BIO-3910

Master's Thesis in Biology

Potential effects of two stressors on morphological traits of Northern Goshawk (*Accipiter gentilis*) nestlings

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January 2009

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In collaboration with
Norwegian Institute of Nature Research (NINA)
Tromsø

Title photo: Northern Goshawk nestlings of the breeding season 2008 in Troms County, Norway. Photo: Lisbeth Schnug

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Acknowledgments

Throughout my study period several people have contributed to this project in one or another way and without their help I could not have completed it.
First, I want to thank my supervisors Jan Ove Bustnes, Sveinn Are Hanssen and Rolf Anker Ims for their support and helping advices.

I wish to give my special thanks to:

- Trond Johnsen, without whom the field work for this study would not have been realizable. I not only learned a lot from him but also appreciate his great company during many weeks in the field

- Dorte Herzke for the instructions in the laboratory and for quantifying the pollutants in the plasma samples

- Nigel G. Yoccoz for great help with statistics

- Sandra Siefken who helped out with doing field work

- my parents, who always have supported me financially and personally

- and last but certainly not least my dear Inge for all his love, support and understanding throughout these times

Abstract

Anthropogenic and natural stressors can affect ecosystems negatively and it is therefore important to have a clear understanding of the responses of organisms to stressors. The Northern Goshawk (*Accipiter gentilis*) is a common raptor species of the boreal forest ecosystem and its location at the top of its food chain makes it a special target for two important stressors; endoparasitism and environmental contaminants. The present study examined potential effects of these two stressors on morphological traits of Northern Goshawk nestlings in Troms County, Norway. A randomly assigned group of nestlings was treated with an anti-helminthic drug while a control group was placebo treated. In all nestlings, blood residues of a set of environmental contaminants were measured. The effects of anti-helminth treatment and contaminant concentrations on the size of morphological traits were assessed using statistical models. Nestlings of the treated group showed increased tail growth while the other morphological traits were unaffected by anti-helminth treatment. Further, no negative effects of the measured contaminant concentrations on morphological traits were found. There was, however, a positive relationship between tail growth and contaminant concentrations which may be interpreted as a result of the quality or quantity of the diet rather than an effect of contaminants. The findings may either imply that stress due to both helminths and environmental contaminants was rather low in the Northern Goshawk nestlings in the study area or that morphological traits are poor indicators for the stressors investigated.

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1. Introduction

The concept of stress is commonly applied to analyse the responses of systems at different levels of biological organization in order to understand the stability properties of ecosystems. It is essential to distinguish between the stressors that are a natural part of the ecosystem and those that are added by humans, as the induced effects may be different (AUERBACH 1981). Important factors are the timing and magnitude of the stresses, causing different responses by populations (UNDERWOOD 1989). By definition, stressors are external constraints that may limit the rates of resource acquisition, growth or reproduction of organisms (GRIME 1989). Regardless of which stressor involved, an interference with the normal function of an ecosystem may occur (AUERBACH 1981). Ecosystems face plenty of different stressors, both natural and human induced (SIH *et al.* 2004). The anthropogenic stressors add on to the natural stressors the organisms already have to cope with, and the combined effects of multiple stressors may be more harmful than the effect of each stressor alone (FOLT 1999, SIH *et al.* 2004).

In order to have effective ecological policy designs for protecting ecosystems it is important to have a clear understanding of the responses of organisms to stress (AUERBACH 1981). When studying stress effects on the forest ecosystem in Norway, the Northern Goshawk (*Accipiter gentilis*) is a suitable study organism as it is a widespread raptor species in Fennoscandia (KENNTNER *et al.* 2003, TORNBERG *et al.* 2006). Several studies showed that impacts such as intensified forestry and hunting led to a decline in the Northern Goshawk populations in Fennoscandia in the second half of the twentieth century (WIDÉN 1997, NYGÅRD *et al.* 1998). Although the Northern Goshawk today is protected in all northern European countries (TORNBERG *et al.* 2006) its populations are affected by other stressors of both natural and anthropogenic origin (NYGÅRD 2005, WIELICZKO *et al.* 2003). Being at the top of their food chains, Northern Goshawks are often the final and main hosts in the indirect life cycles of many parasite species (SANNMARTÍN *et al.* 2004). Further, being at this high trophic level they may accumulate high concentrations of pollutants (KENNTNER 2002, MOVALLI *et al.* 2008). Thus, the effects of these two stressors, parasitism and environmental contaminants, may be suitable candidates for studying the effects of stressors in Northern Goshawks.

Parasites are natural stressors exploiting their hosts for resources for their own maintenance and reproduction and thereby cause damage to their hosts (PRINCE 1980). Resources the host could have used to increase its own fitness are not only lost due to the parasites exploiting them directly, but also by the host spending them on parasite defence (DE LOPE *et al.* 1998, HANSSEN *et al.* 2003). Detrimental effects of parasites on bird host body mass, fitness and reproductive success have been demonstrated in several studies (e.g. MOSS and CAMIN 1970, RÄTTI *et al.* 1993, DE LOPE *et al.* 1993 & 1998). Yet, while many studies focus on the effects of feather mites and blood parasites, only a few deal with the effects of helminths on avian host. For instance, CONNORS and NICKOL (1991) documented a significant detrimental impact of acanthocephalan parasites on the flow of food energy through the European Starling (*Sturnus vulgaris*). Further, helminths were shown to account for reduced reproductive output in female Red Grouse (*Lagopus lagopus scoticus*) to the extent that the population dynamics were affected (HUDSON 1986). Endoparasites such as helminths are assumed to be common in Northern Goshawks (SQUIRES and REYNOLDS 1997), but to the knowledge of the author no studies on the effects of helminths on Northern Goshawks have yet been conducted.

Environmental contaminants represent an important and well known group of anthropogenic stressors. In the last decades, the occurrence of environmental contaminants throughout the global ecosystems and their toxic effects on a wide range of organisms have caused concerns (BEYER *et al.* 1996, HOFFMAN *et al.* 2003). Perfluorinated compounds (PFCs) and persistent organic pollutants (POPs), such as organochlorines (OCs) and brominated flame retardants (BFRs), denote chemicals that degrade slowly in the environment, bioaccumulate in living organisms, biomagnify through the food chain, and have a potential for long-range air transport and deposition (KANNAN *et al.* 2001, UBA 2007 a & b). Being amphiphilic, PFCs bind to blood proteins and accumulate in the liver and gall bladder (HAN *et al.* 2003, BOSSIA *et al.* 2005). POPs, on the contrary, are characterized by high lipophilicity, leading to an accumulation in the fatty tissue of animals. Due to the biomagnifying properties of both contaminant groups, largest concentrations of contaminants are found at high trophic levels. Raptors may therefore be subject to high exposure and thus to potentially severe effects of environmental pollutants (HOFFMAN *et al.* 2003, KENNTNER *et al.* 2003). Several studies have reported detrimental effects of pollutants on birds, e.g. delayed reproduction, reduced foetal growth and hatching condition of chicks (e.g. BUSTNES *et al.* 2003 & 2007), eggshell thinning (e.g. RATCLIFFE 1967, HEATH *et al.* 1969) and disruption of endocrine physiology (e.g. TANABE 2002) due to OCs. Possible impacts of BFR- and PFC concentrations on

reproduction, physiology, and behaviour of birds are not well understood to date and only few publications exist. In a recent study, changes in reproductive courtship behaviour in American Kestrels (*Falco sparverius*) have been linked to polybrominated diphenyl ether (PBDE) exposure (FERNIE *et al.* 2008). In Norway, levels of pollutants in raptors have been monitored using eggs. It could be shown that levels decreased over the last years, leading to a stabilization or even increase in raptor populations (NYGÅRD *et al.* 2006). Concentrations of POPs in Northern Goshawk eggs in Norway have been rather intermediate (HERZKE *et al.* 2002 & 2005). However, levels of POPs or PFCs in the plasma of Northern Goshawk nestlings have not been assessed before.

Many indicators can be chosen to evaluate stress on vertebrates. Morphological traits are commonly used indicators to assess the condition and health status of birds as they are indicative of both survival and reproductive success (DAUWE *et al.* 2006). This is because the phenotype of an organism reflects the outcome of equilibria and trade-offs between physiological processes (TALLOEN *et al.* 2008). Stressors may cause deviations from the expected size of morphological traits or affect the rate at which the final size of these traits is attained. Hence, morphological traits may reflect stress during development (SAINO *et al.* 1998, TALLOEN *et al.* 2008).

To study the effects of a stressor, one possibility is to increase the stress level and measure the reduced performance. However, such an approach in wild and protected bird species is not accomplishable. An alternative approach is to relieve individuals from one of the stressors and to observe if this affects performance positively (HANSSEN *et al.* 2003, BUSTNES *et al.* 2006). Thus, the purpose of this study was to experimentally reduce the helminth burden in Northern Goshawk nestlings and to measure effects on morphological traits and body mass (hereafter condensed to *morphological traits*). Further, concentrations of environmental contaminants in blood samples of Northern Goshawk nestlings were measured and potential effects on morphological traits estimated. Anti-helminth treatment was carried out by treating a randomly assigned group of young nestlings with an anti-helminthic drug, while the nestlings of the other group served as a control group. Morphological traits of nestlings were compared between the treatment groups and in relation to the pollutant levels in blood.

The hypothesis of the present study was that both helminths and environmental contaminants have negative effects on the size of morphological traits of Northern Goshawk nestlings. The

predictions were that anti-helminth treatment would improve the growth performance of nestlings, and negative effects induced by helminths should thus be reduced in medicated nestlings compared with nestlings from the control group. Moreover, environmental contaminant concentrations were expected to have adverse effects on nestling growth performance, and these negative effects should be reduced in medicated nestlings if parasites enhanced such negative effects.

2. Material and methods

2.1 Study area and species

The study was conducted in the Northern Goshawk (*Accipiter gentilis*) population in Troms County, Norway, from April to the end of June 2008. The study area ranged from N 69° to 70° and from E 18° to 19° (Fig. 1).

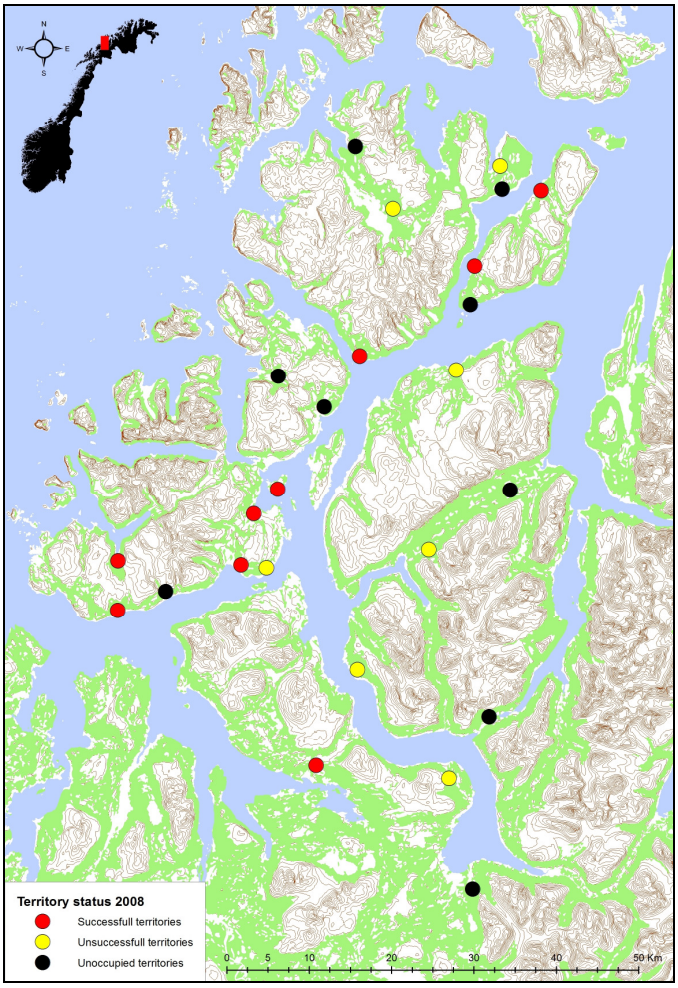

Fig. 1 Map over the study area (Troms County, Norway) and the Northern Goshawk territories included in the study in 2008. The red rectangle on the map in the upper left corner shows the location of the study area in Norway Map: Trond Johnsen.

Troms County is the second northernmost county in Norway and faces the Norwegian Sea. The study area is therewith located in the sub-arctic birch forest ecosystem of Fennoscandia. This ecosystem is characterized by both continental and maritime climatic components

(depending on the distance from the coast) and a natural large-scale fragmentation due to topographical factors such as fjords and mountain ranges (YOCCOZ *et al.* 2001). The forests in the study area occur as narrow belts along the coastline or between mountain areas (Fig. 1). Potential prey species for the Northern Goshawk in this ecosystem are among others Willow Grouse, passerines, corvids, gulls, waders and ducks, but also small mammals (HOGSTAD 1991). Some of these species, in particular small herbivores such as rodents, hares and grouse species have a temporal variability in abundance due to population cycles (YOCCOZ *et al.* 2001).

Twenty-five Goshawk territories in Troms County were initially included in the present study. Nine territories were unoccupied, and of the 16 remaining breeding pairs only 9 reproduced successfully. The successful breeding pairs nested in the northern part of the study area and close to the coastline (Fig. 1). The nests were located in pine (*Pinus sylvestris*) and birch (*Betula pubescens*) trees (Fig. 2).

The Northern Goshawk is one of the most common and widespread raptor species in Fennoscandia (WIDÉN 1985, TORNBERG *et al.* 2006). It is a sedentary top predator in the family Accipitridae and inhabits the forested areas in the boreal zone and parts of the deciduous zone of the Palearctic and Nearctic regions (FISCHER 1980). The Northern Goshawk populations in Troms- and Finnmark County represent the northern extension of the European breeding population (CRAMP and SIMMONS 1983). Throughout this distribution range the Northern Goshawk is a generalist predator on medium sized birds and mammals (CRAMP and SIMMONS 1983, WIDÉN 1987, SELÅS 1998 and KENWARD 2006). Its diets across Europe seem to vary according to availability. In Fennoscandia, grouse is assumed to be the most important prey for the Northern Goshawk (KENWARD 2006). However, no particular studies of diets of the Northern Goshawk in the study area have been published.

The breeding density of the Northern Goshawk in Norway is around three pairs per 100 km^2, equivalent to 2700 breeding pairs (WIDÉN 1997). One breeding pair can have up to eight nests in their territory, which are build in older forest trees (FISCHER 1980). The same breeding sites are used relatively regularly year after year (TORNBERG *et al.* 2006).

Fig. 2 Breeding sites of the Northern Goshawk in the sub-arctic birch ecosystem in Troms, Norway. Nest in pine (left) and birch tree (right). Photos: Lisbeth Schnug, 2008.

The breeding season for the Northern Goshawk in Fennoscandia begins in early spring (HUHTALA and SULKAVA 1981). Egg-laying date varies with latitude, but is considered to be around mid/ late April in Fennoscandia. After the first egg, the female Goshawk lays a new egg every second to fourth day. Clutch size is on average three eggs (1 to 5). Breeding usually begins as soon as the second egg is laid and the incubation period is on average 38 days. At the start of the third week the young have reached about half their full weight and their main flight feathers are emerging. In the sixth week their primaries are two-thirds grown, while their main tail feathers are less than half exposed (KENWARD 2006). The young leave the nest after approximately 44-46 days and reach independence at the age of 75-82 days (KENWARD *et al.* 1993 a. & b.).

2.2 Data collection and analyses

2.2.1 Breeding activity

The nests were checked for breeding activity from late April to the middle of May using binoculars and telescopes keeping as long as possible distance to avoid disturbance of the breeding pairs. The presence of at least one adult bird lying on the nest was used as a confirmation for breeding activity (Fig. 3).

Fig. 3 Adult Northern Goshawk on the nest during the incubation period in Troms County, Norway, breeding season 2008. Photo: Trond Johnsen.

2.2.2 Timing of the visits

In order to time the first visit, behavioural characteristics of the adult birds were used to estimate potential hatching date as this can vary accordingly to the age of the breeding pair. Differences can partly be seen in the way adults behave on the nest (TROND JOHNSEN, *pers. comms.* 2008). However, actual deviations from the estimated hatching date made the timing of the visits difficult and nestling age varied more strongly between the clutches than expected. The second visit was carried out approximately two weeks (14 ± 2 days) after the first visit, shortly before nestlings fledged (Appendix A).

2.2.3 Morphological traits, age and sex determination

In June, nestlings were visited for the first time. The nestlings were brought down from the nest in a nylon bag. All measurements were carried out on the ground (Fig. 4). Nestlings were given numbered steel rings and thereafter weighted to the nearest 5 g by a Pesola® spring balance. Using a calliper with a precision to 0.1 mm, bill length and depth, tarsus length, width and depth, and hind claw length were measured. Lengths of wing and tail (from the cloak to the tip of the tail feathers if already present) were recorded using a 50 cm ruler (±1 mm). The measurements were repeated at the second visit in the same way.

Fig. 4 Procedures on the visits of Northern Goshawk nestlings in the breeding season 2008 in Troms County, Norway: Climbing on the trees to take nestlings down to the ground (left) and measuring the nestlings (right). Photos: Trond Johnsen.

Nestling age was estimated using a photographic guide (BOAL 1994) and varied between <10 and approximately 19 days at the first visit. In order to reduce disturbance, nestlings younger than 10 days were not measured which led to reduction in sample size (Appendix A). Fig. 5 illustrates the appearance of nestlings younger than the minimum age and at the maximum age for measuring. Sex was determined visually by comparing the tarsus size of the nestlings, female having clearly bigger tarsus than males (BYHOLM *et al.* 2002).

Fig. 5 Northern Goshawk nestlings of the breeding season 2008 younger than the minimum age (approx. 4-7 days) to the left and at the maximum age for measuring (approx. 40 days) to the right. Troms County, Norway. Photos: Lisbeth Schnug.

2.2.4 Anti-helminth treatment

Since one aim of this study was to record effects of parasite removal on living birds, and the Northern Goshawk is protected by the *Convention on the Conservation of European Wildlife and Natural Habitats* (1979), individuals in this study could not be sacrificed. However, JENNSVOLL and KØHLER (2001) examined 47 Northern Goshawks from Norway that were found dead for gastrointestinal parasites, and 39 were infested with one or more of the following helminths: nematodes (*Porrocaecum* sp., *Capillaria falconis*/ *Capillaria* sp., *Synhimantus* sp., *Cyrnea* sp., and 3 unidentified nematode species), cestodes (unidentified), and eggs from trematode and strongylide species. According to JENNSVOLL and KØHLER (2001) more juvenile than adult Goshawks had helminths in their intestines; of the 22 examined young birds 21 were infested with one or several helminth species. Thus, it can be assumed that the majority of the Northern Goshawk nestlings included in this study had gastrointestinal parasites.

Nestlings were assigned randomly to two groups, one receiving medicine and the other remaining untreated. In order to do so, every second nestling albeit sex, nest belonging or condition that was taken out of the bag was treated. The nestlings that were too young for measuring were excluded from the experiment (Appendix A). At the first visit, nestlings of the treatment group were treated with a 2 mL (equivalent to 50 mg active ingredient fenbendazole) oral dose of 2.5% PANACUR® (Hoechst Roussel Vet GmbH) which is an anthelmintic drug against endoparasites such as nematodes, cestodes and lungworms (YAZWINSKI *et al.* 1992 & 1993, HANSSEN *et al.* 2003). The nestlings in the control group were given 2 mL distilled water as a placebo treatment. No negative side effects of fenbendazole have been shown in other bird species (SHORT *et al.* 1988, PEDERSOLI *et al.* 1989, HANSSEN *et al.* 2003) and disadvantages for the nestlings of the treated group were thus not expected.

As stated above, nestling age at the first visit differed. Age might influence the effect of treatment on morphological traits, but since both groups were assigned randomly there is no indication of different age distribution in the two groups. Further, although sample size was reduced due to missing measurements of some nestlings, the remaining nestlings were approximately evenly balanced on the treatment and control group.

2.2.5 Blood sampling

Blood was sampled with a syringe from the wing vein at the second visit (between 0.1 and 4.0 mL). The blood was centrifuged at 8000 rpm for 10 minutes and the supernatant plasma transferred to a new Eppendorf® tube. The plasma was frozen on the same day.

2.2.6 Collection of prey remains

Prey remains (feathers, bones and carrion) at nests and the closer surroundings were collected at both visits in order to assess diet composition. This is the quickest and most commonly used technique for studying the diet composition of nestlings. However, the findings may represent less than 30-40 % of the actual prey the parents bring to the nests. In particular, mammals and young bird prey are likely to be underestimated by this technique (KENWARD 2006).

2.2.7 Environmental contaminants and sample analyses

Plasma samples (between 0.04 and 1.0 mL) of Northern Goshawk nestlings were analyzed for a set of POPs and PFCs (Appendix B). The analyses of the environmental contaminants were carried out at the Norwegian Institute of Air Research (Norsk Institutt for Luftforskning, NILU) in Tromsø and followed standard procedures. Briefly, in order to extract POPs from the plasma, an aliquot of each sample was denatured with ethanol after the addition of an internal standard solution. Saturated ammonium sulphate solution was added in order to enhance the extraction process. The lipids storing the target compounds were bound to n-hexane. The samples were cleaned up by Florisil® column chromatography and POPs recovered by elution with dichloromethane in n-hexane. To all samples a recovery standard solution was added. Samples were quantitatively analyzed by gas chromatography (GC) coupled with a mass selective detector. PFCs were extracted and quantified as follows. An internal standard solution was added to an aliquot of each sample. The PFCs were bound to acetronile (ACN) and the supernatant ACN-phase was transferred to tubes containing Envi-Carb™ graphitized carbon adsorbent and glacial acetic acid to clean up samples. An aliquot of the supernatant solution was spiked with a recovery standard solution and PFCs quantified using high pressure liquid chromatography (HPLC).

As part of the quality control, blanks and reference material (1589a human serum) were run concurrently with the samples. Details regarding the analyses can be found in HERZKE *et al.* 2005 and GÖTSCH *et al.* 2004.

2.3 Statistics

Statistical analyses were performed using the program R 2.7.2. (R Development Core Team 2008).

2.3.1 Predictor variables quantifying pollutants

Wet weight concentrations of the pollutants were used and the POP and PFC concentration values were Log-transformed in order to achieve normal distributions. To reduce the number of predictor variables quantifying the concentrations of pollutants a principal component analysis (PCA) was used and composite measures of pollutant concentrations produced (principal components, PCs). The first two principal components, PC1 and PC2, accounted for 70 % of the variation in pollutant concentrations and were therefore used as predictor variables in the statistical models. Tetrabromobisphenol-A (TBA), compound 1 and compound 2 (undetermined compounds), dichlorodiphenyl-dichloroethylene (p,p'-DDE), PBDE-100 and PBDE-154 were most correlated with PC2 ($0.74 < r < 0.92$). Most of the remaining PBDEs, PCBs, hexachlorobenzene (HCB), heptachlor epoxide, chlordanes, nonachlors, Mirex, and most of the PFCs had the highest correlation with PC1 ($0.65 < r < 0.98$). Only β-hexachlorocyclohexane (β-HCH), PCB-28, PBDE-99, perfluorononanoic acid (PFNA) and perfluuoroundecanoic acid (PFDcA) were not correlated to either of the two principal components (Appendix C).

2.3.2 Estimating effects on morphological traits

The distribution of the morphological measurements fulfilled the assumptions of normality. To assess potential effects of the anti-helminth treatment and environmental contaminant concentrations on morphological traits of the nestlings, linear mixed models (lme) were used. The variable "nest" was included as a random effect controlling for potential dependence

between nestlings within clutches. As response variables the morphological traits at the second visit and the difference in traits between both visits (Δ-trait) were used. The effect of anti-helminth treatment could not have been expressed in the morphological traits before the second visit, and the first visit measurements were thus not used as a response variable in the analysis.

When modelling the effects on Δ-traits it would be desirable to control for nestling age at the first visit and time lag between the two visits. However, the age of the nestlings at the first visit was only roughly estimated and the maximum difference in age between the nestlings included in the experiment was only approximately seven days (Appendix A); the age estimations are thus too imprecise to be used in the analysis. Yet, as both treatment groups were assigned randomly, there is no indication of different age distribution in the two groups. Further, time lags between the two visits were approximately equal for the nestlings in the treatment (14.0 days on average) and the control group (13.4 days on average) (Appendix A). As the sample size was small (n = 11) and it thus was necessary to reduce predictor variables in order to maintain degrees of freedom, the time lag was not included in the models. To investigate whether the differences in the size of morphological traits might be driven by sexual differences, the variable "sex" was considered in model selections for all morphometric measures in spite of tarsus measures (as the sex determination of the nestlings was based on tarsus size). Hence, the four predictor variables anti-helminth treatment, environmental contaminant concentrations (condensed to PC1 and PC2) and sex were used in different combinations in the models, i.e. it was also tested for interactions between the predictor variables. The treatment variable was always included in the models as it reflects the experimental part of the study.

To choose the best model for the effects on each trait, Akaike's Information Criterion corrected for small sample sizes (AICc) was used as a model selection criterion. AICc is used when overall sample size divided by total parameter units examined is < 40 (BURNHAM and ANDERSON 2003). However, as a model best at predicting observations is not necessarily the best model at estimating effect sizes, the information from different models were compared.

3. Results

3.1 Breeding success

Brood size was on average 2.8 (2-4) nestlings. All 26 Northern Goshawk nestlings of the 9 breeding pairs survived until the end of the study.

3.2 Diet composition

Prey remains collected varied between the nests. Willow Grouse (*Lagopus lagopus*) dominated the prey findings (Fig. 6). Further, remains of Black Grouse (*Tetrao tetrix*), Redwing (*Turdus iliacus*), Snow Bunting (*Plectrophenax nivalis*), Brambling (*Fringilla montifringilla*), Common Cuckoo (*Cuculus canorus*), Common Magpie (*Pica pica*), Northern Hawk Owl (*Surnia ulula*), Eurasian Woodcock (*Scolopax rusticola*) and Common Gull (*Larus canus*) were found at nests. The material is too small to allow any evaluation of potential differences in diets among breeding pairs.

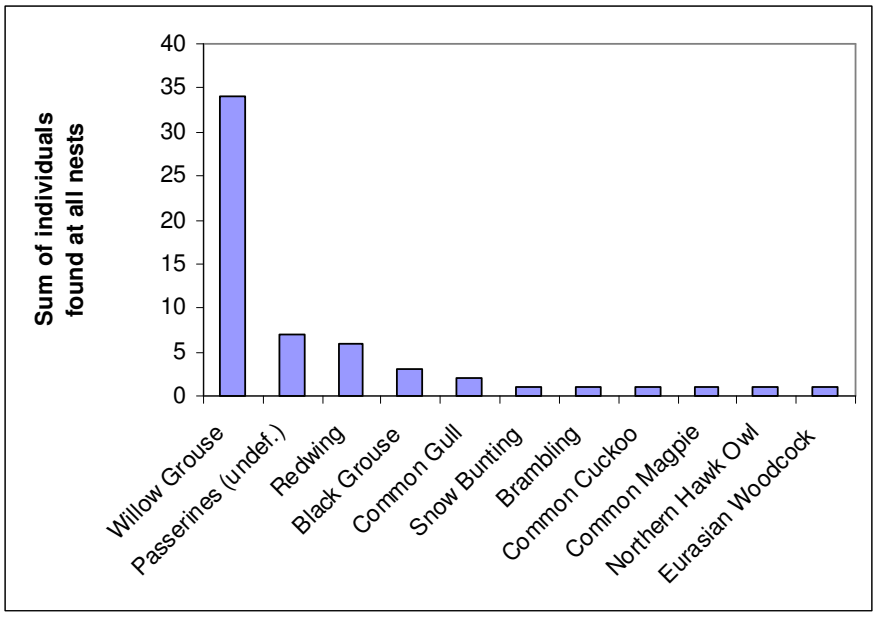

Fig. 6 Composition of total prey findings at Northern Goshawk nests in Troms County, Norway, in the breeding season 2008. The bars show the sum of prey items found at all nests (*n* = 9).

3.3 Environmental contaminants

The results of the residues of a set of environmental contaminants measured in blood plasma of Northern Goshawk nestlings are presented in Appendix B. Total PCBs and p,p'-DDE accounted for 89 % of the sum of all measured pollutants (Fig. 7). However, concentrations varied between the nestlings (Appendix B).

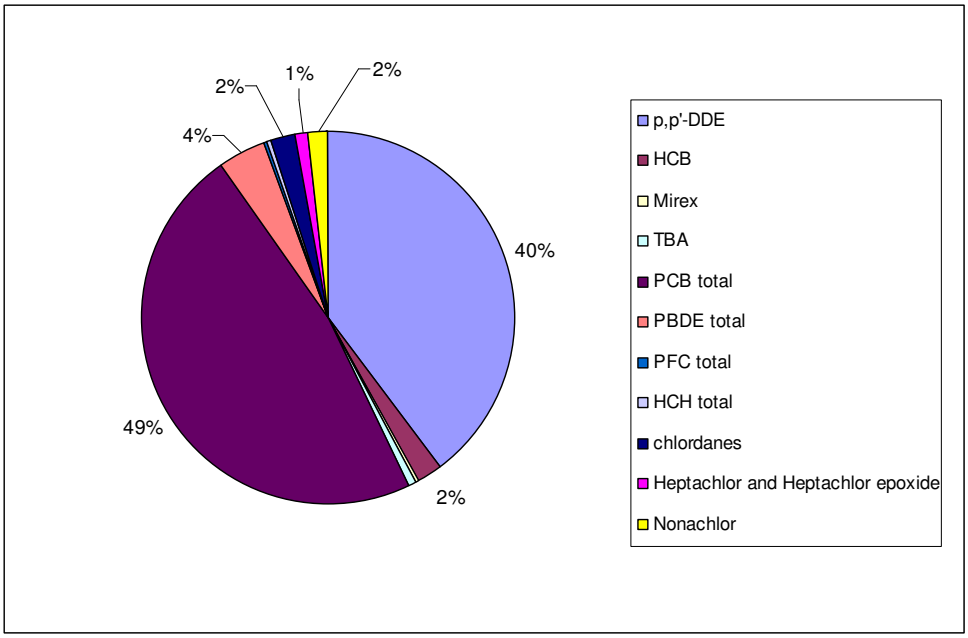

Fig. 7 Relative composition of the measured environmental contaminants in the blood plasma of Northern Goshawk nestlings in Troms County, Norway, in the breeding season 2008 in percent. The percentage values are based on the mean wet weight concentration over all nestlings. Mirex, TBA, total PFCs, and HCH make out less than 1% of the total composition and are not labelled in the chart for reasons of clearness.

3.4 Effects on morphological traits

According to AICc and estimate values from the best models, the difference (Δ) in tail length (hereafter referred to as *tail growth*) was the only morphological trait that could be explained by the stress predictor variables. Except for wing measures and bill heights, sex was a strong and consistent predictor of the morphological traits (Appendix D and E).

3.4.1 Effects of anti-helminth treatment on tail growth

The model including anti-helminth treatment as the only predictor variable was one of the best models explaining the variation in tail growth (Appendix D). In this model treatment had a positive effect on tail growth: Tail growth in the treated group was 19.2 ± 8.7 % (estimate ± SE) higher than in the control group (Tab. 1, Fig. 8). The statistical uncertainty of the estimate is large, and the estimate is not strictly significant ($p>0.05$) as could be expected from the small sample size. However, the effect size is so large that it can be considered as potentially biologically important.

Tab. 1 Linear mixed model output for the relationship between anti-helminth treatment and the difference (Δ) in tail length (tail growth) in mm of Northern Goshawk nestlings in Troms County, Norway, in the breeding season 2008. The upper and lower bound of the 95% confidence intervals are given as "upper CI" and "lower CI". "Treatment" gives the difference in tail growth in mm to the control group (Intercept). *n*=11 (5 treated, 6 control). Groups (nests) = 5.

Fixed effects: tail growth ~ treatment

	estimate	SE	Lower CI	upper CI	d.f.	t-value	p-value
Intercept	59.00	5.14	47.05	70.95	5	11.48	0.0001
treatment	11.34	5.11	-0.55	23.23	5	2.22	0.0773

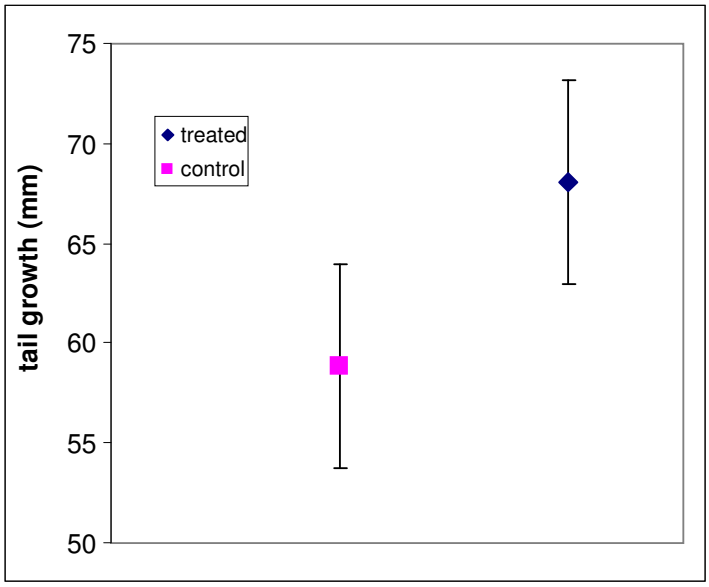

Fig. 8 Difference in tail growth (Δ tail length) in mm (±SE) of Northern Goshawk nestlings in Troms County, Norway, in the breeding season 2008 between the control and the treated group. *n* = 11 (5 treated, 6 control), groups (nests) = 5.

3.4.2 Effects of environmental contaminants on tail growth

An almost equally good candidate model (according to AICc) compared to the one with treatment as the only fixed effect was a model that in addition included PC1 of the environmental contaminants (Appendix D). This model estimated a positive, but quite uncertain, relationship between PC1 and tail growth (Tab. 2, Fig. 9). The three individuals with the highest values for PC1 may be considered as outliers (see Fig. 9). Yet, due to small sample size, three individuals make out 27 % of the total sample and a removal is also questionable.

Tab. 2 Linear mixed model output for the relationship between the factor values of the first principal component (PC1) of a principal component analysis of concentrations of 37 environmental contaminants in blood plasma and the difference (Δ) in tail length (tail growth) in mm of Northern Goshawk nestlings in Troms County, Norway, in the breeding season 2008. The upper and lower bound of the 95% confidence intervals are given as "upper CI" and "lower CI". The intercept gives the mean tail growth for nestlings in the control group when PC1 is assumed to be zero. *n*=11 (5 treated, 6 control), groups (nests) = 5.

Fixed effects: tailgrowth ~ PC1 + treatment

	estimate	SE	lower CI	upper CI	d.f.	t-value	p-value
Intercept	60.97	4.34	50.70	71.25	4	14.05	0.0001
PC1	1.52	0.64	0.01	3.02	4	2.39	0.0754
treatment	6.50	4.57	-4.31	17.32	4	1.42	0.2275

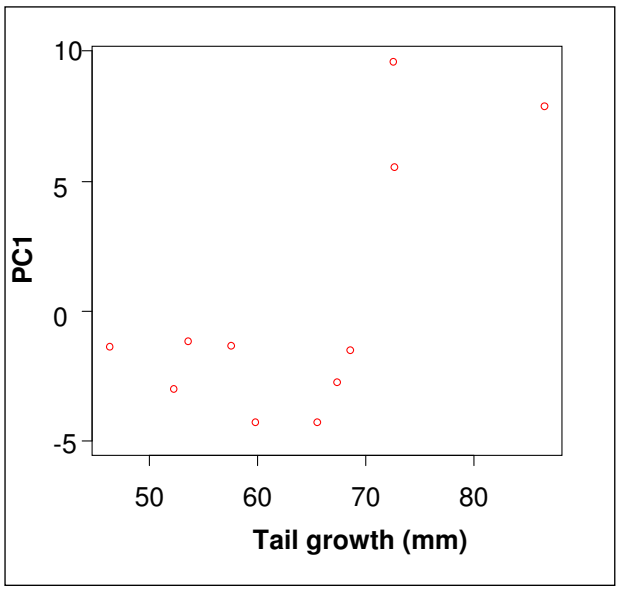

Fig. 9 Scatterplot of the PC1 scores of the principal component analysis of the concentrations of 37 environmental contaminants in blood plasma against tail growth (difference (Δ) in tail length) in mm of Northern Goshawk nestlings in Troms County, Norway, in the breeding season 2008.

According to the estimate values of the model in Tab. 2 tail growth increases with 2.5 ± 1 % (estimate ± SE) when PC1 increases with one unit. However, using the principal component in the model makes it difficult to interpret the actual effect size and thus the strength of the relationship. To further assess the relationship between environmental contaminants and tail growth, the concentrations of environmental contaminants that were correlated with PC1 were plotted against tail growth. There was a strong positive and statistically significant (r=0.84, p=0.0012) correlation between tail growth and perfluorohexane sulfonate (PFHxS) and perfluoroundecanoic acid (PFUnA) (r=0.71, p=0.0136), respectively. These relationships are depicted in Fig. 10.

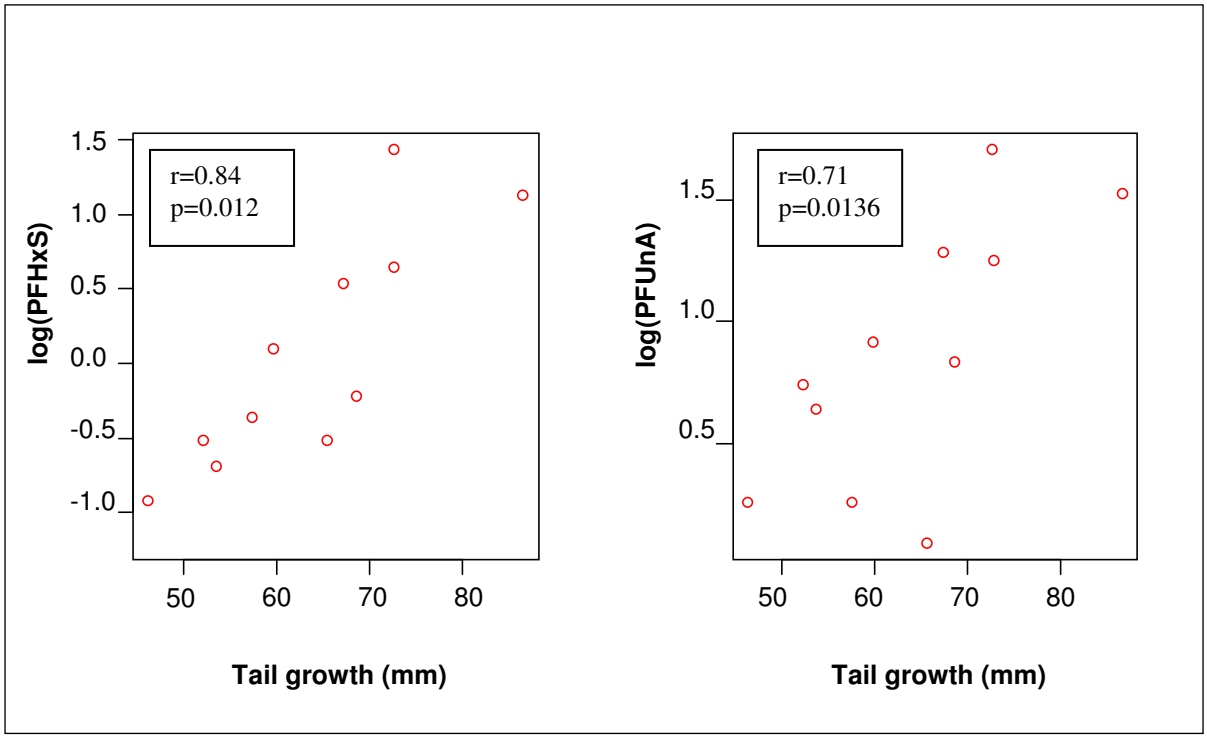

Fig. 10 Scatterplots of the concentrations of PFHxS and PFUnA in blood plasma and tail growth in mm of Northern Goshawk nestlings in Troms, Norway, in the breeding season 2008. Note that concentration values are given on the Log-scale.

4. Discussion

The goal of this study was to estimate stress effects on morphological traits of Northern Goshawk nestlings in Troms County, Norway. The two stressors that were investigated were environmental contaminants and endoparasitism (helminths). The hypothesis of the present study was that both helminths and environmental contaminants have negative effects on morphological traits of Northern Goshawk nestlings. Anti-helminth treatment was thus expected to improve the performance of Northern Goshawk nestlings, and to mitigate adverse effects induced by pollutants if helminths enhanced such potentially negative effects.

Sex, but neither anti-helminths treatment nor environmental contaminant concentrations explained hind claw length, bill length and body mass. This was not unexpected as sexual dimorphism in Northern Goshawks is distinct (BÄHRMANN 1937 *et al.*, FISCHER 1980). This does of course not exclude the possibility that stress may have an impact on these traits. Previous studies revealed that the rate at which the final size of skeletal traits is attained can be changed due to parasitism (e.g. SAINO *et al.* 1998, O'BRIEN and DAWSON 2008). Skeletal growth measures are however highly heritable in birds (GEBHARDT-HENRICH and van NOORDWIJK 1991, O'BRIEN and DAWSON 2008) and it is therefore not surprising that these traits may be unaffected by a short-term alteration in stress as it was done in the present study by anti-helminth treatment; especially if the magnitude of the stress was low.

To the knowledge of the author no study has yet focused on a relationship between anti-helminth treatment and morphological traits in birds, but previous studies have shown that treating birds against helminths can have other positive effects: HANSSEN *et al.* (2003) showed that treating Common Eiders (*Somateria mollissima*) with fendabenzole increased return rates in females not completing reproduction. Another study revealed that nesting success of Glaucous Gulls (*Larus hyperboreus*) was enhanced when treated with the anti-helminthic drug (BUSTNES *et al.* 2006).

In this study there was a positive relationship between the tail growth of Northern Goshawk nestlings and anti-helminth treatment. Although somewhat statistically uncertain, tail growth appeared to be substantially higher in nestlings of the treated group. If anti-helminth treatment really had an effect on tail growth, but on none of the other traits, allocation to feathers might

be reduced under energetic constraints induced by parasites, while allocation to skeletal growth and body mass is not affected. This was also shown in a study by O'BRIEN and DAWSON (2008): Mountain Bluebird (*Sialia zeaxanthin*) nestlings grew shorter wing feathers in the presence of the ectoparasitic blow flies (*protocalliphora* spp.). But why did the anti-helminth treatment in this study only affect the rate of tail but not wing growth? One explanation may be that the primaries of Northern Goshawk nestlings are exposed at an earlier nestling stage compared to tail feathers: while the primaries are already two-thirds grown at the age of six weeks, the tail feathers are less than half exposed (KENWARD 2006). As the nestlings were treated with the anti-helminthic drug at the age of two to three weeks, and the effect of the treatment was expected to occur with a time delay, wing feathers may already have grown to a large extend when the drug started to affect the performance of the nestlings. Thus, by the time the drug had relieved the nestlings from some of the energetic constraints, resources were mainly needed for tail growth. Further, animals will give priority of resource allocation to maintenance functions to guarantee survival (COOP and KYRIAZAKIS 1999). Reducing allocation due to stress may therefore firstly occur to traits that are less crucial for the general performance of the bird, saving resources for maintaining body mass and flight ability. Hence, body mass, skeletal growth and wing development are more important than tail growth, as tail feathers are crucial for steering but beside of providing lift not for the flight as such (e.g. THOMAS and BALMFORD 1995, TUBARO 2003). As only tail growth was affected, stress induced by helminths may have been rather low. Accordingly, as anti-helminth treatment appeared to have relieved Northern Goshawk nestlings from the parasitic stress, more resources could be allocated to tail feather development, which resulted in an increased tail growth.

The second prediction of the present study was that pollutants would affect the performance of nestlings negatively, resulting in e.g. reduced growth or size of morphological traits. However, there was no negative relationship between the concentration of pollutants and any of the morphological traits of the nestlings. On the contrary, the principal component PC1 showed an unexpected positive relationship with tail growth but none of the other morphological traits. This positive relationship appeared to be driven by a strong correlation between tail growth and the two specific contaminants PFHxS and PFUnA. It would go too far to interpret this relationship as a direct effect of the pollutants, i.e. a positive impact of certain contaminants on nestling growth. One can only speculate on an explanation for this effect. First of all, it would be essential to know if the levels of contaminants found in the

present study are high or low compared with other Northern Goshawk individuals or raptor species. In Norway, pollutants in Northern Goshawks and other raptor species were assessed using egg samples. The contamination levels of Northern Goshawk eggs are rather low compared with other raptor species (HERZKE *et al.* 2002 & 2005, NYGÅRD *et al.* 2006). Thus, it can be assumed, that contamination of nestlings is low as well. It is however difficult to classify the present results due to a lack of studies on pollutants in plasma of raptor nestlings. In several studies the concentrations of pollutants in blood plasma of Bald Eagle (*Haliaeetus leucocephalus*) nestlings in America were measured (e.g. CESH *et al.* 2008). Due to high variations in pollutant concentrations in Bald Eagle nestlings between the single study sites, the concentrations of the Northern Goshawk nestlings of this study were somewhat on an intermediate level. ELLIOTT and HARRIS (2001/2002) derived a critical level of p,p'-DDE and total PCBs in Bald Eagle plasma that was based on declines in productivity below the minimal sustainable rate (27.8 µL kg^{-1} for p,p'-DDE and 189 µL kg^{-1} for total PCBs). The PCB and p,p'-DDE concentrations in the plasma of the Northern Goshawk nestling of this study were by far lower than these critical levels. Yet, sensitivity to contaminations varies considerably between species (HOFFMAN *et al.* 1998) and critical levels cannot be applied to other species without former testing.

The absence of negative effects of any of the pollutants may suggest that the contamination levels of the Northern Goshawk nestlings were below any effect limit and thus did not affect nestling morphological traits. This may be linked to the fact that the Northern Goshawk is a terrestrial predator and may accordingly be less exposed to environmental contaminants. Habitat differences between species can lead to interspecific variation in the uptake of pollutants, i.e. predators feeding in aquatic environments take up higher proportions of pollutants than predators of terrestrial ecosystems (LARSSON *et al.* 1990). Although the breeding pairs of the present study were located close to the coast (Fig. 1), the prey findings indicated that not much marine prey was incorporated. Moreover, WIDÉN (1997) proposed that the Northern Goshawk may not directly take up contaminants from other regions due to its own sedentary behaviour and the sedentary behaviour of its most important prey species. The diet composition of the Northern Goshawks in this study shows that even though migratory bird species were part of the prey composition, the main prey was probably Willow Grouse. This supports WIDÉN's (1997) suggestions. Yet, this study does not provide investigations of potential other effects of the detected pollutant concentrations. Thus, there may be detrimental effects owing to the concentrations found, e.g. on reproduction or

survival, as shown in studies on other bird species (e.g. BLUS *et al.* 1983, BUSTNES *et al.* 2006). Therefore, this requires further investigations.

But how can the positive relationships between tail growth and PC1 be interpreted? The most likely explanation may be that the concentrations of the pollutants correlated with PC1 rather are indicators for the diet of the nestlings, i.e. prey species that contain nutrients affecting feather growth positively also contain much of these pollutants. To verify this assumption a detailed analysis of the diet would be desirable. The collected prey items at nests in this study provide an insight into the diet of the Northern Goshawk nestlings. However, it is difficult to derive a relationship between contamination level and diet from it, as diet differences between the single individuals could not be assessed. Hence, it can only be assumed that both the increased tail growth and the contamination level are a result of the quality or quantity of the diet, but that there is no direct connection between contamination and tail growth.

Overall, it can be assumed that there were no measurable effects of the found contaminant concentrations on morphological traits of Northern Goshawk nestlings. This is in agreement with other studies that failed to find significant effects of pollutants on morphological traits in birds (e.g. DAUWE *et al.* 2006).

The findings of the present study are somewhat ambiguous: They may either imply that stress due to both helminths and pollutants have been rather low, affecting Northern Goshawk nestlings less than expected. Another possibility is that effects occur stronger on others traits than morphometrics. The assumption that stress levels induced by both pollutants and helminths might have been low does however not imply that stress levels are generally low in Northern Goshawk nestlings. Other stressors, such as diseases or nutrient deficiency may affect nestlings more severely or interact with the stressors focused on in this study.

The study failed to reveal strong and reliable effects of anti-helminth treatment and environmental contaminant concentrations on Northern Goshawk nestlings. The reasons may be as stated above. However, results were weakened due to a small samples size and missing replicates. Further, as morphological traits in the growth phase of nestlings may be strongly dependent on prey quality and quantity a more precise assessment of the diet is desirable, such as isotope analyses of nestling body feathers. Moreover, blood concentrations of pollutants are point samples in time of dynamic processes, and differences in pollutant

burdens between individuals should be interpreted with care, as recent feeding might be a confounding factor (HENRIKSEN *et al.* 1998).

5. Conclusions and future studies

The results of the present study may indicate that stress due to both helminths and environmental contaminants on morphological traits of Northern Goshawk nestlings in the breeding season 2008 in Troms County, Norway, was low. While helminths did not seem to affect any essential morphological traits of nestlings, anti-helminth treatment had a positive effect on tail growth. The absence of any measurable negative effects of pollutants on nestling morphometrics shows that although pollution is an environmental perturbator, the measured concentrations may not cause stress on nestling growth performance. Even though the Northern Goshawk is a common raptor species in Norway, it is classified as "vulnerable (VU)" on the *Norwegian Red List* due to decreased numbers of reproductive individuals compared with earlier population densities (KÅLÅS *et al.* 2006, NYGÅRD *et al.* 2006). Thus, in order to protect the Northern Goshawk population in Norway, it is crucial to have a clear understanding of the responses of Northern Goshawks to stressors. The present study provides first insights into the effects of an anthropogenic and a natural stressor on the growth performance of Northern Goshawk nestlings. The study emphasizes the importance of assessing other potential stress factors that may affect nestlings more severely and to investigate possible effects of the present stressors on other traits than morphometrics.

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Appendix A

Overview over the Northern Goshawk nestlings in the breeding season 2008 in Troms County, Norway, included in the present study. The age of the nestlings on the first visit is a rough estimation and serves only the illustration of age differences between the nestlings. "Time lag" gives the number of days between the first and the second visit. Nestlings treated with fendabenzole are indicated with "1" and nestlings of the control group with "0" in the treatment column. Nestlings that were too young for measuring were not included in the study and are indicated with "-".

nest-ID	nestling-ID	sex	age on the first visit (days)	time lag	treatment
I	1.1	f	< 10	16	-
	1.2	f	< 10	16	-
	1.3	f	< 10	16	-
II	2.1	f	17	16	1
	2.2	m	15	16	0
	2.3	f	19	16	1
III	3.1	m	14	13	0
	3.2	m	15	13	1
IV	4.1	f	13	14	0
	4.2	m	12	14	1
V	5.1	f	14	15	1
	5.2	f	18	15	0
	5.3	m	16	15	1
VI	6.1	f	17	13	0
	6.2	m	19	13	1
VII	7.1	f	< 10	13	-
	7.2	f	< 10	13	-
	7.3	m	< 10	13	-
VIII	8.1	f	19	12	1
	8.2	m	13	12	0
	8.3	m	17	12	0
	8.4	m	15	12	1
IX	9.1	f	< 10	14	-
	9.2	m	< 10	14	-
	9.3	m	< 10	14	-
	9.4	f	< 10	14	-

Appendix B

Wet weight concentrations of environmental contaminants (pg g^{-1}) in blood plasma of Northern Goshawk nestlings in the breeding season 2008 in Troms County. "< DL" indicates values below detection limit. Values marked with an asterisk were between the limit of detection and limit of quantification. For calculating the means and median, <DL-values were treated as 0.5xDL. The average lipid content (extracted organic matter, EOM) of the plasma samples was 0.64 %.

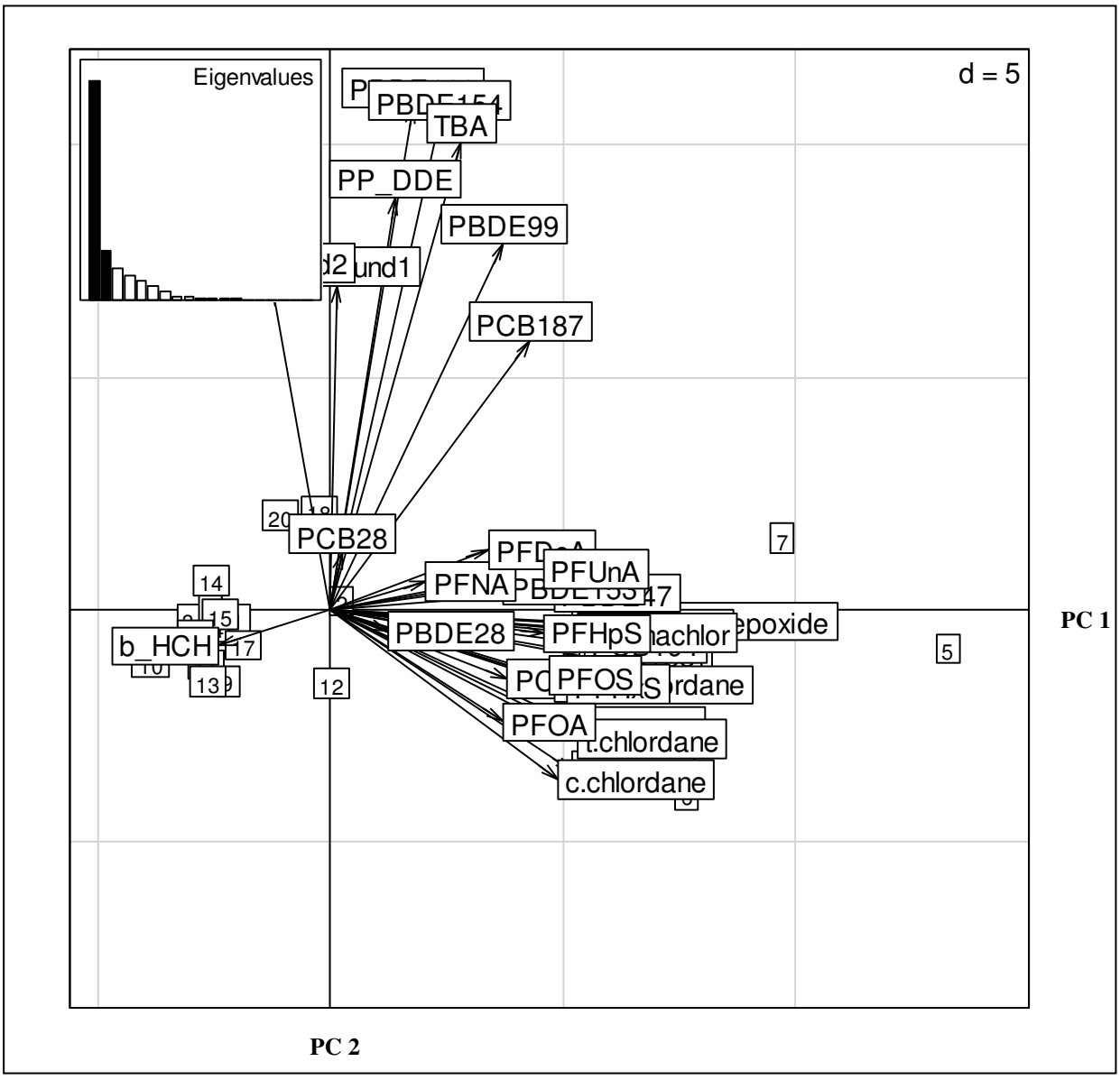
	mean	median	geo. mean	SE (mean)	min.	max.
			n=18			
Organochlorines						
Polychlorinated diphenyls(PCB)						
PCB-28	27.7	4.2	13.01	30.68	< DL	100.0
PCB-99	482.7	327.0	322.5	485.8	< DL	1644.0
PCB-101	263.3	121.7	128.57	414.03	< DL	1781.0
PCB-105	202.1	74.6	81.99	303.31	< DL	1050.0
PCB-118	695.7	299.0	321.0	985.3	< DL	3097.0
PCB-138	2432.6	1469.0	1767.2	2421.0	658.0	9852.0
PCB-153	3272.9	2078.0	2486.2	2749.7	929.0	9782.0
PCB-180	1653.1	1052.0	1276.6	1346.4	441.0	5030.0
PCB-183	241.8	156.4	192.7	192.1	109.2	686.0
PCB-187	863.2	743.5	693.3	566.0	193.2*	1989.0
Dichlorodiphenyldichloroethane						
p,p'-DDE	8727.6	7211.0	6758.0	6540.4	1769.0	24718.0
o,p'-DDE	< DL	< DL	< DL	< DL	< DL	< DL
Dichlorodiphenyltrichloroethane						
p,p'-DDT	< DL	< DL	< DL	< DL	< DL	< DL
o,p'-DDT	< DL	< DL	< DL	< DL	< DL	< DL
Hexachlorocyclohexanes (HCH)						
α-HCH	< DL	< DL	< DL	< DL	< DL	< DL
β-HCH	68.0	74.4	42.52	54.58	< DL	143.0
γ-HCH	< DL	< DL	< DL	< DL	< DL	< DL
Hexachlorobenzene (HCB)	481.5	412.0	275.0	373.7	0.1	1754.0
Heptachlor epoxide	286.6	263.0	186.4	232.2	1.8	818.0
trans-chlordane	43.7	23.0	19.4	54.9	0.9	185.0
cis-chlordane	9.4	1.9	4.1	14.9	1.9	53.0
oxy-chlordane	407.5	299.0	212.4	354.4	2.5	1173.0
trans-Nonachlor	240.3	184.0	148.3	207.7	0.9	772.0
cis-Nonachlor	96.0	84.0	63.3	76.8	0.5	270.0
Mirex	108.8	86.0	59.4	104.7	8.3	362.0

B continued

	mean	median	geo. mean	SE (mean)	min.	max.
Brominated Flame Retardants (BFR)						
Polybrominated diphenyl ethers (PBDE)						
PBDE-28	9.6	1.4	3.4	16.9	1.4	57.0
PBDE-47	220.1	20.2	30.9	504.6	1.6	1686.0
PBDE-99	219.0	154.9	109.5	206.5	1.6	683.0
PBDE-100	345.1	136.0	106.1	524.1	1.5	2196.1
PBDE-153	48.0	1.6	6.5	88.0	1.6	271.0
PBDE-154	107.6	60.0	32.1	147.0	1.5	584.0
Tetrabromobisphenol-A (TBA)	73.4	43.0	19.5	98.5	1.0	371.0
Perfluorinated Compunds (PFC)						
Perfluorohexane sulfonate (PFHxS)	1.1	0.7	0.8	1.0	0.3	4.2
Perfluoroheptane sulfonate (PFHpS)	0.4	0.2	0.3	0.3	0.1	1.6
Perfluorooctanesulfonic acid (PFOS)	19.5	15.0	16.2	15.9	6.6	78.4
Perfluorodecane sulfonate (PFDcS)	0.1	0.1	0.1	0.1	< DL	0.5
Perfluorobutanoic acid (PFBA)	< DL	< DL	< DL	< DL	< DL	< DL
Perfluoropentanoic acid (PFPA)	< DL	< DL	< DL	< DL	< DL	< DL
Perfluorohexanoic acid (PFHxA)	< DL	< DL	< DL	< DL	< DL	< DL
Perfluoroheptanoic acid (PFHpA)	< DL	< DL	< DL	< DL	< DL	< DL
Perfluorooctanoic acid (PFOA)	1.2	1.1	1.1	0.6	0.7*	3.1
Perfluorononanoic acid (PFNA)	2.8	2.3	2.3	1.4	0.1	5.6
Perfluorodecanoic acid (PFDcA)	1.1	1.1	1.0	0.5	0.4	2.1
Perfluoroundecanoic acid (PFUnA)	2.8	2.6	2.6	1.1	1.1*	5.5
Undetermined compounds						
Compound 1	43.3	38.3	25.60	34.11	< DL	123.0
Compound 2	52.6	58.0	26.44	43.93	< DL	140.0

Appendix C

Scatterplot of the first two principal components of a principal component analysis of concentrations of 37 environmental contaminants in the blood plasma of Northern Goshawk nestlings in the breeding season 2008 in Troms County, Norway. The closer the arrow to the axis and the longer the arrow length, the stronger is the pollutant correlated to the principal component. The numbers in the outlined boxes show the location of the nestlings in relation to the PCs. The screeplot in the upper left corner shows how much the single components (bars) explain of the total variation (x-axis: PCs, y-axis: Eigenvalues).



Appendix D

Model selections for the effects of sex, anti-helminth treatment (treat) and environmental contaminants (condensed to PC1 and PC2 from a principal component analysis of concentrations of 37 environmental contaminants in blood plasma) and morphological traits of Northern Goshawk nestlings in the breeding season 2008 in Troms County, Norway. The left tables give the best models for the difference in traits between the first and the second visit (Δ (trait)) and the right tables the best models for traits at the second visit.

	d.f.	AIC	AICc
Δ (tail length)			
constant model	3	89.63	93.05
treat	4	87.87	94.53
PC1 + treat	5	83.97	95.97
Δ (wing length)			
constant model	3	136.54	138.72
treat	4	136.42	140.42
sex + treat	5	137.74	144.40
Δ (tarsus breadth)			
constant model	3	49.72	51.90
treat	4	51.70	55.70
PC1 + treat	5	53.70	60.36
Δ (tarsus width)			
constant model	3	50.42	52.60
treat	4	48.76	52.76
PC1 + treat	5	49.76	56.42
Δ (hind claw length)			
constant model	3	67.12	69.29
treat	4	68.01	72.01
PC1 + treat	5	69.10	75.77
Δ (bill length)			
sex + treat	5	64.47	71.14
constant model	3	69.75	71.94
treat	4	68.62	72.62
Δ (bill heights)			
constant model	3	73.26	75.44
sex + treat	5	75.59	82.26
PC1 + treat	5	75.75	82.42
Δ (body mass)			
sex + treat	5	200.57	207.24
constant model	3	205.71	207.89
treat	4	206.17	210.17

	d.f.	AIC	AICc
tail length			
constant model	3	154.68	156.68
treat	4	154.42	158.06
sex + treat	5	154.07	160.07
wing length			
constant model	3	161.05	163.05
treat	4	163.01	166.65
sex + treat	5	162.996	168.96
tarsus breadth			
constant model	3	54.45	56.45
treat	4	55.47	59.11
PC2 + treat	5	57.47	63.47
tarsus width			
constant model	3	56.81	58.81
treat	4	56.94	60.57
PC1 + treat	5	58.50	64.50
hind claw length			
sex + treat	5	71.43	77.43
constant model	3	77.36	79.36
treat	4	79.09	82.73
bill length			
sex + treat	5	47.28	53.28
sex * treat	6	49.19	58.53
constant model	3	63.91	65.91
bill heights			
constant model	3	63.05	65.05
treat	4	64.16	67.80
sex + treat	5	62.01	68.01
body mass			
sex + treat	5	202.68	208.68
sex * treat	6	204.67	214.01
treat	4	220.42	224.06

Appendix E

Linear mixed model output for the relationship between anti-helminth treatment, sex and morphological traits of Northern Goshawk nestlings in the breeding season 2008 in Troms County, Norway. The intercept gives the morphological trait in mm (g for body mass) for females when not treated. m=male, *n*=11 (5 treated, 6 control). Groups (nests) = 5.

Fixed effects: hind claw length (second visit) ~ sex + treatment

	estimate	SE	d.f.	t-value	p-value
Intercept	26.63	0.85	4	31.39	< 0.0001
sex (m)	-3.66	1.01	4	-3.63	0.0084
treatment	0.60	0.91	4	0.65	0.5349

Fixed effects: bill length (second visit) ~ sex + treatment

	estimate	SE	d.f.	t-value	p-value
Intercept	22.18	0.51	4	43.25	< 0.0001
sex (m)	-3.09	0.41	4	-7.62	0.0001
treatment	-0.55	0.33	4	-1.65	0.1437

Fixed effects: body mass (second visit) ~ sex + treatment

	estimate	SE	d.f.	t-value	p-value
Intercept	1126.63	67.17	4	16.77	< 0.0001
sex (m)	-377.37	51.33	4	-7.35	0.0002
treatment	-7.01	42.22	4	-0.17	0.8729

Fixed effects: wing growth ~ sex + treatment

	estimate	SE	d.f.	t-value	p-value
Intercept	124.91	14.71	4	8.49	0.0001
sex (m)	-7.46	5.47	4	-1.37	0.2211
treatment	5.11	6.83	4	-0.75	0.4826

Fixed effects: Δ (bill heights) ~ sex + treatment

	estimate	SE	d.f.	t-value	p-value
Intercept	3.18	0.94	4	3.38	0.0149
sex	-0.91	1.44	4	-0.63	0.5537
treatment	-0.92	1.42	4	0.65	0.5409