy and parasitological examination.

Parasitological examination of birds

The dissection was performed according to Skírnisson & Kolářová (2008) with some modifications: the gizzard was cut open, muscles and content were checked by the naked eye, while heart, liver, lung and pancreas were pressed carefully and individually placed in a sieve and washed with tap water. The samples were transferred to 1000 ml beakers filled with approximately 700 ml tap water. After 60 min, the water and the suspension was then examined under the dissecting microscope (Leica CLS 150X) for recovery of fragments or complete adult worm. The spinal cord and cerebral membranes were also carefully removed and placed in a petri dish with phosphate-buffered saline and examined. Direct faecal and nasal mucosal tissue examination were performed by placing the stool or mucosa sample on a slide covered with a coverslip and subsequently checked under a compound microscope (Leica DM5000B, Leica Microsystems, Wetzlar, Germany). Digital images was obtained using a Leica MC170 HD camera.

DNA extraction and polymerase chain reaction (PCR)

DNA extraction from cercariae

DNA was extracted from fresh or alcohol-preserved single furcocercaria with a QIAamp® DNA Mini Kit (QIAGEN GmbH, QIAGEN strasse, Hilden, Germany) following the manufacturer's instruction protocol, with a minor modification (DNA was eluted in 50 μl of elution buffer instead of 200 μl).

DNA extraction from waterfowl positive faecal and mucous samples

DNA was extracted and purified from the individual positive faecal samples using the QIAamp® Fast DNA Stool Mini Kit (QIAGEN), while the QIAamp® DNA Mini Kit was used for DNA extraction from the positive mucus samples (one mallard was positive for parasite eggs in the nasal mucosa), as described above.

Molecular identification

By PCR, internal transcribed spacer 1 (ITS1)-5.8S-ITS2 regions of the ribosomal DNA (rDNA) was amplified using the forward primer BD1 (5'AGG AAT TCC TGG TAA GTG CAAG'3) and the reverse primer BD2 (5'TAT GCT TAA ATT CAG CGG GT'3) (Galazzo *et al.*, 2002). Reactions were run in a T100™ Thermal Cycler (BioRad, Copenhagen, Denmark) in a total reaction volume of 60 μl containing: 1 μM of each primer (TAG Copenhagen, Denmark), 1 mM of dNTP Mix (Life Technologies, Copenhagen, Denmark), 1.25 units of BIOTAQ DNA polymerase (Saveen Werner Aps, Copenhagen, Denmark), 1.5 mM magnesium chloride, and 6 μl 10 \times PCR buffer and 2 μl DNA template. Finally, DNase- and RNase-free water (Invitrogen™, Life Technologies, Copenhagen, Denmark) was added to a final reaction volume of 60 μl . Sterile water was used as negative control. The following PCR protocol was used: initial denaturation at 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 30 s and elongation at 72°C for 75 s. After cycling, a final elongation step at 72°C for 7 min was performed. The PCR products were visualized by gel electrophoresis and subsequently purified using Illustra™ GFX™ PCR and Gel Band Purification Kit (VWR, Copenhagen, Denmark). DNA concentration and purity were measured using a Nanodrop 2000 spectrophotometer (Saveen & Werner ApS, Copenhagen,

Table 1. Lake names, locations in Danish main regions (Zealand, Jutland, Funen and Lolland), coordinates and surface area of freshwater lakes examined.

Lakes	Abbreviation	Location	Coordinates	Surface area (km ²)
Ringen Sø	Rin	Zealand	55.632178, 12.081913	0.007
Bagsværd Sø	Bag	Zealand	55.770882, 12.460077	1.21
Furesø	Fur	Zealand	55.796893, 12.415352	9.41
Farum Sø	Far	Zealand	55.802409, 12.360115	1.20
Esrum Sø	Esr	Zealand	56.006314, 12.378258	17.29
Arresø	Arr	Zealand	55.967530, 12.113605	39.87
Søndersø-Z	Søn-Z	Zealand	55.775017, 12.353888	1.44
Sjælsø	Sjæ	Zealand	55.866349, 12.443419	2.93
Himmelsøen	Him	Zealand	55.614694, 12.056722	0.1
Buresø	Bur	Zealand	55.824841, 12.220360	0.76
Tissø	Tis	Zealand	55.574686, 11.285756	12.33
Utteslev mose	Utt	Zealand	55.717188, 12.505587	2.00
Haraldsted Sø	Har	Zealand	55.485526, 11.799915	2.10
Gyrstinge Sø	Gyr	Zealand	55.504606, 11.686293	2.63
Svogerslev Sø	Svo	Zealand	55.642510, 12.003539	0.25
Søndersø Maribo	Søn-L	Lolland	54.759204, 11.494309	8.52
Røgbølle Sø Maribo	Røg	Lolland	54.722582, 11.568850	2.01
Lynby Sø	Lyn	Zealand	55.774150, 12.485206	0.57
Brommelille Sø	Bro	Zealand	55.481158, 11.514709	0.125
Horsholm Kirke Sø	Hør	Zealand	55.873825, 12.499957	0.3
Sankt Jørgens Sø	San	Zealand	55.675971, 12.557444	0.66
Sortedam Sø	Sor	Zealand	55.693424, 12.573894	1.44
Skanderborg Sø	Ska	Jutland	56.030073, 9.943827	8.60
Stubbe Sø	Stu	Jutland	56.256753, 10.692496	3.76
Mossø	Mos	Jutland	56.037874, 9.779143	16.90
Glenstrup Sø	Gle	Jutland	56.595083, 9.852658	3.84
Slivso	Sli	Jutland	55.172519, 9.472084	1.64
Tange Sø	Tan	Jutland	56.323872, 9.582629	5.40
Kvie Sø	Kvi	Jutland	55.725525, 8.769138	0.3
Hampen Sø	Ham	Jutland	56.018553, 9.393947	0.75
Egå engsø	Egå	Jutland	56.218479, 10.223994	1.15
Tjele langsø	Tje	Jutland	56.531729, 9.626501	4.09
Hald Sø	Hal	Jutland	56.380309, 9.355170	3.42
Søndersø-J	Søn-J	Jutland	56.442607, 9.413825	1.45
Almind Sø	Alm	Jutland	56.149603, 9.542246	0.53
Vedsted Sø	Ved	Jutland	55.191228, 9.364211	0.08
Julso	Jul	Jutland	56.117859, 9.677438	5.65
Arreskov Sø	Ars	Funen	55.157691, 10.308423	3.17
Langesø	Lan	Funen	55.434449, 10.194496	0.16

Denmark). Fragments were sequenced by Macrogen Inc. (Amsterdam, the Netherlands) using the PCR primers used for the amplification process.

In order to identify species with high molecular identities, the obtained sequences were subjected to BLAST analysis at the

National Center for Biotechnology Information (NCBI). Further, the annotations were established by a combination of using the two web-based tools Rfam at <http://rfam.xfam.org/search> – tabview=tab1 at Nucleic Acids Research web site (2020) and the ITS Database at <http://its2.bioapps.biozentrum>.

uni-wuerzburg.de/ (Ankenbrand et al., 2015). In cases where 18S and 28S ribosomal sequences were too short, alignment to closely related and highly identical sequences with sufficient parts of 18S and 28S supplemented the web-based tools.

Phylogenetic and molecular evolutionary analyses were conducted using the software MEGA version X (Kumar et al., 2018). Initially, an alignment was constructed using Muscle and including three non-*Trichobilharzia* trematodes species (*Tyloodelphys clavata* (MW135143), *Diplostomum paracaudum* (MW135073) and *Posthodiplostomum cuticola* (MW135111)) as an outgroup. Muscle was chosen because Clustal W was not capable of aligning the included 5.8S ribosomal RNA (rRNA) properly. Due to the presence of a variable number of repeats in the ITS1 of *Trichobilharzia anseri* and *Trichobilharzia szidati*, some obvious editing of the alignment was necessary. Subsequently, the best fitting model of the 24 models tested by MEGA version X was, when using the Akaike criterion, General Time Reversible + G. The evolutionary history was inferred by using the maximum likelihood method and General Time Reversible model (Nei & Kumar, 2020). The tree with the highest log likelihood (−8272.16) was chosen. Initial tree for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (five categories (+G, parameter = 1.4441)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

Lake physicochemical parameters

Online data (ODA) at <https://odaforalle.au.dk>, delivered by the municipalities for the lakes investigated, were accessed and six parameters (temperature, pH, alkalinity, nitrogen, phosphorus, chlorophyll and conductivity) were extracted. Data in ODA are collected as part of the National Monitoring Program for the Aquatic Environment and Nature (NOVANA), the Danish Environmental Protection Agency. The data have been through quality assurance before being made available online. We then performed a Spearman correlation coefficient test between the parameters and the occurrence of infections (infected prevalence of snails and number of human cases).

Results

Lakes and snails

A total of 10,225 freshwater snails from 39 different freshwater localities distributed in Zealand (22), Jutland (15) and Funen (2) were collected (fig. 1). The freshwater snails collected and examined in this study were belonging to three families and ten different species: Lymnaeidae (*L. stagnalis* (2524), *R. balthica* (3175), *R. auricularia* (72), *S. palustris* (1078) and *O. glabra* (10)), Planorbidae (*P. corneus* (899), *P. planorbis* (27), *A. vortex* (1851)) and Bithyniidae (*B. leachii* (66), *B. tentaculata* (523)). The samples were dominated by *R. balthica*, followed by *L. stagnalis* and *S. palustris*. Eight snail species were sampled in Furesø, seven species in Farum Sø, six species in Bagsværd Sø and Esrum Sø, five species from Søndersø Zealand, Buresø,

Haraldsted Sø, Hørsholm kirke Sø and Skanderborg Sø. Other lakes, such as the artificial lakes Ringen Sø and Svogerslev Sø, merely contained one snail species, *L. stagnalis* and *R. balthica*, respectively.

Prevalence of *Trichobilharzia* infection in snails in the investigated lakes

Thirteen lakes were positive with regard to *Trichobilharzia* spp. (cercarial shedding), seven of them located in Zealand and six in Jutland. The remaining 26 locations were negative for *Trichobilharzia* spp. – as judged from cercarial shedding from snails. Lakes (written in bold in fig. 1) were positive for *Trichobilharzia* cercarial shedding. Of the 10,225 snails, 37 released *Trichobilharzia* cercariae, resulting in an overall prevalence of 0.4%. *Lymnaea stagnalis* and *Radix balthica* were the only snail species found infected with *Trichobilharzia* spp. (overall prevalences 1 and 0.4%, respectively). The highest total prevalence (6%) was recorded in Ringen Sø, followed by Arresø (4.9%). Cercariae released from snails in the positive lakes were identified both morphologically and molecularly.

Furesø and Hørsholm Kirke Sø were the only lakes in which two *Trichobilharzia* species were found. In those lakes, *T. franki* and *T. regenti* were isolated from four individual snails (prevalence 0.4%, 0.4% and 0.75% and 0.75%, respectively). The remaining positive lakes hosted only one species of *Trichobilharzia* (table 2). Cercariae released from snails in Ringen Sø, Mossø and Tjele langsø were identified as *T. szidati*, while cercariae from Bagsværd Sø, Furesø, Hørsholm kirke Sø, Himmelsø, Glenstrup Sø, Kvie Sø and Egå Engsø were *T. franki*.

Prevalence of infection in birds

Four of the 12 birds (33.3%) were positive for avian schistosome eggs. Three *A. platyrhynchos* (one male and two females) were positive for *Trichobilharzia* spp. (egg screening). Subsequent molecular sequencing of DNA recovered from faeces showed that one female bird and one male bird carried *T. szidati*, while the other female carried eggs identified as *T. regenti* in the nasal mucosa (table 3). One swan (*C. olor*) was positive for *Trichobilharzia* sp. by faecal examination. Identification to the genus level was done based on the egg morphology and molecular characteristics (Skírnisson & Kolářová, 2008), as the PCR was not conclusive at the species level.

Molecular identification of cercariae

Cercariae released from 15 infected snails in the positive lakes and three samples from birds were identified molecularly and have been assigned GenBank accession numbers from MW482435 to MW482450, MW538530 and MW538531. At NCBI, the BLAST tool was used to reveal similarity/identity to sequences submitted to GenBank prior to this study (supplementary table S1). In addition, phylogenetic analysis was performed (supplementary fig. S2). Seven cercariae were identified as *T. franki* in Furesø, Bagsværd Sø, Hørsholm Kirke Sø, Himmelsøen, Kvie Sø, Egå Engsø and Glenstrup Sø. Five sequences identified the samples as *T. szidati*. Two of these were from cercariae in Ringen Sø, and one in Mossø. Two *T. szidati* sequences were from birds samples in Roskilde municipality. Three sequences identified the samples as *T. regenti* in Furesø, Hørsholm Kirke Sø and Roskilde municipality (bird sample). Three cercariae were identified as

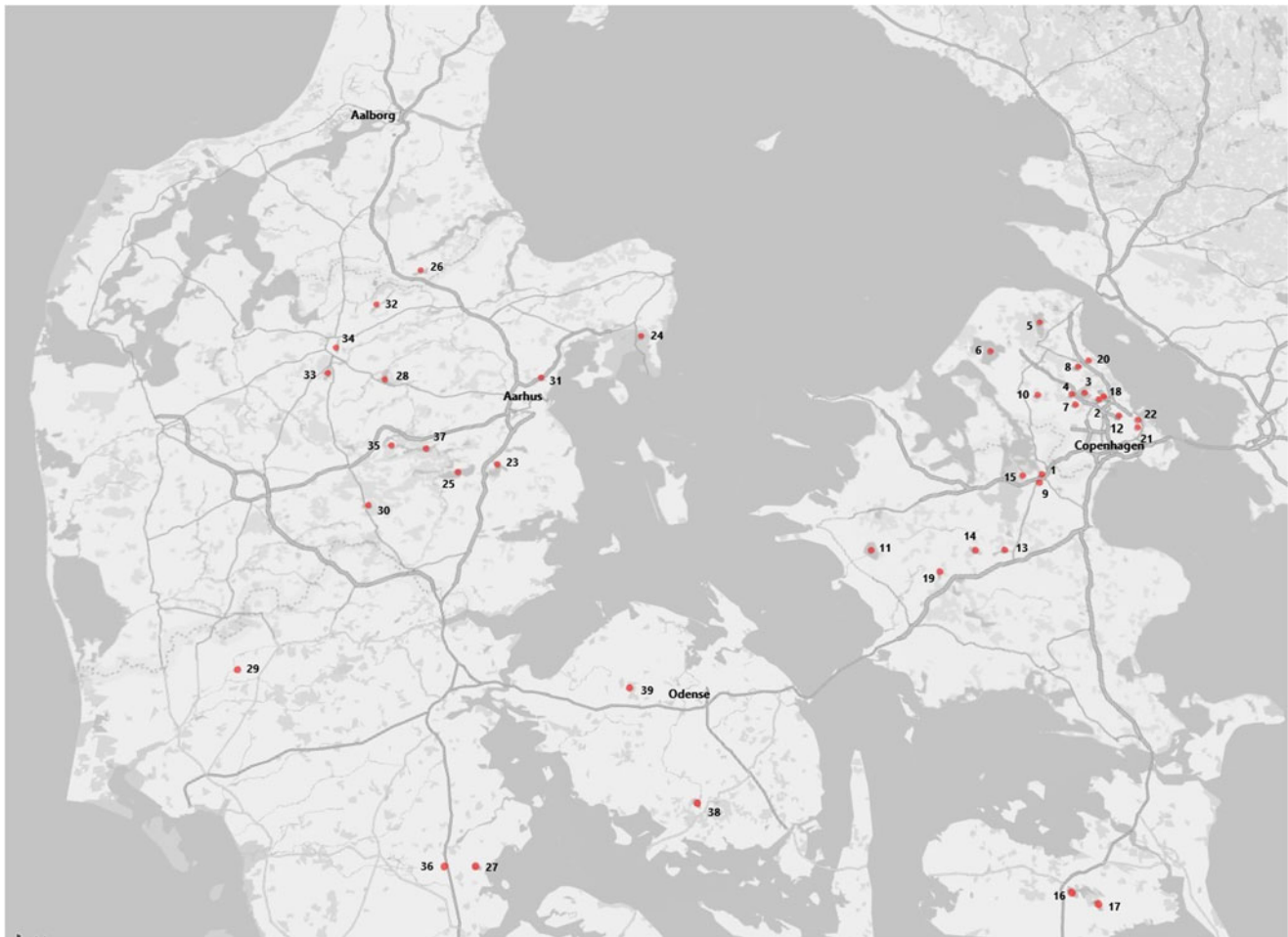


Fig. 1. Sampling locations (number) in Danish (natural and artificial) freshwater lakes. Lake name and sampling size (x) reported as follows (lakes written in bold are negative): **1. Ringen (359)**, **2. Bagsværd Sø (495)**, **3. Furesø (1270)**, **4. Farum Sø (766)**, 5. Esrum Sø (1600), **6. Arresø (92)**, 7. Søndersø-Z (197), 8. Sjælsø (203), **9. Himmelsoen (30)**, 10. Buresø (88), 11. Tissø (296), 12. Utterslev mose (81), 13. Haraldsted Sø (234), 14. Gyrstinge Sø (30), 15. Svogerslev Sø (8), 16. Søndersø Maribo (201), 17. Røgbølle Maribo (217), 18. Lyngby Sø (250), 19. Brommelille Sø (135), **20. Horsholm kirke Sø (182)**, 21. Sankt Jørgens Sø (255), 22. Sortedam Sø (169), 23. Skanderborg Sø (496), 24. Stubbe sø (116), **25. Mossø (100)**, **26. Glenstrup Sø (490)**, 27. Slivso (137), 28. Tange Sø (209), **29. Kvie Sø (162)**, **30. Hampen Sø (140)**, **31. Egå Engsø (183)**, **32. Tjele langso (287)**, 33. Hald Sø (181), 34. Søndersø-J (151), 35. Almind Sø (18), 36. Vedsted Sø (36), 37. Julso (22), 38. Arreskov Sø (183), 39. Langesø (156).

T. anseri in Arresø, Hampen Sø and Farum Sø (for more details, see [table 3](#)). The sequences from the *T. anseri* specimens were difficult to align to each other. First of all, sequences designated *T. anseri* covering either ITS1 or ITS2 – but not both – were available at GenBank. However, three sequences (FJ469784, FJ469785 and FJ469791), designated as *Trichobilharzia* sp., contained parts that were identical to ITS1 and ITS2 sequences of *T. anseri* at GenBank. In addition, these sequences included a part of 18S, the complete 5.8S and a part of 28S. Secondly, a repeated sequence (105 bp long) in ITS1 occurred in one, three, five or seven copies. Thirdly, MW482445 of this study included a 151-bp-long insertion in ITS1. Finally, another sample (MW538530) from Farum Sø identified as *T. anseri* (MW538530) had a stretch of 503 bp double sequence across the insertion mentioned above, thus resulting in a gap. The obtained parts of ITS1 and ITS2 of MW538530 were identical to MW482435 from Arresø, including two heterozygote bases in ITS1. In the case of MW482435 (recovered from Arresø), we obtained parts of 18s and 28S; these rRNAs were identical to the 18S and 28S parts of FJ469784, FJ469785 and FJ469791 from GenBank. Therefore, based on the molecular analysis of

this study, the three sequences FJ469784, FJ469785 and FJ469791 previously deposited at GenBank having the designation *Trichobilharzia* sp. in GenBank may be designated *T. anseri*.

Abiotic parameters

We found a clear association between lake temperature and occurrence of infected snails. During the first months of the year the water temperature was low, and no shedding occurred from sampled snails. When temperature increased in spring the infection prevalence increased and peaked in high and late summer (August) ([fig. 2](#)). However, other abiotic parameters were not clearly associated with infection. [Table 4](#) lists the physicochemical parameters measured from May to September, including pH, alkalinity, nitrogen, phosphorus, chlorophyll and conductivity from 18 different freshwater lakes from where snails were collected and from which data were available online (ten lakes from Zealand, five from Jutland, two from Funen and one from Lolland). We found no significant correlation between

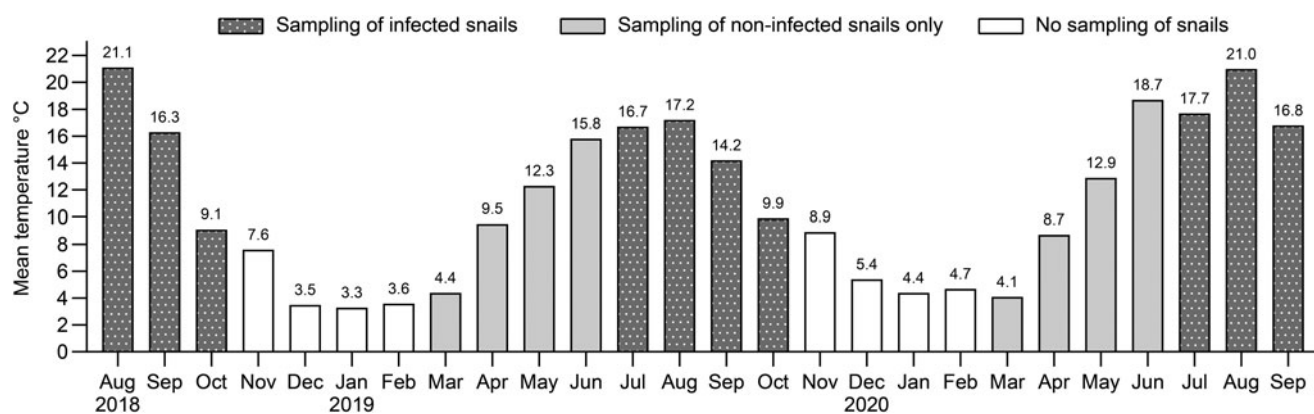
Table 2. Overall prevalence of 13 *Trichobilharzia* spp.-positive lakes (snails shedding) with species identification.

Snail	Parasite	Lake													
		Rin	Bag	Fur	Far	Arr	Him	Hør	Mos	Gle	Kvi	Ham	Egå	Tje	
Lymnaeidae															
<i>Lymnaea stagnalis</i>	Examined	<i>T. szidati</i>	359	54	198	63	33	–	11	52	52	39	37	–	214
	Prevalence		6	–	–	–	–	–	–	1.9	–	–	–	–	0.5
<i>Radix balthica</i>	Examined	<i>T. regenti</i>	–	196	261	190	41	30	129	46	193	123	103	115	73
	Prevalence		–	–	0.4	–	–	–	0.75	–	–	–	–	–	–
	Prevalence	<i>T. franki</i>	–	0.5	0.4	–	–	3.3	0.75	–	0.5	0.8	–	0.8	–
<i>Radix auricularia</i>	Examined	<i>T. anseri</i>	–	–	–	0.5	4.9	–	–	–	–	–	1	–	–
	Prevalence		–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Stagnicola palustris</i>	Examined		–	21	30	–	–	–	10	–	–	–	–	68	–
	Prevalence		–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Omphiscola glabra</i>	Examined		–	–	10	–	–	–	–	–	–	–	–	–	–
	Prevalence		–	–	–	–	–	–	–	–	–	–	–	–	–
Planorbidae															
<i>Planorbarius corneus</i>	Examined		–	178	296	45	18	–	–	1	–	–	–	–	–
	Prevalence		–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Planorbis planorbis</i>	Examined		–	–	–	–	–	–	–	–	–	–	–	–	–
	Prevalence		–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Anisus vortex</i>	Examined		–	25	452	321	–	–	29	1	–	–	–	–	–
	Prevalence		–	–	–	–	–	–	–	–	–	–	–	–	–
Bithyniidae															
<i>Bithynia leachii</i>	Examined		–	–	10	56	–	–	–	–	–	–	–	–	–
	Prevalence		–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Bithynia tentaculata</i>	Examined		–	21	–	28	–	–	3	–	245	–	–	–	–
	Prevalence		–	–	–	–	–	–	–	–	–	–	–	–	–

Consult [table 1](#) for abbreviations of lake names.

Table 3. Overview of isolated specimens of *Trichobilharzia* spp. identification, lake and GenBank accession number.

GenBank acc. no.	Species	Lake	Host
MW482445	<i>Trichobilharzia anseri</i>	Hampen Sø	<i>Radix balthica</i>
MW538530	<i>Trichobilharzia anseri</i>	Farumsø	<i>Radix balthica</i>
MW482439	<i>Trichobilharzia franki</i>	Furesø	<i>Radix balthica</i>
MW482440	<i>Trichobilharzia franki</i>	Bagsværd Sø	<i>Radix balthica</i>
MW482441	<i>Trichobilharzia franki</i>	Hørsholm Kirke Sø	<i>Radix balthica</i>
MW482443	<i>Trichobilharzia franki</i>	Himmelsøen	<i>Radix balthica</i>
MW482444	<i>Trichobilharzia franki</i>	Kvie Sø	<i>Radix balthica</i>
MW482446	<i>Trichobilharzia franki</i>	Egå Engsø	<i>Radix balthica</i>
MW538531	<i>Trichobilharzia franki</i>	Glenstrup Sø	<i>Radix balthica</i>
MW482438	<i>Trichobilharzia regenti</i>	Furesø	<i>Radix balthica</i>
MW482442	<i>Trichobilharzia regenti</i>	Hørsholm Kirke Sø	<i>Radix balthica</i>
MW482450	<i>Trichobilharzia regenti</i>	Roskilde Municipality	<i>Anas platyrhynchos</i>
MW482436	<i>Trichobilharzia szidati</i>	Ringene at Roskilde	<i>Lymnaea stagnalis</i>
MW482437	<i>Trichobilharzia szidati</i>	Ringene at Roskilde	<i>Lymnaea stagnalis</i>
MW482447	<i>Trichobilharzia szidati</i>	Mossø	<i>Lymnaea stagnalis</i>
MW482448	<i>Trichobilharzia szidati</i>	Roskilde Municipality	<i>Anas platyrhynchos</i>
MW482449	<i>Trichobilharzia szidati</i>	Roskilde Municipality	<i>Anas platyrhynchos</i>

**Fig. 2.** Water temperature recorded during the period of investigation (August 2018–September 2020). Months with positive and negative snails are indicated (dark and grey, respectively), together with months where snails could not be recovered (white).

physicochemical parameters and the prevalence of infection in snails or number of cercarial dermatitis cases reported (table 5). The range of pH was from 5.8 in Kvie Sø to 10.5 in Sortedam Sø. The range of alkalinity was from 50 μ M in Kvie Sø to 4.4 mM in Haraldsted Sø. The range of nitrogen measure recorded was from 0.49 mg/l in Esrum Sø to 4.8 mg/l in Tissø. The range of phosphorus was from 0.01 mg/l in Tissø to 0.36 mg/l in Sønder sø Jutland. The range of chlorophyll was from 1.6 μ g/l in Tange Sø to 210 μ g/l in Slivsø. The range of the conductivity measurements was from 10 mS/m in Kvie Sø to 90 mS/m in Sortedam Sø.

Biotic parameters in lakes

During snail collection, the occurrence of waterfowl (mallards and swans) was recorded in all of the freshwater habitats visited – both

infected and non-infected. The only biotic parameter associated with infection in lakes was the presence of intermediate hosts (snails) and final hosts (waterfowl).

Discussion

During the warm summer of 2018, Danish dermatologists reported an unusually high number of clinical cases of cercarial dermatitis (swimmer's itch) (Tracz *et al.*, 2019). Children and adults became infected due to contact with water in several freshwater lakes. Although the disease is considered benign and the invading cercariae are supposed to die in the skin of the patient, it cannot be ruled out that some cercariae of at least two species may migrate further in a mammalian host (Horák & Kolářová, 2000; Horák *et al.*, 2009). We have, therefore, performed an extensive survey of the occurrence of different species of

Table 4. Abiotic parameters recorded during summertime (May–September 2018–2020) in 18 lakes, with records of previous and current cercarial dermatitis clinical cases.

Lake	Location	Cercarial dermatitis recorded	pH geometric mean/GSD	Alkalinity mM Mean \pm SD	Nitrogen mg/l Mean \pm SD	Phosphor mg/l Mean \pm SD	Chlorophyll μ g/l Mean \pm SD	Conductivity mS/m Mean \pm SD
Arresø	Zealand	–	8.6 /1.01	2.7 \pm 0.2	2.3 \pm 0.1	0.09 \pm 0.0	65.4 \pm 13.4	58.98 \pm 0.8
Furesø	Zealand	+	8.6 /1.02	2.0 \pm 0.2	0.7 \pm 0.1	0.04 \pm 0.01	14.6 \pm 7.9	46.62 \pm 2.0
Bagsværd Sø	Zealand	–	7.7 /1.0	3.3 \pm 0.0	0.8 \pm 0.0	0.13 \pm 0.0	3.5 \pm 0.0	44 \pm 0.0
Tissø ^a	Zealand	–	8.4 /1.008	3.6 \pm 0.3	2.8 \pm 1.2	0.01 \pm 0.0	9.9 \pm 2.9	57.62 \pm 2.4
Søndersø-Z ^b	Zealand	+	8.2 /1.02	2.3 \pm 0.3	0.8 \pm 0.2	0.02 \pm 0.02	7.6 \pm 8.8	54.54 \pm 3.7
Haraldsted Sø ^a	Zealand	+	8.1 /1.005	3.9 \pm 0.3	1.8 \pm 1.0	0.03 \pm 0.02	6.2 \pm 4.0	53.24 \pm 5.7
Søndersø-L ^c	Lolland	+	8.5 /1.05	1.8 \pm 0.6	1.6 \pm 0.1	0.04 \pm 0.0	12.4 \pm 3.4	33.62 \pm 4.3
Kvie Sø	Jutland	+	6.9 /1.17	0.08 \pm 0.02	1.8 \pm 0.4	0.15 \pm 0.08	65.7 \pm 76.7	10.68 \pm 0.7
Egå engsø	Jutland	–	8.7 /1.08	2.1 \pm 0.6	1.2 \pm 0.2	0.09 \pm 0.05	26.8 \pm 26.8	52.9 \pm 16.7
Esrum Sø	Zealand	+	8.6 /1.02	2.2 \pm 0.1	0.5 \pm 0.1	0.06 \pm 0.02	5.4 \pm 3.0	35.72 \pm 1.5
Sjælsø	Zealand	–	8.0 /1.02	3.0 \pm 0.4	0.7 \pm 0.05	0.08 \pm 0.04	7.0 \pm 3.9	44 \pm 11.2
Sortedam Sø	Zealand	–	10.0 /1.03	1.5 \pm 0.04	1.0 \pm 0.3	0.14 \pm 0.02	14.7 \pm 8.6	80.58 \pm 7.6
BrommeLille Sø	Zealand	–	7.8 /1.02	3.0 \pm 0.05	1.4 \pm 0.1	0.04 \pm 0.0	24.2 \pm 7.8	39.24 \pm 3.9
Slivsø	Jutland	–	8.6 /1.04	2.8 \pm 0.1	1.1 \pm 0.4	0.15 \pm 0.11	79.0 \pm 88.7	46.7 \pm 2.1
Søndersø-J	Jutland	–	8.9 /1.03	1.8 \pm 0.05	1.3 \pm 0.6	0.18 \pm 0.12	66.0 \pm 47.7	38.44 \pm 2.2
Tange Sø	Jutland	+	7.0 /1.02	0.9 \pm 0.1	1.2 \pm 0.2	0.08 \pm 0.04	9.8 \pm 11.5	25.34 \pm 1.6
Arreskov Sø	Funen	–	8.2 /1.005	2.9 \pm 0.08	1.0 \pm 0.1	0.05 \pm 0.03	10.5 \pm 3.3	48.96 \pm 3.5
Langesø	Funen	–	8.6 /1.04	3.1 \pm 0.01	1.7 \pm 0.8	0.16 \pm 0.15	33.6 \pm 34.2	45.66 \pm 3.1

The first nine lakes listed contained *Trichobilharzia* spp.-positive snails revealed by cercarial shedding, and the subsequent nine lakes did not contain snails with *Trichobilharzia* spp.-positive snails.

^aLarsen *et al.* (2004); ^bChristiansen *et al.* (2016); ^cunpublished data (Jørgensen & Buchmann). GSD, geometric standard deviation; SD, standard deviation.

Trichobilharzia in freshwater lakes. The low prevalence corresponds well with several extensive studies from other freshwater bodies in Europe (Kolářová *et al.*, 1992; Loy & Haas, 2001; Ferte *et al.*, 2005; Skírnisson *et al.*, 2009) and the infection level previously found in Danish lakes (Larsen *et al.*, 2004). However, since the snails were sampled in different seasons (from spring until autumn) (fig. 2), and infection is highly temperature-dependent, precaution is required with respect to interpretation of data as the infection level may vary considerably over a season (Loy & Haas, 2001; Zbikowska & Nowak, 2009; Brown *et al.*, 2011; Al-Jubury *et al.*, 2020). The species identified were *T. franki*, *T. szidati*, *T. regenti* and *T. anseri*. The first three species have been previously reported from several countries in Europe, including Denmark (Horák *et al.*, 2009, 2015; Cichy *et al.*, 2011; Soldánová *et al.*, 2013; Christiansen *et al.*, 2016; De Liberato *et al.*, 2019). However, this is the first report on the identification of *T. anseri* from Danish lakes, which links this work to a few previous findings in Europe (Jouet *et al.*, 2015).

We showed that three schistosome species may use the same snail host, *R. balthica*, corresponding to previous reports showing this species as the most important snail host in the life cycle of avian schistosomes (Jouet *et al.*, 2008, 2010, 2015; Skírnisson & Kolářová, 2008). In the present work, *T. szidati* appeared only in *L. stagnalis*, supporting previous Danish reports (Al-Jubury *et al.*, 2020). However, *T. szidati* and *T. regenti* may infect and,

under certain circumstances, establish in other related snail species, at least under experimental settings (Kock, 2001; Korsunen *et al.*, 2010; Hunova *et al.*, 2012). This suggests that expanded field investigations may be able to expand the list of intermediate hosts for this species. Further systematic analyses of the snail genus *Radix*, including *R. balthica*, would also enlighten the problem of some cryptic species possibly covered under the same name. A molecular approach would be suitable in this regard.

It is noteworthy that several cercarial dermatitis cases have been reported from the lake Esrum Sø (Tracz *et al.*, 2019). Although in this particular lake we sampled the highest number of snails from one location, no *Trichobilharzia*-positive snails were detected. This may be explained by the fact that Esrum Sø is the second largest lake in Denmark, whereby the precise habitat with infected snails may be difficult to locate. It may also illustrate that snail sampling is not always sufficient for mapping the occurrence of the parasite.

We selected a number of freshwater lakes for our investigations, seeking to cover the main areas of Denmark (Zealand, Lolland, Funen and Jutland). *Trichobilharzia regenti* was distributed only in Zealand, while *T. szidati*, *T. franki* and *T. anseri* were noted in lakes also from Jutland and Zealand. However, as mentioned above, this does not exclude that all species may occur nationwide. In some cases, only a low number of snails were collected at some locations (Almind Sø, Julsø, Vedsted Sø,

Table 5. Correlation between physicochemical parameters and occurrence of parasite infection (snails and human cases). *r* and *P* indicate Spearman correlation factors and *P*-values obtained, respectively.

	pH		Alkalinity		Nitrogen		Phosphorous		Chlorophyll		Conductivity	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Prevalence (%) infected snails	-0.174	0.49	0.187	0.459	0.471	0.049	-0.526	0.025	-0.193	0.443	0.328	0.184
No. of cercarial dermatitis cases	-0.160	0.526	-0.363	0.139	-0.261	0.295	-0.465	0.052	-0.376	0.124	-0.323	0.191

Probability level applied: $P < 0.01$.

Svogerslev Sø and Gyrsting Sø) due to limited access to private property, physical inaccessibility, elevated water levels and inferior weather conditions (Duan *et al.*, 2021).

In Europe, a number of waterbird species, including mallard and mute swan, serve as definitive hosts and have been reported to host both visceral and nasal species of *Trichobilharzia* (Horák *et al.*, 2002; Rudolfová *et al.*, 2002; Skírnisson & Kolářová, 2008; Soldánová *et al.*, 2013). This study identified two final hosts of bird schistosomes. We observed, by light microscopic examination, crescentic eggs in three *A. platyrhynchos* (two in stool and one in nasal mucosa), giving a prevalence of 33%. This finding corresponds to previous records in wild freshwater birds from Poland and Czechia, with prevalences of 29% and 23%, respectively (Rudolfová *et al.*, 2007). In addition, Skírnisson & Kolářová (2008) reported 35.5% prevalence of avian schistosomes in anseriform birds in Iceland. Two schistosome species were found in birds in our study. Their identification was based on sequencing of the ITS region, which showed that eggs recovered from the stool were *T. szidati*, while the parasite from the nasal region was *T. regenti*. This connects the final hosts for *T. regenti* and *T. szidati* to infections of snails in the Danish freshwater bodies.

The presence of intermediate snail hosts and final bird hosts is the main biotic factor crucial for the life cycle of the parasites, and in all lakes examined both types of hosts were observed. However, snails and released infective cercariae are directly exposed to and may be affected by environmental biotic and other abiotic factors (Pietroock & Marcogliese, 1992; Al-Jubury *et al.*, 2020). Temperature was clearly associated with infection, as documented previously (Al-Jubury *et al.*, 2020). However, we also included data on other physicochemical parameters and evaluated any connection between the occurrence of infective parasites and pH, alkalinity, nitrogen, phosphorus, chlorophyll and conductivity. A low pH was suggested to affect the presence of host snails (Hunter, 1988, 1990; Okland, 2007), whereas Cañete *et al.* (2004) claimed that pH is rarely a factor affecting the distributions of aquatic snails. The pH range (6.9–10) presented in this study did not seem to affect the occurrence of snails. The values recorded in this study correspond to values presented by other reports (Ntonifor & Ajayi, 2007; Njoku-Tony, 2011; Usman *et al.*, 2017). Another factor analysed for an effect on the development and existence of snail infection was chlorophyll. The mean concentration recorded in the summer period from May to September was between 3.5 and 67 µg/l, which complies with the finding that snails and cases of cercarial dermatitis can occur under 5 µg/l (Van Donk *et al.*, 1989), but otherwise no clear association was noted. This study showed that snails could live and release cercariae in water lakes with a conductivity of 10–80 mS/m, which supplements the range of 56–344 mS/m reported by Dida *et al.* (2014). The contents of nitrogen and phosphorus, in this and other studies, did not impact snail distribution in the water bodies (Krist *et al.*, 2014; Hill & Griffiths, 2017). We cannot exclude that some species of snails, as well as parasites, have different tolerance levels for abiotic factors, but the physicochemical parameters investigated in this study did not exclude snail and parasite occurrence. The distribution of freshwater snails may be a result of more complex interactions of different factors (Utzing *et al.*, 2017), and with regard to the occurrence of parasites, it was clear that the presence of intermediate snail hosts and the final hosts determine the infection risk. As long as the abiotic conditions allow the presence of snails and birds, there is a risk for bird schistosome infections. With climate change, there is a risk of an increased occurrence of the parasites because of temperature elevation, which leads to increased shedding of

cercariae in water bodies (Al-Jubury et al., 2020). In addition, cercariae may be predated upon by aquatic invertebrates such as copepods (Courmes et al., 1964; Holliman & Mecham, 1971; Bulaev, 1982). This was supported by recent studies showing that ambient communities can lower disease risk for hosts, via predation on free-living stages of parasites (predation effect). This suggests that a higher biodiversity may influence the occurrence of infective cercariae in water bodies (Goedknecht et al., 2015; Al-Jubury et al., 2020). Future studies should, therefore, focus on the importance of predatory plankton organisms and their influence on infection risk.

In conclusion, recreational freshwater lakes in Denmark are known to have highly favourable natural conditions for avian schistosomes. We documented the occurrence of at least four different avian schistosome species in Danish freshwater lakes. Cercariae belonging to the species *T. franki*, *T. szidati*, *T. regenti* and *T. anseri* were detected from east to west in Danish lakes commonly used for recreational activities such as bathing, swimming and fishing. The wide distribution is of public health concern due to the skin pathologies that may accompany bathing in infested water. It cannot be excluded that at least some *Trichobilharzia* species may perform extra-dermal migrations in the human host. Particularly noteworthy in this context is the finding of the neurotropic species, *T. regenti*. The parasite occurrence was associated with presence of final bird hosts (mallards and swans) and first intermediate lymnaeid host snails, but a nationwide bird survey is recommended to elucidate the role of other avian host species. The measurement of the physico-chemical parameters under natural conditions of the Danish freshwater lakes documented an association between temperature and infections, whereas other abiotic parameters were of low or no importance. It was clear that the presence of intermediate snail hosts and the final hosts determines the infection risk. As long as the abiotic conditions allow the presence of snails and birds, there is a risk for bird schistosome infections. The possible neurotropic pathology of *T. regenti* and other avian schistosomes in humans (Lichtenbergová & Horák, 2012) calls for further controlled studies on the fate of cercariae in the human host. Routine surveillance of lakes used for recreational activities should be implemented and sensitive and species-specific molecular methods for detection of infective agents should be developed (Bass et al., 2015; Sengupta et al., 1970).

Supplementary material. To view supplementary material for this article, please visit <https://doi.org/10.1017/S0022149X21000122>

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