

Genetics and Biodiversity Journal

Journal homepage: https://ojs.univ-tlemcen.dz/index.php/GABJ e-ISSN: 2588-185X P-ISSN: 2602-5582



Original Research Paper



Detecting bird's eggs in the diet of raccoon dog (Nyctereutes procyonoides)

Bahlke S. Ø¹, Nash D.R¹, Pedersen J.S¹, Hansen M.S², Chriél M³, Pagh S⁴

¹Department of Biology, University of Copenhagen, Universitetsparken 15, 2100 Copenhagen ∅, Denmark;

²Department of Veterinary and Animal Sciences, University of Copenhagen, Grønnegårdsvej 15, 1870 Frederiksberg C, Denmark;

³Department of Health Technology Center for Diagnostics, Technical University of Denmark, Kgs, Lyngby, Denmark

⁴ Department of Chemistry and Bioscience - Section of Biology and Environmental Science, Aalborg

University, Fredrik Bajers Vej 7H, 9220 Aalborg, Denmark

Corresponding Author: Pagh, S., Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark, **Email**: sup@bio.aau.dk

Article history; Received: March 1st 2021; Revised: March 25th 2021; Accepted: June 2nd 2021

Abstract

Diet studies of carnivores are often based on analysis of prey remains found in scats or stomachs. In these analyses differences in decomposition rate and the degree of digestion of the prey item must be taken into consideration. In studies of raccoon dog diets, eggshells are rarely found. This may be due to three possible scenarios, which may act in combination: 1) raccoon dogs rarely eat bird's eggs in the wild, 2) raccoon dogs eat eggs without swallowing shell parts, 3) rapid digestion of swallowed eggshells, so that they are only present briefly in stomach contents. In this study, the feeding behaviour of raccoon dogs on eggs of different sizes was observed under semi-natural conditions in an enclosure and the dissolution time of eggshells was examined. Dissolution time of eggshells of different thicknesses was tested experimentally using different concentrations of hydrochloric acid. The study showed a negative relationship between egg size/shell thickness and the amount of shell ingested by the raccoon dog. No eggshell, independent of bird species, was degraded beyond detectability after 12 hours in hydrochloric acid at pH 3.3. A correction factor estimating the mass of eggshell (g) ingested by the raccoon dog was calculated from the dry mass of eggshell found in the stomach or scat. Also the amount of eggshell estimated to be degraded by gastric acid is given. This correction factor ranged from 14 to 282 depending on eggshell thickness and egg size. From this study, it can be concluded that raccoon dogs do ingest some eggshell and that it is possible to detect eggshells in stomachs and scats of raccoon dogs, suggesting that the lack of eggshell in diet samples probably reflects a low proportion of eggs in the diet and/or preying more heavily on large eggs that are cracked open and have their contents eaten, rather than small eggs that are eaten shell-and-all.

Keywords: Invasive species, predation, diet, correction factor

Introduction

The raccoon dog (*Nyctereutes procyonoides*) is an opportunistic omnivore native to Eastern Asia (Helle and Kauhala 1991, Saeki 2009). The raccoon dog was released in the European part of the former Soviet Union in 1929-1955 (Helle and Kauhala 1991), and has since spread to many European countries, where it is considered an invasive species and a threat to biodiversity and especially to ground-nesting birds (Baagøe 2007, Kauhala and Kowalczyk 2011, Dahl and Åhlén

2016). Diet studies have been conducted in several countries to reveal the consequences of the invasion of the raccoon dogs for the native fauna in order to adapt management plans for the species (Convention on Biological Diversity 2018). Diet studies have shown that the raccoon dog is a generalist and that its basic diet consist of small mammals, birds, amphibians, invertebrates, fruit, seeds, and carrion (Drygala and Zoller 2013, Kauhala and Ihalainen 2014, Mikkelsen et al. 2016, Elmeros et al. 2018). These diet studies are often based on analysis of stomach contents or scats, and the results of the analysis rely on the ability to find and identify partially digested food items and the interpretation of these results (Reynolds and Aebischer 1991). In the stomach, prey items are subjected to mechanical and chemical degradation and the amount of the prey found in the stomach or scat, i.e. their detectability, will depend on the resistance of individual items to the digestion process. Yolk and egg whites leave no trace that can be recognized under the microscope and eggshells will break easily and are expected to dissolve relatively quickly in the acidic stomach fluid. Previous diet studies of raccoon dogs from Europe have found little evidence that bird's eggs make up an important part of their diet (0-14% frequency of occurence; Appendix 1). Sutor et al. (2010) found very small eggshells in the stomachs of raccoon dog and concluded that this was due to the complete dissolution of the eggshells when passing through the digestive tract. It is possible that the amount of egg in the diet of the raccoon dogs has previously been underestimated, due to the feeding behaviour of raccoon dogs and detectability of eggshell in the stomach. To assess the influence of the raccoon dog in areas with rare ground-nesting birds it is essential to be able to interpret to what extent it is possible to detect remains of eggs in stomachs and scats of raccoon dogs. To our knowledge, such attempts have not been made previously.

The aim of this study is to: 1) examine if and how eggshells are ingested by raccoon dogs fed width eggs of different sizes and 2) examine how eggs with different shell thickness will dissolve in different concentrations of hydrochloric-acid over a 12 hours period. 3) to calculate a correction factor for future diet analysis of raccoon dogs for estimating the amount of fresh egg eaten based on the dry weight of the eggshell found in the stomach contents and scats.

Methods

Study A: Raccoon dogs feeding on bird eggs

In a feeding experiment eggs were fed to captive raccoon dogs housed by the Danish Nature Agency. Eggs from domestic ground nesting bird species with different egg sizes and eggshell thicknesses were used to examine the feeding behaviour of raccoon dogs and to estimate the amount of eggshell ingested when an egg was eaten. Eggs chosen for this experiment were selected so they represented a wide range of Danish wild ground-nesting birds. Measurements of egg length and shell thickness of 23 Danish wild bird species protected by the Bird Directive were extracted from the "Handbuch der Oologie" (Schönwetter and Meise 1967), and the domestic birds eggs used in this study were plottet together with the measurements of wild birds (Figure 1).

The domestic species chosen for this study were common quail (*Coturnix coturnix*), domestic chicken (*Gallus gallus domesticus*), Muscovy duck (*Cairina moschata*), and Indian running duck (*Anas platyrhynchos domesticus*).

Length and width of eggs in the experiment were measured with a Vernier calliper (to an accuracy of 0.02 mm) and their mass determined with an A&D FA-2000 balance (to an accuracy of 0.01g). In all, 50 eggs were used: 24 common quail eggs, ten ducks' eggs (two Indian runner duck eggs, eight Muscovy duck eggs), and 16 chicken eggs. Half of the eggs (25 eggs) were placed on the ground in each of two pens (15 m x 6.6 m) with five raccoon dogs (Pen 1) and three raccoon dogs (Pen 2), respectively.

The pens were enclosed with mesh on all four sides (mesh size approximately 50 mm), up to a height of 1.8 m, but were uncovered at the top. Eggs were placed so that they were visible to the observers

sitting in a hide about 2 m above the ground. A surveillance camera (Bushnell 5mp Mini Trail Camera) was mounted in pen 1 to overlook the area with eggs. Two observers noted the feeding behaviour of the raccoon dogs from 8:00 pm to 10:45 pm. The following morning, the SD card from the camera was collected and all visible leftovers of pieces of eggshell down to the size of a few millimetres were carefully collected in the enclosures. The total time the raccoon dogs had access to the eggs was 10 hours, but they lost interest after a few hours, when all eggs were emptied. Shell remnants were collected in bags, marked with the enclosure number and stored in a -20°C freezer. The eggshells were sorted to species (recognizable by colour and thickness of the shell) and weighed on an A&D FA-2000 balance to an accuracy of 0.01g. Theoretically birds and mice could have removed eggshell during the night, however, no other animals were observed around the enclosures during daylight hours and twilight, or on the videos on the surveillance camera. Therefore, it was assumed that no eggs or shells were taken by other animals.

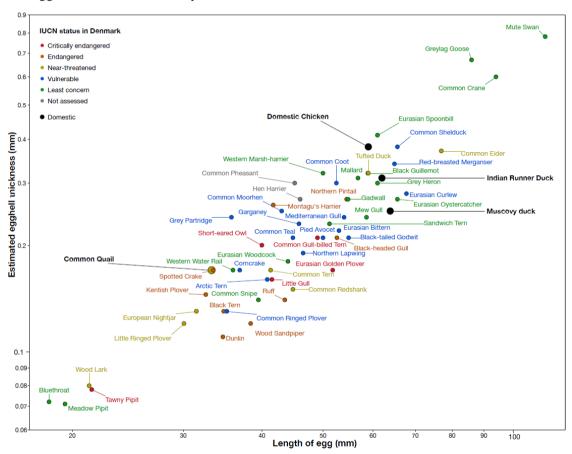


Figure 1. Egg proportions, length and shell thickness of Danish ground-nesting birds (Schönwetter 1967) and of domesticated species used in this study.

To estimate the relative shell mass of the different eggs, four chicken eggs, four Muscovy duck eggs and 16 common quail eggs were weighed and then emptied using a Blas-Fix Egg Blower Kit. The eggshells were dried in an oven at 38°C for 24 hours and then weighed to find the mean dry shell mass for the three species. The mass of the remaining eggshells and the mass of the dry empty eggshell of the same species were used to estimate the percentage of eggshells that had been ingested. As it was not possible to measure the dry eggshell mass for Indian runner duck directly, the relative dry eggshell mass (9.6%) for mallard (*Anas platyrhynchos*), the wild form of Indian runner duck was used as measured by Ricklefs (1977). The eggshell thickness of common quail, chicken, and Muscovy duck were measured with Vernier callipers to an accuracy of 0.02 mm. The eggshell thickness of Indian runner duck was extracted from the "Handbuch der Oologie" (Schönwetter and Meise 1967),

assuming the wild form (mallard) and the domestic form (Indian runner duck) to have the same eggshell thickness.

A paired t-test was used to compare the consumption of eggs of the different species in Pen 1 and 2. Least squares linear regression was used to test the relationship between eggshell thickness and percentage eggshell ingested, and between eggshell thickness and the measured correction factor. In both cases the dependent variable (percentage eggshell eaten and the correction factor) were log-transformed to homogenize variance. Based on the linear regression, predictions of the correction factor with estimated 95% confidence intervals for eggs of different shell thickness could be made. All analyses were conducted using JMP version 14.2 (©SAS institute, 2018).

Study B: Degradation time of eggshell in the stomach of raccoon dogs

The digestion of calcareous eggshell is primarily through the action of hydrochloric acid rather than digestive enzymes. The degradation rate is expected to depend on temperature, pH of the hydrochloric acid and thickness of the eggshell. Therefore, four different sized eggs from three different species of domestic ground-nesting birds (common quail, domestic chicken and Muscovy duck) were used. The eggs were exposed to a temperature and pH concentration expected to correspond to the temperature and pH concentration found in the raccoon dog stomach. The body temperature for both male and female raccoon dogs in snowless periods is on average between 37.8 °C and 38.3 °C (Mustonen et al. 2007). The pH of raccoon dog stomachs is not known and is expected to fluctuate according to the content. However, the pH is expected to be in the range found for other scavenging mammals, i.e. between 1.5 and 4.5 (values for the common brushtailed possum (*Trichosurus Vulpecula*) and dog (*Canis lupus familiaris*), respectively (Beasley et al. 2015). Hence, an intermediate pH of 3.3 was chosen.

Preliminary test with hens' eggs

To select the time scale for measuring shell thickness at different pH, a preliminary test was conducted with hens eggs. Two pH values within the expected spectrum, a lower at 2.3 and a higher at 4.3, were chosen for this preliminary test. Seven eggs for each pH were emptied with a Blas-Fix Egg Blower Kit, rinsed with water and put in an incubator (at 38°C) for 24 hours to dry. After incubation the shells were weighed using a A&D FA-2000 balance to an accuracy of 0.01g. The 14 eggs were then placed into individual 500 mL beakers and crushed with the handle of a wooden hand whisk to mimic the mechanical process of being eaten and to increase the surface area. 500 mL of 38°C hydrochloric acid was added to each beaker. In seven of the beakers the solution was adjusted too pH 2.3, and in the other seven to pH 4.3. All the beakers were incubated at 38°C. As the hydrochloric acid dissolves the calcium carbonate from the eggshell, the pH will rise, thus making the solution more alkaline. In the stomach, acid production is regulated to keep the pH steady with minor fluctuations. To simulate the natural condition in the stomach of a raccoon dog, the pH was regulated by checking the solution with a pH pen (LLG-pH pen) and adding hydrochloric acid to the beaker every half an hour, ensuring that the pH did not differ more than 0.1 from the starting pH. One egg from each pH was taken out of the acid every hour for 7 hours, and the shells were rinsed with demineralized water to stop the dissolution process and placed in the incubator to dry off excess water for 24 hours. The shell was weighed, and the percentage loss of shell mass calculated.

Test of dissolution and degradation time of eggshell

Two eggs of each species of domestic fowl were emptied with a Blas-Fix Egg Blower Kit, rinsed with water and put in an incubator (at 38°C) to dry off any liquid. After shells had been dried for 24 hours, the shells were weighed on a A&D FA-2000 balance to an accuracy of 0.01g. The shells were put in a 500 mL beaker and crushed to mimic the mechanical process of being eaten and create more surface area. Then 500 mL 38°C hydrochloric acid with the pH of 3.3 were added to the beakers, and the beakers were put in a 38°C incubator. The pH was kept at a constant value, by checking the solution with a pH-pen (LLG-pH pen) and adding hydrochloric acid to the beaker every half an hour. One egg

from each species was taken out of the acid after 6 hours and the other after 12 hours. The shell was rinsed with demineralized water to stop the dissolution process and the shells were put in the incubator to dry off excess water for 24 hours. The shell was weighed, and the percentage loss of shell mass calculated.

Calculating correction factor for eggs

A correction factor (CF) was calculated using the fresh weight of eggs for the species (EWs), divided by the weight of eggshell expected to be in the stomach or scat for each bird species. The weight of eggshell in the stomach or scat was estimated using the ingested weight of the egg of the species (Is) (study A) multiplied by the percent eggshell left in the stomach after acid digestion (D%)(study B).

 $CF = EW_s / (Is) \times (D\%)$

D= ingested eggshell x (1- eggshell dissolved under digestion).

Results

Behaviour study of raccoon dogs ingestion of eggshells-study A.

All eggs offered to the raccoon dogs in Pen 1 (25 eggs) and Pen 2 (25 eggs) were eaten, and fragments of eggshell were left behind. Observations and footage from the surveillance camera showed that the raccoon dogs ingest some shell when eating eggs, swallowing small eggs intact, whereas eggs of larger sizes are cracked open with teeth and the content licked up (Figure 2). In Pen 1, 98% quail eggshell, 31% chicken eggshell, 20.3 % Indian runner duck eggshell and 13.4% Muscovy duck eggshell were ingested. In Pen 2 29% quail eggshell, 51% chicken eggshell, 9 % Indian runner duck eggshell and 4% Muscovy duck eggshell were ingested (Figure 3). There was no significant difference in the amount of eggshell ingested between the pens (paired t-test: t3 = 0.93, p = 0.42), but a significant relationship between the egg size and the amount of egg eaten (i.e. (log of the amount of eggshell ingested) and eggshell thickness was found (linear regression: $F_{1,6} = 12.94$, $R^2 = 0.68$, p = 0.011; Figure 4). The thinner the eggshell, the more eggshell was ingested.



Figure 2. Raccoon dog eating a chicken egg. The egg has been cracked open with the teeth and the content is being licked up. Photo from surveillance camera.

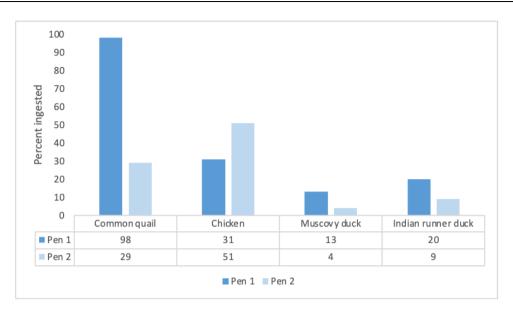


Figure 3. Percent eggshell ingested from eggs of common quail, chicken, Muscovy duck, and Indian runner duck for Pen 1 and Pen 2. ($t_3 = 0.77$, p = 0.50)

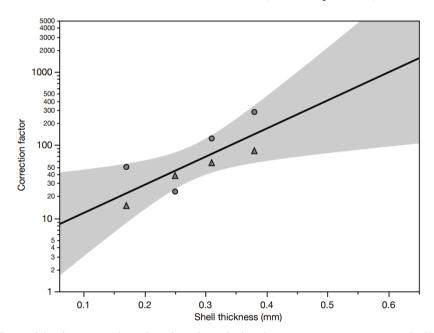
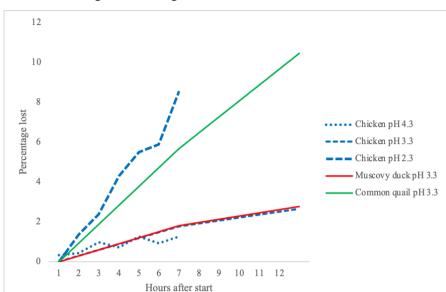


Figure 4. Logarithmic regression showing the relation between percentage eggshell ingested in relation to eggshell thickness (mm) for Pen 1 (circles) and Pen 2 (diamonds) ($R^2 = 0.68$, $F_{1,6}=12.94$, p=0.011).

Degradation of eggshell in hydrochloric acid-study B

The preliminary test with chicken eggs showed that degradation of eggshell in HCl is faster at a pH of 2.3 than 4.3 (Figure 5). Only 9% of the total mass of the eggshell was lost after 7 hours at pH 2.3, and less than 1.25 % at pH 4.3 (Figure 5).

No eggshell, independent of species, was degraded beyond detectability after 12 hours in hydrochloric acid at pH 3.3. After six hours and 12 hours of degradation, common quail eggs had lost 5.7 % and 10.5 % in mass respectively, Muscovy duck eggshells had lost 1.8% and 2.8 in mass and chicken eggshells had lost 1.8 % and 2.7% in mass. In the test experiment with a pH of 2.3, the eggshells had



lost 9.2 % after 7 hours of degradation (Figure 5).

Figure 5. Percent eggshell dissolved in hydrochloric-acid. Chicken eggshells (dashed blue line: 7-hour hydrochloric acid treatment at 2,3 pH, dotted blue line: 7-hour hydrochloric acid treatment at pH 4.3 and dashed blue line:12-hour hydrochloric acid treatment at pH 3.3), common quail (green solid line: 12-hour hydrochloric acid treatment at pH 3.3) and Muscovy duck (red solid line: 12-hour hydrochloric acid treatment at pH 3.3)

Correction factor for eggs of different shell thickness

Based on results from experiment A and B the correction factor (CF) could be calculated for the four domestic fowl species in pen 1 and 2. The correction factor for common quail (14-50%) was low compared to larger species as ducks (57-282%) (table 1).

Table 1. Eggshell thickness (mm), number of eggs used per species, total fresh weight of eggs ingested per species (g) total dry eggshell weight per species (g) the amount of eggshell which were not eaten by the raccoon dogs for the behavioural experiment. All of these values are measured, besides all eggshell thickness and Indian runner duck shell weight, which is derived values. Correction factor (CF) is calculated for each species.

	Species	Shell	No. of	Total	Total shell	Shell left in	Shell	CF
		thickness	eggs	weight	weight (g)	the pen (g)	ingested %	
Pen 1	Common quail	0.17	12	143.0	10.97	0.2	98.2	14.75
	Domestic chicken	0.25	8	512.0	48.16	33.2	31.1	38.03
	Indian runner duck	0.31	1	57.5	5.52	4.4	20.3	57.04
	Muscovy duck	0.38	4	317.6	31.75	27.5	13.4	83.05
Pen 2	Common quail	0.17	12	143.0	10.97	7.8	28.9	50.10
	Domestic chicken	0.25	8	512.0	48.16	23.5	51.2	23.07
	Indian runner duck	0.31	1	57.5	5.52	5	9.4	122.86
	Muscovy duck	0.38	4	317.6	31.75	30.5	3.9	282.37

Using D = 0.9, CF was calculated for common quail eggs to lie between 14.75 and 50.10 (Pen 1 and Pen 2, respectively) and for Muscovy duck between 83.05 and 282.37 (table 1).

The thicker the shell, the larger the correction factor, which means that less eggshell is ingested for thick shells compared to thinner ones ($R^2 = 0.611$, $F_{1,6} = 9.46$, p = 0.022; Figure 6).

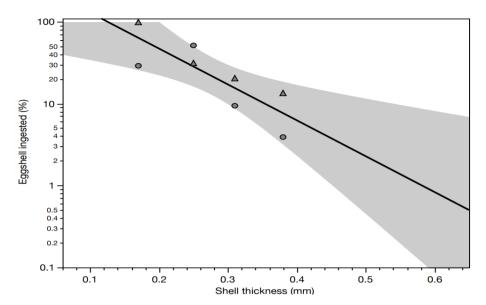


Figure 6. Correction factor for eggs ingested by raccoon dog in relation to eggshell thickness (mm) for Pen 1 and Pen 2. Logarithmic regression showing the relation between eggshell thickness and correction factor ($R^2 = 0.612$, $F_{1,6} = 9.46$, p = 0.022).

The logarithmic relation found can be used to estimate the correction factor for eggshell of a particular thickness found in stomach analyses. This can be expressed as:

 $CF_{shell\,thicknes} = 4.6086 \times e^{8.8536} \times \textit{Eggsh ell th ickness}$ of shell found in stomach or scat

With the shell thickness (in mm) found in the stomachs or scats, a more species specific CF can be found using $CF_{\text{shell thicknes}}$ (table 2).

Table 2. Estimated correction factors for eggshells of different thickness, plus their 95% confidence limits estimated for the range of eggshell thicknesses found in Danish birds (Figure 1).

Eggshell	Correction	95% confidence limits			
thickness (mm)	factor	Lower	Upper		
0.05	7.58	1	74.6		
0.10	11.8	1.51	92.1		
0.15	18.4	2.85	118		
0.20	28.6	5.11	160		
0.25	44.5	8.59	230		
0.30	69.2	13.4	357		
0.35	108	19.5	595		
0.40	168	26.5	1060		
0.45	261	34.1	1990		
0.50	406	42.3	3900		
0.55	632	50.9	7840		
0.60	983	60	16100		

Discussion

Behavioural study of raccoon dogs ingestion of eggshell

Although shells of bird's eggs are rarely found in the stomach content of raccoon dogs, this study shows that the raccoon dog does ingest eggshell when eating eggs. A larger proportion of shell from smaller eggs with thin egg shells, such as common quails, was ingested than that larger eggs with thicker shell, such as Muscovy ducks. We found a significant negative relationship between the amount of eggshell that was ingested and the shell thickness of the egg - i.e. the thinner the shell, the relatively more eggshell was ingested (Figure 5). This may be due to physical limitations: larger eggs with thicker shells are more difficult to swallow whole than smaller eggs, handling time for thicker eggs is longer than that for smaller eggs, and it pays to use more time to separate egg contents from the thicker shell. Even though there was no significant overall difference in the amount of eggshell ingested between the pens, in three out of four egg types used, a greater proportion was ingested in the pen with more raccoon dogs (Figure 3). In pen 1, there may have been more competition and eggs may have been eaten faster to minimise handling time. Feral raccoon dogs, under natural conditions, will more likely behave as the animals in Pen 2, as the raccoon dog is a monogamous species often found hunting alone or in pairs (Kauhala et al. 1993, Kauhala and Saeki 2004, Drygala et al. 2008).

Dahl and Åhlén (2016) observed that raccoon dogs in archipelagos of northern Sweden, when preying on eggs of shorebirds, duck and geese, would leave eggshell. Ducks will typically have eggs with thicker eggshells and as a result, it is likely that it is mostly the contents of the eggs and little shell that is ingested, making detection in the stomach difficult. For smaller birds like shore bird species with small and thin shelled eggs, the raccoon dog is likely to ingest a relatively larger amount of shell.

Degradation of eggshell in hydrochloric acid

Eggshells were only degraded very little within 12 hours (Figure 5). Our preliminary test showed that even at a low pH of 2.3 only 9.2 % of the total mass of the eggshell was lost after 7 hours. Diet studies for artic fox (*Vulpes lagopus*) show that food items take a maximum 24 hours to pass through the whole digestive tract (Pagh 2014). The gastrointerstinal transit time of red fox (*Vulpes vulpes*) has been found to be 5 to 6 hours (Kowalska et al. 2015. The stomach processes of the raccoon dog and foxes are expected to be similar.

The eggshell type which was degraded the most after 12 hours was common quail, which had lost 10.5% of its mass. Common quail had the thinnest shell of all the types of eggs used in the study (0.17 mm) which compares to both the native corn crake (0.17 mm) and the spotted crake (0.17 mm) (Figure 1). As approximately 90% of the common quail eggshell remained after 12 hours of hydrochloric acid-treatment, it should be possible to find most of the ingested eggshell from smaller birds (common quail: 89.53%) and larger birds (chicken: 97.34 % and Muscovy duck: 97.24 %) in stomach content analysis if thickness of the eggshell is the deciding factor. This means that future diet studies will be able to detect eggs from protected species, even though they have as thin an eggshell as the common quail. Another factor that could explain that eggshell is rarely found in stomach content analysis is the prolonged digestion time after the animal is killed and the emptying of the stomach into the intestine stops. The egg will therefore keep dissolving in the stomach acid, before the animal is put into a freezer. However, post mortem the stomach acid production will stop. A measure of the stomach acid ph in 17 dead raccoon dogs at autopsy was 5.9±0.6sd, range 5-7, showed that stomach acid is neutralized post mortem, possibly due to bones and feathers in the stomach.

Correction factor

In a previous diet study of faecal biomass of prey items from foxes, a correction factor of 9000 was found for chicken eggs (total fresh egg mass)/(dry mass of eggshell+ membrane), which is the largest conversion factor for food items in fox scats (Reynolds and Aebischer 1991. The very large correction factor calculated for foxes may be due to loss of some eggshell in the process of stomach or scat analysis, where samples are usually washed through a 0.5 mm sieve.

To improve the correction factor in this study, it would be necessary to conduct more feeding experiments with eggs from the species used in the experiment along with other species that have different eggshell thickness. It would also be important to run the experiment with different numbers of animals in the pens, to test whether competition between the animals influences the amount of eggshell ingested. In the hydrochloric acid experiments more replicates of the experiment with eggs from the species used in the experiment along with other species that have thinner eggshells than common quail, with individual eggshell thickness taken into account, could shed light on the importance of shell thickness for degradation of eggshell.

Based on this study, we can conclude that raccoon dogs do ingest eggshells when feeding on eggs, although this depends on egg size and eggshell thickness, and that it is possible to find most of the eggshell in the stomach after more than 12 hours of digestion. Stomach content analysis is considered a plausible method for detecting eggs in the raccoon dog diet if raccoon dogs are frozen quickly after they have been killed.

Acknowledgements

We would like to thank Aalborg Zoo Conservation Foundation and the Danish Environmental Agency for support of the project. Also Jan Bolding and Jon Fjeldså from the Natural History Museum of Denmark for their expertise regarding birds eggs.

References

- **Baltrūnaitė** L **2002**. Diet composition of the red fox (*Vulpes vulpes* L.), pine marten (*Martes martes* L.) and raccoon dog (*Nyctereutes procyonoides* Gray) in clay plain landscape, Lithuania. Acta Zoologica Lituanica 12:362-368.
- **Baltrūnaitė** L **2006**. Diet and winter habitat use of the red fox, pine marten and raccoon dog in Dzūkija National Park, Lithuania. Acta Zoologica Lituanica 16:46-53.
- **Beasley DE. Koltz AM. Lambert JE. Fierer N. Dunn RR 2015**. The evolution of stomach acidity and its relevance to the human microbiome. PloS one 10:e0134116.
- **Baagøe HJ 2007**. Dansk Pattedyratlas [Atlas of Danish Mammals]. Gyldendal A/S. ISBN 978-87-05506-1
- **Dahl F. Åhlén PA 2016.** Nest predation by raccoon dog *Nyctereutes procyonoides* in the archipelago of northern Sweden. Biological Invasions:1-13.
- **Convention on Biological Diversity 2018**. Invasive Alien Species: What needs to be done? https://www.cbd.int/invasive/done.shtml.
- **Drygala F. Stier N. Zoller H. Mix HM, Bögelsack K. Roth M 2008.** Spatial organisation and intraspecific relationship of the raccoon dog *Nyctereutes procyonoides* in Central Europe. Wildlife biology 14:457-466.
- **Drygala F. Zoller H 2013.** Diet composition of the invasive raccoon dog (*Nyctereutes procyonoides*) and the native red fox (*Vulpes vulpes*) in North-East Germany. Hystrix, the Italian Journal of Mammalogy 24:190-194.
- Elmeros M. Mikkelsen DMG. Nørgaard LS. Pertoldi C. Jensen TH. Chriél M 2018. The diet of feral raccoon dog (*Nyctereutes procyonoides*) and native badger (*Meles meles*) and red fox (*Vulpes vulpes*) in Denmark. Mammal Research:1-9.
- **Helle E. Kauhala K 1991.** Distribution history and present status of the raccoon dog in Finland. Ecography 14:278-286.
- **Kauhala K. Auniola M 2001.** Diet of raccoon dogs in summer in the Finnish archipelago. Ecography 24:151-156.
- **Kauhala K. Ihalainen A 2014.** Impact of landscape and habitat diversity on the diversity of diets of two omnivorous carnivores. Acta Theriologica 59:1-12.
- **Kauhala K. Kaunisto M. E. Helle E 1993.** Diet of the raccoon dog, *Nyctereutes procyonoides*, in Finland. Zeitschrift für Säugetierkunde 58:129-136.
- Kauhala K. Kowalczyk R 2011. Invasion of the raccoon dog Nyctereutes procyonoides in Europe:

- history of colonization, features behind its success, and threats to native fauna. Current Zoology 57:584-598.
- **Kauhala, K. Saeki M 2004.** Raccoon dog. Pages 136–142 Canids: foxes, wolves, jackals and dogs. Status survey and conservation action plan. IUCN/SSC Canid Specialist Group, Gland, Switzerland. Dorota
- **Kowalska D. Piórkowska M. Zoń A 2015**. Comparison of selected metric traits of the digestive system in farmed and wild fox populations. Scientific Annals of Polish Society of Animal Production 3: 23-30.
- Mikkelsen DMG. Nørgaard LS. Jensen TH. Chriél M. Pertoldi C. Elmeros M 2016. Mårhundens (*Nyctereutes procyonoides*) føde og fødeoverlap med hjemmehørende rovdyr i Danmark. [Diet of Danish raccoon dog and their niche overlap with native predators] Flora og Fauna 122:101-114.
- Mustonen AM. Asikainen J. Kauhala K. Paakkonen T. Nieminen P 2007. Seasonal rhythms of body temperature in the free-ranging raccoon dog (*Nyctereutes procyonoides*) with special emphasis on winter sleep. Chronobiology International 24:1095-1107.
- **Pagh S 2014.** Beregning af fisk i føden hos polarræv (*Vulpes lagopus*) [Estimating fish in the food of arctic fox *Vulpes lagopus*]. Flora og Fauna 119:120-122.
- **Pagh S. Chriél M 2017.** Mårhund-risikovurdering, biologi og erfaringsgrundlag for en" best practice" i forhold til regulering [Raccoon dogs: Best practice recommendations for population control based on risk assessment, biology and experience]. DTU-Veterinærinstituttet.
- **Reynolds JC. Aebischer NJ 1991.** Comparison and quantification of carnivore diet by faecal analysis: a critique, with recommendations, based on a study of the fox *Vulpes vulpes*. Mammal Review 21:97-122.
- Ricklefs RE 1977. Composition of eggs of several bird species. The Auk 94:350-356.
- **Saeki M 2019.** Nyctereutes procyonoides (Gray, 1834). https://www.cabi.org/isc/datasheet/72656; retrieved 10 May 2021.
- Schönwetter M 1967. Handbuch der Oologie. Akademie-Verlag Berlin, Germany.
- **Sidorovich VE. Solovej IA. Sidorovich AA. Dyman AA 2008.** Seasonal and annual variation in the diet of the raccoon dog *Nyctereutes procyonoides* in northern Belarus: the role of habitat type and family group. Acta Theriologica 53:27-38.
- **Sutor A. Kauhala K. Ansorge H 2010**. Diet of the raccoon dog *Nyctereutes procyonoides*—a canid with an opportunistic foraging strategy. Acta Theriologica 55:165-176.

Appendix

Appendix 1 Diet studies of the raccoon dog in Europe in spring and summer. Food items are given in frequency of occurrence (FO), volume percentage (V) and biomass percentage (BC). After Pagh and Chriél (2017).

Study	Country	Invertebrates	Plant material	Birds	Eggs	Reptiles and Amphibians	Carrion	Small mammals
Baltrūnaitė (2002)	Lithuania	BC: 5 % FO: 61 %	BC: 31 % FO: 76 %	BC: 10% FO: 7%	Not Calculated	BC: 9 % FO: 10 %	BC: 18 % FO: 10%	BC: 15 % FO: N/A
Baltrūnaitė (2006)	Lithuania Dzûkija Nationalp ark	BC: 11 % FO: 61 %	BC: 21 % FO: 18 %	BC: 18% FO: 12%	Not Calculated	BC: 22 % FO: 14 %	BC: 9% FO: 4 %	<13
Drygala and Zoller (2013)	Germany NØ	BC: 53 % FO: 6 %	BC: 37 % FO: 63 %	BC:26 FO:18	Not Calculated	BC: 18 % FO: 16 %	BC: 24 % FO: 17 %	BC: 6% FO: 17%
Kauhala and Auniola (2001)	Finland SW	BC: 6 % 9 %	BC: 36 % 16 %	BC: 32% 20%	FO: 14%	BC: 1% 10%	BC: 1% 10%	BC: 11% 34%
Kauhala and Ihalainen (2014)	Finland	FO: 60-90%	FO: 20-90 %	FO: 25- 65 %	Not Calculated	FO: 5-50 %	BC: N/A FO: 10-25%	FO: 30-65 %
Mikkelsen et al. (2016)	Denmark	V: 12 %	V: 19 %	V: 11%	FO: 1 %	V: 9 %	V: 23 %	V: 22%
Sidorovich et al. (2008)	Belarus	BC: 10 %	BC: 23 %	BC: 25 %	Not Calculated	BC: 10-27 %	Carrion and Mammals: BC: 23 %	
Sutor et al. (2010)	Germany	FO: 25 %	FO: 18 %	FO: 8 %	Few egg residues	FO: 69 %	FO: 15 %	FO: 8 %
Elmeros et al. (2018)	Denmark	FO: 63.6%	FO: Cereal: 18 % Fruit:14 %	FO: 45 %	1 of 249 stomachs	FO: 27.3 %	Ungulate: 13.6 9	% FO: 68.2 %