Ectoparasite load in growing young and adult barnacle geese in the Dutch delta

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Abstract: *By extending their arctic breeding locations to more temperate latitudes such as the Dutch delta, barnacle geese expose themselves to "dirtier" environments in which a higher pressure of ectoparasites could occur. Ectoparasites make up a very diverse group in the animal kingdom, and every vertebrate organism carries one or more species. However, they are highly unappreciated by biologists. As far as we know this is the first detailed study investigating ectoparasite load in barnacle geese. In this study we sampled ectoparasites on barnacle geese caught in the Dutch delta in order to make an indication of the ectoparasite load carried by the barnacle goose and the ectoparasite species which inhabit it. Five species of ectoparasites where found (2 Amblycera and 3 Ischnocera. The blood-feeding Amblycera accounted for merely 6% of the total. The Ischnoceran Anaticola anseris was most numerous and accounted for 45% of the total. For the 5 ectoparasite species we show densities, presence / absence, co-occurrence. We also show that as goslings mature, the ectoparasite composition in their plumage changes. We conclude that although Amblycera, are blood-feeding and scarce, they are still more harmful to the goose by acting as an intermediate host and vector, spreading more harmful endoparasites. Ischnocera are indicative to the goose's health and are not presumed to transfer any diseases.*

Introduction: Despite being regarded as an obligate arctic breeder, a portion of barnacle geese (*Branta leucopsis)* in the flyway have started a breeding colony in the Dutch delta since 1982 (Van Der Jeugd et al. 2009, Voslamber *et al.* 2007). By extending its breeding distribution from the original arctic breeding colony to the temperate Dutch floodplains potentially exposes the arctic-adapted barnacle geese to "exotic" new ectoparasite species (Kutz, Dobson, & Hoberg, 2009). Moreover breeding in the arctic is "clean", the long harsh winters deter the growth of ectoparasites (Kutz *et al.* 2009) which can have detrimental effects on their avian hosts (Walther & Clayton, 1997). Potentially, arctic species have a reduced immunocompetence (Piersma 1997) due to the fact that there are less parasites in arctic regions (Dobson *et al.* 2008), and therefore may be particularly vulnerable to parasitic invaders.

Parasites make up a very broad group (≈ 40% of the species known to science are parasitic) but sampling of parasitic diversity, and published literature is thin at best (Dobson *et al.* 2008) and mostly very outdated. In this study we provide a baseline to future work on barnacle geese ectoparasite loads.

Ectoparasites: Climate is an important factor determining the diversity and abundance of parasites (Kutz *et al.* 2009). The main group of ectoparasites found on birds around the world are feather lice, order: *Phthiraptera*, formerly known as *Mallophaga* (Lyal 1985), and little is known about the ecology of the individual

Phthiraptera species. In general, ectoparasites are very sensitive to temperature fluctuations and have a narrow range of preference (Ash, 1960). It is conceivable that various stages of the ectoparasite life-cycle require slightly different temperature conditions. The eggs of some ectoparasite Species are laid against the skin, on the base of the birds head feathers, thus the skin temperature is probably the optimum for this stage. Might a nymph require a slightly lower temperature, it only needs to crawl a short way up the feather (Ash, 1960). Because feather lice have poor mobility, transmission often occurs during periods of direct contact between hosts, like that between parents and offspring in the nest (Rothschild and Clay, 1952; Marshall, 1981).

The ectoparasites in the order of *Phthiraptera* have essentially biting mouthparts and are unable to pierce their hosts' skin (Ash, 1960). Feather lice can roughly be divided in two sub-orders: the *Ischnocera* are wingless, permanent ectoparasites on birds that complete all stages of their life cycle on the host's body (Marshall, 1981) and solely feed on feathers and the debris of feather shafts. The *Amblycera* are mostly dependant on tissue fluid for feeding. When examined closely, blood can clearly be seen through the integument, and although it is not quite clear how this is obtained, it is probably drawn by scratching or nibbling at the soft skin at the base of the feathers (Ash, 1960). A normal feather louse population appears to have little effect on the avian host, for by means of preening, dust bathing, sunning and waxing, the bird is able to keep ectoparasite numbers in check and nearly all birds carry ectoparasites (for more on mechanisms of birds controlling for ectoparasites see: Clayton *et al.* 2010). Sick or injured birds are often found with heavy infestations which are due to the inability of the weakened bird to remove excess ectoparasites. It seems unlikely that ectoparasite increase alone will weaken the bird (Ash, 1960), but ectoparasites can be an intermediate cyclodevelopmental host of endoparasites. The Amblycera, *Trinoton anserinum* is found to spread the filarial heartworm *Sarconema eurycerca*, in whistling swans *Cygnus colombianus* in North America and mute swans *Cygnus olor* in the Russian Black Sea (Seegar *et al.* 1976; Cohen *et al.* 1991). This characteristic can make amblyceran ectoparasites quite dangerous to their host.

As most ectoparasites are confined to one group or one host species and have evolved together, they are adapted to the life cycle of their host. Some ectoparasite species winter in the egg stage between the birds warm plumage (Boyd 1951), while others show an increase in numbers prior to migration (Dogel & Karolinskaya 1936). It may be reasoned that an increase in population of ectoparasites prior to the birds breeding season is an excellent colonisation mechanism to infest the bird's offspring. Therefore juvenile birds might be expected to be as heavily parasitized as their parents (Ash, 1960). If this is the case, then Amblycera should be the first to appear on the nestlings as they are able to find food before the feathers appear (Ash, 1960). This is particularly so in nidifugeous young, to which barnacle geese belong, which are well covered with down upon hatching (Ash, 1960). The philopatry displayed by barnacle geese create conditions that are highly conductive to the maintenance and amplification of ectoparasites in the breeding colony (Kutz *et al.,* 2009).

Barnacle goose: Before 1980 all barnacle geese used to follow the so called "Green Wave" of spring from their Dutch wintering grounds and travel north along the climatic gradient, through the Baltic to the Russian tundra's, taking advantage of the spring growth flush in forage plants at each stopover site along the gradient (Drent *et al.* 1978). The "green wave" hypothesis accounts for the northerly migration of geese from temperate latitudes and states that while travelling to their arctic breeding grounds on the Russian tundra the geese take advantage of the best forage in each stopover location (Graaf et al. 2006).

Barnacle geese are specialised herbivores depending on forage of high nutritional quality (prop en Vulink 1992), which is mainly found in monocotyledonous plants (grasses). On the intensely farmed meadows in the Netherlands the grass is mowed 3-5 times per annum and fertilised, making the short fast growing grass highly rich in nutrients and a feast to barnacle geese (van Eerden *et al.* 2005*).* The intense hunting pressure on foxes to protect meadow birds has led to a very low and stable fox population in the Dutch delta (Van der Jeugd et al. 2009; Voslamber *et al.* 2007). This combined with a decrease in goose hunting all over Europe, are major contributing factors to the increase in goose numbers (Ebbinge 1991). Nowadays, the Dutch barnacle goose population is the fastest growing goose population found in the world, with a breeding population of 6000 pairs and 25000 individuals (2005 census). The population's centre of gravity lies around South-Holland's delta region (Van der Jeugd et al. 2009; Voslamber *et al.* 2007).

Study site: The Westplaat (51.790°N / 4.129°E) near the village Dirksland on the Dutch delta island Goeree-Overflakkee is bordered by the light brackish estuary river "Haringvliet" in the north (Fig. 1). To the south the Westplaat is surrounded by vast meadows of heavy intensified dairy and cattle farms. The river's fluctuating water levels and the tidal influence of the sea together with the creeks, gullies and the extensive pastureland make the Westplaat a safe haven for waterfowl and meadow birds in this highly intensified farming landscape. The Westplaat is a nature reserve, protected under the 1971 "Convention on Wetlands of International Importance especially as Waterfowl Habitat", Signed in the city of Ramsar, Iran (Davis 1994) and owned and managed by Staatsbosbeheer (Dutch state forestry department).

The Westplaat is part of the 3000 hectare comprising project "Delta nature" in which sections of farmland / floodplain are being rewilded by the rivers Maas and Rhine in order to restore the delta's natural beauty which was scared by the build of the Haringvliet sluices in 1970 (Schmit 2003). The wetlands Slijkplaat, Scheelhoek, Korendijkse- and Beeninger Slikken are also incorporated in the Delta-nature project. These are situated in close proximity of the Westplaat and exchange individual geese and goslings (Ouweneel, 2001). All this new linked nature makes suitable habitat for geese to breed (Van der Jeugd et al. 2009; Voslamber *et al.* 2007).

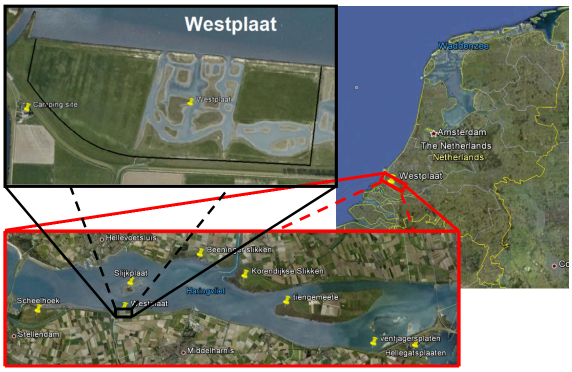

Figure 1: The Westplaat (black lines): situated in the South Holland Delta region (Red lines) in the Netherlands (Google Earth)

Methods: A variety of methods have been used over the years to quantify ectoparasite loads of live birds, the more accurate methods can only be preformed on dead birds (D. H. Clayton & Drown, 2001). The method we used; *Dustruffling* is designed to kill ectoparasites *in situ*, leaves the bird alive and is more effective than previously described methods. It is also more accurate than visual examination (Walther & Clayton 1997).

Dustruffling starts with the “dusting” phase by placing the goose into a 60x40x10cm (LxWxH) vinyl lined box and dusting Beaphar knock down flea powder onto the feathers and rubbing it in with gloved hands. Special care was taken not to rub the powder into the goose’s bill or onto its eyes but still giving thorough attention to the head. If necessary the legs of the goose were restrained from kicking by taping them together with tape. All dustruffling took place in June and July 2012. During catching, special care was taken to make sure that geese belonged to different families to assure independence of the sampled data.

Beaphar Knockdown flea powder is a fast acting powder on a natural basis, which works not only on fleas but also on ticks, lice and other ectoparasites. The active component Pyrethrum extract (25% / 3,0% w/w) is harvested from dried chrysanthemum flowers (Casida & Quistad 1995). Pyrethrum is a fast-knockdown, slow-killing insecticide that is completely safe for use on birds and mammals (Casida 1973, Jackson 1985) but still, a paper dust mask and gloves were worn by the researchers. The second active compound Pyperonyl butoxyde (90% / 1,7% w/w) is an organic synergist which helps the pyrethrum to increase in effectiveness by making it “stick” to the ectoparasites victim (Walther & Clayton, 1997). Beaphar Knockdown flea powder is cheap and comes in a handy shaker, making it ideal for basic field study sites. Dustruffling is best suited for sampling permanent ectoparasites, such as *Phthiraptera* (chewing lice) which pass their entire lifecycle on the body of the host (Walther & Clayton 1997). The time needed to cover the entire goose in flea powder and work the powder in its feathers, the “dusting time”, was taken by stopwatch and noted down.

After the dusting phase we started the first ruffling bout, in which the bird was ruffled thoroughly over the vinyl lined box. The geese where categorized into 3 weight categories: small pulli (150-400g) ruffled for 3 minutes, medium juvenile geese (700-1200g) ruffled for 5min and Adults (1300-2000g) ruffled for 7min. After the bout the goose was put in another cardboard box with vinyl on the bottom from which it could not escape and set aside to rest. The “dust” (flea powder, down and feather scabs) was left in the dusting box and the ectoparasites were collected by using a magnifying glass and special fine insect tweezers, not to damage the specimens beyond recognition. The ectoparasite specimens were stored in vials on 70% alcohol. The slow killing component in the flea powder is handy because twitching ectoparasites are easier to spot then sessile ones (Walther & Clayton, 1997). After all ectoparasites were collected and stored, the dusting box was cleaned with a wet cloth and a new (second) dusting bout was started.

Dusting bouts were continued until Diminishing result. Diminishing result was reached if 0 ectoparasites were found after a ruffling bout or if the found ectoparasite yield of a consecutive bout was lower than <5% of the highest previous bout (Walther & Clayton, 1997). This criterion provides a more accurate comparative estimate of ectoparasite load then when hosts are sampled for an arbitrarily period of time (Clayton & Walther 1997). Between ruffling bouts the goose was set aside in the resting box. After the last ruffling bout the goose was individually marked (web-tagged in small chicks or colour ringed in large chicks and adults), released on land near water and followed till it was assumed to be healthy and safe in the water. Geese feel safer on water and dustruffling with pyrethrin does not delay plumage drying (Walther & Clayton, 1997). The vinyl was taken from the resting box and inspected for ectoparasites. Found ectoparasites were counted and noted down as “rest”, and stored in the 70% alcohol vials with the rest of the ectoparasites of that particular individual.

The start and end time of each dustruffling session was noted down in order to know the handling time per individual barnacle goose.

After the fieldwork ended, the ectoparasite samples were recounted in the lab using a stereo microscope. This was done to make sure that solely ectoparasites were counted and not insects, dust or feather debris, in order to come up with a total and very accurate count.

Throughout the hatching period a sample of 521 hatchlings were individually marked with web-tags placed in the foot web, leaving enough space for the foot web to grow. From this we inferred the age of recaptured goslings and, furthermore, could build a predictive model to estimate age of unmarked goslings based on morphological measurements (see results). Body size measurements of captured geese included (bent) tarsus length, measured with calipers to the nearest 0.1 mm, maximum wing length (flattened wing from wrist to tip of longest primary) and head length (from back of skull to tip of bill), measured with a ruler at 1 mm accuracy. Statistical analyses were performed in SPSS.

Results: A total of 60 (40 juveniles and 20 adult) geese where dustruffled with a combined body mass of 62.25 Kg and an average weight of 1037 g per goose (ranging from 160 – 2250 g). All geese combined carried a total of 4167 ectoparasites with an average of 70 ectoparasites (ranging from 2-422 ectoparasites). Each of the examined geese was found to carry ectoparasites. Species determination of the found ectoparasites was done by Dr. H.J. Cremers, (University Utrecht – Veterinary parasitology) and turned out to be: 5 species of ectoparasite (*Phthiraptera*) of which 2 belonged to the group of Amblycera: *Trinoton anserinum* (Fabricius, 1805), & *Anseriphillus pectiniventris* (Harrison, 1916).
3 species belonged to the Ischnocera: *Ornithobius hexophthalmus* (Giebel 1861), *Anatoecus dentatus brunneopygus* (Mjöberg, 1910), and *Anaticola anseris* (Linnaeus, 1758) (see appendix 1 for microscopic photos and taxonomy). For convenience, we will use generic names of the lice when referring to the species. In the literature we could find no evidence of the host / parasite relation of *Anseriphillus* being reported to be found parasitizing on barnacle geese ever before, however Dr Cremers has determined the species on a barnacle geese sample collected on Svalbard in the summer of 1995. *Trinoton* (Waterston I922), *Ornithobius* and *Anaticola* were recorded to parasitize barnacle geese on Svalbard (Hackman & Nyholm 1968), and *Ornithobius, Anatoecus* and *Anaticola* were in general described to be found parasitizing on barnacle geese (Prince *et al.* 2003).
When looked at the age of establishment of the lice on the goslings we found that *Trinoton*, by far our largest ectoparasite (it was sometimes up to 1cm in length), was found only on geese older than 40 days (see Fig. 2). *Anseriphillus* was found on geese older than 24 days, but showed a decrease around day 40, when the pulli started to loose their down and grow their first feathers. *Ornithobius* was present in goslings from a very young age onwards (i.e. already in the youngest measured gosling at 18 days old). Unlike *Ornithobius, Anatoecus* was found to establish itself rather late, from day 35 onwards. *Anaticola* was present in goslings 30 days of age and older but became well established around the 40th day of life. In one of the adult geese which were dustruffled we found 416 *Anaticola*. Although this number was quite high (10% of all ectoparasites and 21% of found *Anaticola*) we did not consider this goose / ectoparasite sample to be an outlier. *Anaticola* samples often exceeded 100

individuals, and another goose harboured 230 *Anaticola.* The goose carrying the 416 *Anaticola* weight 1895 g and was our 5th heaviest adult goose and seemed to be in good condition (see appendix 2 for frequency distributions).

The age of 7 out of 40 goslings, dustruffled for ectoparasites was known accurately (± onto 1day) as they were among the recaptures of birds marked at hatch. The age of the remaining 33 goslings was estimated from a combination of length measurements of tarsus, head and wing. Each of these body parts follows a different nonlinear growth trajectory. For instance, tarsal length seems a good predictor of age during early growth but varies little with age at later growth stages. Wing length, on the other hand, varies little with age during early growth, but after that and throughout most of the age window studied here it seems a good predictor of age (see figures of biometric measurements in appendix 2). Because each body part follows a different growth trajectory we used a principal component analysis to combine the biometric measurements (n = 208 cases, including recaptures, from n= 170 goslings captured within this study) of tarsus, head and wing length to a single structural size variable: the first principal component (PC1).

The PC1explained 93% of the total observed variance. The advantage of this procedure is that PC1 is the best single and linear predictor for all gosling ages studied here. The relationship between age and PC1 was established from single recaptures of 54 web-tagged goslings and used to predict age of other goslings (Fig. 3).

Capture and handling stress during recapture events may negatively affect gosling growth and potentially bias the age-body size relationship. Therefore, we included only data from first recaptures. Using PC1, the age of an individual gosling could be predicted at approximately ± 10 days accuracy (note 95% individual prediction intervals in Fig. 3).

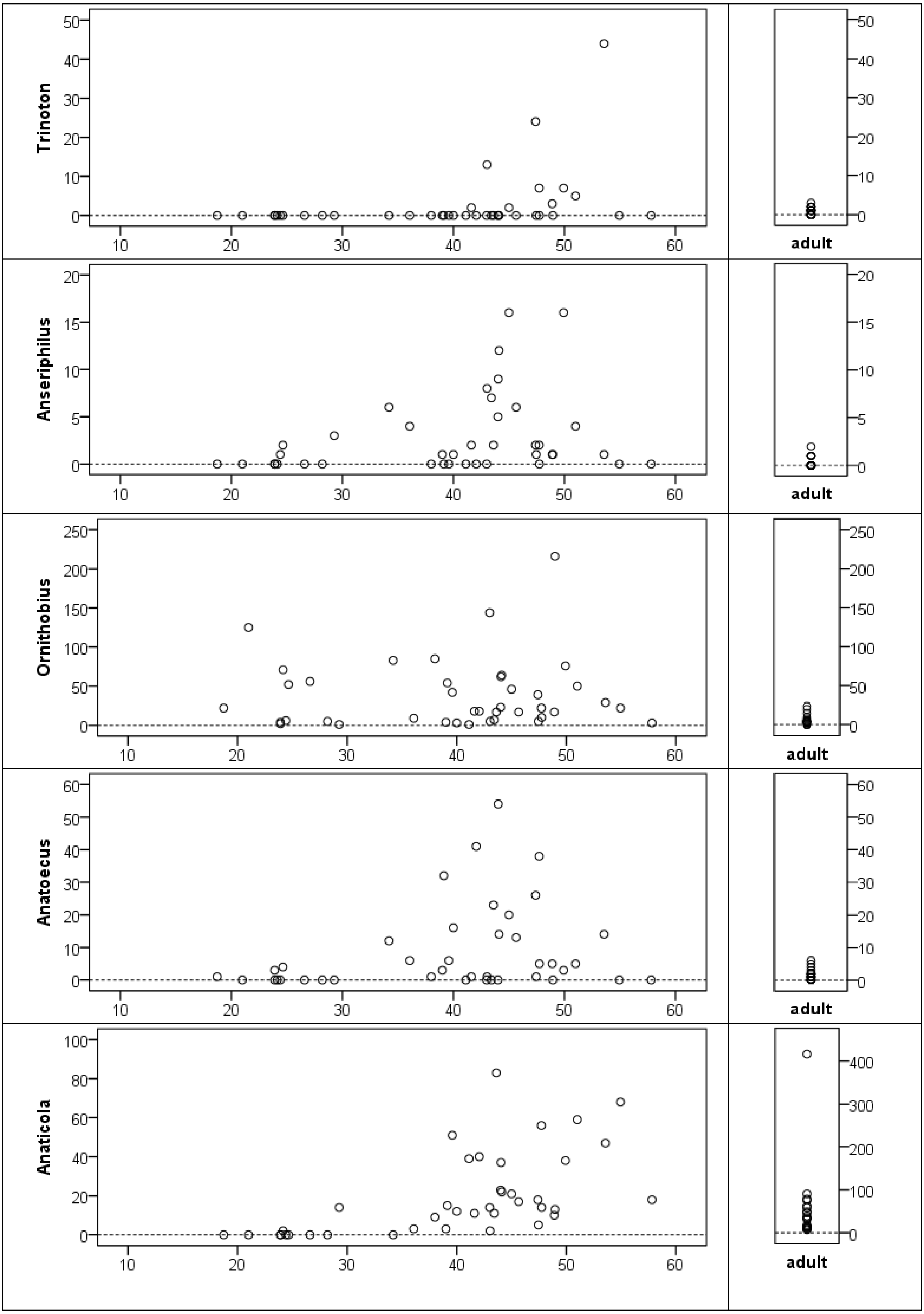

Figure 2: Count data of five species of ectoparasites plotted against age of young (left panels) and for moulting adult (right panels) barnacle geese.

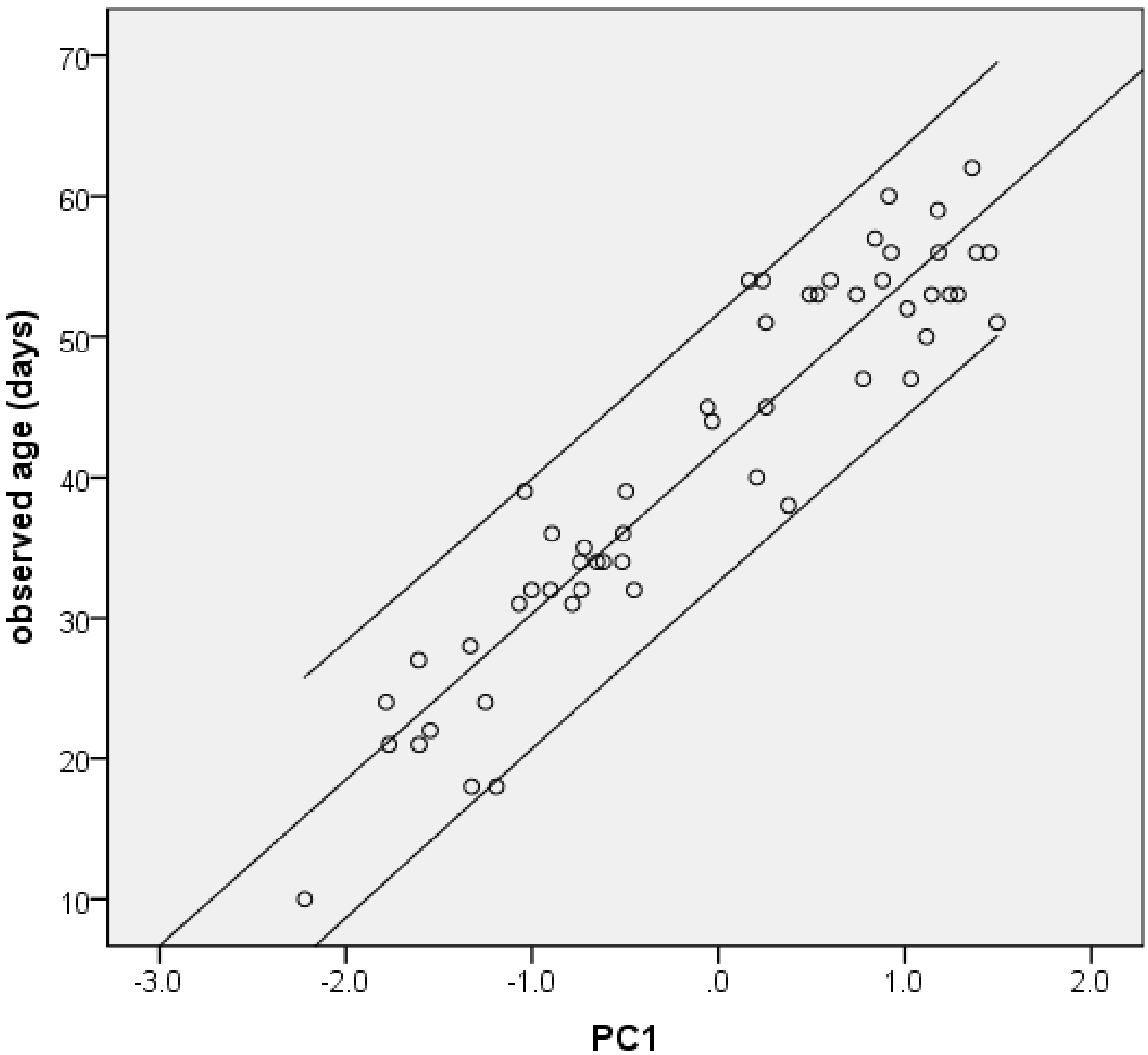

Figure 3: Relationship between known age of web-tagged goslings (R^2 = 0.88) and PC1 from a principal component analysis including length of tarsus, head and wing. The regression line is described by age = 42.12 (SE=0.64) + 11.81 (SE=0.62) * PC1 ($F_{1,53}$=366.1, P<0.001). The outer lines mark the 95% individual prediction intervals.

Based on known or predicted age, we grouped the examined host geese into three groups based on their age: Pulli (< 6 weeks old, N=19), juveniles (≥ 6 weeks old, N=21) and adults (> 1 year old, N=20). For the cut between pulli and juvenile goslings we were led by the time of transition of the down to feather plumage. All 5 species of ectoparasite were found in each age class but with a different intensity. From the count data we could immediately see that the blood feeding group of Amblycera accounted only for 6% of the total ectoparasite load (see tab.1). We found only 125 individuals of the Amblycera *Trinoton* which was only 3% of the ectoparasite species load. We scored presence of each louse over the individual geese to come up with a percentage of the geese in the age categories which carried a particular ectoparasite (see fig. 4). When looked at occurrence, *Trinoton* was found in 60% of adult goose but only in 5% of pulli. The other 3 % of the total ectoparasite yield (and half of the total Amblycera) was contributed by *Anseriphillus* which was found 119 times. 76% of the juveniles but only 25% of the adults harboured *Anseriphillus*. 94% of the total found ectoparasites were Ischnocerans which are feeding on feathers and feather debris. *Ornithobius* was the second most abundant species of all lice found. It contributed to 40% of the total ectoparasite load. It was the ectoparasite which seemed best in colonising and was found in nearly all geese, except for 2 adults. *Ornithobius* was carried in 100% of the pulli and juvenile goslings and in 90% of the adult geese. *Anatoecus* contributed to 9% of the total ectoparasite load and 58% of pulli and 70% of adults harboured 1 or more *Anatoecus*. The most numerous louse, *Anaticola* accounted for 45% of total ectoparasite yield. *Anaticola* was present in only 53% of pulli but in 100% of juvenile and adult geese.

Table 1: Ectoparasite (*Phthiraptera*) load, in counts and percentage, per found species over pulli, juvenile and adult geese. The number of individuals per group is illustrated. Total N=60, total found ectoparasites = 4167. Amblycera (blood feeding) are red and Ischnocera (feather feeding) are green.

	N	*Trinoton*	*Anseriphillus*	*Ornithobius*	*Anatoecus*	*Anaticola*	Total
Pullus	19	2	20	643	85	159	**909**
Juvenile	21	105	93	892	263	616	**1969**
Adult	20	18	6	116	33	1116	**1289**
Total	**60**	**125**	**119**	**1651**	**381**	**1891**	**4167**
%		3	3	40	9	45	100 %

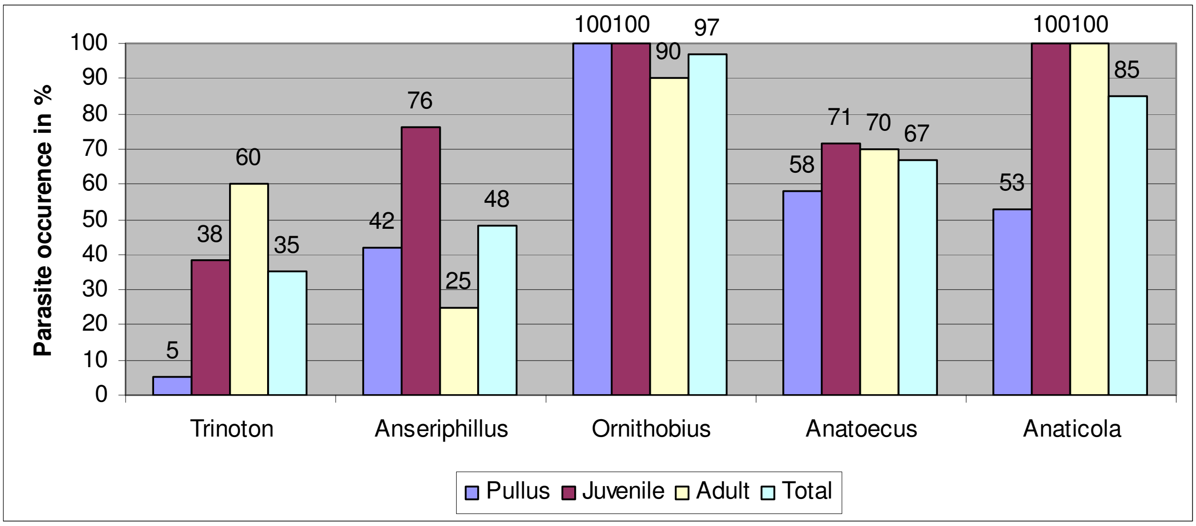

Figure 4: Presence of five ectoparasite (*Phthiraptera*) species in % of geese belonging to three age groups (Pulli, Juvenile, adult)

We ran a binary logistic regression model in SPSS 20 in which the presence of each ectoparasite was scored as 1=present and 0=absent. We took the adult geese as a reference category and compared this to the juveniles and pulli. We used an Omnibus test to test for the strength of this Binary logistic model through a Chi-square test. It showed that the differences between the age groups (as observed in fig. 4) are highly significant for *Trinoton* (p=0,001/ df=3), *Anseriphillus* (p=0,007/ df=3) and *Anaticola* (p=0,00/ df=3). No significant values could be obtained through this test for *Ornithobius* and *Anatoecus* because for each age group, ectoparasite presence was nearly equal and therefore the distribution of *Ornithobius* and *Anatoecus* over the age categories was rather homogenous.

The external surface of the goose is of course its skin, but also the feathers; the habitat in which the ectoparasites live. As barnacle geese inhabit cold climates and follow Allan's rule (Allan 1877) which states that animals of colder latitudes are stockier and more spherical to conserve heat we looked at the goose's surface as dependant on its weight. We calculated the external plumage surface area (S_{ext}) (Walsberg & King, 1978) of each dustruffled goose to come up with a good measurement of habitat available to ectoparasites to inhabit (see equation 1).

$$S_{ext} = 8.11\ M \wedge 0.667 \qquad (1)$$

In which M = mass in g, and the output of S_{ext} is the geese's surface measured in cm^2. The allometric equation S_{ext} ignores the non feathered parts of the bill and the legs, as the equation was created to calculate heat transfer the tail is also ignored as it is considered of minor thermal significance. We considered the non feathered parts of the goose to be negligible as we could not visually observe any ectoparasites in these regions. S_{ext} basically gives a good estimation for the external plumage surface of a resting bird. And it is proven a better measure than assuming birds to be a perfect sphere (Walsberg & King, 1978).
The small pulli had a S_{ext} of 239-526 cm^2 (average 369 cm^2), the larger juveniles had a S_{ext} of 555-910 cm^2 (average 727 cm^2) and the adult geese had a S_{ext} of 978-1396 cm^2 (average 1183 cm^2). In total all geese combined had a S_{ext} of 48091 cm^2 and harboured on average 0,09 ectoparasites per cm^2. The juvenile category proved to have the highest ectoparasite Pressure over S_{ext} with on average 0,126 ectoparasites per cm^2 (Pulli 0,103 and Adult 0,05 ectoparasites / cm^2).

When goslings grow from pulli to juvenile to adult, their age, BM and thus S_{ext} increases and a strong correlation between the variables can be observed. *Anaticola* density significantly increased with S_{ext} (p=0,00248) and with BM (p=0,002194). *Ornithobius* significantly decreased in density when the S_{ext} became larger (P=0,035391) and also decreased significantly in density when the goose's BM increased (P=0,02457). When looked at the (Pulli, Juvenile, Adult) age-groups we saw the same pattern that *Anaticola* density significantly increased with age (P=0,006941) but when the geese grew older *Ornithobius* numbers significantly decreased (P=0,02415). For the remaining ectoparasite species no good assumptions could be made based on S_{ext}.

Although we realized that we could never catch all ectoparasites on the goose by dustruffling, for each age category we assumed our catch to be a 100% score. As we found a total of 909 ectoparasites in the pullus category (see tab. 1) and assumed this to be 100%, we could say that the 2 *Trinoton* found in the pulli category accounted for 0,2% of the pulli total (see fig. 5). The ectoparasite which occurred most in Pulli was *Ornithobius* with 71%. In the juvenile category, *Ornithobius* occured for 45% and *Anseriphillus* for 31% of the total paracite species build up.

The main parasitie in the adult catagory was *Anaticola* with 87%, *Ornithobius* occurred for a mere 9% in the adults. *Ornithobius* seemed to prefer the smaller geese over the adults and when the feathers of the goslings changed into their juvenile / yearling plumage, *Anaticola* took over and increased in numbers. This transition between the two Ischnoceran species could be contributed by different dietary preferences as a result of changes in the down to feather structure of the growing goslings. But competition could also be an option, Both *Ornithobius* and *Anaticola* are Ischnocerans and prefer the same food source; feather barbules and debris.

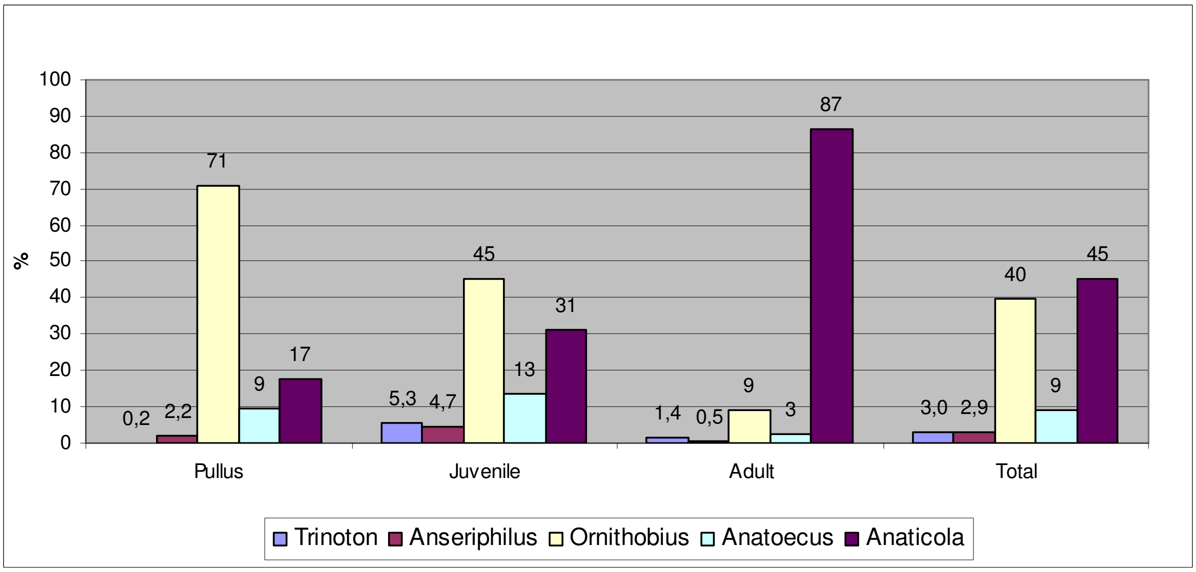

Figure 5: The presence in % per ectoparasite (*Phthiraptera*) species over 3 age groups: Pulli, Juvenile and adults and the total of all geese combined, in which the percentages of each age group add up 100%.

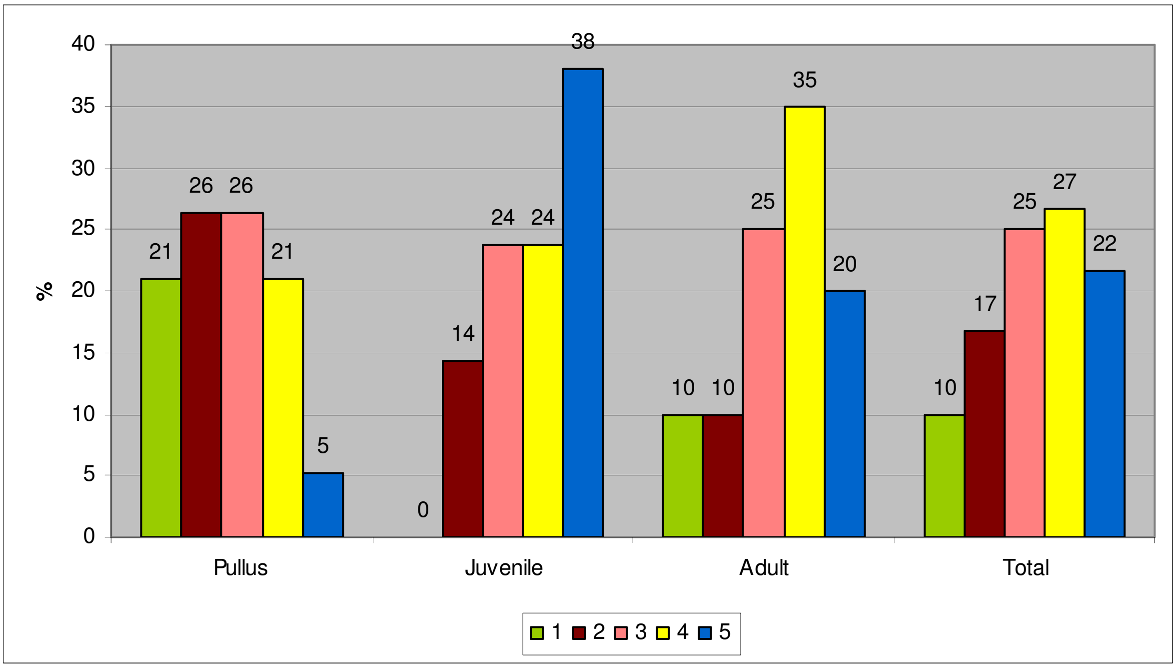

Figure 6: Co-occurrence of 5 species of ectoparasites (*Phthiraptera*) in % over 3 age groups: Pulli, Juvenile and adults and the total of all geese combined, in which the percentages of each age group add up 100%. One can see that the “juvenile” category with its heterogeneous plumage harbours the most diverse ectoparasite species assemblage. 5 species where found in 38% of juveniles.

We looked at the co-occurrence of our 5 species of parasites (see Fig. 6) and assumed each age category to add up to 100%. We found that only 5% of pulli carried 5 species of parasite, but the chance that a pulli carried 1-4 parasites was nearly similar. All juveniles proved to harbour at least 2 species of ectoparasites on their skin, not a single juvenile carrying only a single species of parasite was observed. A staggering 38% of the juveniles carried 5 species of parasite. This finding could potentially be contributed to the transition of down to feathers in the juvenile category and hereby the occcurence of a more heterogenic habitat for ectoparasites to exploit. Most adults (35%) carried 4 species of ectoparacites in their feathers. It was less likeley for adults to carry less then 3 ectoparacites although this occurred in 20% (carrying 1 ectoparasite = 10%, carrieng 2 ectoparacites = 10%) of cases. In all sampled geese combined (the "Total" group in fig. 6) 6 geese (10 %) carried 1 species of ectoparacite, 10 geese (17%) carried 2 species of ectoparacite. 15 individuals (25%) carried 3 ectoparacites, 16 individuals (27%) carried 4 species of ectoparacite and 13 individuals (22%) of them carried 5 ectoparacites.

Discussion: This study had a short duration and was only a snapshot in time on a single location, therefore we could not show seasonal fluctuations in ectoparasite densities which occur throughout the year. *Phthiraptera* are extremely food and temperature specific (Ash, 1960). Lice feeding on feathers of a particulair part of the hosts body will thrive on these feathers, but if presenteted only with feathers from other parts of the body, they will eat them but fail to breed and soon die (Ash, 1960). This propable explains the differences found between the age catagories, as pulli have down and juveniles make the transition to their juvenile plumage, the habitat to which the ectoparasite is subdued changes gravely with the age of its host.

The fact that the Juvenile category was most of the time moulting its feathers from down into feathers gave the juveniles a "mixed" exterior. This heterogenous habitat which the ectoparasite could exploit possibly explains the high ocurrences and co-occurences found in this category.

Ectoparasites are indicative for the condition of their hosts. When the condition of the host declines due to some external factor (e.g. unbalanced diet, diseases, wounds, etc), it loses the ability to preen itself well and in extreme cases feathers growing weakley and twisted (Ash, 1960). In weakend birds the ectoparasite concentration will be higher amplificating the deleterious effects of the initial underlying problem. In this study we used seemingly healthy barnacle geese and no deformaties in plumage structure, besides wear, were observed.

There are virtually no references in literature to the normal degree of parasitation on birds (Ash, 1960). But it is proved that when starlings (*Sturnus vulgaris*) are caught by mist netting 95% of them harbours ectoparasites and seem to be in good health (Ash, 1960). No evidence is found that the ectoparasite solely by itself causes any harm towards its host. But as literature on ectoparasites is scarce and *Phthirapteran* ectoparasites are a very large and diverse group, this remains open to debate.
The bloodsucking Amblycera pose a lager threat towards their host than the feather feeding Ischnocera by being an intermediate host for worms and other endoparasites. *Trinoton* is widley acknowledged for being an intermediate host for filarial heartworm in swans (Seegar *et al.* 1976; Cohen *et al.* 1991). In an other study (Stone 1967) a different type of *Trinoton* (*querquedulae*) was found

to run on the calm surface of the water and be attracted to disturbances in the water, such as swimming waterfowl, swimming mice and buret drips. They seemed to find the source of disturbance by rheotaxis and reached speeds of 15cm/sec (Stone 1967). If there is no disturbance of the water, the *Trinoton* moved slowly on the surface and made small loops on the surface as if to orientate, they readily sought out and climbed upon live mallard ducks swimming in the pool (Stone 1967). This is extremely unusual behaviour for a *Phthirapteran,* which are thought to be feeble in their mobility. This behaviour could potentially explain the low abundances of *Trinoton* found in our geese. *Trinoton* inhabits the breast feathers of its host (Stone 1967). *Anatoecus* is believed to live on the head of its host most of the time and *Anaticola* inhabits the wing (Stone 1967). Both Ischnocera *Anatoecus* and *Anaticola* had the ability to float on water but where incapable of movement or rheotaxis behaviour (Stone 1967). They where found in higher densities then *Trinoton*, which are perhaps necessary because of their inability to move about. However so little is known about the Ischnocera that they might be intermediate hosts or vectors to other diseases and parasites as well. It seems logical that with increased ectoparasite species loads there will be a greater risk of disease.

Conclusions: we found that *Ornithobius* and *Anaticola* make up for 85% of the total found ectoparacite load on Barnacle geese on the Dutch Westplaat (Delta region), but they seem to have different preferences towards their host and hence their diet in such a way that as the goose grows *Anaticola* prevails over *Ornithobius* . Both *Ornithobius* and *Anaticola* are in the iscnocera group and make their living on feathers and their debris. We don't consider the bloodfeeding amblycera to pose a major threat to the geese by drawing to much blood as they only make up 6% of the total ectoparasite load. We did not find a clear picture that the bodyfluid-feeding amblycera were faster to settle on a downy freshley hatched gosling than did the feather-feeding ischnocera. *Ornithobius* occurred in all but 2 geese but Anaticola was most abundant.
The goslings in the juvenile catagory with their heterogenous "mixed"exterior where parasitized most in number and in species.

The Surface Exterior Measure (S_{ext}) proved to be a good measure to compare different ectoparasites over different groups of geese.

We can conclude that dustruffling is a cheap suitable method for quantifing ectoparasite load, which can be preformed under field conditions on Barnacle geese.

Opportunities to future research: As all biological studies the answers found in this study raise a lot of new questions. To answer the question whether the Ischnocera *Ornithobius* and *Anaticola* competed with each other and did not like to coexist, as a result of changes in the down to feather structure of the growing goslings some experiments with captive growing geese could be conducted. In these experiments ectoparasite-free geese could be "infected" with ectoparasites and checked for competition of ectoparasites in each age group.

Different characteristics of plumage and courtship in birds have been suggested to be indicators of parasite load (Moreno-Rueda, 2005). Recent work has shown that white patches in the plumage attract *Phthiraptera* in the barn swallow (*Hirundo rustica*) (Kose *et al.* 1999 ; Kose & Møller 1999) and in the house sparrow (*Passer domesticus*) (Moreno-Rueda, 2005). The males in these species which display larger white patches are in better condition and have better underlying defence mechanisms against *Phthiraptera*. This might give rise to a sexual selection handicap mechanism in which males with more or lager white patches in their plumage are in better health and poses better genes (Kose *et al.* 1999; Kose & Møller 1999). Feathers containing melanin, the pigment responsible for black and gray plumage colorations (McGraw 2006), are more resistant to mechanical abrasion (Burtt 1986; Bonser 1995), wear and tear and may also deter feather feeding lice (Clayton *et al.* 2010; Kose & Møller 1999, Kose *et al.* 1999). As barnacle geese possess a lot of white, and it is believed that male geese display lager white patches in their face mask (Dr Henk van der Jeugd, personal communications), this sexual selection hypothesis could hold true for barnacle geese as well.

The Barnacle goose population in the whole flyway experienced a potential genetic bottleneck due to the severe hunting pressure in the beginning of the 20th century. After hunting diminished, the world population of barnacle geese grew exponentially but the breeding success decreased (Ebbinge, 1991). The only explanation for the growth in population size is the lowering of the mortality rate (Ebbinge, 1991). The Dutch barnacle geese population was founded by only a couple of individuals which had escaped or where released from a captive population. This small founding population had input from wild geese but still a genetic founder effect can be expected which functions as another genetic bottleneck (Hartl & Clark 2007). When an ectoparasite forages on a barnacle goose, it challenges the goose's immune system (Hoeck & Keller, 2012). Populations that have undergone historical processes of inbreeding may have successfully purged some of their immunity-related genetic load (Crnokrak and Barrett 2002, Ross-Gillespie et al. 2007), resulting in a weaker association between inbreeding and immunity against ectoparasites (Hoeck & Keller, 2012). Mating with genetically dissimilar mates is a way in which females might be able to increase the parasite resistance of their offspring (Owen *et al.* 2010). It was found that genetic diversity was negatively correlated with louse load (*Colpocephalum turbinatum* and *Degeeriella regalis*) in an inbred population of Galapagos hawk (*Buteo galapagoensis*) (Whiteman *et al.* 2006). Overall; inbred populations have been shown to exhibit a decrease in parasite and pathogen resistance or a lowered immune response (Hoeck & Keller, 2012). It would be interesting to establish the degree of inbreeding in the delta and Russian population and compare this to their ectoparasite pressures, to see if the Dutch population harbours more ectoparasites and if this is really due to inbreeding or to other external factors. It can be true that goslings of parents with a lower fitness, and higher inbreeding coefficient carry more parasites than do the offspring of fitter more outbred parents.

Although we found no evidence for this in this study; it could be the case that male barnacle geese carry more parasites than female geese (Owen *et al.* 2010) or the other way around. The sex-hormone testosterone has been linked to impaired immune function and increased parasite susceptibility in a number of vertebrate groups (Owen *et al.* 2010) whereas oestrogen is often associated with increased resistance against infection (Matthysse *et al.* 1974; Klein 2004). During incubation, female geese in the temperate regions deplete their body mass more than incubating females on arctic latitudes (Eichhorn *et al.* 2010). Depletion of body stores weakens an organism and could potentially make it more vulnerable to parasitism. As the Dutch delta population chicks are born and raised in a temperate climate and because seasonality plays a large role in the annual cycle of ectoparasites, it could be the case that Dutch-born Barnacle geese carry different ectoparasites than Russian ones. Geese raised in the Dutch delta, might, when paired to a Russian mate, introduce new parasite species into Russia.

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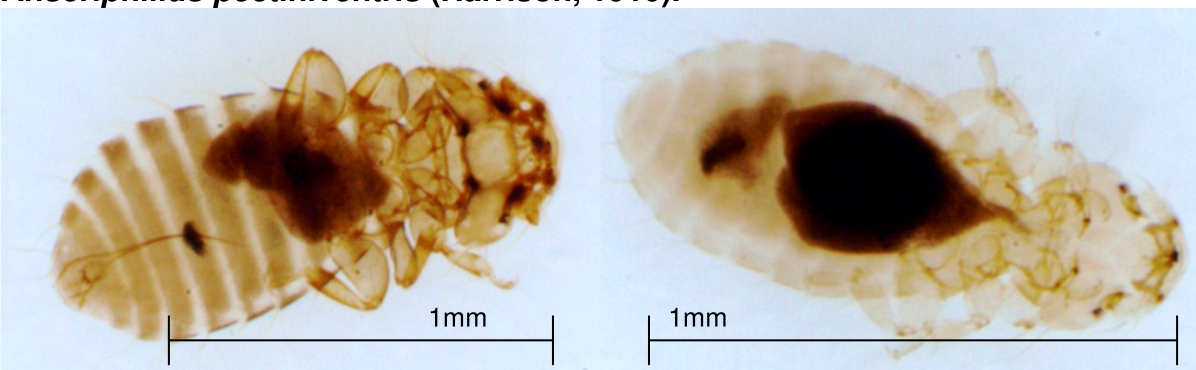
Appendix 1: Microscopic photos of ectoparasites (*Phthiraptera*) found in this study.

Taxonomy:
Insecta -> ***Phthiraptera* -> *Amblycera* -> *Menoponidae* ->**

***Trinoton anserinum* (Fabricius, 1805).**

Length: 9.7 mm
Sex: Male
View: Ventral

***Anseriphillus pectiniventris* (Harrison, 1916).**



Length: 1,4 mm
Sex: Male
View: Ventral

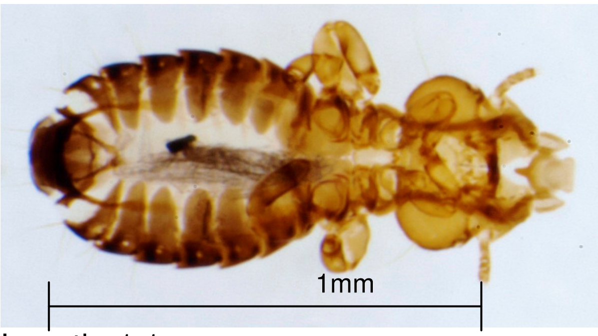
Length: 1,1 mm
Sex: Female
View: Ventral

Taxonomy:
Insecta -> ***Phthiraptera* -> *Ischnocera* -> *Philopteridea* ->**

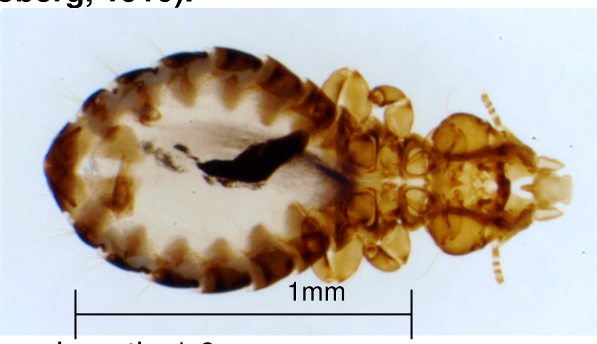
Ornithobius hexophthalmus **(Giebel 1861).**

Length: 3,6 mm
Sex: Female
View: Ventral

Anatoecus dentatus brunneopygus **(Mjöberg, 1910).**



Length: 1,1 mm
Sex: Male
View: Ventral



Length: 1,6 mm
Sex: Female
View: Ventral

Anaticola anseris **(Linnaeus, 1758).**

Length: 3,6 mm
Sex: Female (both)
View: Ventral

Appendix 2: Frequency distributions:

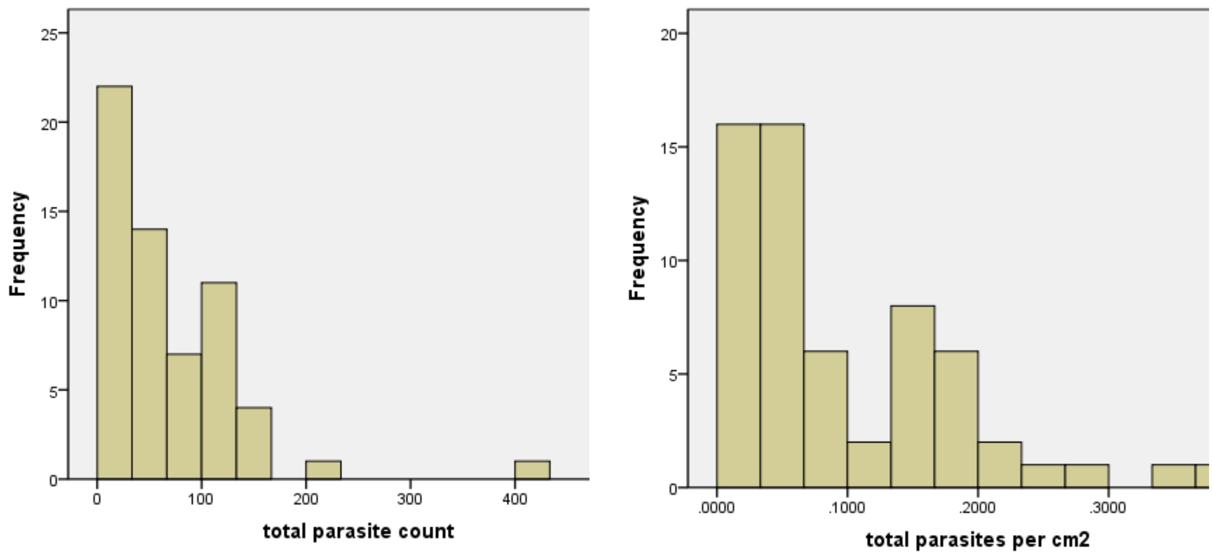
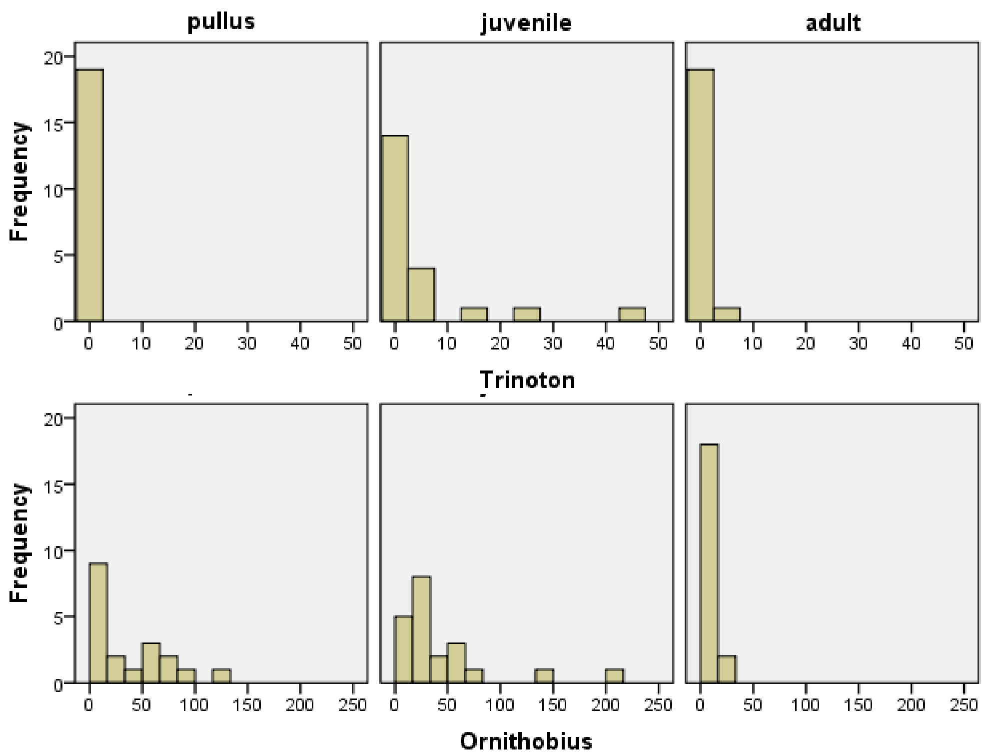


Figure A1. Frequency distributions of total (five species pooled) ectoparasite counts (left panel) and total ectoparasite count per cm2 of external plumage surface area (right panel). Surface area was estimated from body mass after Walsberg & King 1978.



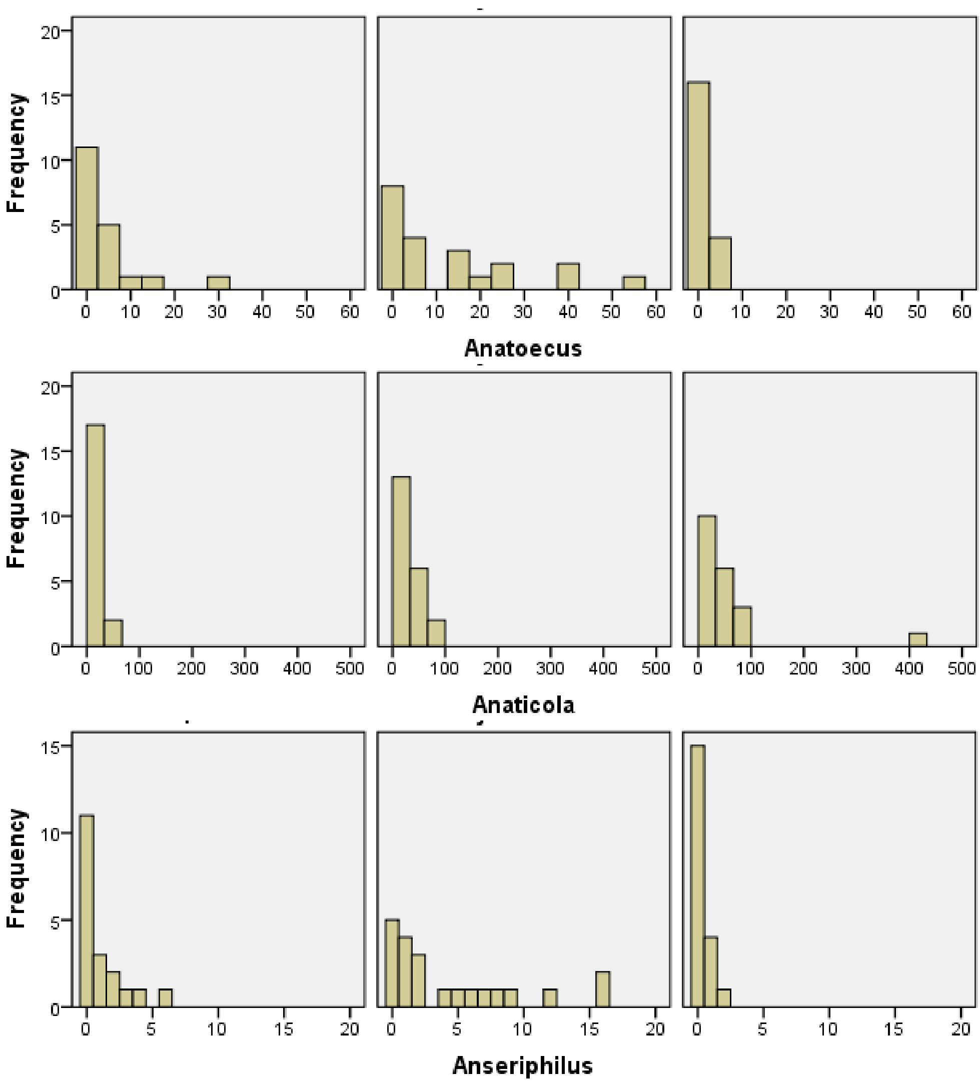


Figure A2. Frequency distributions for five species of ectoparasites counted on barnacle geese belonging to three different age groups: pulli (< 6 weeks old, left panel), juveniles (≥ 6 weeks old, central panel) and adults (> 1 year old, right panel). Note the differences on the X-axis's.

Appendix 3: Biometric measurements:

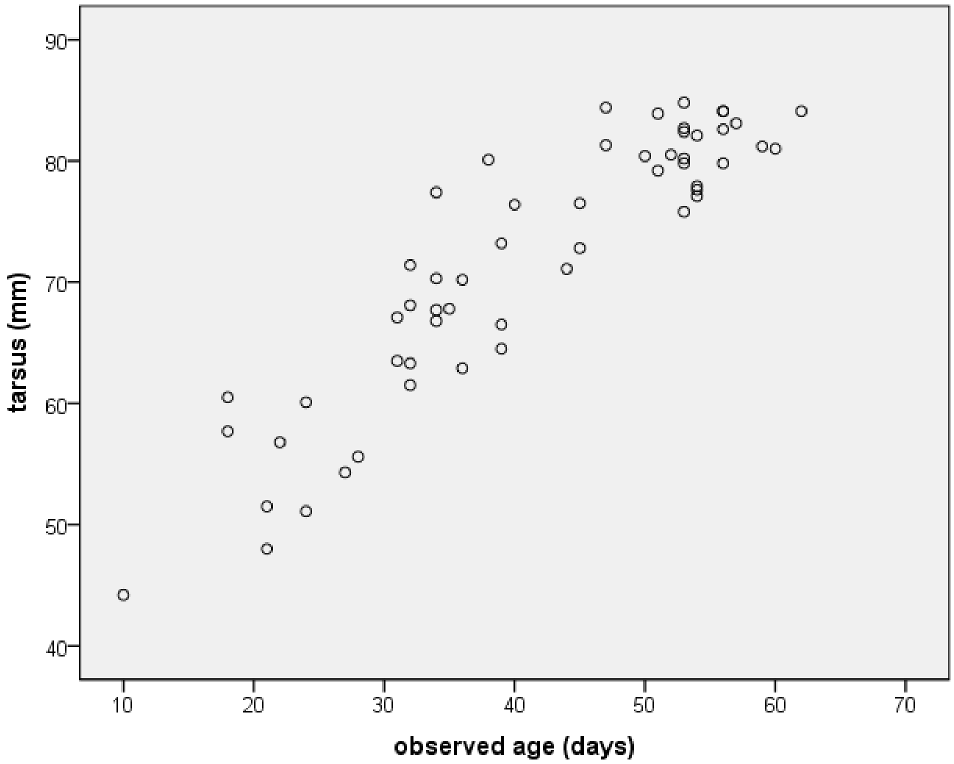


Figure: AA1. Development of tarsus length in growing barnacle goose. Each data point presents one individual (web-tagged) gosling. Only data from first recaptures are included.

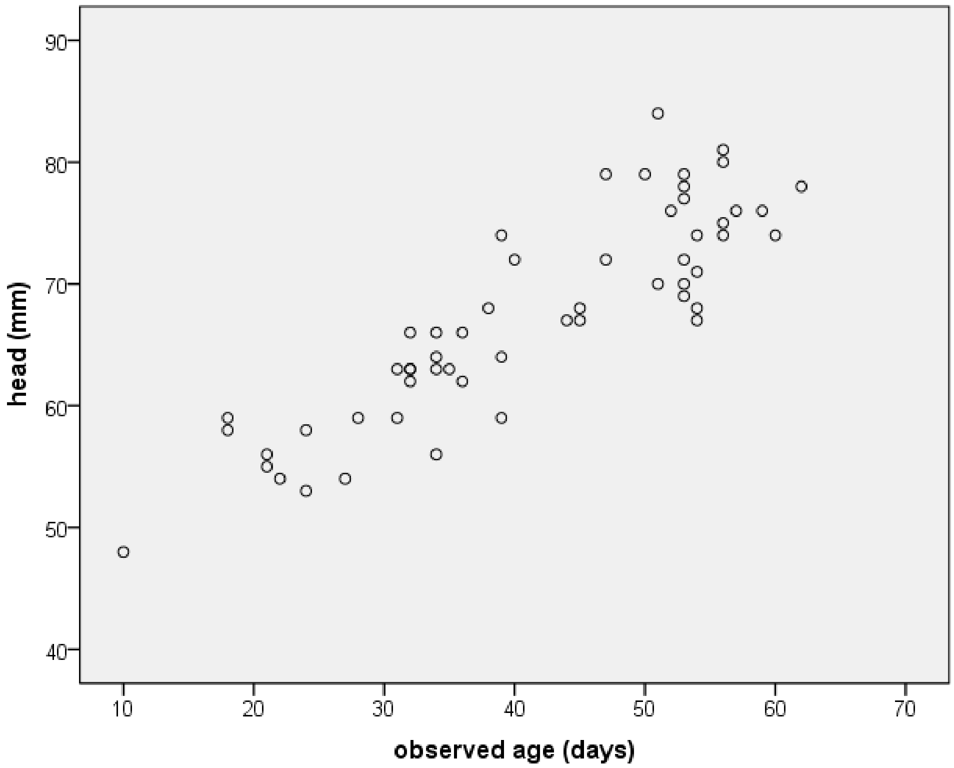


Figure: AA2. Development of head length in growing barnacle goose. Each data point presents one individual (web-tagged) gosling. Only data from first recaptures are included.

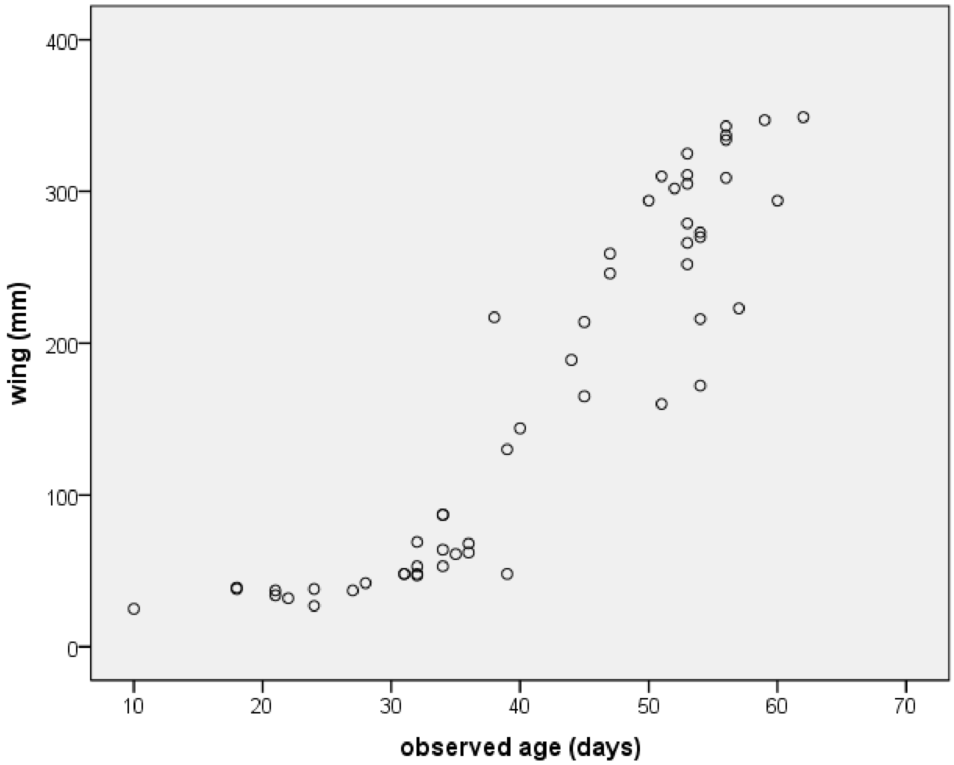


Figure: AA3. Development of wing length in growing barnacle goose. Each data point presents one individual (web-tagged) gosling. Only data from first recaptures are included.