Anti-parasite treatment removes negative effects of environmental pollutants on reproduction in an Arctic seabird

Jan O. Bustnes^{1,*}, Kjell E. Erikstad¹, Sveinn A. Hanssen^{1,2},
Torkild Tveraa¹, Ivar Folstad² and Janncehe U. Skaare³

¹Norwegian Institute for Nature Research, The Polar Environmental Centre, 9296 Tromsø, Norway

²Biology Department, Faculty of Science, University of Tromsø, 9037 Tromsø, Norway

³National Veterinary Institute and Norwegian School of Veterinary Science, PO Box 8156 dep., 0033 Oslo, Norway

Recent studies have shown that the detrimental effects of anthropogenic pollutants may be worse if organisms are exposed to natural stress. In this study, we examined whether negative effects of organochlorines (OCs) could be influenced by parasites. In two breeding seasons, we administered an anti-helminthic drug to groups of breeding glaucous gulls (*Larus hyperboreus*), whereas control groups were placebo treated. In all birds, blood residues of the most important OCs in the study population (hexachlorobenzene, oxychlordane, *p,p'*-dichlorodiphenyldichloroethylene and polychlorinated biphenyl), were measured. The relationships between OCs and fitness components (i.e. nesting success and return rate between breeding seasons) were then compared between the birds receiving anti-parasite treatment and the controls. Among untreated males, higher blood residues of OCs were associated with lowered nesting success, while in males receiving anti-parasite treatment, there was no detrimental effect of OCs on fitness. Return rate was not affected by treatment or OCs. Our findings suggest that parasites may be an important factor in triggering reproductive effects of such pollutants, and that relatively low levels of OCs may have serious reproductive consequences in natural populations when stress from other sources is high.

Keywords: glaucous gull; organochlorines; parasites; experiment; breeding success; survival

1. INTRODUCTION

An important aspect of the present ecology is to investigate the effects of anthropogenic stressors on organisms in nature. Human-induced stress is adding to existing natural stress and the combination of different stressors may be more detrimental than single stressors acting alone (Sih *et al.* 2004). A well-known anthropogenic stress factor is environmental pollutants, such as pesticides and industrial chemicals. Recent experimental studies have provided evidence that the effects of environmental pollutants are more detrimental when combined with natural stressors. For example, laboratory studies of several frog species have found that the impact of pesticides on larval survival increases dramatically when combined with predation stress (Relyea & Mills 2001; Relyea 2003). In juvenile chinook salmon (*Oncorhynchus tshawytscha*), the combination of polychlorinated biphenyl (PCB) and a fluke (Trematoda) had greater negative effects on immune function than either of the stressors alone (Jacobson *et al.* 2003). In addition, other laboratory experiments on invertebrates, rodents and birds have found that environmental contaminants may have different effects when individuals were exposed either to pathogens, nutritive stress or low temperatures (Rattner & Franson 1984; Keith & Mitchell 1993; Sibly *et al.* 2000; Leung & Furness 2001). However, in natural bird

populations, the joint effects of environmental pollutants and natural stress have not, to our knowledge, been experimentally studied.

In the glaucous gull (*Larus hyperboreus*) population at Bear Island (Norwegian Arctic), blood residues of different organochlorines (OCs) are adversely related to behaviour, reproductive performance, survival and other factors (reviewed in Bustnes 2006), suggesting that OCs cause considerable stress. However, the levels of OCs, such as PCB ($\bar{x} = 15$, maximum = $30 \mu\text{g g}^{-1}$, wet weight in brains) and *p,p'*-dichlorodiphenyldichloroethylene (DDE; $\bar{x} = 3$, maximum = $6 \mu\text{g g}^{-1}$) in birds found dead, or dying after showing abnormal behaviour, have been below concentrations normally assumed to be lethal (Gabrielsen *et al.* 1995), suggesting that mortality is caused by a combination of several interacting stress factors. For example, birds with high OC concentrations had altered immune status, poor immune function (Bustnes *et al.* 2004) and higher parasite burdens (Sagerup *et al.* 2000), suggesting complex interactions (trade-offs) between the ability to cope with OCs and the immune system. In birds, parasites are costly (Hudson & Dobson 1997) and a combination of high burdens of parasites and OCs may thus have great impact on individuals. The objective of this study was to experimentally examine interactive effects between OCs and parasites by testing whether effects of OCs could be mitigated by a reduction in parasite burdens. We randomly assigned individual glaucous gulls to anti-helminthic drug treatment or

* Author for correspondence (jan.o.bustnes@nina.no).

placebo treatment and recorded their levels of the four most abundant OCs (hexachlorobenzene (HCB), oxy-chlordane, DDE and PCB) in the blood of glaucous gulls (Bustnes 2006). We then compared nesting success (i.e. probability of hatching young) and the return rate to the next breeding season, between the treatment groups, as a function of the average blood residues of the four OCs. If parasites were enhancing the adverse impact of OCs, our prediction was that the effects of OCs should be reduced in medicated birds compared with placebo-treated birds.

2. MATERIAL AND METHODS

The study was carried out at Bear Island (74°30' N, 19°01' E) in late May and early June 2000 and 2001. All details about the study area have been described by Bustnes *et al.* (2000, 2001a). Breeding glaucous gulls were caught on their nests about two weeks into the incubation period (Bustnes *et al.* 2001a). Blood was sampled from the wing vein (*ca* 10 ml) with a syringe and frozen within 5 h. All individuals were given numbered steel rings and letter-coded PVC rings.

Body condition was indexed using body mass (± 5 g), controlling for body size (head + bill; ± 0.5 mm; Garcia-Berthou 2001). However, body size had no impact on our results and conclusions, and was thus removed from the final analyses. Sex was determined by size; males being larger than females (see details in Bustnes *et al.* 2001a).

During incubation, the nests were checked every third day and predation recorded. Nest predation was recorded as both partial predation (loss of eggs) and complete nest predation (all eggs lost). In the following analyses, however, we only used complete nest predation, owing to few cases of partial nest predation. In the hatching period, nests were checked daily.

The breeding areas were thoroughly searched for returning birds in the breeding seasons 2001 and 2002 to record the return rate of adults. Glaucous gulls usually occupy the same, or nearby, nest sites for several years (Bustnes *et al.* 2003a, 2005). We are thus confident that we were able to record a very high percentage of returning individuals.

(a) *Anti-parasite treatment*

Since we were recording fitness components of living birds, individuals in this study could not be sacrificed. However, Sagerup *et al.* (2000) killed and examined 40 nesting glaucous gulls for intestinal parasites in our study area. All gulls had intestinal helminths; 12 species in total. We thus have reliable information suggesting that all gulls included in this study had intestinal parasites. Moreover, critical to our experiment was a reliable method for removing parasites. In birds, several anti-helminthic drugs are known to be effective against intestinal parasites (Hanssen *et al.* 2003) and application of such drugs has also become an established method in ecological studies of wild birds (Hudson 1986; Fox & Hudson 2001; Hanssen *et al.* 2003; Seivwright *et al.* 2005). In 2000 and 2001, every second individual caught was administered a 2 ml (50 mg fenbendazole, approx. 30 mg kg⁻¹ body mass) oral dose of 2.5% PANACUR (Hoechst Roussel Vet GmbH), active ingredient fenbendazole (a benzimidazole; 25 mg ml⁻¹). The drug is effective against various intestinal parasites in birds, e.g. nematodes, lungworms and cestodes (Norton *et al.* 1991; Yazwinski *et al.* 1992, 1993), and has also been reported to cure Acanthocephala infections in monkeys (Weber & Junge 2000).

Moreover, one study showed that one treatment with fenbendazole eliminated all nematode parasites in 221 out of 230 birds from 38 species of six orders (Lawrence 1983). At recommended doses, negative side effects of fenbendazole are few (Short *et al.* 1988; Pedersoli *et al.* 1989), but toxic effects may be found in some bird species (Howard *et al.* 2002; Bonar *et al.* 2003). It is thus very unlikely that the drug will have any positive effects apart from parasite removal, either alone or in interaction with OCs.

The birds in the control group were given 2 ml of distilled water by the same procedure. Nesting pairs were always given the same treatment.

Age may be a factor influencing parasite burdens (Hudson & Dobson 1997), but since the medicated and placebo groups were randomly assigned and equal in all respects apart from treatment, there is no indication of different age distributions in the two groups.

(b) *Environmental contaminants and chemical analysis*

In this study, we measured blood residues of the four most important OC components in glaucous gulls at Bear Island, which make up more than 95% of the sum of measured OCs (table 1): HCB (3–4%), oxychlordane (3–4%), DDE (17–18%) and PCB (73–74%) (Bustnes *et al.* 2003a).

Manipulating the pollutant levels, as well as the parasite burden would be ideal. However, apart from the ethical aspect of poisoning animals in nature, the glaucous gulls already have high and greatly varying OC residues. It would thus be very difficult to disentangle the effects of contaminants occurring already from the effects of those experimentally given to the birds. Nevertheless, persistent organic pollutants that have no natural sources can be considered a 'treatment' on a continuous scale, since long-lived glaucous gulls have had only a limited time to adapt to this environmental stress, i.e. analyses from sediment cores have shown that OCs started to reach Bear Island in the 1950s, but high levels were not found before the late 1960s and early 1970s (Christensen *et al.* 2004). Moreover, OC concentrations in the blood of glaucous gulls seem largely unrelated to age (Bustnes *et al.* 2003b), further suggesting that age of the individuals is not a factor influencing the result of this study.

The OC analyses were carried out at the Environmental Toxicology Laboratory at the Norwegian School of Veterinary Science/National Veterinary Institute. All details regarding analyses of OCs can be found in Andersen *et al.* (2001). The following PCB-congeners were determined (IUPAC numbers: 99, 118, 138, 153, 170 and 180). In the analyses of ecological variables, we used the sum of these congeners, denoted PCB.

Other studies suggest a relatively high short- and long-term stability of the blood levels of OCs in incubating glaucous gulls under stable conditions, such as at Bear Island, indicating that blood concentration is a reliable and relative (compared with other individuals) measurement for body burden of an individual (Henriksen *et al.* 1998; Bustnes *et al.* 2001b).

(c) *Statistical analysis*

OC values were log-transformed to approximate a normal distribution. The levels of HCB, oxychlordane, DDE and PCB within an individual were highly positively correlated (range in *r*-values: 0.83–0.89). Inclusion of these four components as independent predictor variables consequently resulted in large variance inflation factors (greater than 10).

Table 1. Egg-laying dates (date since 1st May), catching date, head and bill length (mm), body mass (g), clutch size and concentrations in blood of four organochlorines (ng g^{-1} , wet weight) in experimental groups (treated with anti-parasite drug and placebo treated) in female and male glaucous gulls. (Data from Bear Island in 2000 and 2001.)

	2000					2001				
	untreated		treated			untreated		treated		
compound	$\bar{x}$	s.e.	$\bar{x}$	s.e.	p^a	$\bar{x}$	s.e.	$\bar{x}$	s.e.	p^a
<i>females</i>										
<i>n</i>	21		22			16		19		
egg-laying date	26.7	1.0	25.2	0.9	0.25	33.5	0.7	32.3	1.3	0.41
catching date	37.8	1.9	37.1	1.7	0.81	44.6	1.4	42.7	1.5	0.35
head and bill	134.5	0.6	135.6	0.5	0.17	135.2	0.6	134.6	0.6	0.52
body mass	1425.7	19.3	1433.0	20.4	0.79	1399.1	15.6	1409.2	23.5	0.73
clutch size	2.8	0.1	2.9	0.1	0.20	2.6	0.2	2.4	0.2	0.37
HCB	9.6	1.2	12.1	1.5	0.22	14.0	2.3	16.4	2.1	0.40
Oxychlordane	7.6	0.9	9.3	1.1	0.26	8.6	1.3	12.1	1.3	0.07
DDE	42.9	5.9	47.5	5.4	0.33	48.3	7.3	70.3	7.9	0.08
PCB	245.3	42.5	298.5	37.7	0.22	206.2	50.7	308.7	50.6	0.11
<i>males</i>										
<i>n</i>	20		17			18		16		
egg-laying date	26.6	1.0	26.3	1.1	0.84	32.6	0.8	31.6	0.8	0.38
catching date	38.7	1.9	37.9	1.7	0.79	44.0	2.0	44.1	1.8	0.96
head and bill	148.2	0.7	147.8	0.8	0.77	147.2	0.7	147.3	0.4	0.88
body mass	1766.0	17.5	1796.5	28.5	0.35	1799.7	18.2	1764.4	19.6	0.60
clutch size	2.9	0.1	2.8	0.1	0.28	2.4	0.2	2.8	0.1	0.18
HCB	14.6	2.1	14.1	1.9	0.87	19.0	2.1	17.2	2.6	0.47
Oxychlordane	11.8	1.8	13.1	2.4	0.69	14.6	1.7	14.6	2.8	0.63
DDE	74.6	9.6	74.3	12.8	0.99	100.5	10.6	86.0	16.1	0.25
PCB	373.6	59.2	454.8	101.7	0.59	396.7	60.0	392.0	92.1	0.50

^a *t*-test.

Rather than performing separate analyses for each compound, we calculated the mean of the four OC compounds and used this measure as an index of overall OC level. This index was highly correlated to the different compounds (range in *r*-values: 0.94–0.96). Only nests where eggs had been laid were included in the sample. *Nesting success* was modelled as a binomial response: hatching any number of chicks (1) or hatching no chicks (0), using generalized linear models (R development core team 2005). We examined interactions using type I likelihood ratio statistics. However, this was a planned experiment with one-tailed predictions, i.e. removal of parasites would enhance individuals' performance. We thus refitted the final model using type II Wald statistics (Fox 2002) and present one-tailed tests when examining the impact of treatment on breeding success. Both individuals could only be caught on 37 out of the 112 nests (females only: *n*=41, males only: *n*=34), and owing to this limited number each sex was treated separately when analysing nesting success. For each sex however, we took into account whether one or two birds had been treated, but this factor did not affect our results and conclusions. For simplicity, we omitted these results in the presentation. *Return rate*, i.e. the probability of returning from one year to the next in relation to treatment, year and OC, was modelled in a similar manner as nesting success, but in this analysis both sexes were analysed in the same models.

To examine whether any nonlinear relationship between OC level and nesting success and return rate existed, we used both generalized linear models with polynomials and generalized additive models (Wood 2006). However, no such relationship was evident.

3. RESULTS

(a) *Nesting success*

There was no significant difference in egg-laying dates, date of catching, clutch size, body mass and body size between the two experimental groups, either in males or in females (table 1). Moreover, for males in both years and for females in 2000, there was no significant difference between the experimental groups in OC levels (table 1). In females, in 2001, however, two individuals had extremely high levels of oxychlordane (290 and 410% higher than the mean level) and DDE (250 and 315% higher than the mean level), which gave a significantly ($p<0.05$) higher level in treated birds. To make the OC distribution in the two groups more equal, these two individuals were removed as outliers (table 1).

Nesting success varied significantly between years in both males and females ($p<0.01$), and we controlled for year in all statistical models. Body mass ($p=0.45$) did not affect the probability of nesting success after controlling for year and treatment, and this variable was excluded. Similarly, the interactions between year and treatment, and year and OC level were insignificant and hence removed from further analyses. There was, however, a significant main effect of treatment on nesting success. Furthermore, a significant interaction with OC level revealed that when the males were relieved from their parasite burden, OC level had no negative impact on nesting success (table 2 and figure 1). For females, no significant effect of treatment ($p=0.62$), OC level ($p=0.81$) or interaction between these two predictors on nesting success was evident ($p=0.45$).

Table 2. The effects of year, anti-parasite treatment and OC level on log-odds of nesting success in male glaucous gulls. (Insignificant interactions were removed and the model refitted. Results are from generalized linear models using a binomial error structure and the logit link function. *p*-values are based on Wald statistics and *p*-values with asterisk are one-tailed (see §2). (Data from Bear Island, 2000 and 2001).)

predictor	estimate	(95% CI)	Wald χ^2	<i>p</i>
intercept	5.90	(0.80, 12.32)		
year (2001)	-1.44	(-2.70, -0.29)	5.59	0.01
treatment (anti-parasite)	-6.73	(-15.55, 1.23)	2.85	0.045*
OC level	-2.83	(6.48, 0.21)	0.42	0.52
treatment (anti-parasite) $\times$ OC level	4.84	(0.03, 10.31)	3.50	0.03*

(b) Return rate between breeding seasons

Altogether, 25 out of 37 (68%) control females and 30 out of 41 (73%) treated females returned to the colony the next breeding season. For males, 32 out of 38 (84%) controls and 25 out of 33 (75%) treated individuals returned. Year ($p=0.28$), sex ($p=0.23$) and body mass ($p=0.83$) had no significant effect on return rate, and were thus excluded from further analysis. Nor was there any significant interaction between year and OC level ($p=0.44$) or sex and OC level ($p=0.64$) on return rate.

Treatment had no effect on the probability of returning and there was no interaction between treatment and OC level (table 3).

4. DISCUSSION

Both pollutants and parasites may have serious fitness consequences in birds, affecting both reproductive output and survival (Hudson & Dobson 1997; Bustnes *et al.* 2003a; Hanssen *et al.* 2003). This is, however, the first study in a wild bird population to provide experimental evidence that interactive effects between these factors have fitness consequences. That is, reductions of parasite burdens by anti-parasite treatment also removed the negative effects of the OC level on nesting success. However, in the present study, OC level did not affect the return rate between breeding seasons, although such relationships have been found in previous studies (Bustnes *et al.* 2003a, 2005). Moreover, anti-parasite treatment had no effect on return rate suggesting that parasite removal had only a short-term effect which disappeared as parasite burdens were rebuilding.

The fact that positive effects on nesting success were only found in males is not surprising since glaucous gull males have a greater protective role during the nesting stage than females; i.e. in addition to being larger and more aggressive than females, males were only away from the nest for about 20% of the time when not incubating, while females spent as much as 65% of this time away from the nest (Bustnes *et al.* 2001a). Moreover, the lack of effects in females may also depend on the fact that they have lower OC levels than males (Bustnes *et al.* 2003a) owing to sequestering of OCs into eggs (Norstrom *et al.* 1986).

Combined effects between pollutants and natural stressors have been found both in amphibians and in fish (Relyea & Mills 2001; Jacobson *et al.* 2003; Relyea 2003), but in birds, there are few studies demonstrating such interactive effects. One central study of captive ringed turtle doves (*Streptopelia risoria*) showed that the negative effects of food deficiency on reproductive performance were much

Figure 1. The estimated log-odds of nesting success among glaucous gull males receiving anti-parasite treatment relative to controls, in relation to $\log_{10}$ OC level in their blood. Dashed lines indicate approximate confidence intervals. The figure is based on the model presented in table 2. Data from Bear Island, 2000 and 2001.

Table 3. The effects of anti-parasite treatment and OC level on the log-odds of returning to the next breeding season. (Insignificant interactions were removed and the model refitted (see §3). *p*-Values are based on Wald statistics and *p*-values with asterisk are one-tailed (see §2). (Data from Bear Island, 2000 and 2001).)

predictor	estimate	(95% CI)	Wald χ^2	<i>p</i>
intercept	-0.53	(-3.34, 2.82)		
treatment (anti-parasite)	1.67	(-2.96, 5.92)	0.09	0.38*
OC level	1.12	(-1.25, 2.94)	0.57	0.45
treatment (anti-parasite) $\times$ OC level	-1.17	(-3.88, 1.84)	0.66	0.21*

stronger when the birds were exposed to DDE (Keith & Mitchell 1993). Rattner & Franson (1984) also found that cold intensified the toxicity of methyl parathion in American kestrel (*Falco sparverius*). Moreover, in fish-feeding glaucous gulls with long foraging flights (i.e. high feeding costs), there was a negative relationship between chick growth and the mothers' blood concentration of OCs. For females feeding in a seabird colony near the nest site (i.e. low feeding costs),

no such relationship was found (Bustnes *et al.* 2005). Hence, high energy expenditure capturing food seemed to trigger the negative effects of the OCs.

Calow (1991) suggested that there were considerable metabolic costs related to a set of pathways through which the damage from toxins could be eliminated. A potential mechanism for how parasite removal mitigated the effects of OCs is thus an energetic trade-off between reducing the damage of OCs and coping with parasites. Walker *et al.* (2001) also acknowledged that there may be energetic costs associated with enzyme induction, the main detoxification pathway of PCB and some other OCs in birds, but concluded that such costs were small compared with the total energetic stress encountered by organisms in natural environments. Therefore, energetic trade-offs could exist, but there may also be other explanations for different effects of OCs when combined with natural stressors. Funseth *et al.* (2002a) recently demonstrated that mice subjected to dioxins and virus pathogens downregulated the detoxifying process in favour of acute-phase protein synthesis, which could be a result of a trade-off for vital resources necessary both in the detoxifying process and in coping with virus infections. Moreover, in a concurrent study, it was shown that mortality from dioxins increased in virus-infected individuals compared with non-infected individuals. Proposed explanations for the changes in the effects of dioxins in infected individuals were an infection-induced increase in dioxin toxicity to vital organs and/or dioxin-induced changes in tissue affinity and virulence of the virus (Funseth *et al.* 2002b).

We have no information about the actual mechanism causing improved nesting success in anti-parasite-treated males, since we were unable to monitor behaviour of the birds. However, in a previous study in the same population, we monitored nesting behaviour of both male and female glaucous gulls and found that high OC loads were associated with reduced nest attendance (Bustnes *et al.* 2001a). A possible chain of events for how the reproductive performance was improved may be that parasite removal released energy, or other vital resources previously needed for immune functions, which then could be used for mitigating the effects of pollutants. This may have resulted in lesser feeding time and higher nest attendance, which again increased nesting success.

This study indicates that parasites enhance the detrimental effects of OCs, but other natural stressors, such as nutrient deficiency, disease, thermal stress and predation may function in concert and cause synergies which worsen the effects of the anthropogenic pollutants even further. Such synergetic effects may explain why glaucous gulls with non-lethal OC levels die at Bear Island (Gabrielsen *et al.* 1995). Hence, statements about levels in nature being too low to have adverse effects may be premature if the hazard thresholds are based on laboratory studies where animals are kept under optimal conditions. This also suggests that the hazardous levels of contaminants in nature will vary greatly both temporally and spatially. Finally, in this study, we tested the effects of the four OCs separately, but all individuals have a wide variety of OCs and other pollutants in their bodies (Verreault *et al.* 2005). This mixture may exert even greater effects on reproduction which we may not have been able to record through this experiment.

We thank Magnus Fjeld and Øystein Miland for great help during fieldwork, and Anuschka Polder and her team for conducting the analyses of pollutants. We also thank three anonymous referees for suggestions that greatly improved earlier drafts of the manuscript. The project was funded by the Norwegian Research Council.

REFERENCES

- Andersen, M., Lie, E., Derocher, A. E., Belikov, S. E., Bernhoft, A., Bolotov, A. N., Garner, G. W., Skaare, J. U. & Wiig, O. 2001 Geographic variation of PCB congeners in polar bears (*Ursus maritimus*) from Svalbard east to the Chukchi Sea. *Polar Biol.* **24**, 231–238. (doi:10.1007/s003000000201)
- Bonar, C. J., Lewandowski, A. H. & Schaul, J. 2003 Suspected fenbendazole toxicosis in 2 vulture species (*Gyps africanus*, *Torgos tracheliotus*) and marabou storks (*Leptoptilos crumeniferus*). *J. Avian Med. Surg.* **17**, 16–19. (doi:10.1647/1082-6742(2003)017[0016:SFTIVS]2.0.CO;2)
- Bustnes, J. O. 2006 Pinpointing potential causative agents in mixtures of persistent organic pollutants in observational field studies: a review of glaucous gull studies. *J. Toxicol. Environ. Health Part A* **68**, 97–108. (doi:10.1080/15287390500259301)
- Bustnes, J. O., Erikstad, K. E., Bakken, V., Mehlum, F. & Skaare, J. U. 2000 Feeding ecology and the concentration of organochlorines (OCs) in glaucous gulls. *Ecotoxicology* **9**, 175–186. (doi:10.1023/A:1008938603257)
- Bustnes, J. O., Bakken, V., Erikstad, K. E., Mehlum, F. & Skaare, J. U. 2001a Patterns of incubation and nest site attentiveness in relation to organochlorine (PCB) contamination in glaucous gulls. *J. Appl. Ecol.* **38**, 791–801. (doi:10.1046/j.1365-2664.2001.00633.x)
- Bustnes, J. O., Skaare, J. U., Erikstad, K. E., Bakken, V. & Mehlum, F. 2001b Whole blood concentrations of organochlorines as a dose metric for studies of the glaucous gull (*Larus hyperboreus*). *Environ. Toxicol. Chem.* **20**, 1046–1052. (doi:10.1897/1551-5028(2001)020<1046:WBCOOA>2.0.CO;2)
- Bustnes, J. O., Erikstad, K. E., Skaare, J. U., Bakken, V. & Mehlum, F. 2003a Ecological effects of organochlorine pollutants in the Arctic: a study of the glaucous gull. *Ecol. Appl.* **13**, 504–515.
- Bustnes, J. O., Bakken, V., Skaare, J. U. & Erikstad, K. E. 2003b Age and accumulation of persistent organochlorines: a study of arctic breeding glaucous gulls. *Environ. Toxicol. Chem.* **22**, 2173–2179. (doi:10.1897/02-456)
- Bustnes, J. O., Hanssen, S. A., Folstad, I., Erikstad, K. E., Hasselquist, D. & Skaare, J. U. 2004 Immune function and organochlorine pollutants in arctic breeding glaucous gull. *Arch. Environ. Contam. Toxicol.* **47**, 530–541. (doi:10.1007/s00244-003-3203-6)
- Bustnes, J. O., Miland, Ø. O., Fjeld, M., Erikstad, K. E. & Skaare, J. U. 2005 Relationships between ecological variables and four organochlorine pollutants in an Arctic glaucous gull (*Larus hyperboreus*) population. *Environ. Pollut.* **136**, 175–185. (doi:10.1016/j.envpol.2004.09.026)
- Calow, P. 1991 Physiological costs of combating chemical toxicants: ecological implications. *Comp. Biochem. Physiol.* **100**, 3–6. (doi:10.1016/0742-8413(91)90110-F)
- Christensen, G. N., Evenset, A., Zaborska, A., Berger, U. & Carroll, J. 2004 Datering av sediment og historisk utvikling av miljøgifter i Ellasjøen, Bjørnøya. Akvaplan-niva Report no: APN-514.2934, 29 pp. In Norwegian.
- Fox, J. 2002 *An R and S-PLUS companion to applied regression*. London, UK: Sage Publication, Inc.
- Fox, A. & Hudson, P. J. 2001 Parasites reduce territorial behaviour in red grouse (*Lagopus lagopus scoticus*). *Ecol. Lett.* **4**, 139–143. (doi:10.1046/j.1461-0248.2001.00207.x)

- Funseth, E., Pahlman, M., Eloranta, M. L., Friman, G. & Ilback, N. G. 2002a Effect of Coxsackievirus B3 infection on the acute-phase protein metallothionein and on cytochrome P-4501 A1 involved in the detoxification process of TCDD in mouse. *Sci. Total Environ.* **284**, 37–47. (doi:10.1016/S0048-9697(01)00864-6)
- Funseth, E., Wesslén, L., Lindh, U. & Ilback, N. G. 2002b Effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin on trace elements, inflammation and viral clearance in the myocardium during Coxsackievirus B3 infection in mice. *Sci. Tot. Environ.* **284**, 135–147. (doi:10.1016/S0048-9697(01)00874-9)
- Gabrielsen, G. W., Skaare, J. U., Polder, A. & Bakken, V. 1995 Chlorinated hydrocarbons in glaucous gulls (*Larus hyperboreus*) in the southern part of Svalbard. *Sci. Total Environ.* **160/161**, 337–346. (doi:10.1016/0048-9697(95)04367-A)
- García-Berthou, E. 2001 On the misuse of residuals in ecology: testing regression residuals vs. the analysis of covariance. *J. Anim. Ecol.* **70**, 708–711. (doi:10.1046/j.1365-2656.2001.00524.x)
- Hanssen, S. A., Folstad, I., Erikstad, K. E. & Oksanen, A. 2003 Costs of parasites in common eiders: effect of antiparasite treatment. *Oikos* **100**, 105–111. (doi:10.1034/j.1600-0706.2003.12162.x)
- Henriksen, E. O., Gabrielsen, G. W. & Skaare, J. U. 1998 Validation of the use of blood samples to assess tissue concentrations of organochlorines in glaucous gull *Larus hyperboreus*. *Chemosphere* **37**, 2627–2643. (doi:10.1016/S0045-6535(98)00162-3)
- Howard, L. L., Papendick, R., Stalis, I. H., Allen, J. L., Sutherland-Smith, M., Zuba, J. R., Ward, D. L. & Rideout, B. A. 2002 Fenbendazole and albendazole toxicity in pigeons and doves. *J. Avian Med. Surg.* **16**, 203–210. (doi:10.1647/1082-6742(2002)016[0203:FAA-TIP]2.0.CO;2)
- Hudson, P. J. 1986 The effect of a parasite nematode on the breeding production of red grouse. *J. Anim. Ecol.* **55**, 85–92. (doi:10.2307/4694)
- Hudson, P. J. & Dobson, A. P. 1997 Host–parasite processes and demographic consequences. In *Host–parasite evolution. General principles and avian models* (ed. D. H. Clayton & J. Moore), pp. 128–153. Oxford, UK: Oxford University Press.
- Jacobson, K. C., Arkoosh, M. R., Kagle, A. N., Clemons, E. R., Collier, T. K. & Casillas, E. 2003 Cumulative effects of natural and anthropogenic stress on immune function and disease resistance in juvenile chinook salmon. *J. Aquat. Anim. Health* **15**, 1–12. (doi:10.1577/1548-8667(2003)015<0001:CEONAA>2.0.CO;2)
- Keith, J. O. & Mitchell, C. A. 1993 Effects of DDE and food stress on reproduction and body condition of ringed turtle doves. *Arch. Environ. Contam. Toxicol.* **25**, 192–203. (doi:10.1007/BF00212130)
- Lawrence, K. 1983 Efficacy of fenbendazole against nematodes of captive birds. *Vet. Rec.* **112**, 433–434.
- Leung, K. M. Y. & Furness, R. W. 2001 Survival, growth, metallothionein and glycogen levels of *Nucella lapillus* (L.) exposed to chronic cadmium stress: the influence of nutritional state and prey type. *Mar. Environ. Res.* **52**, 173–194. (doi:10.1016/S0141-1136(00)00271-3)
- Norstrom, R. J., Clark, T. P., Jeffrey, D. A., Won, H. T. G. & Gilman, A. P. 1986 Dynamics of organochlorine compounds in herring gulls (*Larus argentatus*): I. Distribution and clearance of [¹⁴C] DDE in free-living herring gulls (*Larus argentatus*). *Environ. Toxicol. Chem.* **5**, 41–48.
- Norton, R. A., Yazwinsky, T. A. & Johnson, Z. 1991 Research note—use of fenbendazole for the treatment of turkeys with experimentally induced nematode infections. *Poult. Sci.* **70**, 1835–1837.
- Pedersoli, W. M., Spano, J. S., Krista, L. M., Whitesides, J. F., Ravis, W. R., Kemppainen, R. J. & Young, D. W. 1989 Effects of fenbendazole administration on hematology, clinical chemistries and selected hormones in the white Pekin duck. *J. Vet. Pharm. Therap.* **12**, 200–208.
- R Development Core Team. 2005 R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna.
- Rattner, B. A. & Franson, J. C. 1984 Methyl parathion and fevalerate toxicity in American kestrels: acute physiological responses and effects of cold. *Can. J. Physiol. Pharmacol.* **62**, 787–792.
- Relyea, R. A. 2003 Predator cues and pesticides: a double dose of danger for amphibians. *Ecol. Appl.* **13**, 1515–1521.
- Relyea, R. A. & Mills, N. 2001 Predator-induced stress makes the pesticide carbaryl more deadly to grey treefrog tadpoles (*Hyla versicolor*). *Proc. Natl Acad. Sci. USA* **98**, 2491–2496. (doi:10.1073/pnas.031076198)
- Sagerup, K., Henriksen, E. O., Skorpning, A., Skaare, J. U. & Gabrielsen, G. W. 2000 Intensity of parasitic nematodes increases with organochlorine levels in the glaucous gull. *J. Appl. Ecol.* **37**, 532–539. (doi:10.1046/j.1365-2664.2000.00521.x)
- Seiwright, L. J., Redpath, S. M., Mougeot, F., Leckie, F. & Hudson, P. J. 2005 Interactions between intrinsic and extrinsic mechanisms in a cyclic species: testosterone increases parasite infection in red grouse. *Proc. R. Soc. B* **272**, 2299–2304. (doi:10.1098/rspb.2005.3233)
- Short, C. R., Barker, S. A., Hsieh, L. C., Ou, S.-P., Pedersoli, W. M., Krista, L. M. & Spano, J. S. 1988 The elimination of fenbendazole and its metabolites in the chicken, turkey and duck. *J. Vet. Pharm. Therap.* **11**, 204–209.
- Sibly, R. M., Williams, T. D. & Jones, M. B. 2000 How environmental stress affects density dependence and carrying capacity in a marine copepod. *J. Appl. Ecol.* **37**, 388–397. (doi:10.1046/j.1365-2664.2000.00534.x)
- Sih, A., Bell, A. M. & Kerby, J. L. 2004 Two stressors are deadlier than one. *Trends Ecol. Evol.* **19**, 274–276. (doi:10.1016/j.tree.2004.02.010)
- Verreault, J., Gabrielsen, G. W., Chu, S., Muir, D. C. G., Andersen, M., Hamaed, A. & Letcher, R. J. 2005 Flame retardants and methoxylated and hydroxylated polybrominated diphenyl ethers in two Norwegian Arctic top predators: glaucous gulls and polar bears. *Environ. Sci. Technol.* **39**, 6021–6028. (doi:10.1021/es050738m)
- Walker, C. H., Hopkin, S. P., Sibly, R. M. & Peakall, D. B. 2001 *Principles of ecotoxicology*, 2nd edn. London, UK: Taylor & Francis.
- Weber, M. & Junge, R. 2000 Identification and treatment of *Moniliformis clarki* (acanthocephala) in cotton-topped tamarins (*Saguinus oedipus*). *J. Zool. Wildl. Med.* **31**, 503–507.
- Wood, S. N. 2006 *Generalized additive models. An introduction with R*. London, UK; Boca Raton, FL: Chapman & Hall/CRC.
- Yazwinski, T. A., Johnson, Z. & Norton, R. A. 1992 Efficacy of fenbendazole against naturally acquired *Raillietina cesticillus* infections of chickens. *Avian Pathol.* **21**, 327–331.
- Yazwinski, T. A., Rosenstein, M., Schwartz, R. D., Wilson, K. & Johnson, Z. 1993 The use of fenbendazole in the treatment of commercial turkeys infected with *Ascaridia dissimilis*. *Avian Pathol.* **22**, 177–181.