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DIPLOMARBEIT

Titel der Diplomarbeit

„Patterns of parasite burden in greylag goose families from hatching to fledging "

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Abstract

Both increased transmission of parasites and social stress are challenges for the immune system of group-living individuals. In the present study I investigated relations between social behaviour and parasite burden in greylag goose (*Anser anser*) families. I collected individual faecal samples for determination of intestinal parasite excretion from six families starting at four weeks after hatching to fledging of the offspring. Additionally, behavioural protocols lasting for ten minutes were recorded in four different periods of the day. Overall, geese families were infected with a low burden of nematode eggs (e.g. *Amidostomum sp*., *Ascaridia sp*.). Still, I found significant positive correlations between the duration of ‚resting behaviour', and the occurrence of ‚active agonistic behaviour' and an individual's parasite load. The more geese were loaded by nematode eggs, the more time they spent resting. Individuals frequently engaged in ‚active agonistic behaviour' were less parasitized than frequent targets of agonistic encounters. In sum, I found some relationships between parasite burden and social behaviour. However it remains unclear whether a less active and proactive behaviour is a result of being parasitized or vice versa, whether individuals with low behavioural activity or being prone to be attacked also experience a stress-related increase in parasite load.

Introduction

One of the universal costs of group-living is a potentially increased transmission of parasites (Alexander 1974). Particularly social animals with a high group density which use small home ranges intentensly may be at risk of infection from parasites that are transmitted trough faeces (Freeland 1979; Anderson and May 1982). Additionally, parasites of social hosts tend to be highly virulent (Møller et al. 2001).

Generally the social context affects physiology, behaviour, fertility and immune system of vertebrates (deVries et al. 2003, McEwen & Wingfield 2003, Von Host 1998). Abiotic and biotic stressors, including social environment, activate both the symphatico-adrenomedullary system and the hypothalamic-pituitary-adrenocortical axis, causing an increased concentration of glucocorticoids (GC) in the blood. Short-term increases of GCs help mobilizing energy to cope with a stressful situation, but a chronic activation of the stress axis may constrain the immune system (Creel 2005). A weakened immune system may increase the susceptibility for parasites, also via stress effects on behaviour and physiology (Hanley and Stamps 2000). Willow grouse (*Lagopus lagopus*), for example, show decreased ingestion induced by nematode affection (Moore 2002) and mice (*Mus musculus*) show reduced activity (Moore 2002).
However, the impact of stressors is determined by the ability of the organism to cope with the situation, using different coping styles (Koolhaas et al. 1999; Koolhaas 2008). A coping style can be defined as a coherent set of behavioural and physiological stress responses which is characteristic to a certain group of individuals. In social stress research two different stress response patterns (proactive and reactive) may be distinguished. Proactive individuals are more aggressive, territorial and approach novel objects faster but tend to be less flexibel than reactive ones and contrarivise (Koolhaas et al. 1999).

In greylag geese (*Anser anser*) social stressors have been shown to have a pronounced effect on an individual's physiology e.g. manifest in increased heart rate (Wascher et al. 2008). However, not all individuals of a population are affected by social stressors in the same way. Social support (Frigerio et al. 2000; Scheiber et al. 2005a; Weiß 2000) is considered a key mechanism for coping with social stress. Besides 'active' social support (e.g. interference in agonistic interactions), also 'passive' social support (e.g. the simple presence of a social ally like a family member or the partner) may increase the prospects of winning in agonistic interactions and reduce stress (Scheiber et al. 2005a).

The long-term monogamous greylag geese live in flocks consisting of family units, pairs without offspring, sub-adult sibling groups and singletons. After fledging juvenile geese stay with their parents in primary family bonds (Lorenz et al. 1978). Juveniles benefit from staying in company of their parents in terms of higher dominance, feeding and body condition (Weiss et al. 2008). Juvenile barnacle geese *(Branta leucopsis*) at five months of age living in family units were feeding uninterrupted for longer periods than those who had already left the family unit, and they were in better body condition at ten months of age (Black et al. 2007). Successful parents are more likely to fledge new offspring in successive years (Weiß et al. 2008).

Geese in the Grünau flock have been shown to be infected by various parasites (Hurst & Lambinet 2008, not published). High parasite concentrations may have a major impact on an individual's well-being. The nematode species, *Ascaridia sp., Capillaria sp*. and *Amidistomum sp.* and the protozoan *Coccidia sp*. have a role in this and are at the same time easily monitored from faeces.
In the present study I focus on different nematode species which are all ingested as eggs in faeces-contaminated food, such as *Ascaridia sp*. (Pantchev 2006). The larvae enter the intestines and emigrate into the liver or other visceral parenchyma. Hair-worms (*Capillaria sp*.) with a higher pathogenicity than *Ascaridia sp*. also enter the gut. After a short time the entire gastrointestinal tract can be infected (Foreyt 2001; Pantchev 2006). The first infection of *Capillaria sp*. can be lethal after seven days. *Amidostomum sp*. is the most commonly found stomach worm in geese, which often results in death, especially in young hosts (Pantchev 2006). The larvae are not only ingested by food, but some larval stages swim and may enter goslings percutaneously. Symptoms for all of these parasite

infections are anorexia, diarrhoea and weight loss. Goslings infected by *Amidostomum sp*. may also show retching motions (Foreyt 2001; Pantchev 2006).

In the present study I investigated social stress in greylag geese families against the background of the hypothesis that stress may constrain their immune system, which in turn, may modulate. I expected correlations between parasite egg excretion and behaviour as well as social factors (e.g. rank of the family). For example, agression, which may be energetically costly and stressful for individuals involved in numerous agonistic interactions, is more prevalent in males than in femals and more in families than in pairs or single individuals (Weiß et al. 2011, Kotrschal et al. 1998). Therefore, I expected an increased parasite infection in individuals which are more involved in agonistic interactions than others. In particular, I expected parsite burden to be higher in individuals being frequent targets of attacks. In alingnment with previous studies (Moore 2002; Hurst & Lambinet 2008, not published), I also expected to find resting, preening and feeding to scale with parasite load.
Additionally, I also compared parasite load of goslings and their parents to see whether there would be family-general levels of infection.

Methods

Study species

I worked with a non-migratory, free-ranging flock of greylag geese (*Anser anser*), which was introduced into the valley of the river Alm by Konrad Lorenz in 1973 (Lorenz 1988). In spring, the geese breed in Oberganslbach (OGB), Cumberland Wildpark Grünau and around the Konrad Lorenz research station (KLF). During the rest of the year geese stayed on the meadows around the research station. At the time of my study, the flock consisted of 130 individuals. All individuals, except the goslings, were marked with coloured leg rings and habituated to the presence of humans (Weiss et al. 2000). Each year approximatly 10 % of the individuals are preyed upon, mainly by red foxes (*Vulpes vulpes*) and golden eagle (*Aquila chrysaetos*; Kotrschal et al. 1992). In the year of data collection 19 pairs bred, 80 goslings hatched and 19 goslings were hand-raised in OGB. For all individuals detailed life-history data including complete family pedigrees were available.

Data collection

I collected behavioural protocols and faeces from six families, which were located most of the time at the KLF.

1. Behaviour protocols:

Starting at four weeks of goslings' age, behavioural protocols of the families were collected. Before this, parasite eggs could not be detected in faeces samples because of their prepatent periods (span, from the first infection with parasites to the first time reproduction products can be detected in the host's faeces; Table 1; Pantchev et al. 2006). Additionally, I could not identify the individual goslings earlier. Data has been collected during four periods over the day. First period: from 8:00 to 9:00 during morning feeding, second period: 30 minutes after the morning feeding, third period: from 16:00-17:00 during afternoon feeding and fourth period: 30 minutes after the afternoon feeding. Observations lasted for ten minutes during which the entire family was videotaped. All occurring behaviours were analysed using ‚Observer' (Noldus 2002) where some behaviours were counted by their frequency (Agonistic behaviour: peck/be pecked; comfort: shake tail, shake neck, scratch; other behaviour: defecate) and others by the duration of the behaviour (Agonistic behaviour: threat/receive threat, approach aggressive/withdrawal, chase/submission, fight/escape; resting behaviour: rest, rest with beak under; interactions: greeting partner, triumph ceremony; comfort: preen, shake wings, stretch, shake body, bath, play swim; food: picking grain, nibble, drinking, filter, grazing; locomotion: swim; behaviours in families: nest building female; mixed & other behaviours: bite leg bands, explore object; for further descriptions of behaviour & ethogram see Lorenz, 1991).

2. Parasitological examinations:

Faeces have been collected individually directly after defecation in Eppendorf tubes. From the adult individuals, I collected three faeces samples per week. Sample collection of the goslings started at four weeks of age, when they could be individually distinguished.

For the faecal analysis I used a modified version of the flotation method described in Schnieder et al. 2006. Aliquots (0,5 g) of the faecal sample were weighed into Eppendorf tubes, received 1 ml of sodium-chloride solution (unit weight: 1.195 g/l) and were centrifuged for 5 minutes at 3000 rpm. Afterwards, the Eppendorf tubes were filled up with sodium-chloride solution and a cover slip (18x18mm) was positioned on to the tube. Due to the high density of the sodium-chloride solution the parasite eggs and oocystes floated up and were caught on the cover slip. After 10 minutes the cover slip was transferred to an object slide and the parasite eggs and oocytes were identified by size and shape and counted. We used a binocular (Nikon Alphaplot -2 YS2) with 100-fold and 400-fold amplification.

Data analysis

I used a generalized linear mixed model (GLMM) for each parameter (Tab. 2), analysed and applied the restricted maximum likelihood (REML) procedure for repeated sampling with an unbalanced design. I present Wald statistic for REML, because the change in deviance when dropping a term from the model approximates a χ^2 –distribution (Foerster and Kempenaers 2005). As response variable I took the parasite load (number of nematode eggs per week), as fixed models the following parameter: sex, goslings lifetime, family's rank (calculated by in video-observations from 26.5.-15.6.2008 occurred agonistic behaviour with matman), pair bond duration, hatching date, how many eggs were hatched/how many goslings fletched per year from the adult pairs over their pair bond duration (HJ, FJ) and how many of all eggs, which were laid from the adult females, were hatched/fletched in their lifetime (HE, FE; GLMM 1)

Also with the behavioural observation data I conducted GLMM for each behavioural parameter (see above; Tab. 2). As response variable I took the parasite load (number of nematode eggs per week), as fixed models the behaviour parameters (Tab. 2; GLMM 2).

Both analyses were made with GenStat 10.1 statistical package.

Results

Identified parasites

The most abundant nematode species found in greylag geese was *Amidostomum anserii* (in adults: 55,6 %, in goslings: 56 %) and unidentified nematode species (which were most likely *Amidostomum anserii* or *Ascarida sp*.; in adults: 37,7 %, in goslings; 38,1 %). *Capillaria sp.* were only found in adults, not in goslings (adults: *Capillaria sp*.: 0,7 %; *Heterakis sp*.: 1,5 %; *Trichostrongylus tenius*: 1,2 %; *Ascaridia sp*.: 3,5 %; Fig.1; goslings: *Heterakis sp*.: 4,1 %; *Trichostrongylus tenius*: 1,1 %; *Ascaridia sp*.: 0,7 %; Fig. 2).

Parasite load and social parameters

I found no significant influence of the number of nematode eggs and (social) parameters as sex, gosling's lifetime, hatching date or family's rank. Neither I found a correlation between the amount of parasite eggs and parameters, which determine the quality of an adult pair's relationship as permanence of fix pair bond duration, how many eggs were hatched/how many goslings fletched per year from the adult pairs over their permanence of fix pair bond status or how many of all eggs, which where laid from the adult females, were hatched/fletched in their lifetime. In addition ththere was also no significant difference of the parasite load between families detectable.

Parasite load and behaviour

There was no significant effect on most behaviours, such as 'preening' or 'feeding' (see Tab. 2).

However, there was a significant influence of the number of nematode eggs and ‚resting behaviour' (Fig. 3; Tab. 3) as well as ‚active agonistic behaviour' (Fig. 4; Tab. 3).

Discussion

Although parasite burden in the goose flock investigated is generally low, my data shows that birds with high parasite loads are resting significantly longer compared to geese with low parasite burden. This is in line with Moore (2002), who showed, that mice had a reduced activity due to nematode infection. However, other important symptoms for parasite infection, like lower food ingestion, anorexia, diarrhoea and weight loss (Pantchev 2006) are not apparent in our data, probably because infection rates were still generally low, not causing pathological symptoms, but still subtly affecting behaviour.

Furthermore, I would have expected to find a difference in the amount of nematode eggs between goslings and adult individuals because the immune system of young individuals not being fully developed (Scope 1999). Previous research shows, that sex and other social parameters (as in parental birds: number of goslings fledged; in goslings: family membership) may co-vary with parasite burden (Wascher 2012). None of this was found in the present study.

My main result is that individuals, which were more often the attacker during agonistic encounters than others, had a lower parsite load. This is in alignment with the current views of 'coping styles' in animals, which point out, that proactive coping is associated with high sympathetic reactivity to stressors whereas the reactive or more passiv coping style generally has a higher HPA axis reactivity (Koolhaas et al. 1999, also see Koolhaas 2008). The proactive type (originally described by Cannon 1915 as fight-flight response) is characterized by aggressive behaviour, the reactive type (originally described by Engel and Schmale 1972 as the conseration-withdrawal response) show low levels of aggression. High consistency in aggressiveness over time and across different social situations implies that aggressiveness may be a heritable component of male geese' personality (Kralj-Fišer et al. 2007), whereby these individuals may be proactive.

Koolhaas (2008) mentioned, proactive behaviour control works best under highly predictable conditions. Because our goose flock live under very stable conditions regarding to food approach and environmental challenges proactive coping may be a more successful way to deal with social challenges and hence parasite load remains low in more aggressive individuals compared to less aggressiv ones. This is in line with data on HR in greylag geese. Wascher et al. (2011) could not only show, that greylag geese can discriminate between different kinds of stressors but also estimate the relevance of any event for an individual und modulate their phyiological response accordingly.

Additionally, in family units, passive support plays a major role in handling stress (Scheiber et al. 2005a). Parents win most of their agonistic interactions without active support by other familiy members, but seem to profit from passive social support due to motivational effects (Lamprecht, 1986a). Therefore I assume, that individuals with increased ‘active agonistic behaviour’ benefit strongly from passive social support and therefore their parasite load was significantly lower than in indiviuals, which thereby were more often the target in agonistic encounters.

To sum up, I found parasite burden to be correlated with social behaviour in a highly social bird species. However, it is yet to be investigated whether social behaviour affects an individual’s parasite burden or vice versa. Furthermore, it is of interest if an individuals coping style (proactive/reactive) related to this its ability to deal with social stress, influences parasite infection. Further studies on behavioural stress coping mechanisms and their impact on the immune system would be highly desirable.

Figures and Tables

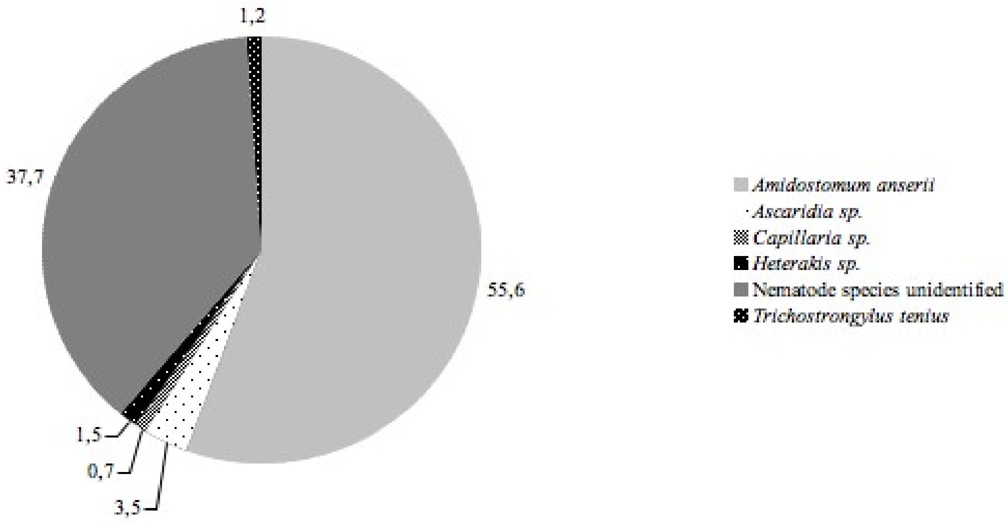

Fig.: 1: Distribution of nematode egg species found in adult greylag geese in % (total number of nematode eggs: 324; *Amidostomum anserii*: 180, *Ascaridia sp.*: 11, *Capillaria sp.*: 2, *Heterakis sp.*: 4, nematode species unidentified: 122, *Trichostrongylus tenius*: 4).

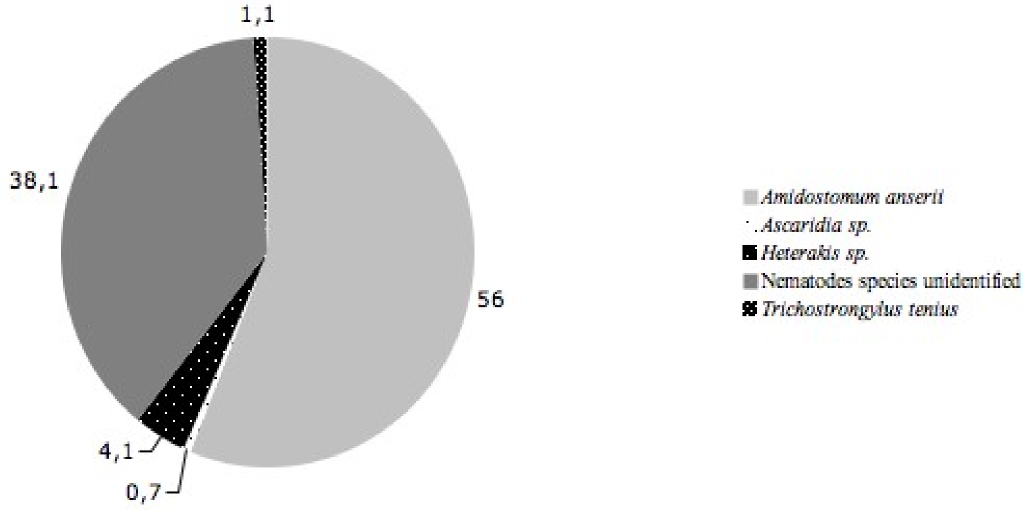

Fig. 2: Distribution of nematode egg species found in goslings in % (total number of nematode eggs: 1477; *Amidostomum anserii*: 827, *Ascaridia sp.*: 11, *Heterakis sp.:* 60, nematode species unidentified: 562, *Trichostrongylus tenius*: 16).

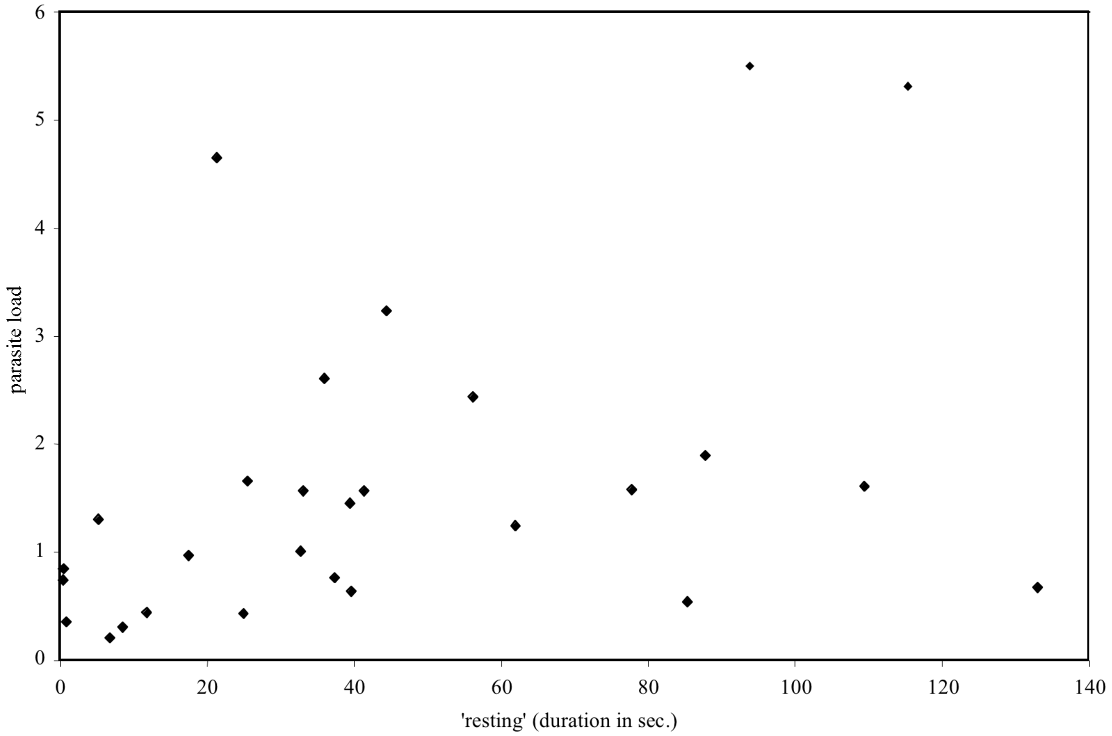

Fig. 3: Parasite load (average number of parasite eggs per week) and duration of resting (average duration per week in seconds) for each individual (n=28).

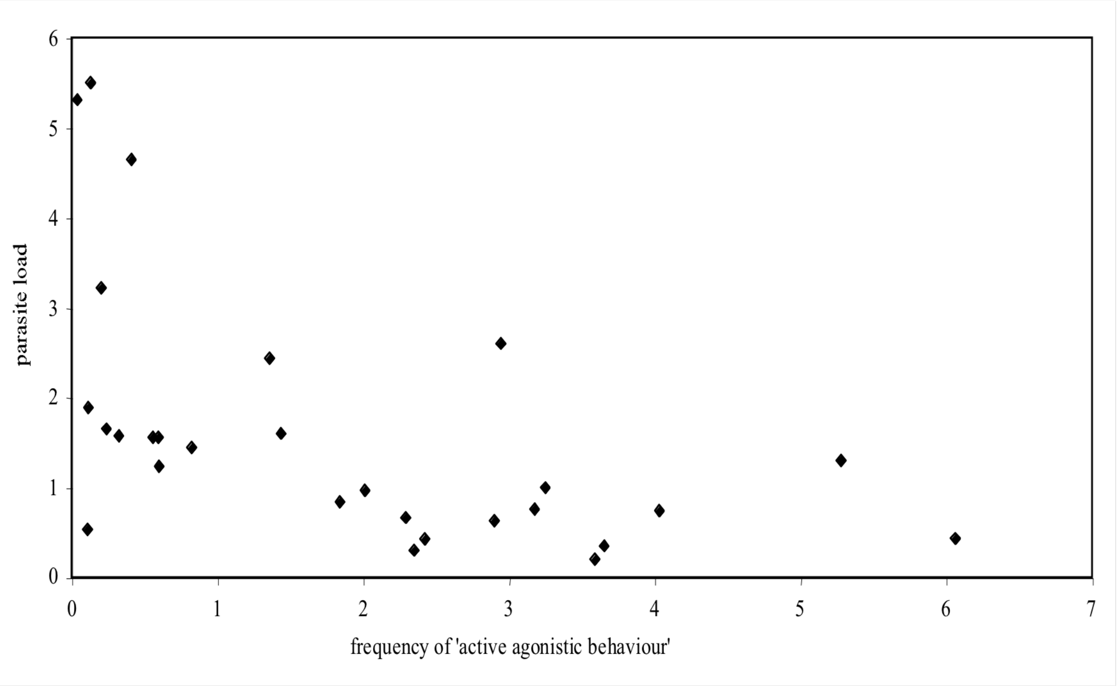

Fig. 4: Parasite load (average number of parasite eggs per week) and frequency of active agonistic behaviour (average frequency per week) for each tested individual (n=28).

Fig. 5: Greylag goose families resting and swimming near the KLF (© Ch. Bauer).

Fig. 6: Goose families in front of the KLF (© Gigi Schlögel).

Table 1: Parasite species with the highest occurrence in this study, their prepatent periods (Pantchev et al. 2006) and the percentage (%) of infected adults in time range from 5.4.2008 to 5.5.2008 (n=17, 3 collected faeces minimum).

parasites	**prepatent period**	**% infected individuals**
Amidostomum sp.	15 – 18 days	82,4
Ascaridia sp.	14 days	17,6
Capillaria sp.	21 – 25 days	11,8

Table 2: Parameters, which were used in the GLMM calculations as response variable and fixed models.

	response variable	**fixed models**
GLMM 1	number of parasite eggs per week per individual	sex, goslings lifetime, family's rank, pair-bond duration, hatching date, HJ, FJ, HE, FE
GLMM 2	number of parasite eggs per week per individual	*Agonistic behaviour*: peck/be pecked, threat/receive threat, approach aggressive/withdrawal, chase/submission, fight/escape; *resting behaviour*: rest, rest with beak under; *interactions*: greeting partner, triumph ceremony; *comfort*: shake tail, shake neck, scratch, preen, shake wings, stretch, shake body, bath, play swim; *food*: picking grain, nibble, drinking, filter, grazing; *locomotion*: swim; behaviours in families: nest building female; *mixed & other behaviours*: defecate, bite leg bands, explore object

Table 3: Statistical results of the GLMM of the video analysis. In the final model only terms with *P* < 0,05 were given.

Fixed term	Full fixed model		Final model	
	Wald statistics	P	Wald statistics	P
Defecate freq.	0,14	0,718		
Shake neck freq.	0,01	0,934		
Shake tail freq.	3,44	0,098		
Passive agonistic behav.	0,53	0,487		
Active agonistic behav.	2,33	0,164	5,96	0,022
Bite leg bands duration	0,29	0,606		
Comfort duration	7,44	0,025		
Drinking/filter duration	2,45	0,154		
Expore object duration	0,8	0,396		
Family business	0,5	0,499		
Food duration	2,83	0,127		
Nest building female duration	1,62	0,238		
Nibble duration	0,77	0,404		
Resting duration	3,21	0,108	17,55	<0,001
Scratch freq.	0,61	0,458		
Shake body freq.	2,5	0,15		
Shake wings freq.	0,43	0,532		
Strech freq.	0,62	0,454		
Vigilance duration	0,01	0,929		

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Zusammenfassung

Der Umgang mit erleichterter Übertragung von Parasiten und sozialem Stress sind beides große Herausforderungen für Individuen, die in Gruppen leben. In der hier vorliegenden Studie wurde die Beziehung zwischen dem Sozialverhalten und der Parsitenbelastung von Graugänsen (*Anser anser*) untersucht. Nachdem die Gössel vier Wochen alt waren bis zum Flügge werden wurden von sechs Grauganslfamilien Kotproben genommen. Zusätzlich wurden Verhaltensprotokolle à 10 min. in vier verschiedenen Konditionen über den Tag verteilt angefertigt. Generell war die Parasitenbelastung der Familien, gemessen an den im Kot gefundenen Nematodeneier (z.B. *Amidostomum sp., Ascaridia sp*.), eher gering. Trotzdem konnte eine signifikante Korrelation zwischen der Dauer vom ‚Ruheverhalten', dem Auftreten von ‚positivem agonistischem Verhalten' und der Anzahl der Parasiteneier der Individuen gefunden werden. Je mehr die Gänse parasitiert waren desto länger dauerten ihr ‚Ruhephasen'. Tiere, die oft aktiv attackierten (‚positives agonistisches Verhalten') waren schwächer von Parasiten belastet als Tiere, die öfter die Ziele von Auseinandersetzungen waren. Zusammenfassend konnte eine Beziehung zwischen sozialem Verhalten und der Parasitenbelastung bei Graugänsen gezeigt werden. Jedoch bleibt unklar, ob das Verhalten selbst den Stress reduziert und somit die damit einhergehende Parasitenbelastung oder umgekehrt.

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Data collection for the diploma thesis (‚patterns of parasite burden in greylag goose families vom hatching to fletching'; ao. Univ. Prof. Mag. Dr. Kurt Kotrschal)

03/06-07/06 Sea turtle conservation project, Cooperation from Universities from Vienna and Turkey; in Calis, Turkey

Project collaborator

2000-03 Biologische Station Lunz am See
3293 Lunz am See

several one month lasting practical trainings

- data collection (collecting and determine insects and drawing the shoreline on a daily basis)
- data collection in a geological labour

Education

2002-now Universität Wien, BIOZENTRUM
Althanstraße 14
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Biology – Zoology (Ethology) studies

1997-2002 HLUW Yspertal (Höhere Lehranstalt für Umwelt und Wirtschaft)
Schulstraße 13
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1993-97 Hauptschule Gaming
3292 Gaming

1989-93 Volksschule Gaming
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