

Aus dem Fachbereich Wildtierkrankheiten
des Leibniz Instituts für Zoo- und Wildtierforschung, Forschungsverbund Berlin e.V.
eingereicht beim Fachbereich Veterinärmedizin
der Freien Universität Berlin

PARASITES AND IMMUNITY IN ANTHROPOGENICALLY IMPACTED WATERFOWL POPULATIONS

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der
Freien Universität Berlin

vorgelegt von
Hanna Prüter
Tierärztin aus Lüneburg

Berlin 2019
Journal-Nr.: 4116

**Aus dem Fachbereich Wildtierkrankheiten
des Leibniz Instituts für Zoo- und Wildtierforschung, Forschungsverbund Berlin e.V.
eingereicht beim Fachbereich Veterinärmedizin
der Freien Universität Berlin**

**PARASITES AND IMMUNITY IN
ANTHROPOGENICALLY IMPACTED
WATERFOWL POPULATIONS**

**Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der
Freien Universität Berlin**

**vorgelegt von
Hanna Prüter**

**Tierärztin
aus Lüneburg**

Berlin 2019

Journal-Nr.: 4116

Gedruckt mit Genehmigung
des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Dekan: Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter: Prof. Dr. Alex Greenwood
Zweiter Gutachter: Univ.-Prof. Dr. Georg von Samson-Himmelstjerna
Dritte Gutachterin: Univ.-Prof. Dr. Susanne Hartmann

Deskriptoren (nach CAB-Thesaurus): *waterfowl, parasites, immune system, prevalence, biodiversity, lead, helminths, Trichobilharzia regenti, Bilharziella polonica, Riemerella anatipestifer, epidemiology*

Tag der Promotion: 25.06.2019

This dissertation was done in the Leibniz-Institute for Zoo and Wildlife Research in Berlin during the period 01/06/2014 – 01/05/2018 under the supervision of Dr. Gábor Árpád Czirják, Dr. Oliver Krone and Prof. Dr. Alex D. Greenwood and it is submitted to the Department Veterinary Medicine of Freie Universität Berlin.

CONTENT

GENERAL INTRODUCTION	7
Thesis outline	9
Key concepts	10
Life history theory and resource tradeoffs.....	10
Environmental lead pollution and its impact on host-parasite interactions (in birds).....	11
Invasive vertebrate species and their health-related aspects relevant during invasion.....	13
Avian helminth infections affecting humans– bird schistosomes as an example.....	14
Study species	15
Mallard (<i>Anas platyrhynchos</i>)	15
Egyptian goose (<i>Alopochen aegyptiacus</i>)	16
References	19
CHAPTER 1	
Chronic lead intoxication decreases intestinal helminth species richness and infection intensity in mallards (<i>Anas platyrhynchos</i>).....	27
CHAPTER 2	
The immunological plasticity hypothesis explains contradictory immunity patterns in invasive species	39
CHAPTER 3	
Having bird schistosomes in mind—the first detection of <i>Bilharziella polonica</i> (Kowalewski 1895) in the bird neural system.....	67
CHAPTER 4	
Sane and sound - A serologic and molecular survey for selected infectious agents in neozootic Egyptian geese (<i>Alopochen aegyptiacus</i>) in Germany	75
GENERAL DISCUSSION	85
Impact of anthropogenic changes to parasite and host community structures	85
Potential threats for native species, poultry and humans.....	87
Outlook.....	88
References	90
ZUSAMMENFASSUNG	94
SUMMARY	96
PUBLIKATIONSLISTE	98
ACKNOWLEDGEMENT	99

GENERAL INTRODUCTION

Central European freshwater habitats, which provide breeding, roosting and molting habitats for different waterfowl species, are highly anthropogenically impacted ecosystems. Among others, human impact on freshwater habitats and its biodiversity include eutrophication, restructuring, environmental pollution and introduction of non-native species (Meybeck 2003; Framing Committee of the Global Water System Project 2004; Strayer 2010; Vörösmarty et al. 2010). Particularly species biodiversity and quality of freshwater habitats are threatened by environmental pollutants originating from industry, agriculture and hunting. Additionally, native species biodiversity, community structures and ecosystem functioning may be threatened by the introduction of non-native species (Dudgeon et al. 2006; Carpenter et al. 2011).

Both environmental pollution and vertebrate invasion can interact with health indices of native populations such as parasite biodiversity, exposure to pollutants, parasite transmission and immunity. Environmental habitat pollution can result in acute or chronic intoxication of vertebrates. Such intoxication can result in a decrease of vertebrate immune competences, which subsequently facilitates disease outbreaks and parasite transmission (Franson 1986; Vallverdú-Coll et al. 2015b, a, 2016). Additionally, biodiversity loss may increase the transmission rate and risk of infectious diseases (Keesing et al. 2010). Moreover, parasite transmission originating from invasive species can be of high risk for the health of native species (Callaway and Ridenour 2004; Crowl et al. 2008; Pyšek and Richardson 2010). Thus, studying the epidemiological roles of native and invasive waterfowl species is needed to broaden the understanding of anthropogenic impact on health indices of specific species and species communities in freshwater habitats.

Out of the complex interplay of anthropogenic impact on freshwater ecosystems and health indices of freshwater species, parasite indices and immunity of two waterfowl species of the family Anatidae (waterfowl: ducks, geese and swans) were investigated. These species were chosen as they are characteristic species of freshwater habitats. Thus, an interplay between anthropogenic impacted fresh water habitat conditions and health indices such as parasite-host interactions, prevalence and abundance of parasites/infectious agents, immunity and disease transmission of Anatids (Figure 1) can be expected.

Out of the family Anatidae, the mallard (*Anas platyrhynchos*) as the most common native breeding duck and the most heavily hunted game bird in Germany was selected as study species (Gedeon et al. 2015; Deutscher Jagdverband e.V. 2018). Mallards have been shown to be affected by environmental pollution, predominantly caused by lead leading to both acute and chronic lead intoxication (Birkhead and Perrins 1985; Guitart et al. 1994; Mateo et al. 1997, 1998; Kelly and Kelly 2004; Degernes et al. 2006; Mateo 2009; Binkowski et al. 2013; Ferreyra et al. 2014, 2015). Moreover, mallards occur in all types of freshwater habitats including those of recreational use and are final hosts for bird schistosomes, whose cercaria might cause swimmer’s itch in humans (Cort 1928).

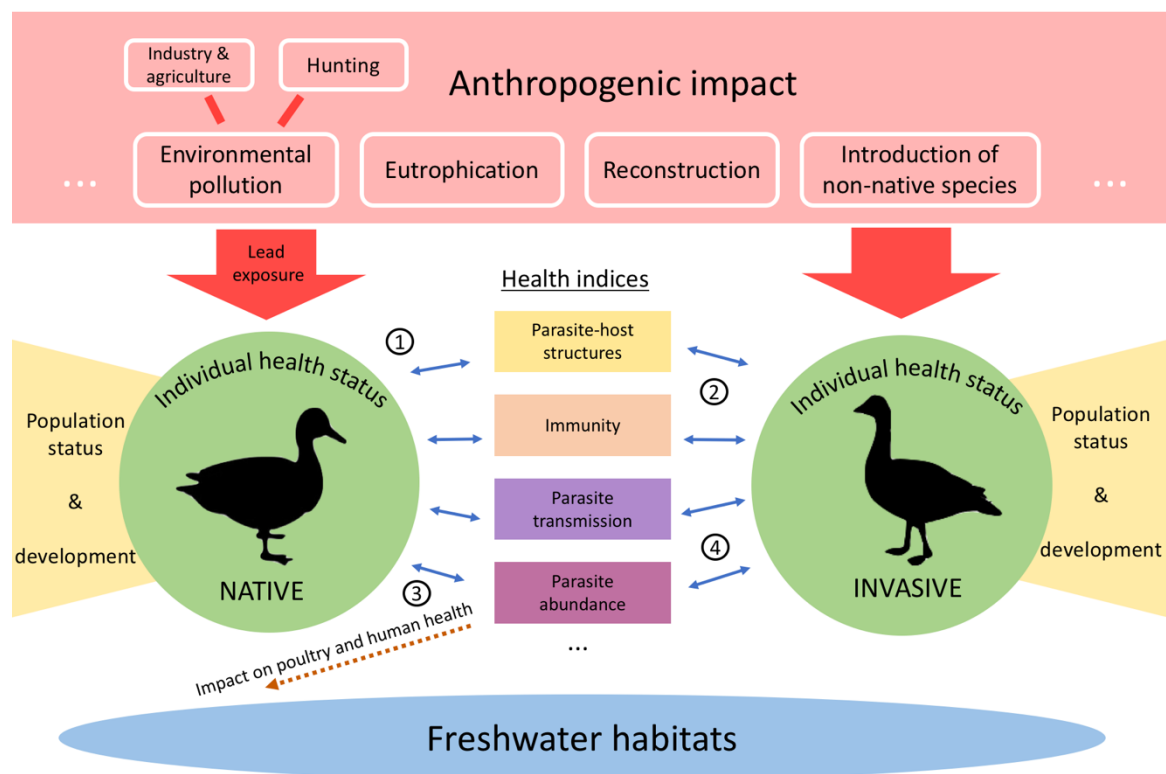


Figure 1: Schematic illustration of the interplay between anthropogenic impact and health indices of the two study species in freshwater habitats. Approaches of the thesis are highlighted with numbers of the different chapters (1-4).

Additionally, several species of the family Anatidae were introduced to non-native regions and successfully established as invasive populations (Duncan et al. 2003). Several health-related hypotheses have been formulated to characterize the interaction of health-indices of non-native species with the probability to become invasive for anthropogenically introduced species (Blossey and Notzold 1995; Torchin et al. 2003; Callaway and Ridenour 2004; Lee and Klasing 2004). The Egyptian goose (*Alopochen aegyptiacus*) was chosen as second study species as one of the most successful invasive

goose species in Europe (Gedeon et al. 2015) to investigate potential health related mechanisms behind the successful invasion of this species as well as its epidemiological role in the infection cycles of pathogens, which could be of relevance for native species, livestock and humans.

This thesis aims to highlight the impact of anthropogenic actions on health indices of waterfowl species from freshwater habitats. It evaluates the impact of environmental lead pollution and the introduction of non-native species on health indices of the study species in freshwater habitats. This research has the potential to broaden the general understanding of the complex interplay between humans and wildlife and consequently could be of relevance for nature conservation.

Thesis outline

The main body of this thesis consists of four chapters addressing different subjects related to health indices of both study species, which are either impacted by anthropogenic changes or may impact human and poultry health (Figure 1).

To study the **impact of anthropogenic changes to parasite and host community structures** in freshwater ecosystems, two main approaches were selected. First, to address the long-term effect of environmental lead pollution on parasite biodiversity in waterfowl, the chronic effect of lead burden on intestinal helminthes species richness and infection intensity in mallards were investigated (chapter 1). Second, health-related mechanisms, which might function as underlying mechanisms in the successful invasion of the Egyptian goose, were studied to test and potentially improve existing hypotheses in the field of animal invasion using the Egyptian goose as a model species. Therefore, parasite prevalence and variance of immune marker of Egyptian geese from Germany were compared to those of native Egyptian geese from Namibia to investigate potential differences in the light of the successful invasion of Europe by this species (chapter 2).

To study **potential threats for native species, poultry and humans, originating from waterfowl**, two additional approaches were selected. First, to gain knowledge on the prevalence and abundance of potentially zoonotic helminthic infections, neural bird schistosome infections in mallards, whose cercaria might cause swimmer's itch in humans, were studied. Thus, prevalence and abundance of neural bird schistosomes were investigated to study which species of neural bird schistosomes occur at which prevalence in mallards from German fresh waters (chapter 3). Second, parasites and selected

infectious agents of Egyptian geese in Germany were investigated to gain knowledge on their epidemiological role in transmission cycles of diseases relevant for native species and poultry. Additionally, the prevalence of these selected infectious agents was compared to published data from native geese species, to discuss them in the frame of contrasting ecology of the different geese species (chapter 2 and 4).

Key concepts

Key concepts, which are relevant to the studies presented in chapter 1-4, will be introduced in the following.

Life history theory and resource tradeoffs

Environmental factors, available resources, predators and parasites are important factors determining population growth rate and success of both native and invasive populations (Shea and Chesson 2002). Generally, resources are limited and must be allocated between different life history traits or stages, such as individual growth, dispersion, reproduction and immunity (Sheldon and Verhulst 1996; van der Most et al. 2011). Parasites, which compete for resources with the host, are energetically costly as they cause tissue damage or deprive the host from resources and induce immune reaction. Hosts with a high parasite burden will shift energetic resources into immunity. Thus, parasitic infection may decrease energetic investment of the host into other life history traits and therefore impact hosts population dynamics (Schmid-Hempel 2011).

Moreover, Atkinson, Thomas, and Hunter (2008) state that parasitism and its impact on the hosts should generally be considered in the context it occurs, including the impact of anthropogenic changes to the environment. According to the authors, wild birds are equipped with a suite of evolved traits, which enable them to successfully survive, reproduce and stay healthy under given environmental conditions. However, anthropogenic changes, which affect wild bird populations, may lead to an increased need for other trades, which might not have been developed and therefore are not available. Following this argumentation, Schlaepfer, Runge, and Sherman (2002) state that in changed habitat conditions (e.g. caused by anthropogenic disturbance, altered environments) adaptations to the natural environment can function as “evolutionary

traps”, meaning that naturally evolved trades may no longer be optimal but rather unfavorable for the hosts.

In summary, anthropogenic changes to the environment can be energetically costly for the host. Particularly, environmental pollutants can induce tissue damage and thus resources must be used for reparation directly. Moreover, pollutants may indirectly affect vertebrate hosts by altering parasite community structures and immune defenses of the host or directly affect parasite health (Rocke and Samuel 1991; Grasman and Scanlon 1995; Grasman 2002; Sures 2004; Sures et al. 2017). In addition, species invasion can impact resource tradeoffs as it may result in relatively lower parasitic burden (Enemy release, see below) for the invasive species. Consequently, resources can be allocated from immunity into reproduction and growth. Allocating resources from immunity to other physiological processes, such as reproduction, has been suggested to increase invasion success (Lee and Klasing 2004). Thus, studying parasite-host interactions in the frame of environmental pollution and species invasion allows to investigate resource tradeoffs induced by anthropogenic changes and threats to biological systems, which have been shown to be high in freshwater habitats (Vörösmarty et al. 2010).

Environmental lead pollution and its impact on host-parasite interactions (in birds)

Environmental pollution originating from industry, agriculture and hunting build up in freshwater habitats (Carpenter et al. 2011). Generally, high stability and persistence of pollutants can lead to severe contamination of both sediment and water bodies. Contamination entering food chains can be of risk for livestock and human health. Moreover, wildlife health including the health of plants, invertebrates and vertebrates, habitat quality and vertebrate biodiversity are threatened by environmental pollutants (Acevedo-Whitehouse and Duffus 2009).

One of the most prominent pollutants can be found among heavy metals. Especially, lead is a highly toxic heavy metal that is known to be toxic to living organisms including humans and animals, primary affecting the central nervous system, renal and hepatic function as well as the hematopoietic system. Lead is non-biodegradable and cannot be physiologically excreted from the body. In vertebrates, lead gets mainly stored in bones, which serve as long-time accumulation matrix (Beyer and Meador 2011; Flora et al. 2012).

Beside the negative impact of lead on human health also wildlife health is threatened by lead accumulating in the environment (Armeno et al. 2016). Lead pollution of freshwater habitats originates from different anthropogenic sources such as mining, fishing using lead fishing weights and hunting. Lead based shot gun pellets used for waterfowl hunting form the main source of environmental lead pollution (Birkhead and Perrins 1985; Sears 1988; Scheuhammer and Norris 1995; Mateo et al. 1997, 1998; Meharg et al. 2002; Gómez-Ramírez et al. 2011; Newth et al. 2013; Ferreyra et al. 2014).

Environmental lead pollution leading to intoxications may impact host-parasite interactions by either directly affecting the parasites or the immune system and resource balance of its vertebrate hosts. Depending on the lead dosage, acute (lethal) or chronic (sublethal) symptoms can manifest in vertebrate hosts. In vertebrates, acute intoxication may result in severe clinical symptoms that include central nervous, renal and hepatic dysfunction directly leading to death (Sears 1988; Degernes et al. 2006; Flora et al. 2012; Newth et al. 2013), and thus not directly affecting host-parasite interactions. In contrast, sublethal dosages or chronic intake lead to sublethal effects of lead (Martinez-Haro et al. 2011; Vallverdú-Coll et al. 2016) with important fitness consequences for the vertebrate host. For instance decreased reproductive success and immunosuppression were found in vertebrates affected by chronic lead intoxication (Franson 1986; Trust et al. 1990; Rocke and Samuel 1991; Grasman and Scanlon 1995; Youssef et al. 1996; Singh et al. 2003; Eeva et al. 2005; Pikula et al. 2010; Vallverdú-Coll et al. 2015a, 2016).

Parasites can benefit from weakened immune function leading to an increase of parasite load in vertebrates (Franson 1986). However, parasites might also be directly poisoned by lead in the intestines of the hosts. Intestinal parasites of ducks are often exposed to lead as oral intake of hunting pellets is the main route of lead intoxication in these birds (Mateo et al. 1997; Ferreyra et al. 2014). Moreover, parasite related immune defenses might be enhanced by lead intoxication (Degen et al. 2005; Eeva et al. 2005; Gao et al. 2007; Cizauskas et al. 2014; Vallverdú-Coll et al. 2015b; Bertellotti et al. 2016). Additionally, intestinal helminths were found to accumulate lead in higher dosages than their vertebrate hosts. Thus, intestinal parasites may function as lead sink and reduce lead levels in vertebrate hosts (Sures et al. 2017).

In summary, the impact of anthropogenic induced environmental lead pollution on host-parasite interactions is highly complex. Here, this topic is addressed by studying chronic lead intoxication in mallards and its association with intestinal helminth parasite species richness and infection intensity (**chapter 1**).

Invasive vertebrate species and their health-related aspects relevant during invasion

Invasive species are major threats to native species biodiversity and health (Lövei 1997; McGeoch et al. 2010). With increasing globalisation leading to global biodiversity loss and climate change resulting in habitat changes, studying the mechanisms that promote species invasion is important for risk assessment, conservation and public health (Pyšek and Richardson 2010; Chown et al. 2015). Underlying mechanisms can be anthropogenically induced (such as for instance: number of introduced individuals) or arise from the traits of the introduced species, including its physiology, behaviour or pathogens.

Several health-related hypotheses have been formulated to study and understand species invasion and its impact on native species. The “Novel Weapon” hypothesis states that neozootic species bring novel pathogens to the novel range which harm native species (Callaway and Ridenour 2004). Beside this direct transmission of parasites from invasive to native species, parasites, infectious agents and host immune function of invaders have been considered to play important mechanistic roles during the process of vertebrate invasion. For instance the loss of parasites during invasion (“Enemy Release Hypothesis”) and the ability to shift energetic resources away from immunity into reproduction and growth (“Evolution of Increased Competitive Ability”) enable non-native species to successfully invade new areas (Blossey and Notzold 1995; Torchin et al. 2003; Lee and Klasing 2004).

However, support for the above-mentioned hypotheses are non-consistent among studies of invasive vertebrates, which might be caused by the complexity of the vertebrate immune system as well as parasite-host interactions. Thus, studying parasites, pathogens and immunity of native and invasive Egyptian goose populations can provide new insights into the mechanisms of anthropogenic induced vertebrate invasion. In **chapter 2**, the Egyptian goose was used as a model species for vertebrate invasion and it was aimed to study the above mentioned major hypotheses in the field of invasion biology by investigating immunity and parasites of both a native and an invasive population. Moreover, it was aimed to combine existing hypotheses with the role of newly acquired parasites in the invasive range. Furthermore, in **chapter 4**, antibody seroprevalence against selected viruses and prevalence of selected bacteria in the invasive population of

Egyptian geese were studied to evaluate the role of this species in the transmission and maintenance of pathogens relevant for wild birds and poultry.

Avian helminth infections affecting humans– bird schistosomes as an example

Zoonotic diseases originating from waterfowl can be of risk for human health. Predominantly viral and bacterial infectious agents have been demonstrated to be transmitted from waterfowl to humans sometimes inducing severe clinical symptoms (Peiris et al. 2007; Benskin et al. 2009). One of the most intensively studied viral disease of this kind is the avian influenza virus, where waterfowl species function as reservoir hosts. These viruses can also be transmitted to humans and can result in severe symptoms in patients and occasionally death (Peiris et al. 2007).

Also avian helminth parasites can impact human health. Especially trematodes from the Schistosomatidae family, including bird schistosomes can cause diseases in humans (Kolářová et al. 2010; Soldánová et al. 2013). Human schistosomes of the genus *Schistosoma* (e.g. *Schistosoma mansoni*, *Schistosoma haematobium*) are agents of schistosomiasis, affecting humans in the subtropics and tropics. However, also in Europe, bird schistosomes of the genus *Trichobilharzia*, which cause trichobilharziosis in various bird species – especially species of the family Anatidae – with pathogenesis comparable to human schistosomiasis (Wojcinski et al. 1987), can affect human health. Cercaria of bird schistosomes may induce allergic dermatitis in humans (“swimmers itch”), which occurs after repeated cercaria penetration of the skin of sensitized individuals (Cort 1928). Humans engaged in recreational water activities (e.g. swimming, etc.) are most likely to be exposed to these cercariae (Chamot et al. 1998). “Swimmers itch” occurs worldwide with the exception of Antarctica (Horák et al. 2002) and is regarded as a re-emerging disease in Europe (Horák and Kolářová 2011; Soldánová et al. 2013).

Due to their aquatic habitat, waterfowl of the family Anatidae are most likely the main reservoir species of bird schistosomes. However, only a few studies have examined the prevalence of neural bird schistosomes in waterfowl from Europe (Rudolfová et al. 2002, 2007; Jouet et al. 2008) and so far, *T. regenti* was the only neural bird schistosome species described in European birds. In **chapter 3** neural bird schistosome species prevalence in mallards from German freshwaters was studied.

Study species

Among vertebrates, water birds of the family Anatidae (ducks, geese and swans) are characteristic species of freshwater habitats, which have been shown to be affected by habitat pollution caused by factors such as lead intoxication (Birkhead and Perrins 1985; Guitart et al. 1994; Mateo et al. 1997, 1998; Kelly and Kelly 2004; Degernes et al. 2006; Mateo 2009; Binkowski et al. 2013; Ferreyra et al. 2014, 2015). Additionally, birds of the family Anatidae form one of the six major groups of invasive birds and were mainly introduced for hunting and exhibition purpose (Duncan et al. 2003).

Out of the family Anatidae, the mallard (*Anas platyrhynchos*) as the most common native breeding duck species and the Egyptian goose (*Alopochen aegyptiacus*) as one of the most successful invasive goose species in Europe (Gedeon et al. 2015) were chosen as study species. In Germany, both species share breeding, roosting and molting habitats. Additionally, both can be considered as synanthropic species, which often occur in anthropogenic impacted habitats in Germany.

Mallard (*Anas platyrhynchos*)

The mallard is the most common breeding duck species in Europe (Gedeon et al. 2015) and almost all races of the domestic duck are descended from the mallard (Herre and Röhrs 1990). Wild mallards are highly flexible in breeding habitat choice, which includes almost all freshwater water habitat types in Germany including highly anthropogenic impacted environments. The long-term population trend of breeding mallards in Germany is considered to be stable although it fluctuated in recent decades (Gedeon et al. 2015). European mallards are partial migratory. Recoveries of ringed individuals show that the autumn and winter population is composed of both German breeding birds and animals from mainly North-east and Eastern Europe (Bairlein, Dierschke, and Dierschke 2014).



Figure 2: Male (back) and female (front) mallard (Picture: Susanne Auls)

Mallard populations are impacted by hunting and its indirect effects of environmental lead pollution. The mallard is the most heavily hunted game duck in Germany (Deutscher Jagdverband e.V. 2018) and a general intake of lead from the environment in mallards leading to chronic lead intoxication is likely. Intake from the environment can occur via contamination of the sediments with old ammunition and other anthropogenic lead pollution of aquatic habitats. Furthermore, the mallard is a particularly suitable model species to study lead effects as the species can be immunosuppressed by chronic lead intake (Vallverdú-Coll et al. 2016). Moreover, mallards harbor a variety of parasitic helminth species belonging to all major helminth classes including bird schistosomes (Boch and Schneidawind 1988).

Egyptian goose (*Alopochen aegyptiacus*)

European neozootic Egyptian geese escaped from captivity in the late 19th century and became established as breeding population since the 1970s. Over the last decades, Egyptian geese in central Europe show rapid population growth (Wahl et al. 2011). In Germany, the population increased more than 30-fold over the last 15 years from 250-300 breeding pairs in 1999 to at least 8,000 pairs in 2013 (Arnold et al. 2013; Gedeon et al. 2015). Thus, among birds, the Egyptian goose is one of the most successful neozootic species to Europe (Bauer and Woog 2008). Moreover, in 2017 the Egyptian goose

became listed as invasive species in Germany according to “EU Regulation No 1143/2014 on the prevention and management of the introduction and spread of invasive alien species”. This EU regulation “sets out rules to prevent, minimize and mitigate the adverse impact on biodiversity of the introduction and spread ... of invasive alien species” (Article 1) including the need for a scientific risk assessment (Nehring and Skowronek 2017).



Figure 3: Male (right) and female (left) Egyptian goose (Picture Manuela Merling de Chapa)

European Egyptian geese occur in a variety of freshwater habitats (Dietzen et al. 2015), often come into close contact with humans and frequently use anthropogenic structures (e.g. buildings, bridges, roofs) for nesting. Additionally, they often share their habitats with mallards, which they usually dominate by aggressive behavior (personal observations). Especially the Rhine and Mosel area (50.4°N, 7.6°E), which was chosen for field work, is a favored habitat in Germany. Here, Egyptian geese often use tutored lawns (e.g. turfs, parks) for grazing, where they are frequently fed with bread by humans. Those birds have rather low fear of humans and lawns are often riddled with their feces (personal observations). Thus, if Egyptian geese carry zoonotic infectious agents, pathogen transmission to humans is quite possible.

In their native range in Africa, Egyptian geese are hosts of parasite species from all major parasitic classes (trematodes, cestodes, nematodes, acanthocephalans) (Alexander and McLaughlin 1997). Additionally, they have been reported to carry avian

influenza A virus and avian avulavirus 3 (Shihmanter et al. 1998; Pfitzer et al. 2000; Thompson et al. 2008). Regarding avian influenza A virus, they were described as excellent sentinels having the highest total risk score compared to 15 other anatid species (regarding: range, abundance, mobility, shared roosts, mixed flocks, foraging and anthropogenic association) (Cumming et al. 2008; Abolnik et al. 2010). However, the epidemiological role of Egyptian geese in their invasive range in Europe is largely unknown.

Compared to its native range, the Egyptian goose faces fewer predators in novel European habitats (Gyimesi and Lensink 2010). Therefore, its population size can be expected to continuously increase in the next decades, raising the importance of gaining knowledge on the underlying mechanisms of their invasion success, their health status and on infectious agents and relevant pathogens they may carry.

References

- Abolnik C, Gerdes GH, Sinclair M, Ganzevoort BW, Kitching JP, Burger CE, Romito M, Dreyer M, Swanepoel S, Cumming GS, Olivier AJ (2010) Phylogenetic analysis of influenza A viruses (H6N8, H1N8, H4N2, H9N2, H10N7) isolated from wild birds, ducks and ostriches in South Africa from 2007 to 2009. *Avian Dis* 54:313–322. doi: 10.1637/8781-040109-Reg.1
- Acevedo-Whitehouse K, Duffus ALJ (2009) Effects of environmental change on wildlife health. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364:3429–3438. doi: 10.1098/rstb.2009.0128
- Alexander SJ, McLaughlin JD (1997) A checklist of helminths from the respiratory system and gastrointestinal tracts of African Anatidae. *Onderstepoort J Vet Res* 64:5–16
- Arnold JM, Greiser G, Kampmann S, Martin I (2013) Status und Entwicklung ausgewählter Wildtierarten in Deutschland. Jahresbericht 2013. Wildtier-Informationssystem der Länder Deutschlands (WILD) Deutscher Jagdverband; Berlin, pp 24
- Atkinson CT, Thomas NJ, Hunter DB (eds) (2008) Parasitic diseases of wild birds. Wiley-Blackwell, Ames, Iowa, pp 3
- Bairlein F, Dierschke J, Dierschke V, Salewski V, Geiter O, Hüppop K, Köppen U, Fiedler W (2014) Atlas des Vogelzugs: Ringfunde deutscher Brut- und Gastvögel, 1. Auflage 2014. Aula-Verlag, Wiebelsheim, pp 78
- Bauer H-G, Woog F (2008) Nichtheimische Vogelarten (Neozoen) in Deutschland, Teil I: Auftreten, Bestände und Status - Non-native and naturalized bird species (neozoa) in Germany, part I: occurrence, population size and status. *Vogelwarte* 46, 2008: 157 – 194
- Benskin CMH, Wilson K, Jones K, Hartley IR (2009) Bacterial pathogens in wild birds: a review of the frequency and effects of infection. *Biological Reviews* 84:349–373. doi: 10.1111/j.1469-185X.2008.00076.x
- Bertellotti M, D’Amico VL, Palacios MG, Barbosa A, Coria N (2016) Effects of antihelminthic treatment on cell-mediated immunity in Gentoo penguin chicks. *Polar Biology* 39:1207–1212. doi: 10.1007/s00300-015-1839-0
- Beyer WN, Meador JP (eds) (2011) Environmental contaminants in biota: interpreting tissue concentrations, 2nd ed. CRC Press, Boca Raton, FL, pp 574
- Binkowski ŁJ, Sawicka-Kapusta K, Szarek J, Strzyżewska E, Felsmann M (2013) Histopathology of liver and kidneys of wild living mallards *Anas platyrhynchos* and Coots *Fulica atra* with considerable concentrations of lead and cadmium. *Science of The Total Environment* 450-451:326–333. doi: 10.1016/j.scitotenv.2013.02.002
- Birkhead M, Perrins C (1985) The breeding biology of the mute swan *Cygnus olor* on the river Thames with special reference to lead poisoning. *Biological Conservation* 32:1–11. doi: 10.1016/0006-3207(85)90061-8
- Blossey B, Notzold R (1995) Evolution of Increased Competitive Ability in Invasive Nonindigenous Plants: A Hypothesis. *The Journal of Ecology* 83:887. doi: 10.2307/2261425

- Boch J, Schneidawind H (1988) *Krankheiten des jagdbaren Wildes: mit 19 Tab. Parey, Hamburg Berlin, pp 333*
- Callaway RM, Ridenour WM (2004) Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2:436–443. doi: 10.1890/1540-9295(2004)002[0436:NWISAT]2.0.CO;2
- Carpenter SR, Stanley EH, Vander Zanden MJ (2011) State of the World's Freshwater Ecosystems: Physical, Chemical, and Biological Changes. *Annual Review of Environment and Resources* 36:75–99. doi: 10.1146/annurev-environ-021810-094524
- Chamot E, Toscani L, Rougemont A (1998) Public health importance and risk factors for cercarial dermatitis associated with swimming in lake Lemán at Geneva, Switzerland. *Epidemiol Infect* 120:305–314
- Chown SL, Hodgins KA, Griffin PC, Oakeshott JG, Byrne M, Hoffmann AA (2015) Biological invasions, climate change and genomics. *Evolutionary Applications* 8:23–46. doi: 10.1111/eva.12234
- Cizauskas CA, Turner WC, Wagner B, Küstersrs M, Vance RE, Getz WM (2014) Gastrointestinal helminths may affect host susceptibility to anthrax through seasonal immune trade-offs. *BMC Ecology* 14.: doi: 10.1186/s12898-014-0027-3
- Cort WW (1928) Schistosome dermatitis in the United States (Michigan). *Journal of the American Medical Association* 90:1027–1029
- Crowl TA, Crist TO, Parmenter RR, Belovsky G, Lugo AE (2008) The spread of invasive species and infectious disease as drivers of ecosystem change. *Frontiers in Ecology and the Environment* 6:238–246. doi: 10.1890/070151
- Cumming GS, Hockey PAR, Bruinzeel LW, Du Plessis MA (2008) Wild bird movements and avian influenza risk mapping in Southern Africa. *Ecology and Society* 13 (2): 26
- Degen WGJ, Daal N van, Rothwell L, Kaiser P, Schijns VEJC (2005) Th1/Th2 polarization by viral and helminth infection in birds. *Veterinary Microbiology* 105:163–167. doi: 10.1016/j.vetmic.2004.12.001
- Degernes L, Heilman S, Trogdon M, Jordan M, Davison M, Kraege D, Correa M, Cowen P (2006) Epidemiologic investigation of lead poisoning in Trumpeter and Tundra swans in Washington State, USA, 2000–2002. *Journal of Wildlife Diseases* 42:345–358. doi: 10.7589/0090-3558-42.2.345
- Deutscher Jagdverband e.V. Vereinigung der deutschen Landesjagdverbände für den Schutz von Wild (2018) *DJV-Handbuch Jagd 2018. Jagd und Natur Bonn : DJV*
- Dietzen C, Dolich T, Grunwald T, Keller P, Kunz A (2015) *Die Vogelwelt von Rheinland-Pfalz. Band 2 Entenvögel bis Storchenvögel (Anseriformes-Ciconiformes). In: Gesellschaft für Ornithologie Rheinland-Pfalz. Landau*
- Dudgeon D, Arthington AH, Gessner MO, Kawabata Z-I, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard A-H, Soto D, Stiassny MLJ, Sullivan CA (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* 81:163. doi: 10.1017/S1464793105006950

- Duncan RP, Blackburn TM, Sol D (2003) The ecology of bird introductions. *Annual Review of Ecology, Evolution, and Systematics* 34:71–98. doi: 10.1146/annurev.ecolsys.34.011802.132353
- Eeva T, Hasselquist D, Langefors Å, Tummeleht L, Nikinmaa M, Ilmonen P (2005) Pollution related effects on immune function and stress in a free-living population of pied flycatcher *Ficedula hypoleuca*. *Journal of Avian Biology* 36:405–412. doi: 10.1111/j.0908-8857.2005.03449.x
- Ferreyra H, Beldomenico PM, Marchese K, Romano M, Caselli A, Correa AI, Uhart M (2015) Lead exposure affects health indices in free-ranging ducks in Argentina. *Ecotoxicology* 24:735–745. doi: 10.1007/s10646-015-1419-7
- Ferreyra H, Romano M, Beldomenico P, Caselli A, Correa A, Uhart M (2014) Lead gunshot pellet ingestion and tissue lead levels in wild ducks from Argentine hunting hotspots. *Ecotoxicology and Environmental Safety* 103:74–81. doi: 10.1016/j.ecoenv.2013.10.015
- Flora G, Gupta D, Tiwari A (2012) Toxicity of lead: A review with recent updates. *Interdiscip Toxicol* 5:47–58. doi: 10.2478/v10102-012-0009-2
- Framing Committee of the Global Water System Project (2004) Humans transforming the global water system. *Eos AGU Trans* 513–514
- Franson JC (1986) Immunosuppressive effects of lead. In: J.Scott Feierabend, A.Brooke Russell (eds) *Lead poisoning in wild waterfowl - A workshop*. National Wildlife Federation, Washington, DC, pp 106–109
- Gao D, Mondal TK, Lawrence DA (2007) Lead effects on development and function of bone marrow-derived dendritic cells promote Th2 immune responses. *Toxicology and Applied Pharmacology* 222:69–79. doi: 10.1016/j.taap.2007.04.001
- Gedeon K, Sudfeldt C, Dougalis P (eds) (2015) *Atlas Deutscher Brutvogelarten - Atlas of German breeding birds, neue Ausg.* Dachverband Deutscher Avifaunisten, Münster, Westf, pp 88
- Gómez-Ramírez P, Martínez-López E, María-Mojica P, León-Ortega M, García-Fernández AJ (2011) Blood lead levels and δ -ALAD inhibition in nestlings of Eurasian Eagle owl (*Bubo bubo*) to assess lead exposure associated to an abandoned mining area. *Ecotoxicology* 20:131–138. doi: 10.1007/s10646-010-0563-3
- Grasman KA (2002) Assessing immunological function in toxicological studies of avian wildlife. *Integrative and Comparative Biology* 42:34–42. doi: 10.1093/icb/42.1.34
- Grasman KA, Scanlon PF (1995) Effects of acute lead ingestion and diet on antibody and T-cell-mediated immunity in Japanese quail. *Arch Environ Contam Toxicol* 28:161–167
- Guitart R, To-Figueras J, Mateo R, Bertolero A, Cerradelo S, Martinez-Vilalta A (1994) Lead poisoning in waterfowl from the Ebro Delta, Spain: Calculation of lead exposure thresholds for mallards. *Archives of Environmental Contamination and Toxicology* 27.: doi: 10.1007/BF00213161

- Gyimesi A, Lensink R (2010) Risk analysis of the Egyptian Goose in the Netherlands. In: Bureau Waardenburg BV. The Netherlands
- Herre W, Röhrs M (1990) Haustiere – zoologisch gesehen. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 66
- Horák P, Kolářová L (2011) Snails, waterfowl and cercarial dermatitis: bird schistosomes. *Freshwater Biology* 56:779–790. doi: 10.1111/j.1365-2427.2010.02545.x
- Horák P, Kolářová L, Adema CM (2002) Biology of the schistosome genus *Trichobilharzia*. *Adv Parasitol* 52:155–233
- Jouet D, Ferté H, Depaquit J, Rudolfová J, Latour P, Zanella D, Kaltenbach ML, Léger N (2008) *Trichobilharzia* spp. in natural conditions in Annecy Lake, France. *Parasitology Research* 103:51–58. doi: 10.1007/s00436-008-0926-3
- Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, Holt RD, Hudson P, Jolles A, Jones KE, Mitchell CE, Myers SS, Bogich T, Ostfeld RS (2010) Impacts of biodiversity on the emergence and transmission of infectious diseases. *Nature* 468:647–652. doi: 10.1038/nature09575
- Kelly A, Kelly S (2004) Fishing tackle injury and blood lead levels in Mute swans. *Waterbirds* 27:60–68. doi: 10.1675/1524-4695(2004)027[0060:FTIABL]2.0.CO;2
- Kolářová L, Horák P, Skírnisson K (2010) Methodical approaches in the identification of areas with a potential risk of infection by bird schistosomes causing cercarial dermatitis. *J Helminthol* 84:327–335. doi: 10.1017/S0022149X09990721
- Lee KA, Klasing KC (2004) A role for immunology in invasion biology. *Trends in Ecology & Evolution* 19:523–529. doi: 10.1016/j.tree.2004.07.012
- Lövei GL (1997) Global change through invasion: Biodiversity. *Nature* 388:627–628. doi: 10.1038/41665
- Martinez-Haro M, Green AJ, Mateo R (2011) Effects of lead exposure on oxidative stress biomarkers and plasma biochemistry in waterbirds in the field. *Environmental Research* 111:530–538. doi: 10.1016/j.envres.2011.02.012
- Mateo R (2009) Lead poisoning in wild birds in Europe and the regulations adopted by different countries. *The Peregrine Fund*, pp 2
- Mateo R, Belliure J, Dolz J, Aguilar Serrano J, Guitart null (1998) High prevalences of lead poisoning in wintering waterfowl in Spain. *Arch Environ Contam Toxicol* 35:342–347
- Mateo R, Martínez-Vilalta A, Guitart R (1997) Lead shot pellets in the Ebro delta, Spain: Densities in sediments and prevalence of exposure in waterfowl. *Environmental Pollution* 96:335–341. doi: 10.1016/S0269-7491(97)00046-8
- McGeoch MA, Butchart SHM, Spear D, Marais E, Kleynhans EJ, Symes A, Chanson J, Hoffmann M (2010) Global indicators of biological invasion: species numbers, biodiversity impact and policy responses: Invasive alien species indicator: 2010 Biodiversity Target. *Diversity and Distributions* 16:95–108. doi: 10.1111/j.1472-4642.2009.00633.x

- Meharg AA, Pain DJ, Ellam RM, Baos R, Olive V, Joyson A, Powell N, Green AJ, Hiraldo F (2002) Isotopic identification of the sources of lead contamination for white storks (*Ciconia ciconia*) in a marshland ecosystem (Doñana, S.W. Spain). *Sci Total Environ* 300:81–86
- Meybeck M (2003) Global analysis of river systems: from Earth system controls to Anthropocene syndromes. *Philosophical Transactions of the Royal Society B: Biological Sciences* 358:1935–1955. doi: 10.1098/rstb.2003.1379
- Nehring S, Skowronek S (2017) Die invasiven gebietsfremden Arten der Unionsliste der Verordnung (EU) Nr. 1143/2014. Erste Fortschreibung 2017. Bundesamt für Naturschutz, Germany
- Newth JL, Cromie RL, Brown MJ, Delahay RJ, Meharg AA, Deacon C, Norton GJ, O'Brien MF, Pain DJ (2013) Poisoning from lead gunshot: still a threat to wild waterbirds in Britain. *European Journal of Wildlife Research* 59:195–204. doi: 10.1007/s10344-012-0666-7
- Pain D (2009) A global update of lead poisoning in terrestrial birds from ammunition sources. *The Peregrine Fund*, 3-20
- Peiris JSM, de Jong MD, Guan Y (2007) Avian Influenza Virus (H5N1): a Threat to Human Health. *Clinical Microbiology Reviews* 20:243–267. doi: 10.1128/CMR.00037-06
- Pfitzer S, Verwoerd DJ, Gerdes GH, Labuschagne AE, Erasmus A, Manvell RJ, Grund C (2000) Newcastle disease and avian influenza A virus in wild waterfowl in South Africa. *Avian Diseases* 44:655. doi: 10.2307/1593107
- Pikula J, Bandouchova H, Hilscherova K, Paskova V, Sedlackova J, Adamovsky O, Knotkova Z, Lany P, Machat J, Marsalek B (2010) Combined exposure to cyanobacterial biomass, lead and the Newcastle virus enhances avian toxicity. *Science of The Total Environment* 408:4984–4992. doi: 10.1016/j.scitotenv.2010.07.050
- Pyšek P, Richardson DM (2010) Invasive Species, Environmental Change and Management, and Health. *Annual Review of Environment and Resources* 35:25–55. doi: 10.1146/annurev-environ-033009-095548
- Rocke TE, Samuel MD (1991) Effects of lead shot ingestion on selected cells of the mallard immune system. *Journal of Wildlife Diseases* 27:1–9. doi: 10.7589/0090-3558-27.1.1
- Rudolfová J, Littlewood DTJ, Sitko J, Horák P (2007) Bird schistosomes of wildfowl in the Czech Republic and Poland. *Folia Parasitol* 54:88–93
- Rudolfová J, Sitko J, Horák P (2002) Nasal schistosomes of wildfowl in the Czech Republic. *Parasitol Res* 88:1093–1095. doi: 10.1007/s00436-002-0634-3
- Scheuhammer AM, Norris SL (1995) A review of the environmental impacts of lead shotshell ammunition and lead fishing weights in Canada. Minister of Supply and Services Canada, Ottawa, pp 18
- Schlaepfer MA, Runge MC, Sherman PW (2002) Ecological and evolutionary traps. *Trends in Ecology & Evolution* 17:474–480. doi: 10.1016/S0169-5347(02)02580-6

- Schmid-Hempel P (2011) *Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics*. Oxford University Press, Oxford ; New York
- Sears J (1988) Regional and seasonal variations in lead poisoning in the mute swan *Cygnus olor* in relation to the distribution of lead and lead weights, in the Thames area, England. *Biological Conservation* 46:115–134. doi: 10.1016/0006-3207(88)90095-X
- Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution* 17:170–176. doi: 10.1016/S0169-5347(02)02495-3
- Sheldon BC, Verhulst S (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution* 11:317–321. doi: 10.1016/0169-5347(96)10039-2
- Shihmanter E, Weisman Y, Lublin A, Mechani S, Gruenberg R, Horowitz H, Lipkind M (1998) Avian paramyxoviruses serotype 3 isolated from captive birds in Israel: Clinical signs, pathology, and antigenic characterization. *Avian Diseases* 42:418. doi: 10.2307/1592497
- Singh VK, Mishra KP, Rani R, Yadav VS, Awasthi SK, Garg SK (2003) Immunomodulation by lead. *Immunologic Research* 28:151–166. doi: 10.1385/IR:28:2:151
- Soldánová M, Selbach C, Kalbe M, Kostadinova A, Sures B (2013) Swimmer’s itch: etiology, impact, and risk factors in Europe. *Trends in Parasitology* 29:65–74. doi: 10.1016/j.pt.2012.12.002
- Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* 55:152–174. doi: 10.1111/j.1365-2427.2009.02380.x
- Sures B (2004) Environmental parasitology: relevancy of parasites in monitoring environmental pollution. *Trends in Parasitology* 20:170–177. doi: 10.1016/j.pt.2004.01.014
- Sures B, Nachev M, Selbach C, Marcogliese DJ (2017) Parasite responses to pollution: what we know and where we go in “Environmental Parasitology.” *Parasites & Vectors* 10.: doi: 10.1186/s13071-017-2001-3
- Thompson PN, Sinclair M, Ganzevoort B (2008) Risk factors for seropositivity to H5 avian influenza virus in ostrich farms in the Western Cape Province, South Africa. *Preventive Veterinary Medicine* 86:139–152. doi: 10.1016/j.prevetmed.2008.03.011
- Torchin ME, Lafferty KD, Dobson AP, McKenzie VJ, Kuris AM (2003) Introduced species and their missing parasites. *Nature* 421:628–630. doi: 10.1038/nature01346
- Trust KA, Miller MW, Ringelman JK, Orme IM (1990) Effects of ingested lead on antibody production in mallards (*Anas platyrhynchos*). *Journal of Wildlife Diseases* 26:316–322. doi: 10.7589/0090-3558-26.3.316
- Vallverdú-Coll N, López-Antia A, Martínez-Haro M, Ortiz-Santaliestra ME, Mateo R (2015a) Altered immune response in mallard ducklings exposed to lead through maternal

- transfer in the wild. *Environmental Pollution* 205:350–356. doi: 10.1016/j.envpol.2015.06.014
- Vallverdú-Coll N, Mougeot F, Ortiz-Santaliestra ME, Rodriguez-Estival J, López-Antia A, Mateo R (2016) Lead exposure reduces carotenoid-based coloration and constitutive immunity in wild mallards: Lead exposure, coloration, and immunity in mallards. *Environmental Toxicology and Chemistry* 35:1516–1525. doi: 10.1002/etc.3301
- Vallverdú-Coll N, Ortiz-Santaliestra ME, Mougeot F, Vidal D, Mateo R (2015b) Sublethal Pb exposure produces season-dependent effects on immune response, oxidative balance and investment in carotenoid-based coloration in red-legged partridges. *Environmental Science & Technology* 49:3839–3850. doi: 10.1021/es505148d
- van der Most PJ, de Jong B, Parmentier HK, Verhulst S (2011) Trade-off between growth and immune function: a meta-analysis of selection experiments: Trade-off between growth and immune function. *Functional Ecology* 25:74–80. doi: 10.1111/j.1365-2435.2010.01800.x
- Vörösmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Glidden S, Bunn SE, Sullivan CA, Liermann CR, Davies PM (2010) Global threats to human water security and river biodiversity. *Nature* 467:555–561. doi: 10.1038/nature09440
- Wahl J, Dröschmeister R, Langgemach T, Sudfeldt C (2011) Vögel in Deutschland - 2011. DDA, BfN, LAG VSW, Münster, pp 46
- Wojcinski ZW, Barker IK, Hunter DB, Lumsden H (1987) An outbreak of schistosomiasis in Atlantic brant geese, *Branta bernicla hrota*. *Journal of Wildlife Diseases* 23:248–255. doi: 10.7589/0090-3558-23.2.248
- Youssef SA, El-Sanousi AA, Afifi NA, El Brawy AM (1996) Effect of subclinical lead toxicity on the immune response of chickens to Newcastle disease virus vaccine. *Res Vet Sci* 60:13–16

CHAPTER 1

**Chronic lead intoxication decreases
intestinal helminth species richness and infection
intensity in mallards (*Anas platyrhynchos*)**

Published in *Science of the Total Environment*

ISSN 0048-9697

Volume 644, 10 December 2018, Pages 151-160

DOI 10.1016/j.scitotenv.2018.06.297

You have to purchase this part online.

Prüter, Hanna¹; Franz, Mathias¹; Auls, Susanne¹; Czirják, Gábor Á.¹; Greben, Oksana²; Greenwood, Alex D.^{1,3}; Lisitsyna, Olga²; Syrota, Yaroslav^{2,5}; Sitko, Jilji⁴; Krone, Oliver¹

¹ Leibniz Institute for Zoo and Wildlife Research, Department of Wildlife Diseases, Alfred-Kowalke-Straße 17, 10315 Berlin, Germany

² National Academy of Sciences of Ukraine, I. I. Schmalhausen Institute of Zoology, Vul. B. Khmelnytskogo, 15, 01030 Kiev, Ukraine

³ Freie Universität Berlin, Department of Veterinary Medicine, Berlin, Germany

⁴ Komenský Museum, Horní nám. 7, 750 11 Přešov 2, Czech Republic

⁵ Kyiv Zoological Park of National Importance, prosp. Peremohy, 32, Kyiv, 04116 Uk

CHAPTER 2

The immunological plasticity hypothesis explains contradictory immunity pattern in invasive species

In preparation for submission

Prüter, Hanna¹; Franz, Mathias¹; Twietmeyer, Sönke²; Böhm, Niklas³; Middendorff, Gudrun⁴; Portas, Ruben⁵; Melzheimer, Jörg⁵; Kolberg, Holger⁶; von Samson-Himmelstjerna, Georg⁷; Greenwood, Alex D.^{1,8}; Lüschow, Dörte⁹; Mühldorfer, Kristin¹; Czirják, Gábor Árpád¹

¹ Leibniz Institute for Zoo and Wildlife Research, Department of Wildlife Diseases, Alfred-Kowalke-Straße 17, 10315 Berlin, Germany

² Department of Research and Documentation, Eifel National Park, Urftseestraße 43, D-53937 Schleiden-Gemünd, Germany

³ FÖA Landschaftsplanung GmbH, Auf der Redoute 12, 54296 Trier, Germany

⁴ Namibia Bird Club, Windhoek, Namibia

⁵ Leibniz Institute for Zoo and Wildlife Research, Department of Evolutionary Ecology, Alfred-Kowalke-Straße 17, 10315 Berlin, Germany

⁶ Ministry of Environment and Tourism, Windhoek, Namibia

⁷ Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Robert-von-Ostertag-Str. 7-13, 14163, Berlin, Germany

⁸ Freie Universität Berlin, Department of Veterinary Medicine, 14163, Berlin, Germany

⁹ Freie Universität Berlin, Institute of Poultry Diseases, Königsweg 63, 14163, Berlin, Germany

Abstract

Several related hypotheses have been formulated to explain and understand the success of invasive species. Often, immunity and parasites have been linked to invasion success. While most of the related hypotheses are supported by empirical studies, there are contradictory results for some species. In many cases, hypotheses have been tailored for certain simplified scenarios. Here, we propose a new hypothesis (the Immunological Plasticity Hypothesis) which can explain contradictory findings and help to understand the role of parasites and immunity during vertebrate invasion. This hypothesis states that immune plasticity enables invaders to reduce the overall physiological cost of immunity while maintaining the ability to efficiently defend against novel parasites. Comparing a broad spectrum of immune effectors and parasite prevalences in an invasive and a native Egyptian goose population we find support for our hypothesis and discuss the implications for studying immunity and parasite infection during invasion processes.

Introduction

Invasive species are major threats to global biodiversity (Lövei 1997; McGeoch et al. 2010). Moreover, they may affect animal and public health by playing important epidemiological roles in spreading and maintaining several micro- and macro-parasites (from here on termed ‘parasites’) (Strauss, White, and Boots 2012). With increasing globalisation and biodiversity loss, studying the underlying mechanisms which promote species to become invasive, is essential for risk assessment, species conservation efforts and public health (Pyšek and Richardson 2010).

Although, the introduction of exotic, neozootic species to novel habitats is common, only few become true invaders. After introduction, neozootic species need to become established and spread to successfully invade a new region (Duncan, Blackburn, and Sol 2003). Environmental factors, available resources and natural enemies (i.e. predators and parasites) are important factors determining the population growth rate and invasion success of such species (Shea and Chesson 2002). Resources are typically limited and must be allocated between different life history traits or stages, such as individual growth, dispersion, reproduction and immunity (Sheldon and Verhulst 1996; van der Most et al. 2011). Allocating resources from immunity to other physiological processes, such as reproduction, has been suggested to increase invasion success (Lee and Klasing 2004).

Allocating resources away from immunity is especially beneficial when parasite burden is low. According to the “Enemy Release Hypothesis” (ERH) invading hosts show reduced parasite burdens by escaping the parasites in their native range when these are absent in the new range (Torchin et al. 2003). The ERH has support from studies on vertebrates including different bird species (Shwartz et al. 2009; Lima et al. 2010; Marzal et al. 2011; Valente et al. 2014; Clark et al. 2015; Ellis et al. 2017; Keogh et al. 2017; Diagne et al. 2016; Torchin et al. 2003). Generally, both parasite species richness and prevalence have been found to be lower in invasive than in native populations because specialist enemies are absent in the new region and host switching by specialist parasites of native species is rare (Keane and Crawley 2002; Torchin et al. 2003).

Based on the ERH, the “Evolution of Increased Competitive Ability Hypothesis” (EICA) states that invasive plants that escape from parasites can reduce the investment in defence mechanisms and thereby allocate more energetic resources to reproduction and dispersal (Blossey and Notzold 1995). Lee and Klasing (2004) refined this hypothesis (revised-EICA) focusing on the vertebrate immune system. They suggested that successful vertebrate invaders are more likely to decrease investment into energetically costly immune defences and compensate with immunity that incurs less energetic and pathological costs. Thus, down regulated inflammatory responses which are costly might lead to an increase in less costly responses (e.g. antibody-mediated immunity) (Lee and Klasing 2004).

In contrast to the revised-EICA, Cornet et al. (2016) suggest that newly acquired local parasites might induce an equivalent immune response in invasive and native populations. The authors show that only a subset of studies on invasive vertebrates support the revised-EICA, whereas others could not find evidence supporting the revised-EICA hypothesis. In a common garden experiment, Keogh et al. (2017) showed that invasive shore crabs (*Hemigrapsus sanguineus*) are less infected with rhizocephalan parasites than native crabs but at the same time more susceptible to experimental infection with these parasites. This indicates that reducing immune investment might increase the risk of getting infected by new parasites for invasive species. Also, Brown and Shine (2014) suggest that trade-offs between different immune effectors are more important for invasive species than the general downregulation of costly traits. Moreover, Møller and Cassey (2004) propose that invasive bird species with strong immune response towards novel parasites are potentially more likely to become successful invaders. Thus, several studies indicate that the revised-EICA might underestimate the impact of novel parasites on invasive populations.

The inconsistencies are addressed here by a new hypothesis that combines (1) the existing concepts underlying the enemy release and reduced immune investment in invasive populations with (2) the ability to defend against novel parasites in a new range. Our hypothesis posits that plasticity of immunity as opposed to generally reduced investment in immune function provides invading species with the necessary flexibility to colonize novel environments. Plasticity of immunity could enable invaders to balance reduced immune investment against their original parasites with increased defence against novel parasites. Thus, individuals which do not face novel parasites will reduce their immune investment, whereas individuals who face novel parasites will be able to increase the appropriate immune defense. Accordingly, we name this hypothesis “Immunological Plasticity Hypothesis” (IPH).

Depending on the immune effector, its main role in defence against parasites, and the stage of the invasion process we propose three different scenarios (Figure 1 b-d). If the enemy release effect is dominant (e.g. early stages of invasion; invasion front), immune investment would be down-regulated as suggested by the revised-EICA hypothesis (Figure 1b). In this case, low parasite prevalence and reduced overall costs of immunity can be expected. After introduction, individuals of invasive populations would increasingly face new parasites. Invasive animals must provide effective defences against these novel parasites. Here, low prevalence for some and higher prevalence for other parasite species (more heterogeneous than in the early stages) on a population level are likely. Thus, some individuals must be able to defend against the new parasites requiring higher immune investment than native individuals (Møller and Cassey 2004) whereas others may still invest less in immunity compared to their native relatives. Plasticity of immunity would be most prominent during this time and accordingly we would expect an increased variance in immune measures (Figure 1c). In later phases, prevalence of parasites might increase further and thus, defence against new parasites must increase. Immune effector levels would increase due to increasing need to defend against new parasites (Figure 1d).

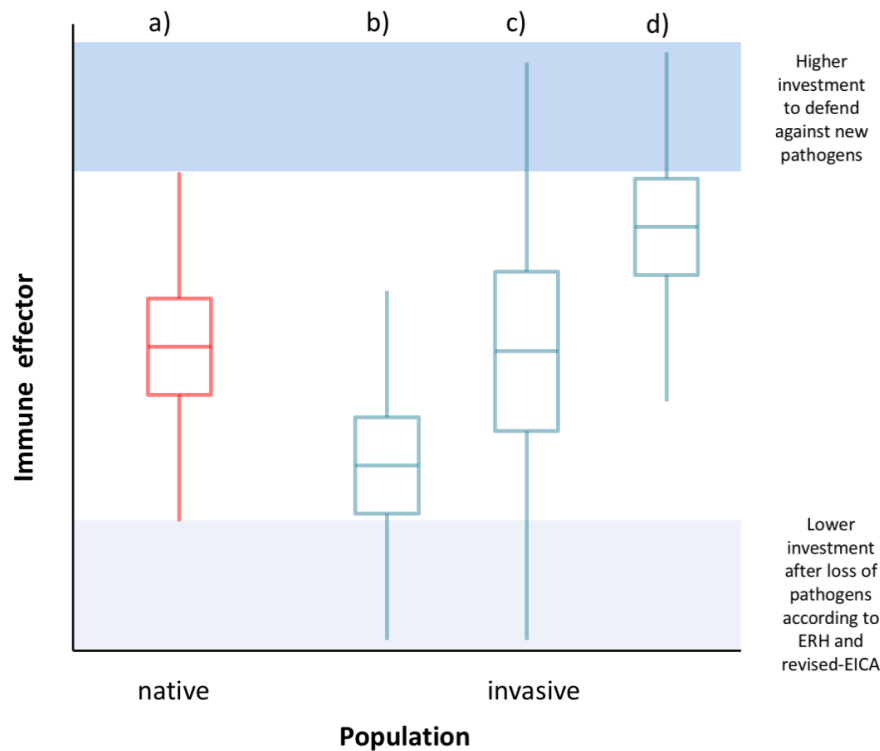


Figure 1: Predictive changes of the means and variances of immune effectors between native (a) and invasive (b-d) populations; (b) decrease in mean according to the “Enemy Release Hypothesis “ (ERH) and “revised- Evolution of Increased Competitive Ability Hypothesis” (revised-EICA), (c) increase in variance if the effect of enemy release allows decreasing investment into immunity but defence against new parasites must increase as new parasites affect the hosts, (d) the effect of increased investment to defend against new parasites is stronger than the enemy release effect.

Based on the IPH, the prediction was that the differences in mean and variance between immune effectors of native and invasive Egyptian geese would depend on the specific pattern of loss and gain of parasites. If only parasite loss is observed, we predict a decrease in the means of high cost and a potential increase in low cost immune effectors as shown in Fig. 1b. If the invasive population is infected with new parasites, an increase of variance in immune effectors as in Fig 1c would be predicted. After having successfully established (later phase of invasion), mean values of specific immune effectors needed to defend against those new parasites can be expected to stay high (Fig 1d). To assess potentially congruent patterns, parasite communities and mean and variance of immune effectors of a native and an invasive population of the Egyptian goose (*Alopochen aegyptiacus*) were investigated. The Egyptian goose is one of the most successful invasive bird species in Europe, whose population continues to increase (Bauer and Woog 2008; Gyimesi and Lensink 2010) and which has been listed as an invasive species in Germany according to “EU Regulation No 1143/2014 on the prevention and management of

the introduction and spread of invasive alien species” and the BfN script 47 in 2017 (Nehring and Skowronek 2017).

Material and Methods

Sampling:

Parasite prevalence and immunity of Egyptian geese from a native population in Namibia were investigated and compared to those of a currently spreading invasive population of the same species in Germany. In both regions, geese were sampled during ringing procedures (live trapped) or dissected after hunting (necropsy).

Live trapping

Twenty-one Egyptian geese (9 male, 12 female) were live trapped in Namibia (22.35°S, 17.05°E) (native range) in February 2016. Additionally, data from a subset of 110 adult Egyptian geese (65 male, 45 female) investigated by Prüter et al. (2018a) were included in this comparative study. German geese were sampled in the Rhine and Mosel areas (50.4°N, 7.6°E) (invasive range) in 2015 ($n=78$) and 2016 ($n=32$) in different months (supplementary data Table S1). Sex and reproductive status were recorded. Reproductive status was defined as breeding (e.g. guiding gosling, showing territorial behavior with a partner, having an egg-laying active cloaca) or non-breeding (e.g. not fulfilling criteria of breeding and/or being part of a non-family-flock). All Namibian birds were non-breeding individuals. Blood was drawn from the vena *metatarsalia plantaris superficialis* using needles with a diameter of 0.06 mm for males and 0.04 mm for females. A fresh blood smear was prepared at capture and air dried. Blood samples kept at cool were centrifuged and sera were frozen in liquid nitrogen within eight hours after blood draw. Pharyngeal swabs were collected using sterile cotton swabs. Sera, blood cell pellets and pharyngeal swabs were kept frozen at -80°C till further analysis. Sampling in Germany was authorized by the Landesuntersuchungsamt Rheinland-Pfalz (G 15-20-005) and Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen (LANUV) (84-08.04.2015.A266). Permission to collect samples in Namibia was granted to GM and HK by the Ministry of Environment and Tourism (MET). Permission to export sample material from Namibia was granted by a MET export permit (No. 107513), and samples were transported to Germany in compliance with the Nagoya Protocol on Access to Genetic Resources.

Necropsy

Additionally to live trapping, twenty-six free ranging Egyptian geese (17 male, 9 female) hunted during the autumn/winter season 2014/2015 and 2015/2016 in the North and West of

Germany and twenty-seven Egyptian geese (11 male, 16 female), which were shot in February 2016 during regular pest control in Central Namibia were dissected. One of twenty-seven was live trapped and sampled before death and is thus included in both groups (live trapped and necropsy). Geese from Germany were kept frozen at -20°C after hunting until further analysis. Namibian geese were dissected immediately *post mortem*. During necropsy, ectoparasites, intestinal helminthes and nasal leeches were collected. Additionally, pharyngeal swabs were taken for molecular analyses.

Determination of parasites:

Both macro-parasites (ectoparasites, nasal leeches (Euhirundidae), intestinal helminthes) and selected micro-parasites (blood parasites (Haematozoa), bacteria, viruses) of Egyptian geese from the two populations were determined. Hereafter we use the term “parasites” combining macro- and micro-parasites and only explicitly distinguish between the type of parasites when differences can be expected and/or occur.

During necropsy, wing and breast feathers were macroscopically checked for the presence of ectoparasites. The upper beak was cut open and macroscopically investigated for the presence of nasal leeches. Intestinal helminthes were extracted from the intestine of the birds and were determined to the family level based on morphology. Additionally, blood smears of all live-trapped animals were investigated for the presence of blood parasites during immunological cell counts (Doster and Goater 1997).

To compare with bacterial prevalence of adult German Egyptian geese (included in Prüter et al. 2018a) (red shaded in Table 2)), the Namibian birds were screened for DNA from *Mycoplasma* spp. and *Riemerella (R.) anatipestifer* using conventional 16S rRNA-based PCR assays as described by Prüter et al. (2018a). To verify the specificity of the *Mycoplasma* PCR assay, products with a clear band were further investigated by sequence analysis, again following the procedure described by Prüter et al. (2018a). Only samples with a clear sequencing result were assessed as positive.

Serological methods were used to investigate the seroprevalence of antibodies (Ab) against Influenza A virus (IAV), *Avian avulavirus 1* (AAvV-1) and *West Nile virus* (WNV). For the detection of Ab against IAV, a commercial competitive enzyme linked immunosorbent assay (ELISA) was used following the manufacturer instructions (ID.vet, Grabels, France, Influenza A Antibody competition, FLUACA ver 0917DE).

A commercial competitive ELISA for detection of Ab against AAvV-1 (former Avian paramyxovirus 1; syn. Newcastle disease virus) was used and evaluated according to the

manufacturer protocol (ID.vet, Grabels, France, Newcastle Disease Competition, NDVC ver 0913 DE). Additionally, sera were tested applying a commercial competitive ELISA for Ab against Flaviviridae including WNV following the manufacture protocol (ID.vet, West Nile Competition, WNC ver 1014-1P DE).

Immunological assays:

Due to the complexity of the immune system, several eco-immunological tests were used to quantify both the cellular and humoral parts of the acquired and innate immune responses of Egyptian geese (Demas et al. 2011). Most of the methods are not species specific and have been used in a wide variety of free-living avian species, including different waterfowl (Matson et al. 2006; Giraudeau et al. 2010; Bourgeon et al. 2010). We quantified the levels of different humoral (natural antibodies, complement, lysozyme and haptoglobin) and cellular (monocytes, heterophils, eosinophils and basophils) effectors of innate immunity. For adaptive immunity we measured the total immunoglobulin Y (IgY) concentration and the number of lymphocytes (Matson et al. 2006). Sample sizes (n) for each assay were dependent on the total amount of serum available from each individual and therefore differ between the tests (Table 1).

Table 1: Total sample sizes (n), sample sizes grouped by sex ($n \♂$, $n \♀$) and year of sampling of blood and serum samples from Namibian (native) and German (invasive) Egyptian geese (*Alopochen aegyptiacus*) for each immunological effector grouped by the costs of immunity (low costs vs. high cost according to (Klasing 2004; Lee and Klasing 2004))

Immunological effectors	Invasive 2015 (Germany)		Invasive 2016 (Germany)		Native 2016 (Namibia)	
	n	$n \♂$, $n \♀$	n	$n \♂$, $n \♀$	n	$n \♂$, $n \♀$
Low cost						
IgY	74	44♂, 30♀	26	16♂, 10♀	21	9♂, 12♀
Lysozyme	76	43♂, 33♀	30	18♂, 12♀	20	9♂, 11♀
Natural antibodies, complement	75	43♂, 32♀	24	16♂, 8♀	21	9♂, 12♀
High cost	n	Sex ratio	n	Sex ratio	n	Sex ratio
Granulocytes (basophil, eosinophil, heterophil), Total leucocytes, Lymphocytes, Monocytes	77	45♂, 32♀	31	19♂, 12♀	21	9♂, 12♀
Haptoglobin	72	42♂, 30♀	23	15♂, 8♀	21	9♂, 12♀

Immunoglobulin Y

Total IgY, the avian equivalent to mammalian IgG, was assessed using a sensitive ELISA with commercial anti-chicken antibodies (Martinez et al. 2003; Bourgeon et al. 2010). 96-well high-binding ELISA plates (82.1581.200, Sarstedt) were coated with 100 μ l of diluted serum sample (2 samples per bird 1:16000 diluted in carbonate–bicarbonate buffer) and incubated first for

1 h at 37°C and then overnight at 4°C. After incubation, the plates were washed with a 200 µl solution of phosphate buffer saline and PBS–Tween, before 100 µl of a solution of 1% gelatine in PBS–Tween was added. Plates were then incubated at 37°C for 1 h, washed with PBS–Tween and 100 µl of polyclonal rabbit anti-chicken IgY conjugated with peroxidase (A-9046, Sigma) at 1:250 (v/v) was added. Following 2 h incubation at 37°C, the plates were washed again with PBS–Tween three times. After washing, 100 µl of revealing solution [peroxide diluted 1:1000 in ABTS (2,20-azino-bis- (3-ethylbenzthiazoline-6-sulphonic acid))] was added, and the plates were incubated for 1 h at 37°C. The final absorbance was measured at 405 nm using a photometric microplate reader (µQuant Microplate Spectrophotometer, Biotek) and subsequently defined as total serum IgY levels (Bourgeon and Raclot 2006).

Lysozyme

To measure lysozyme concentration in serum, we used the lysoplate assay (Giraudeau et al. 2010): 25 µl serum were inoculated in the test holes of a 1% Noble agar gel (A5431, Sigma) containing 50 mg/100 ml lyophilized *Micrococcus lysodeikticus* (M3770, Sigma), a bacteria which is particularly sensitive to lysozyme concentration. Crystalline hen egg white lysozyme (L6876, Sigma) (concentration: 1, 1.25, 2.5, 5, 6.25, 10, 12.5, 20 and 25 µg/ml) was used to prepare a standard curve for each plate. Plates were incubated at room temperature (25-27°C) for 20 h. During this period, as a result of bacterial lysis, a clear zone developed in the area of the gel surrounding the sample inoculation site. The diameters of the cleared zones are proportional to the log of the lysozyme concentration. This area was measured three times digitally using the software ImageJ (version 1.48, <http://imagej.nih.gov/ij/>) and the mean was converted to a semi-logarithmic plot into hen egg lysozyme equivalents (HEL equivalents, expressed in µg/mL) according to the standard curve (Rowe et al. 2013).

Haemolysis–haemagglutination assay

The levels of the natural antibodies and complement were assessed by using a haemolysis–haemagglutination assay as described by (Matson, Ricklefs, and Klasing 2005) adjusted to the limited volume of serum. After pipetting 15 µl of serum into the first two columns of a U-shaped 96-well microtitre plate, 15 µl sterile PBS were added to columns 2-12. The content of the second column wells was serially diluted (1:2) until the 11th column, resulting in a dilution series for each sample from 1/1 to 1/1024. The last column of the plate was used as negative controls, containing PBS only. We then added 15 µl of 1% rabbit red blood cells (supplied as 50% whole blood, 50% Alsever's solution, Envigo) suspension to all wells and incubated at 37°C for 90 min. After incubation, in order to increase the visualisation of agglutination, the plates were tilted at a 45° angle at room temperature. Agglutination and lysis, which reflect the

activity of the natural antibodies and the interaction between these antibodies and complement (Matson, Ricklefs, and Klasing 2005; Pap et al. 2010), was recorded after 20 and 90 min, respectively. Haemagglutination is characterised by the appearance of clumped red blood cells, as a result of antibodies binding multiple antigens, while during haemolysis, the red blood cells are destroyed. Titres of the natural antibodies and complement were given as the log₂ of the reciprocal of the highest dilution of serum showing positive haemagglutination or lysis, respectively (Matson, Ricklefs, and Klasing 2005; Pap et al. 2015).

White blood cell counts

To count leucocytes, blood smears were prepared, air-dried and stained using Giemsa- and May-Grünwald staining. Smears were examined at 1,000×magnification with oil immersion and the relative number of different types of leucocytes was assessed by counting 100 leucocytes. The number of white blood cells of different types was expressed per 10⁴ erythrocytes (Pap et al. 2015).

Haptoglobin

We measured haptoglobin concentrations with a commercial kit (TP801, Tri-Delta Diagnostics, Inc.) following the instructions of the manufacturer. Haptoglobin concentrations (mg/ml) in undiluted serum samples were calculated according to the standard curve on each plate (Matson et al. 2006).

Statistical analyses:

Parasite prevalence

To investigate potential differences in the prevalence of parasites between native and invasive Egyptian geese, we used Fisher's exact tests.

Immunity

The means and variances of the different immune effectors were compared between the invasive and native Egyptian geese populations. To this end, we used linear mixed-effects models (LMMs). These models typically assume that the response variable is a function of a linear combination of some predictor variables, random effects, and a normally and independently distributed error. Importantly, the variance of this error (the so-called residual variance) is usually assumed to be constant. Thus, the estimated effects of the predictors only describe changes in the mean of the response variable, but not to the variance around that mean. Here, models were used in which the variance was allowed to be a linear function of some predictors. Thus, we were able to estimate simultaneously the effect of predictors upon the

variation in the mean of the response variable and also in the residual variation around that mean.

Different immune effectors were used as response variables (Table 1). As predictors for the mean sex (male vs. female), reproductive status (breeding vs. non-breeding) and invasion status (native vs. invasive) were included as fixed effects and month of sampling as a random effect. As predictors for the variance, we included invasion status (native vs. invasive) as the single predictor, which allowed us to test our prediction that the variance in immune effectors is higher among invasive compared to native individuals.

Some of the immune effectors were transformed (see tables supplementary data S2, S3, S4) to ensure normality of residuals. For haptoglobin we were not able to perform a transformation that ensured normality, because of the high proportion of values below the detection threshold. To account for this, we performed a general linear mixed model (GLMM) with a binomial error distribution and with a binary response variable (haptoglobin being either above or below the detection threshold). Thus, for haptoglobin we were only able to test for a change in mean but not for a change in variance. Total leucocytes were analysed followed by a separate analysis of differential white blood cell counts.

The LMMs and GLMMs were implemented using the R package *glmmTMB* version 0.2.0 (Brooks et al. 2017). Potential collinearity of predictors was tested by calculating variance inflation factors using the R package *car* version 2.1-6 (Fox and Weisberg 2011). All statistical analyses were performed using R version 3.3.2 (R Core Team 2016).

Results

Parasite prevalence:

Blood parasites and all groups of macro-parasites (ectoparasites, nasal leeches, intestinal cestodes and trematodes), except intestinal nematodes, were found at lower prevalence in the invasive than in the native population of Egyptian geese. However, none of the observed trends reached statistical significance (Table 2). DNA of the bacterium *R. anatipestifer*, which has a prevalence of 67.0% in adult invasive Egyptian geese from Germany (Prüter et al. 2018a) was not detected in the pharynx of native geese from Namibia. This difference in the prevalence was highly significant for *R. anatipestifer* (Table 2). *Mycoplasma* DNA, which was not detected in the German geese (Prüter et al. 2018a), was detected in the pharynx of two individuals from Namibia. However, the results did not reach statistical significance (Table 2). Seroprevalence of selected viruses (IAV, AAvV and WNV) were all lower in geese from the

invasive population but this effect was only statistically significant for antibodies against IAV (Table 2).

Immunity:

Males showed significantly higher levels of total leucocytes, haptoglobin abundance and lymphocytes than females. Total leucocytes and lymphocytes were significantly higher in birds that were in breeding status than in non-breeding individuals. No statistically significant effects on the other immune effectors were associated with sex or reproductive status (supplementary data Table S2, S3 and S4).

Differences in the mean immune function measures:

Of the four assays measuring 'low cost' immune effectors, only mean haemolysis concentration was significantly higher in the invasive population (Figure 2). The mean of total leucocytes ('high cost' immune effector) was not significantly different between the two study populations (Figure 2). Detailed analyses of the mean of differential white blood cells demonstrated significantly higher mean levels of heterophils and lymphocytes in the invasive population, whereas mean eosinophil concentration was significantly lower in this group. No statistically significant differences in mean monocytes and mean basophil numbers between the two groups were observed. In contrast, haptoglobin concentration (a 'high cost' immune effector) was significantly higher in abundance in the invasive (65%) than in the native (7.3%) population (Figure 3).

Differences in variance in immune function measures:

Variance of all 'low cost' immune effectors was significantly higher in the invasive population (Figure 2). The variance of total leucocytes did not significantly differ between the two groups (Figure 2). The variance in the concentration of eosinophils, heterophils and monocytes was significantly higher in the invasive geese. Variance in basophils and lymphocyte counts did not significantly differ between the groups (Figure 4).

CHAPTER 2

Table 2: Results of the parasite screening and serology of Egyptian geese from Namibia and Germany (Prüter et al. 2018a). Total sample sizes (*n*), number of infected and non-infected individuals (Ratio) and prevalences (%) of macro parasites, bacteria and seroprevalences against selected viruses in the native Namibian and invasive German population of Egyptian geese (*Alopochen aegyptiacus*); Trend: ↓ higher prevalence in the native than in the invasive population; ↑ higher prevalence in the invasive than in the native population; Outcome of Fisher’s exact test comparing prevalences of the two groups (*p* -value).

		Namibia			Germany			Fisher test	
Parasitology:	Group	<i>n</i>	Ratio	Prevalence	<i>n</i>	Ratio	Prevalence	Trend	<i>p</i>
	Ectoparasites	27	11/16	40.74	26	3/23	11.54	↓	0.08
	Euhirundidae	27	1/26	3.85	26	0/26	0	↓	1
	Intestinal helminthes	27	6/21	22.2	26	4/22	15.38	↓	0.74
	Cestoda	27	2/25	7.4	26	1/25	3.8	↓	1
	Nematoda	27	0/27	0	26	1/25	3.8	↑	1
	Trematoda	27	4/23	14.8	26	1/25	3.8	↓	0.36
	Heamatozoa	21	1/20	4.76	110	0/110	0	↓	0.17
Bacteria:	Target genes	<i>n</i>	Ratio	Prevalence	<i>n</i>	Ratio	Prevalence	Trend	<i>p</i>
	<i>Riemerella anatipestifer</i> 16S rRNA gene	47	0/47	0	94	63/31	67.02	↑	<0.001
	<i>Mycoplasma</i> spp. 16S rRNA gene	47	2/45	4.44	94	0/94	0	↓	0.12
Serology:	Antigen	<i>n</i>	Ratio	Prevalence	<i>n</i>	Ratio	Prevalence	Trend	<i>p</i>
	IAV	21	9/12	42.86	105	9/96	8.57	↓	0.003
	AAvV-1	20	2/18	10	102	4/98	3.92	↓	0.27
	WNV	13	1/12	7.69	56	0/56	0	↓	0.2

Abbreviations: IAV= Influenza A virus; AAvV-1= *Avian avulavirus 1*; WNV= *West Nile virus*

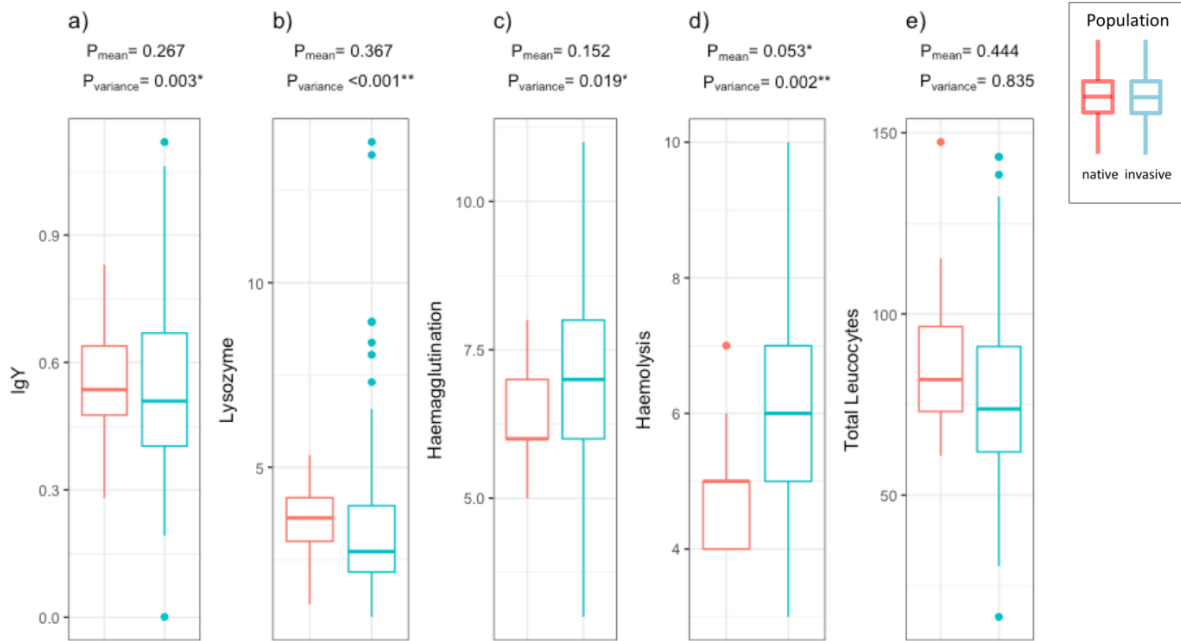


Figure 2: Differences in distributions of low cost (a-d) and high cost (e) immune measures between native and invasive Egyptian geese are shown; red= native; blue=invasive; $P = p$ -values for the effects of population (native vs. invasive) on the mean (P_{mean}) and variance (P_{variance}) of the respective immune measure.

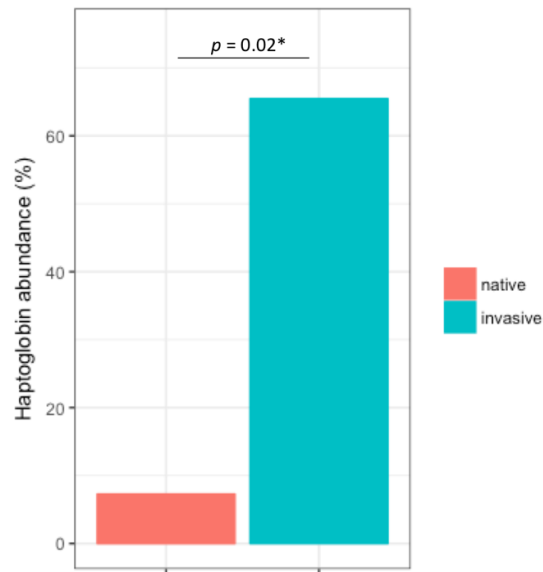


Figure 3: A barplot for the differences in haptoglobin abundance between native and invasive Egyptian geese is shown; red= native, blue=invasive; p -value for the effects of population (native vs. invasive) on the mean haptoglobin from the binomial distribution model. (It was not possible to fit a Gaussian model for haptoglobin concentration. Thus, the assessment of the difference in variance was not possible).

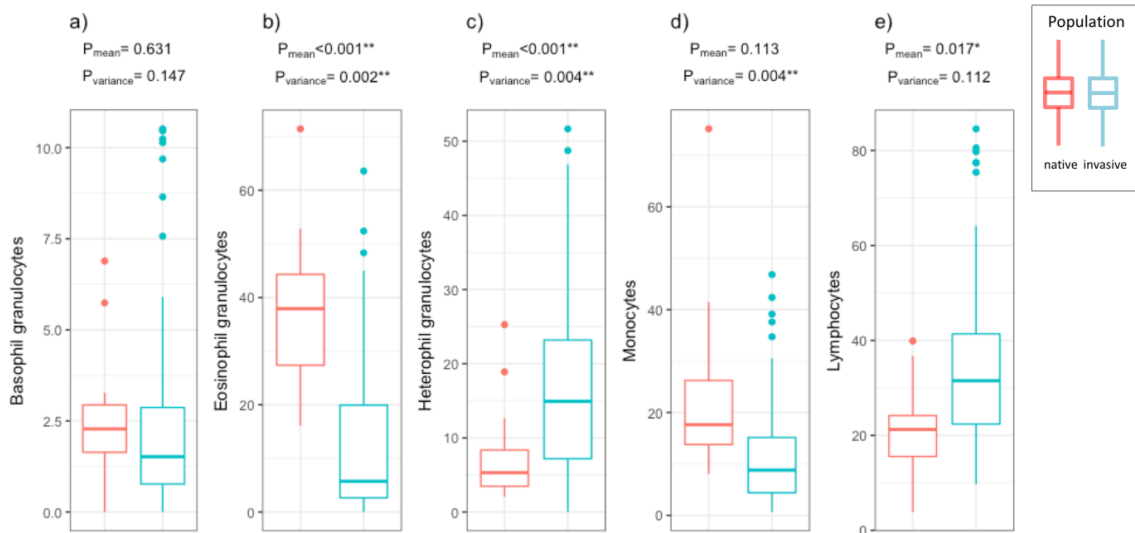


Figure 4: Differences in distributions of the differential white blood cells (a-e) between native and invasive Egyptian geese are shown; red= native; blue=invasive; $P = p$ -values for the effects of population (native vs. invasive) on the mean (P_{mean}) and variance (P_{variance}) of the respective immune measure.

Discussion

Our results support the immune plasticity hypothesis (IPH). All but one of the studied parasite's prevalence or seroprevalence decreased when compared to native and invasive populations of Egyptian geese. Only the bacterium *R. anatipestifer* showed a significant increase in the invasive population, suggesting that this population faces novel pathogens during invasion. Thus, we predicted an increased variance of immune effectors, especially those of relevance for the defence against this novel pathogen, in the invasive compared to the native population (Figure 1c). As expected, the variance for six out of ten immune effectors in the invasive population of Egyptian geese was higher than in the native population. Moreover, five out of eleven immune effectors had higher mean values in the invasive population when compared to their native conspecifics. Most of these immune effectors are considered energetically costly (Lee and Klasing 2004), which is inconsistent with the predictions of the revised-EICA hypothesis. Still, the variance results are consistent with the predictions arising from the IPH demonstrating that existing hypotheses insufficiently covered the complex interplay of loss and gain of parasites in invasive populations.

Most studies of invasive vertebrate hosts and their pathogens focus on helminths, against which hosts respond with a Th2 response and eosinophils are one of the main immune

effectors (Al-Sabi et al. 2013; Romeo et al. 2014; Valente et al. 2014; Diagne et al. 2016). Thus, previous tests of the revised-EICA related predictions, which focused mainly on macro-parasites, might be biased by the pathogens and immune effectors investigated. Here, low mean eosinophil granulocytes in invasive geese indicate a lower impact of macro-parasites on the immune system than in the native population (consistent with the ERH). However, variance of eosinophil granulocytes was significantly higher in the invasive than in the native population. The evidence suggests that Egyptian geese did not only lose parasites (ERH) but gained and are affected by novel ones. Thus, combining the results of mean and variance of immune marker has the potential to detect combined effects in immunological studies (IPH).

The *R. anatipestifer* prevalence, which was significantly higher in the invasive geese in Germany, indicated that invasive Egyptian geese encounter different pathogens in their new environments (Prüter et al. 2018a). *R. anatipestifer* is a bacterium of relevance for domestic ducks and geese, leading to severe clinical symptoms (Hubálek 2004; Hinz et al. 1998). The lack of clinical symptoms in the invasive Egyptian geese infected with *R. anatipestifer* may indicate that they are more tolerant than expected (Raberg, Graham, and Read 2009; Cornet et al. 2016) or that waterfowl may carry *R. anatipestifer* as a commensal bacterium (Ryll et al. 2001; Prüter et al. 2018a). Immune defences effective against micro-parasites, especially bacteria (heterophils, lymphocytes, haemolysis) were found to be higher on average in the invasive than in the native goose populations. Haptoglobin, which is an energetically costly acute phase protein with bacteriostatic function (Matson et al. 2006), was significantly more abundant in the invasive than in the native population of Egyptian geese, inconsistent with the EICA-related predictions. However, a significantly higher mean value of lymphocytes without evidence for higher variance in the invasive population indicate, that bacteria might be particularly important pathogens affecting this invasive population. Thus, immune defences against bacteria needs to be maintained in the invasive population. This is consistent with the predictions arising from the IPH (Fig. 1d).

The complexity of the immune system and the high specificity of different immune traits towards specific parasites may be more important than previously acknowledged (Morand et al. 2015). Furthermore, White and Perkins (2012) suggested that the higher the plasticity of the immune system of individuals of a founder population, the more likely the species becomes a successful invader (pre-selection). Additionally, Ghaleb et al. (2007) hypothesized that the period of persistence of invasive species (period after introduction and before rapid population growth starts) might be dependent on phenotypic plasticity. Thus, changes in immune

investment and immune plasticity in the different stages of invasion are likely (for the different phases of invasion see Duncan et al. 2003).

The Egyptian goose (*Alopochen aegyptiacus*) is a successful invasive bird species having and continuing to spread throughout Western Europe since the mid-20th century (Gyimesi and Lensink 2010). The impact of newly acquired pathogens on this population is most likely still ongoing and potentially increasing. This might explain why plasticity in immunity is prominent. The higher bactericidal investment but less plasticity in bacterial related immunity, might indicate that population growth rates can be expected to slow in the future, as immune investment increases. After the invasion has progressed further or completed, the variance of all effectors could decrease (Figure 1d). Thus, our findings indicate that among-individual variation in immunity is on average higher in invasive compared to native Egyptian geese but that these effects depend on the parasites and therefore differ among immune effectors.

Compared to mallards (*Anas platyrhynchos*), a native species to Germany's avifauna, which shares the same habitat with the invasive Egyptian goose population, both native and invasive Egyptian geese from this study reveal lower parasite prevalence and load (Prüter et al. 2018b). Low parasite prevalence in the invasive population might indicate that the Egyptian goose is not a suitable host for parasites native to German waterfowl. However, this comparably low macro-parasite prevalence and richness in both populations might suggest that the Egyptian goose is highly immunocompetent and thus a prime water bird invasive species (Morand et al. 2015).

Contradictory effects of invasion on immune function have been also reported in amphibians. Immune defences are weaker in invasive cane toads (*Rhinella marina*) that move further distances indicating a trade-off between dispersal and immune investment (Brown and Shine 2014). Cane toads on the invasion-front were found to have higher bactericidal and phagocytic activity than in more established populations consistent with our observations in Egyptian geese (Brown et al. 2015). However, the overall immune investment in cane toads at the invasion front was lower compared to established populations (Llewellyn et al. 2012). In contrast, Cuban treefrogs (*Osteopilus septentrionalis*) at the invasion front were found to have a reduced bactericidal ability compared to frogs from more established areas (Goetz et al. 2018). These contradictory results from amphibians may reflect a complex mechanistic difference in immune investment of invading species. We suggest that studying plasticity of the immune system during range expansion of these amphibians might provide further insights into the drivers of invasion of those species and might help to explain contradictory findings. Based on the IPH we expect that immune plasticity benefits individuals of invasive populations (1) in the

early stages of invasion by reducing investment in the immune system and (2) in later stages of invasion by providing effective defence against novel pathogens.

Invasive populations can have higher or lower genetic diversity than their native conspecifics (Roman and Darling 2007; Edelaar et al. 2015). Plasticity in immunity might either compensate for potentially lower genotypic variation (phenotypic plasticity) of invasive populations or for higher genetic diversity. Thus, the higher plasticity in immunity in the invasive population of this study may be caused by high phenotypic plasticity or potentially be an indicator of increased genetic variation. Studies of the genotypic diversity of the immune system (e.g. MHC and other immune genes) (Acevedo-Whitehouse and Cunningham 2006) of Egyptian geese from both populations could help to clarify the underlying mechanisms of the higher variance in immune effectors between the two populations in this study.

Intraspecific comparisons ('bio geographic' approach), (Roy and Lawson Handley 2012; Cornet et al. 2016) are essential for studying the IPH as interspecific comparisons of immune function are less robust (Matson et al. 2006). Common garden experiments combined with immune challenge or infection experiments would help to clarify the susceptibility to infection during range expansion of invasive species. Moreover, we recommend reanalysing data from studies on the revised-EICA by applying our approach of simultaneously investigating changes in mean – and in the variance – of immune markers. This approach could identify drivers of successful invasion and potentially predict population trends for neozotic species.

Acknowledgments

This research was undertaken as part of the Graduate School IMPact-Vector funded by the Senate Competition Committee grant (SAW-2014-SGN-3) of the Leibniz Association and financially supported by the Ministry of Rhineland-Palatinate (Ministerium für Umwelt, Energie, Ernährung und Forsten, Project Nr: Gz. 105-63 313/2015-40). Hanna Prüter is also an associated doctoral student of the GRK2046 from the German Research Foundation (DFG). Additional support was received from the project AquaVir (Leibniz Association, SAW-2015-IZW-1 440), for which we are grateful.

We want to thank Katja Pohle, Lorena Derezanin, Elke Dyrks, Gabriele Grotehenn, Michaela Mann, Tanja Bartmann, Nadine Jahn, Oliver Krone, Lea Jäger, Felix Prüter, Jannis Twietmeyer, Sophie Ewert and Manuela Merling de Chapa for their assistance with the study in Germany. We thank all the hunters for providing us geese for parasitological investigation. We are thankful to Vera Menges, Bettina Wachter, Rebekka Müller, Neil Thomson, Mark

Middendorff, Hellmuth von Seydlitz and Frikkie Booysen (METCO), Jessica Sack and Mark Boorman for their enormous help in Namibia. We are grateful to Sophie Bourgeon for kindly providing help with the IgY ELISA and to Bart Kempnaers for his valuable comments on an earlier version of the manuscript.

Statement of authorship

GÁC, HP, KM and MF designed the study; HP, ST, JM, HK, ADG, KM, DL, GÁC coordinated different parts of the study; HP, ST and NB collected the data in Germany; HP, GM, JM, RP and GÁC collected the data in Namibia; HP, GvSH, KM, DL, GÁC performed laboratory analysis; HP and MF analysed the data; HP wrote the manuscript with the contributions of all the co-authors.

References

- Acevedo-Whitehouse K, Cunningham A (2006) Is MHC enough for understanding wildlife immunogenetics? *Trends in Ecology & Evolution* 21:433–438. doi: 10.1016/j.tree.2006.05.010
- Al-Sabi MNS, Chriél M, Jensen TH, Enemark HL (2013) Endoparasites of the raccoon dog (*Nyctereutes procyonoides*) and the red fox (*Vulpes vulpes*) in Denmark 2009–2012 – A comparative study. *International Journal for Parasitology: Parasites and Wildlife* 2:144–151. doi: 10.1016/j.ijppaw.2013.04.001
- Bauer H-G, Woog F (2008) Nichtheimische Vogelarten (Neozoen) in Deutschland, Teil I: Auftreten, Bestände und Status - Non-native and naturalized bird species (neozoa) in Germany, part I: occurrence, population size and status. *Vogelwarte* 46, 2008: 157 – 194
- Blossey B, Notzold R (1995) Evolution of Increased Competitive Ability in Invasive Nonindigenous Plants: A Hypothesis. *The Journal of Ecology* 83:887. doi: 10.2307/2261425
- Bourgeon S, Kauffmann M, Geiger S, et al (2010) Relationships between metabolic status, corticosterone secretion and maintenance of innate and adaptive humoral immunities in fasted re-fed mallards. *Journal of Experimental Biology* 213:3810–3818. doi: 10.1242/jeb.045484
- Bourgeon S, Raclot T (2006) Corticosterone selectively decreases humoral immunity in female eiders during incubation. *Journal of Experimental Biology* 209:4957–4965. doi: 10.1242/jeb.02610
- Brooks ME, Kristensen K, van Benthem KJ, et al (2017) Modeling Zero-Inflated Count Data With glmmTMB. -. doi: 10.1101/132753
- Brown GP, Phillips BL, Dubey S, Shine R (2015) Invader immunology: invasion history alters immune system function in cane toads (*Rhinella marina*) in tropical Australia. *Ecology Letters* 18:57–65. doi: 10.1111/ele.12390
- Brown GP, Shine R (2014) Immune response varies with rate of dispersal in invasive cane toads (*Rhinella marina*). *PLoS ONE* 9:e99734. doi: 10.1371/journal.pone.0099734
- Clark NJ, Olsson-Pons S, Ishtiaq F, Clegg SM (2015) Specialist enemies, generalist weapons and the potential spread of exotic pathogens: malaria parasites in a highly invasive

- bird. *International Journal for Parasitology* 45:891–899. doi: 10.1016/j.ijpara.2015.08.008
- Cornet S, Brouat C, Diagne C, Charbonnel N (2016) Eco-immunology and bioinvasion: revisiting the evolution of increased competitive ability hypotheses. *Evolutionary Applications* 9:952–962. doi: 10.1111/eva.12406
- Demas GE, Zysling DA, Beechler BR, et al (2011) Beyond phytohaemagglutinin: assessing vertebrate immune function across ecological contexts: Assessing vertebrate immune function across ecological contexts. *Journal of Animal Ecology* 80:710–730. doi: 10.1111/j.1365-2656.2011.01813.x
- Diagne C, Ribas A, Charbonnel N, et al (2016) Parasites and invasions: changes in gastrointestinal helminth assemblages in invasive and native rodents in Senegal. *International Journal for Parasitology* 46:857–869. doi: 10.1016/j.ijpara.2016.07.007
- Doster GL, Goater CP (1997) Collection and quantification of avian helminths and protozoa. In: *Host-parasite evolution—general principles and avian models*. Oxford University Press, Oxford, p 396–418
- Duncan RP, Blackburn TM, Sol D (2003) The ecology of bird introductions. *Annual Review of Ecology, Evolution, and Systematics* 34:71–98. doi: 10.1146/annurev.ecolsys.34.011802.132353
- Edelaar P, Roques S, Hobson EA, et al (2015) Shared genetic diversity across the global invasive range of the monk parakeet suggests a common restricted geographic origin and the possibility of convergent selection. *Molecular Ecology* 24:2164–2176. doi: 10.1111/mec.13157
- Ellis VA, Medeiros MCI, Collins MD, et al (2017) Prevalence of avian haemosporidian parasites is positively related to the abundance of host species at multiple sites within a region. *Parasitology Research* 116:73–80. doi: 10.1007/s00436-016-5263-3
- Fox J, Weisberg S (2011) *An R companion to applied regression*. CA: SAGE Publications Inc 2nd ed. Thousand Oaks:
- Ghalambor CK, Mc Kay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* 21:394–407. doi: 10.1111/j.1365-2435.2007.01283.x
- Giraudeau M, Czirják GÁ, Duval C, et al (2010) Effect of restricted preen-gland access on maternal self maintenance and reproductive investment in Mallards. *PLoS ONE* 5:e13555. doi: 10.1371/journal.pone.0013555
- Goetz SM, Romagosa CM, Appel AG, et al (2018) Reduced innate immunity of Cuban treefrogs at leading edge of range expansion. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*. doi: 10.1002/jez.2146
- Gyimesi A, Lensink R (2010) Risk analysis of the Egyptian Goose in the Netherlands. In: Bureau Waardenburg BV. The Netherlands
- Hinz K, Ryll M, Köhler B, Glünder G (1998) Phenotypic characteristics of *Riemerella anatipestifer* and similar micro-organisms from various hosts. *Avian Pathology* 27:33–42. doi: 10.1080/03079459808419272
- Hubálek Z (2004) An annotated checklist of pathogenic microorganisms associated with migratory birds. *Journal of Wildlife Diseases* 40:639–659. doi: 10.7589/0090-3558-40.4.639
- Keane R, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* 17:164–170. doi: 10.1016/S0169-5347(02)02499-0
- Keogh CL, Miura O, Nishimura T, Byers JE (2017) The double edge to parasite escape: invasive host is less infected but more infectable. *Ecology* 98:2241–2247. doi: 10.1002/ecy.1953
- Klasing KC (2004) The costs of immunity. *Acta Zoologica Sinica* 50 (6):961–969

- Lee KA, Klasing KC (2004) A role for immunology in invasion biology. *Trends in Ecology & Evolution* 19:523–529. doi: 10.1016/j.tree.2004.07.012
- Lima M, Simpson L, Fecchio A, Kyaw C (2010) Low prevalence of haemosporidian parasites in the introduced house sparrow (*Passer domesticus*) in Brazil. *Acta Parasitologica* 55.: doi: 10.2478/s11686-010-0055-x
- Llewellyn D, Thompson MB, Brown GP, et al (2012) Reduced investment in immune function in invasion-front populations of the cane toad (*Rhinella marina*) in Australia. *Biological Invasions* 14:999–1008. doi: 10.1007/s10530-011-0135-3
- Lövei GL (1997) Global change through invasion: Biodiversity. *Nature* 388:627–628. doi: 10.1038/41665
- Martinez J, Tomas G, Merino S, et al (2003) Detection of serum immunoglobulins in wild birds by direct ELISA: a methodological study to validate the technique in different species using antichickens antibodies. *Functional Ecology* 17:700–706. doi: 10.1046/j.1365-2435.2003.00771.x
- Marzal A, Ricklefs RE, Valkiūnas G, et al (2011) Diversity, loss, and gain of Malaria parasites in a globally invasive bird. *PLoS ONE* 6:e21905. doi: 10.1371/journal.pone.0021905
- Matson KD, Cohen AA, Klasing KC, et al (2006) No simple answers for ecological immunology: relationships among immune indices at the individual level break down at the species level in waterfowl. *Proceedings of the Royal Society B: Biological Sciences* 273:815–822. doi: 10.1098/rspb.2005.3376
- Matson KD, Ricklefs RE, Klasing KC (2005) A hemolysis–hemagglutination assay for characterizing constitutive innate humoral immunity in wild and domestic birds. *Developmental & Comparative Immunology* 29:275–286. doi: 10.1016/j.dci.2004.07.006
- McGeoch MA, Butchart SHM, Spear D, et al (2010) Global indicators of biological invasion: species numbers, biodiversity impact and policy responses: Invasive alien species indicator: 2010 Biodiversity Target. *Diversity and Distributions* 16:95–108. doi: 10.1111/j.1472-4642.2009.00633.x
- Møller AP, Cassey P (2004) On the relationship between T-cell mediated immunity in bird species and the establishment success of introduced populations. *Journal of Animal Ecology* 73:1035–1042. doi: 10.1111/j.0021-8790.2004.00879.x
- Morand S, Bordes F, Chen H-W, et al (2015) Global parasite and *Rattus* rodent invasions: The consequences for rodent-borne diseases. *Integrative Zoology* 10:409–423. doi: 10.1111/1749-4877.12143
- Nehring S, Skowronek S (2017) Die invasiven gebietsfremden Arten der Unionsliste der Verordnung (EU) Nr. 1143/2014. Erste Fortschreibung 2017. Bundesamt für Naturschutz, Germany
- Pap PL, Cziráj GÁ, Vágási CI, et al (2010) Sexual dimorphism in immune function changes during the annual cycle in house sparrows. *Naturwissenschaften* 97:891–901. doi: 10.1007/s00114-010-0706-7
- Pap PL, Vágási CI, Vincze O, et al (2015) Physiological pace of life: the link between constitutive immunity, developmental period, and metabolic rate in European birds. *Oecologia* 177:147–158. doi: 10.1007/s00442-014-3108-2
- Prüter H, Cziráj GÁ, Twietmeyer S, et al (2018a) Sane and sound: a serologic and molecular survey for selected infectious agents in neozootic Egyptian geese (*Alopochen aegyptiacus*) in Germany. *European Journal of Wildlife Research* 64.: doi: 10.1007/s10344-018-1231-9
- Prüter H, Franz M, Auls S, et al (2018b) Chronic lead intoxication decreases intestinal helminth species richness and infection intensity in mallards (*Anas platyrhynchos*). *Science of The Total Environment* 644:151–160. doi: 10.1016/j.scitotenv.2018.06.297

- Pyšek P, Richardson DM (2010) Invasive Species, Environmental Change and Management, and Health. *Annual Review of Environment and Resources* 35:25–55. doi: 10.1146/annurev-environ-033009-095548
- Raberg L, Graham AL, Read AF (2009) Decomposing health: tolerance and resistance to parasites in animals. *Philosophical Transactions of the Royal Society B: Biological Sciences* 364:37–49. doi: 10.1098/rstb.2008.0184
- R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria
- Roman J, Darling J (2007) Paradox lost: genetic diversity and the success of aquatic invasions. *Trends in Ecology & Evolution* 22:454–464. doi: 10.1016/j.tree.2007.07.002
- Romeo C, Wauters LA, Ferrari N, et al (2014) Macroparasite fauna of alien grey squirrels (*Sciurus carolinensis*): composition, variability and implications for native species. *PLoS ONE* 9:e88002. doi: 10.1371/journal.pone.0088002
- Rowe M, Cziráj GÁ, Lifjeld JT, Giraudeau M (2013) Lysozyme-associated bactericidal activity in the ejaculate of a wild passerine: Lysozyme in the ejaculate of a wild bird. *Biological Journal of the Linnean Society* 109:92–100. doi: 10.1111/bij.12044
- Roy HE, Lawson Handley L-J (2012) Networking: a community approach to invaders and their parasites. *Functional Ecology* 26:1238–1248. doi: 10.1111/j.1365-2435.2012.02032.x
- Ryll M, Christensen H, Bisgaard M, et al (2001) Studies on the prevalence of *Riemerella anatipestifer* in the upper respiratory tract of clinically healthy ducklings and characterization of untypable strains. *J Vet Med B Infect Dis Vet Public Health* 48:537–546
- Shea K, Chesson P (2002) Community ecology theory as a framework for biological invasions. *Trends in Ecology & Evolution* 17:170–176. doi: 10.1016/S0169-5347(02)02495-3
- Sheldon BC, Verhulst S (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *Trends in Ecology & Evolution* 11:317–321. doi: 10.1016/0169-5347(96)10039-2
- Shwartz A, Strubbe D, Butler CJ, et al (2009) The effect of enemy-release and climate conditions on invasive birds: a regional test using the rose-ringed parakeet (*Psittacula krameri*) as a case study. *Diversity and Distributions* 15:310–318. doi: 10.1111/j.1472-4642.2008.00538.x
- Strauss A, White A, Boots M (2012) Invading with biological weapons: the importance of disease-mediated invasions. *Functional Ecology* 26:1249–1261. doi: 10.1111/1365-2435.12011
- Torchin ME, Lafferty KD, Dobson AP, et al (2003) Introduced species and their missing parasites. *Nature* 421:628–630. doi: 10.1038/nature01346
- Valente R, Ibañez LM, Lorenti E, et al (2014) Helminth parasites of the European starling (*Sturnus vulgaris*) (Aves, Sturnidae), an invasive bird in Argentina. *Parasitology Research* 113:2719–2724. doi: 10.1007/s00436-014-3928-3
- van der Most PJ, de Jong B, Parmentier HK, Verhulst S (2011) Trade-off between growth and immune function: a meta-analysis of selection experiments: Trade-off between growth and immune function. *Functional Ecology* 25:74–80. doi: 10.1111/j.1365-2435.2010.01800.x
- White TA, Perkins SE (2012) The ecoimmunology of invasive species. *Functional Ecology* 26:1313–1323. doi: 10.1111/1365-2435.12012

Supplementary data

Table S1:

Month of sampling	Status	Origin	non-breeding		breeding	
			male	female	male	female
2015						
April	invasive	Germany	1	0	7	4
June	invasive	Germany	1	1	17	12
July	invasive	Germany	3	0	11	11
October	invasive	Germany	2	3	2	2
November	invasive	Germany	1	0	0	0
2016						
February	native	Namibia	9	12	0	0
June	invasive	Germany	1	0	12	9
July	invasive	Germany	2	0	5	3

CHAPTER 2

Table S2: Summary output of the GLMMs to test for differences in the mean (in the conditional model) and variance (in the dispersion model) of the respective “low cost” immune effector compared between native and invasive Egyptian geese

Effect on the mean						Effect on the variance				
Response	Predictor (fixed effects)	Estimate	Std. Error	z	p		Estimate	Std. Error	z	p
IgY	Intercept	0.75001	0.02141	35.04	<2e-16 ***	Intercept	-3.8156	0.1412	-27.025	< 2e-16 ***
	Status	0.04835	0.04357	1.11	0.2671	status	-1.0285	0.3457	-2.975	0.00293 **
	Sex	-0.04258	0.02446	-1.74	0.0817 .					
	reproduction	-0.04270	0.04169	-1.02	0.3057					
log(Lysozyme)	Intercept	1.11349	0.07420	15.006	<2e-16 ***	Intercept	-1.1904	0.1368	-8.70	< 2e-16 ***
	Status	0.14721	0.16312	0.903	0.367	status	-1.2431	0.3462	-3.59	0.00033 ***
	Sex	-0.07812	0.08457	-0.924	0.356					
	reproduction	0.01005	0.15863	0.063	0.949					
Haemagglutination	Intercept	7.2142	0.1927	37.44	<2e-16 ***	Intercept	0.5771	0.1414	4.080	4.49e-05 ***
	Status	-0.5815	0.4062	-1.43	0.1523	status	-0.9183	0.3396	-2.704	0.00684 **
	Sex	-0.3684	0.2204	-1.67	0.0947 .					
	reproduction	-0.2368	0.3857	-0.61	0.5393					
Haemolysis	Intercept	6.16166	0.17711	34.79	<2e-16 ***	Intercept	0.3872	0.1415	2.736	0.00622 **
	Status	-0.72516	0.37448	-1.94	0.0528 .	status	-0.7976	0.3407	-2.341	0.01922 *
	Sex	-0.03806	0.20527	-0.19	0.8529					
	reproduction	-0.42019	0.35082	-1.20	0.2310					

CHAPTER 2

Table S3 Summary output of the GLMMs to test for differences in the mean and variance of the respective “high cost” immune effector compared between native and invasive Egyptian geese

Effect on the mean						Effect on the variance				
Response	Predictor (fixed effects)	Estimate	Std. Error	z	p		Estimate	Std. Error	z	p
Total leucocytes	Intercept	85.331	5.928	14.396	<2e-16 ***	Intercept	6.19793	0.13976	44.35	<2e-16 ***
	status	-9.760	12.762	-0.765	0.4444	status	-0.07186	0.34425	-0.21	0.835
	sex	-11.555	3.993	-2.894	0.0038 **					
	reproduction	16.122	7.078	2.278	0.0227 *					
log(haptoglobin binomial)	Intercept	1.7129	0.4198	4.080	4.5e-05 ***					
	status	-2.0296	0.8368	-2.425	0.0153 *					
	sex	-0.9708	0.4681	-2.074	0.0381 *					
	reproduction	0.2173	0.7166	0.303	0.7617					

Table S4: Summary output of the GLMMs to test for differences in the mean and variance of the differential white blood cells compared between native and invasive Egyptian geese

Effect on the mean						Effect on the variance				
Response	Predictor (fixed effects)	Estimate	Std. Error	z	p		Estimate	Std. Error	z	p
basophil granulocytes	Intercept	1.42532	0.10786	13.214	<2e-16 ***	Intercept	-0.5348	0.1348	-3.966	7.3e-05 ***
	status	0.11548	0.24031	0.481	0.631	status	-0.4880	0.3368	-1.449	0.147
	sex	-0.19872	0.13009	-1.528	0.127					
	reproduction	-0.03004	0.21373	-0.141	0.888					
eosinophil granulocytes	Intercept	3.03294	0.24407	12.426	< 2e-16 ***	Intercept	1.1671	0.1350	8.645	< 2e-16 ***
	status	2.76064	0.52452	5.263	1.42e-07 ***	status	-1.0498	0.3394	-3.093	0.00198 **
	sex	-0.08153	0.28233	-0.289	0.773					
	reproduction	0.17518	0.50016	0.350	0.726					
sqrt(heterophil granulocytes)	Intercept	3.9632	0.1946	20.362	< 2e-16 ***	Intercept	0.7067	0.1349	5.241	1.6e-07 ***
	status	-1.5726	0.4205	-3.740	0.000184 ***	status	-0.9608	0.3371	-2.850	0.00437 **
	sex	-0.2066	0.2263	-0.913	0.361127					
	reproduction	0.2738	0.3974	0.689	0.490832					
lymphocytes	Intercept	39.444	4.819	8.184	2.74e-16 ***	Intercept	5.1786	0.1397	37.07	<2e-16 ***
	status	-25.537	10.672	-2.393	0.01672 *	status	-0.5684	0.3575	-1.59	0.112
	sex	-8.089	2.351	-3.441	0.00058 ***					
	reproduction	11.680	4.135	2.825	0.00473 **					
log(monocytes)	Intercept	2.16666	0.20471	10.584	<2e-16 ***	Intercept	-0.3884	0.1391	-2.792	0.00523 **
	status	0.68564	0.43262	1.585	0.113	status	-1.0521	0.3607	-2.917	0.00353 **
	sex	-0.04229	0.13565	-0.312	0.755					
	reproduction	0.12671	0.24652	0.514	0.607					

Table S5: Methods of the parasite screening and serology of Egyptian geese from Namibia and Germany.

Parasitology:	Group	Transmission mode	Method	Material
	Ectoparasites	direct	morphology	carcasses
	Euhirundidae	direct	morphology	carcasses
	Intestinal helminthes		morphology	carcasses
	Cestoda	vector	morphology	carcasses
	Nematoda	direct	morphology	carcasses
	Trematoda	vector	morphology	carcasses
	Heamatozoa	vector	morphology	blood smear
Bacteria:	Target genes	Transmission mode	Method	Material
	<i>Riemerella anatipestifer</i> 16S rRNA gene	direct	PCR	pharyngeal swab
	<i>Mycoplasma</i> spp. 16S rRNA gene	direct	PCR	pharyngeal swab
Serology:	Antigen	Transmission mode	Method	Material
	IAV	direct	ELISA	serum
	AAvV-1	direct	ELISA	serum
	WNV	vector	ELISA	serum

Abbreviations: IAV= Influenza A virus; AAvV-1= *Avian avulavirus 1*; WNV= *West Nile virus*; ELISA= Enzyme linked immunosorbent assay; PCR= Polymerase chain reaction

CHAPTER 3

**Having bird schistosomes in mind—
the first detection of *Bilharziella polonica*
(Kowalewski 1895) in the bird neural system**

Published in *Parasitology Research*

ISSN 0932-0113

Volume 116 Number 3

Parasitol Res (2017) 116:865-870

DOI 10.1007/s00436-016-5359-9

You have to purchase this part online.

Prüter Hanna¹, Sitko Jilji², Krone Oliver¹

¹Leibniz Institute for Zoo and Wildlife Research, Department of Wildlife Diseases, Alfred-Kowalke-Straße 17, 10315 Berlin, Germany

²Komenský Museum, Horní nám. 7, 750 11 Přerov 2, Czech Republic

CHAPTER 4

**Sane and sound -
A serologic and molecular survey for selected
infectious agents in neozootic Egyptian geese
(*Alopochen aegyptiaca*) in Germany**

Published in the *European Journal of Wildlife Research*

ISSN (print) 1612-4642 (online) 1439-0574

Volume 64 Number 71

DOI: 10.1007/s10344-018-1231-9

You have to purchase this part online.

Prüter Hanna¹, Czirják Gábor Árpád¹, Twietmeyer Sönke², Harder Timm³, Grund
Christian³, Mühlendorfer Kristin¹, Lüschoff Dörte⁴

¹Leibniz Institute for Zoo and Wildlife Research, Department of Wildlife Diseases, Alfred-Kowalke-Straße 17, 10315 Berlin, Germany; prueter@izw-berlin.de

²Department of Research and Documentation, Eifel National Park, Urftseestraße 43, D-53937 Schleiden-Gemünd, Germany

³Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Südufer 10, 17493 Greifswald – Insel Riems, Germany

⁴Freie Universität Berlin, Institute of Poultry Diseases, Königsweg 63, 14163 Berlin, Germany

GENERAL DISCUSSION

This thesis focuses on two anthropogenic impacted waterfowl species of freshwater habitats, the mallard, as the most common native duck species and the Egyptian goose, which is currently invading Europe. The anthropogenic driven changes to freshwater habitats/ecosystems, which were emphasized were environmental lead pollution (**chapter 1**) and the introduction of the Egyptian goose to Europe (**chapter 2 and 4**). Additionally, neural bird schistosomes in mallards from German freshwaters, whose cercaria might cause swimmer's itch, were investigated (**chapter 3**).

Impact of anthropogenic changes to parasite and host community structures

Using the example of intestinal helminth parasites in mallards, it could be shown that anthropogenic induced environmental lead pollution resulting in long term accumulation of lead in mallards, is related to significant loss of intestinal helminth species richness and infection intensity in mallards (**chapter 1**). Thus, studying intestinal helminths as sentinels for lead pollution in mallards, clearly highlights that lead intoxication not only directly affects waterfowl health (as it has been shown by others before) (Sears 1988; Degernes et al. 2006; Newth et al. 2013; Martinez-Haro, Green, and Mateo 2011; Vallverdú-Coll et al. 2016) but freshwater ecosystem species communities in a more complex manner than previously acknowledged.

Parasite-host systems are coevolved and balanced systems in which the parasites take away energetic resources from the host without running the risk to kill the host. External influences, which throw this parasite-host system out of balance, might lead to severe consequences for the vertebrate host by weakening its immune system and/or affecting its parasite lifecycles (Schmid-Hempel 2011). Here, it is shown that environmental lead pollution negatively impacts biodiversity indices of intestinal helminth parasites of wild mallards.

Moreover, intestinal helminth parasites may accumulate higher lead dosages than the vertebrate host and therefore function as lead sink (Sures et al. 2017). Hence, intestinal helminthes might protect their hosts from higher lead dosages. Results of **chapter 1** indicate that also in mallards, intestinal helminths might function as lead sink. Still, lower infection intensities in ducks showing higher lead levels in bones indicate

susceptibility towards lead also in those parasite species. Consequently, intestinal helminthes, which accumulate lead are presumably threatened by excessive chronic lead intake.

Summarizing, the results of **chapter 1** underline the importance of studying parasite-host interactions in the frame of anthropogenic changes to the environment. Beside the health of vertebrate hosts, parasite biodiversity is a subject of conservation concern (Gómez and Nichols 2013). Vice versa, imbalanced parasite-host interactions might bare health risks for vertebrate hosts. Hence, it can be recommended to generally lower anthropogenic induced environmental pollution to preserve healthy species communities in German freshwater habitats.

Moreover and beside the indirect impact of environmental lead on parasite species richness and infection intensity, this thesis shows that mallards from German fresh waters show comparably high chronic lead burden (Ferreya et al. 2014, 2015). This finding indicates that lead pollution in German freshwater habitats is potentially still high although the use of lead based ammunition for hunting waterfowl is prohibited in most of the German federal states (JWVG, n.d.; BayJG 1978; LJG-NRW 1994; NJagdG 2001; BbgJagdDV 2004; LJG 2010). Still, due to their partly migratory behavior, lead intoxication in mallards could also be caused by high lead burden of freshwater habitats in other European countries. Nevertheless, European studies on lead burden of waterfowl indicate that the compliance of hunters to use lead-free ammunition only increased after enforcement and vigilance of rangers was intensified (Mateo et al., 2013). Since the regulation on the use of lead-free ammunition to hunt waterfowl is not consistent within Germany, a standardization of law and regular controls could help to lower lead burden in freshwater habitats and consequently reduce the risk of species biodiversity losses.

By studying parasites and immunity of native and invasive Egyptian goose a new hypothesis (“Immunological plasticity hypothesis”) was developed, which integrates the role of newly acquired pathogens into existing hypotheses on species invasions. In support of the novel hypothesis the variance of immune marker is significantly higher in the invasive than in the native population of Egyptian goose. Thus, the invasive Egyptian goose population in Germany seems to be both able to reduce investment into immunity if possible (at low parasite burden) and defend against newly acquired parasites when necessary. This flexibility in immune investment might be the fundament of the successful invasion of the Egyptian goose to Europe and is potentially a general mechanism helping non-native species to become invasive.

Potential threats for native species, poultry and humans

Generally, birds can function as vectors for infectious diseases which can affect livestock and are potentially of zoonotic risk. For instance, cercaria of bird schistosomes, including *T. regenti*, whose final hosts are water birds, may cause cercarial dermatitis (swimmer's itch) in humans, which is regarded as a re-emerging disease (Horák and Kolářová 2011; Soldánová et al. 2013). Particularly climate change was shown to increase the risk of re-emerging diseases. Climate change driven changes in behavior traits of migratory birds, shifts in seasonal or temperature dependent processes of intermediate hosts (snails) and the frequency of transmission and intensity of infection, are potential risk factors regarding the dispersal of bird schistosomes to new regions and therefore increases the availability in wetland habitats (Horák and Kolářová, 2011).

In **chapter 3**, a 21% prevalence of *Trichobilharzia regenti*, a neural bird schistosome, was found in native mallards from German freshwaters. This result is consistent with previous studies in water birds, which reported prevalences of *T. regenti* ranging from 24 to 74.5% in different definitive bird hosts (Kolářová, Skirnisson, and Horák 1999; Rudolfová, Sitko, and Horák 2002; Jouet et al. 2008). Moreover, *Bilharziella polonica* was detected either between the brain membranes (meninges) and the brain, in the spinal cord or in the intestine of 12% of the mallards, which is the first description of this species from the birds neural system. The presence of *B. polonica* in the birds neural system indicates that this species has a broader spectrum of target organs in the definitive host than previously described and that the spectrum of neural bird schistosome species is broader than previously acknowledged. Thus, for *B. polonica*, parasite-host interaction is of higher complexity than previously known, which should be further investigated for different definitive host species as well as for potential accidental hosts. Monitoring bird schistosomes in vertebrate hosts in the light of anthropogenic changes to ecosystems (such as climate change, pollution, species invasion) should be considered to increase knowledge on this particular parasite-host system in the view of the fact that swimmer's itch is re-emerging in Europe.

In **chapter 4**, it was shown that Egyptian geese are frequent carriers of *Riemerella anatipestifer* and furthermore provided serological evidence of exposure to selected viral pathogens of relevance for native birds and poultry. This finding indicates that invasive Egyptian geese are involved in native pathogen transmission cycles and therefore might

potentially spillover and spillback pathogens to native species and poultry. Additionally, in **chapter 4**, seroprevalence of viral pathogens were compared between Egyptian geese and published results from studies on other goose species (Kruckenberg et al. 2011; Kistler et al. 2012; Brown et al. 2010; Hlinak et al. 1998; Bradshaw and Trainer 1966; Bönner et al. 2003). Reported differences between different goose species are likely driven by season or ecological differences between the species. Thus, future research of pathogen transmission cycles among wild birds and their epidemiological roles should include both the role of native and invasive species in the maintenance and spread of pathogens. This will help to assess potential changes to health indices of native species (e.g. parasite-host interactions, parasite/pathogen abundance and disease transmission) caused or promoted by invasive species.

Outlook

Freshwater species biodiversity is threatened by human activities and anthropogenic impact on a global scale (Vörösmarty et al. 2010). Waterfowl health and population dynamics have been found to be negatively impacted by climate change, industrialization of agriculture, increasing recreational use of water habitats, invasion of species, intensification of ship traffic, aridification of wetlands and hunting (Wahl et al., 2011). Increasing knowledge on waterfowl health, their epidemiological roles, threats and parasites can help to assess those threats in terms of biodiversity loss in freshwater habitats. Particularly the results of **chapter 1**, which highlight the negative impact of lead pollution on parasite species richness in freshwater ecosystems, are applicable to implement and evaluate strategies of international wetland protection. Thus, reducing lead pollution in freshwater ecosystems could help to protect species biodiversity in German freshwater habitats.

Invasive species can significantly harm native species and biodiversity (Lövei 1997; McGeoch et al. 2010). Especially freshwater ecosystems are vulnerable and threatened by the impact of invasive species (Strayer 2010). Thus, monitoring of non-native species and their potential to become invasive is of great importance for biodiversity conservation especially in freshwater ecosystems. Moreover, invasive species may affect animal and public health by playing important epidemiological roles in spreading and maintaining several micro- and macro-parasites (Strauss, White, and Boots 2012). Thus, studying health related aspects in the frame of species invasion may help to

reduce negative impact of invasive species on native biodiversity. Predictions arising from the “Immunological Plasticity Hypothesis”, proposed in **chapter 2**, should be tested in studies on invasive vertebrates to investigate the predictive power of variability in immune markers for population trends of non-native species including the probability to become invasive.

In Europe, based on §5 Regulation (EU) No 1143/2014, a risk assessment of invasive species conducted by each European Union member state is mandatory. According to the formulation of Article 5, this risk assessment should be based on available scientific knowledge. Regarding this European legislation, the results from **chapter 4**, which indicate that German Egyptian geese are involved in transmission cycles of infectious agents relevant for wild birds and poultry, can be a basis for the evaluation of its health impact on native species (Article 5 f). Moreover, the new hypothesis proposed in **chapter 2** may help to create projections of likely future distributions of invasive species (§5 e) in general by studying variance of immune markers in the different phases of invasion.

In conclusion, this thesis highlights the importance to encounter the complex interplay between health indices of waterfowl and anthropogenic impacted environmental factors such as pollution and species invasion to studies on habitat biodiversity and wildlife health. Moreover, it provides one of the very few studies on infectious agents and immunity of invasive birds and raises a new hypothesis in the field of vertebrate invasion, which will help to study health related drivers of invasion processes.

References

- BayJG (1978) Bayerisches Jagdgesetz. <http://www.gesetze-bayern.de/Content/Document/BayJG>true>. Accessed 10 April 2018
- BbgJagdDV (2004) Verordnung zur Durchführung des Jagdgesetzes für das Land Brandenburg, §5a. <https://bravors.brandenburg.de/de/verordnungen-212978>. Accessed 10 April 2018
- Bönner BM, Jäger S, Reichel U, Lutz W, Wissing J, Knickmeier W, Gerlich WH, Wend UC, Bolte AL, Kaleta EF (2003) Untersuchungen zum Gesundheitsstatus von Kanadagänsen (*Branta canadensis*, Linnaeus, 1758) in Nordrhein-Westfalen an Hand der Analyse von Eiern. *Zeitschrift für Jagdwissenschaft* 49:61–76. doi: 10.1007/BF02192014
- Bradshaw JE, Trainer DO (1966) Some infectious diseases of waterfowl in the Mississippi flyway. *The Journal of Wildlife Management* 30:570–576. doi: 10.2307/3798749
- Brown JD, Luttrell MP, Berghaus RD, Kistler W, Keeler SP, Howey A, Wilcox B, Hall J, Niles L, Dey A, Knutsen G, Fritz K, Stallknecht DE (2010) Prevalence of antibodies to type A influenza virus in wild avian species using two serological assays. *Journal of Wildlife Diseases* 46:896–911. doi: 10.7589/0090-3558-46.3.896
- Degernes L, Heilman S, Trogdon M, Jordan M, Davison M, Kraege D, Correa M, Cowen P (2006) Epidemiologic investigation of lead poisoning in Trumpeter and Tundra swans in Washington State, USA, 2000-2002. *Journal of Wildlife Diseases* 42:345–358. doi: 10.7589/0090-3558-42.2.345
- Ferreya H, Beldomenico PM, Marchese K, Romano M, Caselli A, Correa AI, Uhart M (2015) Lead exposure affects health indices in free-ranging ducks in Argentina. *Ecotoxicology* 24:735–745. doi: 10.1007/s10646-015-1419-7
- Ferreya H, Romano M, Beldomenico P, Caselli A, Correa A, Uhart M (2014) Lead gunshot pellet ingestion and tissue lead levels in wild ducks from Argentine hunting hotspots. *Ecotoxicology and Environmental Safety* 103:74–81. doi: 10.1016/j.ecoenv.2013.10.015
- Gómez A, Nichols E (2013) Neglected wild life: Parasitic biodiversity as a conservation target. *International Journal for Parasitology: Parasites and Wildlife* 2:222–227. doi: 10.1016/j.ijppaw.2013.07.002
- Hlinak A, Müller T, Kramer M, Mühle RU, Liebherr H, Ziedler K (1998) Serological survey of viral pathogens in bean and white-fronted geese in Germany. *Journal of Wildlife Diseases* 34:479–486. doi: 10.7589/0090-3558-34.3.479
- Horák P, Kolářová L (2011) Snails, waterfowl and cercarial dermatitis: bird schistosomes. *Freshwater Biology* 56:779–790. doi: 10.1111/j.1365-2427.2010.02545.x
- Jouet D, Ferté H, Depaquit J, Rudolfová J, Latour P, Zanella D, Kaltenbach ML, Léger N (2008) *Trichobilharzia* spp. in natural conditions in Annecy Lake, France. *Parasitology Research* 103:51–58. doi: 10.1007/s00436-008-0926-3

- JWMG Jagd- und Wildtiermanagementgesetz §31 Abschnitt 1 Satz 5.
<http://www.landesrecht-bw.de/jportal/?quelle=jlink&query=WildTManagG+BW&psml=bsbawueprod.psml&max=true&aiz=true>. Accessed 10 April 2018
- Kistler WM, Stallknecht DE, Deliberto TJ, Swafford S, Pedersen K, Why KV, Wolf PC, Hill JA, Bruning DL, Cumbee JC, Mickley RM, Betsill CW, Randall AR, Berghaus RD, Yabsley MJ (2012) Antibodies to avian influenza viruses in Canada geese (*Branta canadensis*): A potential surveillance tool? *Journal of Wildlife Diseases* 48:1097–1101. doi: 10.7589/2011-02-046
- Kolářová L, Skirnisson K, Horák P (1999) Schistosome cercariae as the causative agent of swimmer's itch in Iceland. *Journal of Helminthology* 73:215. doi: 10.1017/S0022149X99000335
- Kruckenbergh H, Müller T, Freuling C, Mühle R-U, Globig A, Schirrmeier H, Buss M, Harder T, Kramer M, Teske K, Polderdijk K, Wallschläger D, Hlinak A (2011) Serological and virological survey and resighting of marked wild geese in Germany. *European Journal of Wildlife Research* 57:1025–1032. doi: 10.1007/s10344-011-0514-1
- LJG (2010) Landesjagdgesetz §23 Abschnit 1 Satz 3.
http://landesrecht.rlp.de/jportal/portal/t/1dz7/page/bsrlpprod.psml?pid=Dokumentanzeige&showdoccase=1&js_peid=Trefferliste&documentnumber=1&numberofresults=69&fromdoctodoc=yes&doc.id=jlr-JagdGRP2010rahmen&doc.part=X&doc.price=0.0&doc.hl=1%23focuspoint. Accessed 10 April 2018
- LJG-NRW (1994) Landesjagdgesetz Nordrhein-Westfalen, §19 Absatz 1 Satz 4.
https://recht.nrw.de/lmi/owa/br_text_anzeigen?v_id=10000000000000000107. Accessed 10 April 2018
- Lövei GL (1997) Global change through invasion: Biodiversity. *Nature* 388:627–628. doi: 10.1038/41665
- Martinez-Haro M, Green AJ, Mateo R (2011) Effects of lead exposure on oxidative stress biomarkers and plasma biochemistry in waterbirds in the field. *Environmental Research* 111:530–538. doi: 10.1016/j.envres.2011.02.012
- McGeoch MA, Butchart SHM, Spear D, Marais E, Kleynhans EJ, Symes A, Chanson J, Hoffmann M (2010) Global indicators of biological invasion: species numbers, biodiversity impact and policy responses: Invasive alien species indicator: 2010 Biodiversity Target. *Diversity and Distributions* 16:95–108. doi: 10.1111/j.1472-4642.2009.00633.x
- Newth JL, Cromie RL, Brown MJ, Delahay RJ, Meharg AA, Deacon C, Norton GJ, O'Brien MF, Pain DJ (2013) Poisoning from lead gunshot: still a threat to wild waterbirds in Britain. *European Journal of Wildlife Research* 59:195–204. doi: 10.1007/s10344-012-0666-7
- NJagdG (2001) Niedersächsisches Jagdgesetz §24 Absatz 1 Satz 1.
<http://www.voris.niedersachsen.de/jportal/?quelle=jlink&query=JagdG+ND&psml=bsvorisprod.psml&max=true&aiz=true>. Accessed 10 April 2018

- Rudolfová J, Sitko J, Horák P (2002) Nasal schistosomes of wildfowl in the Czech Republic. *Parasitol Res* 88:1093–1095. doi: 10.1007/s00436-002-0634-3
- Schmid-Hempel P (2011) Evolutionary parasitology: the integrated study of infections, immunology, ecology, and genetics. Oxford University Press, Oxford ; New York
- Sears J (1988) Regional and seasonal variations in lead poisoning in the mute swan *Cygnus olor* in relation to the distribution of lead and lead weights, in the Thames area, England. *Biological Conservation* 46:115–134. doi: 10.1016/0006-3207(88)90095-X
- Soldánová M, Selbach C, Kalbe M, Kostadinova A, Sures B (2013) Swimmer’s itch: etiology, impact, and risk factors in Europe. *Trends in Parasitology* 29:65–74. doi: 10.1016/j.pt.2012.12.002
- Strauss A, White A, Boots M (2012) Invading with biological weapons: the importance of disease-mediated invasions. *Functional Ecology* 26:1249–1261. doi: 10.1111/1365-2435.12011
- Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* 55:152–174. doi: 10.1111/j.1365-2427.2009.02380.x
- Sures B, Nachev M, Selbach C, Marcogliese DJ (2017) Parasite responses to pollution: what we know and where we go in “Environmental Parasitology.” *Parasites & Vectors* 10.: doi: 10.1186/s13071-017-2001-3
- Vallverdú-Coll N, Mougeot F, Ortiz-Santaliestra ME, Rodríguez-Estival J, López-Antia A, Mateo R (2016) Lead exposure reduces carotenoid-based coloration and constitutive immunity in wild mallards: Lead exposure, coloration, and immunity in mallards. *Environmental Toxicology and Chemistry* 35:1516–1525. doi: 10.1002/etc.3301
- Vörösmarty CJ, McIntyre PB, Gessner MO, Dudgeon D, Prusevich A, Green P, Glidden S, Bunn SE, Sullivan CA, Liermann CR, Davies PM (2010) Global threats to human water security and river biodiversity. *Nature* 467:555–561. doi: 10.1038/nature09440

ÜBER DIE ROLLE VON PARASITEN UND DES IMMUNSYSTEMS IN ANTHROPOGEN BEEINFLUSSTEN WASSERVOGELPOPULATIONEN

ZUSAMMENFASSUNG

Der menschliche Einfluss auf Süßwasserökosysteme ist immens. Die vorliegende Arbeit befasst sich mit den Wechselwirkungen zwischen menschlichen Einflüssen auf Süßwasserökosysteme und Parasiten sowie die Immunsysteme der dort lebenden Wasservögel. Hierfür wurden Parasitenindizes und Immunparameter von Stockenten als die häufigste heimische Entenart und der Nilgans als eine der häufigsten invasiven Gänsearten in Deutschland untersucht.

In **Kapitel 1** wurde untersucht, welchen Einfluss chronische Bleibelastung bei Stockenten auf deren Parasitenbiodiversität hat. Dazu wurden Stockenten hinsichtlich chronischer Bleibelastung sowie Artenvielfalt und Infektionsintensität von Magendarmhelminthen untersucht. Sowohl Artenvielfalt als auch Infektionsintensität waren signifikant niedriger in Enten mit höherer chronischer Bleibelastung. Dieses Ergebnis deutet darauf hin, dass nicht nur die Ente als Wirt, sondern auch die Parasiten selbst durch Blei negativ beeinflusst werden. Dies zeigt, dass eine menschlich verursachte Bleibelastung der Umwelt, welche zu Langzeitbleibelastungen bei Wasservögeln führt, einen signifikanten Biodiversitätsverlust von Magendarmhelminthen zur Folge haben kann.

In **Kapitel 2** wurden Parasitenprävalenzen und Immunparameter von einer invasiven und einer heimischen Nilganspopulation verglichen. Hierbei konnte gezeigt werden, dass die Varianzen der Immunparameter der invasiven Population signifikant höher sind als die der heimischen Population. Zudem wird eine neue Hypothese („Immunological Plasticity Hypothesis“) beschrieben, die bereits bestehende Hypothesen zum Einfluss von Parasiten und Immunparameter auf Mechanismen der Invasionsbiologie verbindet und um den Einfluss neuerlich erworbener Parasiten erweitert. Diese neue Hypothese kann helfen, gesundheitsbezogene Antriebe von Invasionsprozessen besser zu verstehen.

In **Kapitel 3** wurden Stockenten hinsichtlich des Auftretens und der Prävalenz neuronaler Vogelschistosomen untersucht, deren Zerkarien beim Menschen Badermatitis auslösen können. Mit einer Prävalenz von 21% war *Trichobilharzia*

regenti die häufigste Vogelschistosomenart in den Stockenten. Neben *T. regenti* konnte *Bilharziella polonica* aus verschiedenen Bereichen des zentralen Nervensystems von 12% der Stockenten extrahiert werden. Diese Entdeckung ist die erste Beschreibung *B. polonicas* im Nervensystem von Vögeln und lässt die Schlussfolgerung zu, dass das Spektrum neuronaler Vogelschistosomen größer ist als bisher angenommen. Beide Ergebnisse unterstreichen die Wichtigkeit für ein Monitoring von Vogelschistosomen in Wirbeltierwirten.

In **Kapitel 4** konnte gezeigt werden, dass Nilgänse in Deutschland Träger von *Riemerella anatipestifer* sind. Zusätzlich deutet das Auftreten von Antikörpern gegen Pathogene, die von Bedeutung für heimische Vögel und Hausgeflügel sind (Influenzavirus A, *Aviäres Avulavirus 1*, Aviadenoviren, *Enten-Atadenovirus A* (syn.: egg drop syndrome 1976 virus), auf einen Kontakt mit diesen Erregern hin. Diese Ergebnisse zeigen, dass invasive Nilgänse in Deutschland in Zyklen heimischer Krankheitserreger eingebunden sind und diese Art möglicherweise als Überträger der untersuchten Erreger fungieren kann.

Mit dieser Dissertation wird deutlich, dass das komplexe Zusammenspiel zwischen menschlichen Einflüssen auf die Umwelt und Gesundheitsaspekten von Wasservögeln in Studien zur Artenvielfalt in Süßwasserökosystemen einbezogen werden sollten. Zusätzlich wurde eine neue Hypothese vorgestellt, anhand derer zukünftige Studien die gesundheitsbezogenen Mechanismen der Invasion von Wirbeltieren besser beurteilen können und die helfen kann, das Feld der Invasionsbiologie generell weiter zu entwickeln.

PARASITES AND IMMUNITY IN ANTHROPOGENICALLY IMPACTED WATERFOWL POPULATIONS

SUMMARY

This thesis aimed to investigate interactions between human driven ecosystem changes and waterfowl species in the frame of immunity and parasitic infections. This interplay was investigated studying parasite indices and immunity of the mallards and Egyptian geese in the context of lead pollution in mallards, helminthic parasites of zoonotic potential in mallards, the role of immunity and parasites in the invasion process of Egyptian goose and infectious agents of Egyptian goose which could be of relevance for native species, livestock and humans.

In **chapter 1**, it was studied how lead pollution affects parasite diversity by investigating intestinal helminth species richness and infection intensity in mallards exposed to environmental lead. Parasite species richness and infection intensity was found to be significantly lower in birds with higher chronic lead levels suggesting both host and parasites respond to lead exposure. Thus, it was shown that anthropogenic induced environmental lead pollution resulting in long term intoxication of mallards, is related to significant biodiversity loss in intestinal helminth species communities of mallards.

In **chapter 2**, parasite prevalence and immunity of an invasive and a native Egyptian goose populations were compared, showing that the variance of immune marker is significantly higher in the invasive than in the native population. Moreover, a new hypothesis (“Immunological Plasticity Hypothesis”) was raised in the field of vertebrate invasion, which aims to combine existing hypothesis with the impact of newly acquired pathogens on invasive species. The new hypothesis will help to study health related drivers of invasion processes.

In **chapter 3**, prevalence of neural bird schistosomes, whose cercaria might cause swimmer’s itch in humans, in mallards was investigated. Showing a prevalence of 21%, *Trichobilharzia regenti* was the most prevalent neural bird schistosome. Beside *T. regenti*, *Bilharziella polonica* was detected either between the brain membranes (meninges) and the brain, in the spinal cord or in the intestine of 12% of the mallards, which is the first description of this species from the birds neural system. This finding

indicates that the spectrum of neural bird schistosome species is broader than previously acknowledged. Both findings highlight the importance of monitoring this parasitic group in vertebrate hosts.

Chapter 4 shows that Egyptian geese are frequent carriers of *Riemerella anatipestifer* and furthermore provides serological evidence of exposure to selected viral pathogens of relevance for native birds and poultry. Thus, invasive Egyptian geese are involved in native pathogen transmission cycles and therefore might potentially spillover and spillback pathogens to native species and poultry.

In conclusion, this thesis highlights the importance to encounter the complex interplay between health indices of waterfowl and anthropogenic impacted environmental factors such as pollution and species invasion to studies on species biodiversity in freshwater ecosystems. Moreover, it provides one of the very few studies on infectious agents and immunity of invasive birds and proposes a new hypothesis in the field of vertebrate invasion, which will help to study health related drivers of animal invasion.

PUBLIKATIONSVERZEICHNIS

Scientific publications:

Prüter H, Sitko J, Krone O (2016): Having bird schistosomes in mind- The first detection of *Bilharziella polonica* (Kowalewski 1895) in the bird neural system. *Parasitology Research*, doi: 10.1007/s00436-016-5359-9

Prüter H, Franz M, Auls, S, Czirják GÁ, Greben O, Greenwood AD, Lisitsyna O, Syrota Y, Sitko J, Krone O (2018): Chronic lead intoxication decreases intestinal helminth species richness in mallards (*Anas platyrhynchos*). *Science of the total Environment*, doi: 10.1016/j.scitotenv.2018.06.297

Prüter H, Czirják GÁ, Twietmeyer S, Harder T, Grund C, Mühldorfer K, Lüscho D (2018): Sane and sound - A serologic and molecular survey for selected infectious agents in neozootic Egyptian geese (*Alopochen aegyptiacus*) in Germany. *European Journal of Wildlife Research*, doi: 10.1007/s10344-018-1231-9

Scientific presentations:

2015, Talk, Poster: Impact of invasive bird species on ecosystems in Europe and its human drivers, Endoparasites and immunity of native and invasive waterfowl in Germany, 6th European Wildlife Disease Association student Workshop, Verrier-du-lac, France

2015, Poster: Endoparasites and immunity of native and invasive waterfowl in Germany, 10th International Conference on Behaviour, Physiology and Genetics of Wildlife, Berlin, Germany

2015, Talk: Endoparasitenfauna und Immunfunktion bei heimischen und invasiven Wasservögeln im Vergleich, Deutsche Ornithologen-Gesellschaft (DO-G) 148. annual meeting – 3rd price of young scientist talk, Konstanz, Germany

2015, Invited speaker: Egyptian goose (*Alopochen aegyptiacus*) – a new European citizen, Scientific society Windhoek, Namibia

2015, Talk: Abundance of nasal bird schistosomes in Mallards from German wetlands, Tagung der DVG-Fachgruppe “Parasitologie und parasitäre Krankheiten”, Berlin, Germany

2016, Talk, poster: Immune function of the Egyptian goose (*Alopochen aegyptiacus*) – a highly successful neozootic waterbird species in Europe, Serological survey of selected viral pathogens in Egyptian geese (*Alopochen aegyptiacus*) from Germany, 12th Conference of the European Wildlife Disease Association (EWDA), Berlin, Germany

ACKNOWLEDGEMENT

I thank Oliver Krone for giving me the opportunity to work on this topic at the Leibniz Institute for Zoo- and Wildlife Research (IZW) in Berlin. I am particularly grateful to my supervisor Gábor Czirják, who by accident slipped into this role and did a great job hereafter. I also want to thank Alex Greenwood, who partly took over the supervision too and supported me to finish my projects and thesis. Furthermore, I am grateful to Mathias Franz not only for his help with the statistics but also for all the fruitful discussions and unvarnished comments on my concepts, ideas, approaches and manuscripts. Mathias taught me how to improve my scientific writing and was my sparring partner for scientific discussions.

I also want to thank my close colleagues from the IZW: First, thanks to Manuela Merling de Chapa being office mate and graduate school “partner” during the last years. Manuelas’ solidarity and support both mentally and workwise helped me a lot to keep it rolling. Furthermore, I am extremely grateful to Susanne Auls and Ines Lesniak for their motivation, support, patience and humor. Additionally, I thank Anisha Dayaran for her feedback and comments on this thesis.

Thanks to all the supporting hands in the field, necropsy hall and laboratory. I particularly thank Lorena Derezanin for her enormous help with the necropsies and laboratory work. Thanks to Susanne Auls, Katja Pohle and Nina Hartmann for their help and methodological support in the laboratory. Furthermore, I want to thank Kristin Mühlendorfer and Dörte Lüschoff for their enthusiasm and friendly support of the Egyptian goose related chapters.

Regarding the field work in Germany, I am grateful to Sönke Twietmeyer whose Egyptian goose ringing project I could join to take the necessary samples for my projects. Additionally, I thank Niklas Böhm and Lea Jäger for their friendship and enormous help during fieldwork in Germany.

During the field season in Namibia, there were several people who supported my work. At first, I want to thank Jörg Melzheimer and Bettina Wachter for giving me the opportunity to go on this field trip to Namibia. Moreover, I want to thank Gudrun Middendorff and Neil Thomson, who gave me a friendly and comfortable home during the time and helped me with catching and sampling in the African heat. Additionally, I

ACKNOWLEDGEMENT

thank Ruben Portas for his help and all members of the IZW team in Namibia for keeping my samples under correct conditions and sending them to Germany.

Additionally, I want to thank all my co-authors of the four chapters for their input and support. Thanks to Prof. Petr Horák and Dr. Jana Bulantová for their friendly and thorough introduction into bird schistosome research and methodology.

I thank Martin Bulla, who made me start my scientific “career” by introducing me into science, always being enthusiastic about ornithology research. I am grateful to Bart Kempenaers for being a mentor and friend.

Last but not least, I am very thankful to my family and friends for their mental support and love. Especially my husband Felix, who gave me space, backed me up whenever needed and reassured me to keep on writing. I am thankful to my father Johannes, who helped me a lot structuring my ideas for this thesis and in general.

SELBSTSTÄNDIGKEITSERKLÄRUNG

Hiermit bestätige ich, dass ich die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Berlin, den 25.06.2019

Hanna Prüter

