

# Guideline for Sampling and Sample Treatment

Feral pigeon (Columba livia f. domestica)



Umwelt

**Bundes** 

Für Mensch und Umwelt

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## Guidelines for Sampling, Transport, Storage and Chemical Characterization of Environmental and Human Samples

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# 1 German Environmental Specimen Bank

The German Environmental Specimen Bank an instrument of environmental (ESB) is monitoring for the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) underlying specialized and administrative co-ordination of the Federal Environmental Agency (Umweltbundesamt, UBA). The ESB collects ecologically representative environmental specimen in addition to human samples. maintains and examines them concerning relevant environmental substances (BMU 2008).

Long term storage is accomplished under conditions, which exclude condition change or loss of chemical characteristics, over a period of numerous decades. The archive stores samples for retrospective examination of such substances whose danger potential for the environment or for human health is today unknown.

Comprehensive information of the ESB is available at www.umweltprobenbank.de.

# 2 Guideline Objective

Sampling is the first and most important step to safeguard the quality of samples and data. It is the result of science-based and standardized methods, to avoid contamination and inhibit loss of chemical information. The exceptionally high demand of true quality results derives from the extraordinary value of the samples as archive material. Representativeness and reproducibility of the samples are the basis for spatial and temporal comparison.

The current guideline is an update of the ALTMEYER & PAULUS (1995) version.

Transport, further sample treatment and storage as well as chemical analysis have to be done following the actual guidelines of the ESB.

# 3 Function of the Specimen Type

As a representative of the herbivorous trophic level, the domestic pigeon lends itself to be used as accumulation indicator like almost no other species.

Comprehensive experience with the feral pigeon as accumulation indicators exist, based on monitoring studies in numerous cities (e.g. TANSY & ROTH 1970; SIEGFRIED et al. 1972; OHI et al. 1974; JENKINS 1975; HUTTON & GOODMAN 1980; KAPHALIA et al. 1981; JOHNSON et al. 1982; KENDALL & SCANLON 1982; ALTMEYER 1987, 1993; DRASCH et al. 1987; GRACIA et al. 1988; HAAG-WACKERNAGEL et al. 1998; NAGEL et al. 2001). In all studies, significant regional variations in the pollution burden could be shown for the populations studied.

The following criteria underline the use of the feral pigeon as an accumulation indictor in the scope of the ESB:

- as a cosmopolitan, is widely spread and occurs throughout in all regions of Germany,
- continuously available in large numbers: Variations within the population are few, thus monitoring continuity is guaranteed. Data relating to population trends, growth and mortality rates are available. (MURTON et al. 1972; GLUTZ VON BLOTZHEIM & BAUER 1980; HAAG 1984),
- relatively resident with a limited home range. This ensures sufficient spatial relation of the pollution burden,
- the feeding habits of the feral pigeon is thoroughly investigated (e.g. GOODWIN 1960, 1978; MURTON & WESTWOOD 1966; DILKS 1975; HAVLIN 1979; VOGEL 1980; HAAG 1984). Feeding habits are as contamination path of primary concern, because the substance intake of terrestrial animals results primarily from the intake of food and water. Including town centers, natural nourishment (e.g. seeds, leaves,) constitutes an important element for the food spectrum of the pigeons, despite of multiple feedings,

- the sampling is relatively easy to perform. Breeding in enclosures or aviaries and population steering or field colonization are feasible without problems. Moreover, wild nesting sites are often available for the sampling,
- the availability is neither restricted by the shooting rights nor by nature conservation regulations (WOHLFARTH 1993),
- the species is easy to identify.

# 4 Target Compartments

Comprehensive studies revealed that especially liver-, kidney-, plumage- and egg samples are suited as accumulation indicators. The use of eggs has the advantage that through the determination of biometric characteristics and derived indices (e.g. Ratcliffe-Index) useful information on effects of chemical substances can also be ascertained. Thus they can be utilized both as accumulation- and effect indicator. The egg contents serve as sample for substance investigations.

The following criteria underline the use of eggs as target compartment if birds are to be used in monitoring studies (e.g. BECKER 1989; ALTMEYER 1995; HAHN & HAHN 1995):

- the eggs have a sufficient biomass,
- date and location of the egg sample can be exactly defined,
- eggs mirror the contamination of the hatching females,
- the animals need not be killed,
- the time spent on collection is minimal compared to catch campaigns,
- the eggs are easy to handle during the sampling and the sample preparation,
- the shell is excellent protection and inhibits contamination of the sample (egg contents),
- according to the current level of knowledge the chemical composition of eggs is constant in comparison to viscera,
- eggs constitute an important pathway for the excretion of lipophilic persistent pollutants and some heavy metals,
- in specific stages of development their reaction towards toxic chemicals is very sensitive.

When evaluating the analysis data attention must be drawn to the fact that the ovary builds a sort of barrier to many heavy metals. This barrier inhibits higher concentrations of e.g. lead and cadmium to the burden of the egg.

# 5 Predefinitions for the Sampling

#### 5.1 Selection and Definition of Sampling Sites

Especially in urban-industrial areas, more than one sampling site is usually defined so that the burden spectrum of a regionally differentiated pollution is sufficiently justified. First of all the pollutant content of the available wild pigeons is analyzed during а screening. eggs Subsequently, the number and location of the sampling sites is defined with regard to regionally varying pollution burdens. In case there are no free living populations available, the sampling site(s) will be defined following possibly existing pollution data ascertained in other monitoring studies and by land use criteria.

A sampling site can consist of several sampling locations. As sampling locations for the eggs either existing nesting sites (in attics or bridgeniches) will be selected or pigeonries will be constructed.

The selected sampling sites containing the egg sampling locations are specified in the area-related sampling scheme (chap 0).

## 5.2 Selection of Individuals and Sample Size

The pigeon clutch normally consists of two eggs. Clutches with one or three eggs are extremely scarce (VOGEL 1980; HAAG 1984; DABERT 1987). In course of the sampling all fresh, not incubated if possible, (Fig. 1, chap. 6.2), eggs are gathered from the nests.

For the description of one sampling site a random sample number of at least 25 eggs should be

reached. With his random sample number the biometric as well as the analytical variability of the egg samples is sufficiently taken into account.

25 eggs with an average egg content of 15 g multiply to a total sample quantity of at least 375 g egg contents. The needed sample quantity of 2.200 g egg contents for ESB storage requires sampling of 150-180 eggs. However, further eggs should be collected per sampling site to reject incubated or damaged eggs (chap. 6.2).

If the sampling region consists of several sampling sites a screening should be performed to check them for different pollution levels. If the pollution burden differs the eggs shall be collected equally distributed from the individual sampling sites.

## 5.3 Sampling Period and Frequency

Despite the fact that the broodiness of feral pigeons is very pronounced almost year-round, due to better environmental conditions a main incubation period from March to September can clearly be defined (e.g. GOODWIN 1960; RIDDLE 1971; MURTON et al. 1972; DABERT 1987). This period is chosen for repeated annual sampling, because then a relatively large number of fresh eggs is available.

The collection of eggs should be performed at least twice a year (once in the first and once in the second half of the main incubation period) with an equal amount of each sampling to represent the whole incubation period. In case of small breeding swarms, the sampling period needs to be enlarged until a sufficient sample amount is reached. To secure that no eggs originating from post-laying activities (stress situation) will be collected, an interval of three to four weeks shall at least intervene between two sampling dates, if possible. Number and sequence of the sampling dates need to be defined in the area-related sampling scheme.

With a random sample number as mentioned above, sampling can be conducted in an annual rhythm without drastic interferences in the populations to be expected.

#### 5.4 Area Related Sampling Scheme

Based on the sampling guidelines, specific definitions for the individual sampling areas and sites must be made and documented in an area related sampling scheme. This includes amongst others:

- location and demarcation of the sampling sites,
- required sample size (depending on the weight of the eggs in the specific breeding colonies),
- time frame for sampling,
- sampling frequency,
- addresses of the appropriate authorities,
- addresses of respective owners of land including the nesting sites,
- assured sample identification.

Describing the characteristic elements of the sampling sites within the area related sampling scheme secures long-term continuous sampling. In the case of changes within the sampling site or the sampled population the document has to be updated.

In case of major changes, so that comparability of the samples could not be guaranteed anymore, a new site has to be selected.

# 6 Sampling Procedure

All data collected in the course of sampling and through the biometric sample description are documented in the respective specimen data sheets (see appendix). A record is kept for each sampling with the following contents:

- all persons involved in the sampling,
- chronological procedure of the sampling,
- the underlying version of the sampling guideline and the area related sampling scheme for the current sampling,
- alterations to the sampling guideline and the area related sampling scheme.

#### 6.1 Required Equipment and Cleaning Procedures

#### **Technical Preparations:**

Pigeoneries offering species compliant nesting sites will be installed on sampling sites with an insufficient number of natural sites or where those are not within reach. Thus the sample collection is always possible at identical sites, from stable and easily to characterize populations and in comparable sampling periods. Pigeons which live in those pigeoneries normally retain the nesting site once occupied lifelong.

The pigeoneries should provide individual breeding locations for at least 15-20 pigeon pairs. Beside specially constructed pigeon towers, little wooden pigeon houses can be used, or pigenories can be installed in attics. The pigeoneries should stand several meters (at least 3 m) above ground. Alternatively other safety measures against natural enemies (marten, cat, rat) are required. The locations must be selected in a way, that they offer the possibility for the birds to find their feed themselves.

A new pigeonery is colonized with fledged squabs (feral pigeons). To support the development of a close relationship of the birds to the pigeonery, it is necessary to provide enough feed during the first months. Therefore a standard feed offered in the respective specialized trade should be used. After six months (sexual maturity of the birds) the feed amount should be reduced to 5g per pigeon and day. This feed amount covers approx. 20% of the daily feed requirement. The birds are hereby urged to search for additional natural feed sources.

The pigeoneries should be built at least one year preceding the intended begin of the sampling, to have a sufficiently stable pigeon population (PAULUS & NENTWICH 1999).

#### Field work:

- specimen data sheets for documentation during the sampling, for description of the sampling site and nesting sites
- protective clothing (i.e. protective face mask with filter class A2P3, disposable gloves, overall, long boots)
- disinfectant

- if necessary, extendable ladder (5 to 8 m working-height)
- torches
- polyethylene bags (i.e. 12x17 cm)
- pen to label the polyethylene bags
- cooling device (5 °C (+/-3°)) for egg transportation.

#### Laboratory:

- specimen data sheets for the biometric sample description and for the documentation of the sample treatment
- cooling device (5°C (+/- 3°)) for the storage of the eggs until further processing
- glass beaker with deionized water to determine incubation stage
- disposable gloves
- paper tissues to clean the eggshells
- clean bench with particles- and activated carbon filtration
- stainless steel containers (5.5 l) with lids and fasteners
- stainless steel scalpel to open the eggs
- stainless steel sieve
- Petri-dishes to dry the eggshells
- Identity cards to label the Petri-dishes
- precision scales (reading accuracy 0.1 mg) to determine the weight of the fresh egg (FW) and weight of dry shell (DW)
- slide gauge (reading accuracy 0.1 mm) to determine the size of the egg
- micrometer caliper (reading accuracy 0.001 mm) to measure the thickness of the egg shell
- liquid nitrogen
- tools and protective clothing for liquid nitrogen handling
- cooling device (dewar vessel) for the rapid deep-freezing and storage of the samples in the gas phase above liquid nitrogen (LIN), corresponding to the number of required stainless steel containers.

For the packing and immediate deep freezing of the eggs contents, the stainless steel containers are inserted directly during the sample preparation in the gas phase above liquid nitrogen

#### **Cleaning procedures:**

Sample containers and all equipment is cleaned in a laboratory washer using a chlorine-free powerful washing agent in a first step. After cold and hot (90-95°C) rinsing, neutralization using 30 % phosphorus acid in warm water is performed, followed by hot and cold rinsing with deionized water. After this procedure the containers are dried in a cabinet dryer at  $130^{\circ}$ C (+/– 5°) for a minimum of an hour (sterilization). The containers remain closed while they are left to cool. Sterilization is not applied to synthetic materials.

## 6.2 Sampling Technique

All undamaged eggs from the existing clutches are removed if an advanced incubation stage can be excluded when candled by means of a torch. The eggs will be packed in labeled polyethylenebags (sampling location, sampling site, date) and placed in a cooling device (refrigerator) at  $5^{\circ}$ C (+/–  $3^{\circ}$ ) for transport and subsequent storage. An interim storage until further sample processing should not exceed 2 weeks. To prevent the shells from cracking, the eggs should not be frozen.

Protective clothing and breathing protection have to be worn at the nesting sites the whole time.

Sample preparation and biometric sample description are carried out in the laboratory. First the incubation stage is determined analogous to the method of HAYS & LECROY (1971) by inserting the eggs in a glass beaker filled with deionized water). This guarantees that only fresh eggs are used. Only those eggs which comply with the situation a) to d) (Fig. 1) are considered. After immersion the eggs are cleaned and dried from dirt particles and water, using tissues.

For biometric sample description (chap. 7) the first 25 eggs are measured, length, diameter and fresh weight (FW) prior to the removal of contents from the calcium shell.

The separation of the calcium shell and the egg content is carried out under clean air conditions. Using a scalpel the shell is cut open between its equator and its pointed end. The content of the egg is in its entirety, emptied (approx. five seconds) through a stainless steel sieve hanging above a stainless steel container which is filled with liquid nitrogen. Due to the immediate shockfreezing the nitrogen prevents the egg contents from adhering to the container walls. After visual survey of the sample quality (egg yolk without noticeable solid body structures!) the egg contents are gradually transferred to the stainless steel collecting containers also filled with liquid Afterwards the contents of nitrogen. this containers are used for further sample processing (creation of the homogenate in the cryo-mill).

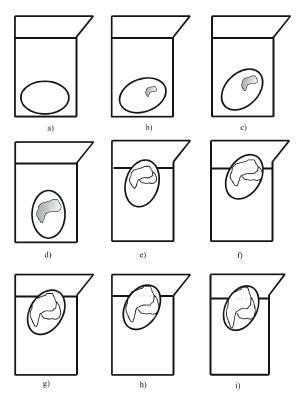


Fig. 1: Incubation stages of bird's eggs (HAYS & LECROY 1971)

The individual fast-freezing of egg content in the stainless steel sieve, prior to the transfer to the sample container provides the advantage - apart from the opportunity of for visual survey - of preventing the individual egg content from combining and freezing together which considerably simplifies the subsequent homogenization.

The required amount of nitrogen to cater for the eggs depends on the sample quantity. After all the eggs have been transferred to the stainless steel container, the liquid nitrogen has to be removed.

After sample preparation the shells are washed again to eliminate residuals of egg content remaining on the insides of the shells. Then the egg shells are transferred individually to clearly labeled Petri-dishes for drying at room temperature. After the drying for at least 7 days the thickness of the dry shell is weighed (DW) and measured.

# 7 Biometric Characterization

For each sampling site a detailed biometric characterization is carried out using the first 25 eggs. The following parameters are ascertained:

- length of the egg (0.1 mm reading accuracy),
- diameter of the egg (0.1 mm reading accuracy),
- fresh weight of the egg (0.1 g reading accuracy),
- dry weight of the egg shell (0.001 g reading accuracy),
- thickness of the egg shell (0.001 mm reading accuracy).

The determination of the lengths, diameters and fresh weights takes place preceding the separation of egg contents and calcium shell (chap. 0).

After weighing of the shells dried in the Petridishes (dry weight of the egg shells) the thickness of the egg shells is determined using a micrometer indicator caliper as follows. Four fragments (both of the pole caps and two parts from the equatorial region) are separated from the egg shell (shell with undamaged membrane). On each of these four fragments five point measurements are carried out. Of the five point measurements an average thickness of the egg shell is derived upon for the pointed pole and the edgeless pole, from the ten measurements (equatorial region 1 and 2) the thickness of the equatorial region is defined. The average thickness of the entire egg shell is calculated, based on the 20 measurements per egg.

Apart from consideration of the biometric characteristics mentioned, the derived here from, established by RATCLIFFE (1967, 1970) "Ratcliffe-Index" (also "eggshell-index", "eggshell-thinning-index by RATCLIFFE") is well proven as an effective indicator of bird's eggs. It is reached by:

 $R = \frac{\text{Weight of the egg shell [mgDW]}}{\text{Length [mm] x Width [mm]}}$ 

## 8 References

- ALTMEYER, M. (1987): Vergleichende rückstandsanalytische Untersuchungen an Stadttauben (*Columba livia;* Gmelin, 1789). Diplomarbeit, FR. Biogeographie, Universität Saarbrücken.
- ALTMEYER, M. (1993): Biomonitoring mit Stadttaubeneiern zur Erfassung von Chemikalien und deren Wirkungen in Verdichtungsräumen. Dissertation, Universität des Saarlandes, Saarbrücken.
- ALTMEYER, M. (1995): Stadttaube (*Columba livia f. domestica*). In: KLEIN, R. & PAULUS, M. (Hrsg.): Umweltproben für die Schadstoffanalytik im Biomonitoring - Standards zur Qualitätssicherung bis zum Laboreingang. Gustav Fischer Verlag, Jena, Stuttgart. S. 218-237.
- ALTMEYER, M. & PAULUS, M. (1995): Richtlinie zur Probenahme und Probenbearbeitung: Stadttaube (*Columba livia* f. *domestica*). In: UMWELTBUNDESAMT (Hrsg.) (1996): Umweltprobenbank des Bundes. Verfahrensrichtlinien für Probenahme, Transport, Lagerung und chemische Charakterisierung von Umwelt- und Human-Organproben. Erich Schmidt Verlag, Berlin.
- BECKER, P.H. (1989): Seabirds as monitor organisms of contaminants along the german north sea coast. *Helgoländer Meeresunters*. 43: 395-403.
- Bundesministerium für Umwelt, naturschutz und Reaktorsicherheit (Bmu) (Hrsg.) (2008): Umweltprobenbank des Bundes – Konzeption (Stand: Oktober 2008); www.umweltprobenbank.de
- DABERT, J. (1987): Breeding ecology of the feral pigeon Columba livia f. domestica in Poznan, Poland. Acta Ornithologica 23(2): 177-195.
- DILKS, P.J. (1975): Diet of feral pigeons (*Columba livia*) in Hawkes Bay, New Zealand. *N.Z.J. Agric. Res.* 18(1): 87-90.
- DRASCH, G.A.; WAALSER, D. & KÖSTERS, J. (1987): The urban pigeon (*Columba livia* forme urbane) - a biomonitor for the lead burden of the environment. *Environmental Monitoring and Assessment* 9: 223-233.
- GLUTZ VON BLOTZHEIM, U.N. & BAUER, K. (1980): Handbuch der Vögel Mitteleuropas, Bd. 9 Columbiformes -Piciformes. Akademische Verlagsgesellschaft, Wiesbaden.
- GOODWIN, D. (1960): Comparative ecology of the pigeons in inner London. *British Birds* 53: 201-212.

- GOODWIN, D. (1978): Birds of man s world. University of Queensland press, St. Lucia Queensland.
- GRACIA, M.T.A.; Martinez-Conde, E. & Corpas Vazquez, I. (1988): Lead levels of feral pigeons (*Columba livia*) from Madrid (Spain). *Environmental Pollution* 54: 89-96.
- HAAG, D. (1984): Ein Betrag zur Ökologie der Stadttaube (*Columba livia*, Gmelin 1789). Dissertation, Universität Basel.
- HAAG-WACKERNAGEL, D.; SMREKAR, G. & NAGEL, P. (1998): Trace elements and age in the feral pigeon *Columba livia. Fresenius Environ. Bull.* 7: 376-378.
- HAHN, E. & HAHN, K. (1995): Standards für ausgewählte Probenarten: Greifvögel. In: KLEIN, R. & PAULUS, M. (Hrsg.): Umweltproben für die Schadstoffanalytik im Biomonitoring: Standards zur Qualitätssicherung bis zum Laboreingang. Gustav Fischer Verlag, Jena, Stuttgart: S. 238-254.
- HALVIN, J. (1979): Die Flüge der Stadttauben in die Umgebung von Brno. *Folia zoologica* 28: 125-146.
- HAYS, H. & LECROY, M. (1971): Field criteria for determining incubation stage in eggs of the common term. *Wilson Bull.* 83: 425-429.
- HUTTON, M. & GOODMAN, G.T. (1980): Metal contamination of feral pigeons *Columba livia* from the London area: part
   1 - tissue accumulation of lead, cadmium and zinc. *Environmental Pollution* Ser. A 22, No. 3: 207-217.
- JENKINS, C. (1975): Utilisation du pigeon biset (*Columba livia* Gm.) comme temoin de la pollution atmospherique per le plomb. *Comptes rendus hebdomadaires des seances de l'Academie de Sciences*, Ser. D 281: 1187-1189.
- JOHNSON, M.S.; PLUCK, H.; HUTTON, M. & MOORE, G. (1982): Accumulation and renal effects of lead in urban populations of feral pigeons, *Columba livia. Archives of Environmental Contamination and Toxicology* 11: 761-767.
- KAPHALIA, B.S.; HUSSAIN, M.M.; SETH, T.D.; KUMAR, A. & MURTI, R.K. (1981): Organochlorine pesticide residues in some indian wild birds. *Pesticides Monitoring Journal* 15(1): 9-13.
- KENDALL, R.J. & SCANLON, P.F. (1982): Tissue lead concentration and blood characteristics of rock doves from an urban setting in Virginia. Archives of Environmental Contamination and Toxicology 11: 265-268.
- LEVESQUE, H. & MCNEIL, R. (1986): Deplacements du pigeon biset (*Columba livia*) dans le Vieux-Port de Montreal. *Le Naturaliste Canadien* 113(1): 47-54.
- MURTON, R.K. & WESTWOOD, N.J. (1966): The foods of the rock dove and feral pigeon. *Bird Study* 13: 130-146.
- MURTON, R.K.; COOMBS, C.F. & THEARLE, R.J.P. (1972): Ecological studies of the feral pigeon II. Flock behaviour and social organisation. *Journal of Applied Ecology* 11: 61-81.
- NAGEL, P.; SMREKAR, G. & HAAG-WACKERNAGEL, D. (2001): Use of ferap pigeon eggs for urban biomonitoring. *Fresenius Environ. Bull.* 10(1): 18-25.

- OHI, G.; SEKI, H.; AKIYAMA, K. & YAGW, H. (1974): The pigeon, a sensor of lead pollution. *Bulletin of Environmental Contamination and Toxicology* 12: 92-98.
- PAULUS, M. & NENTWICH, K. (1999): Biomonitoring mit Stadttaubeneiern - Langzeitstudie. UWSF – Z. Umweltchem. Ökotox. 11(5): 281-287.
- RATCLIFFE, D.A. (1967): Decrease in egshell weight in certain birds of prey. *Nature* 215: 208-210.
- RATCLIFFE, D.A. (1970): Changes attributable to pesticide in egg breakage frequency and eggshell thickness in some British birds. *J. Appl. Ecol.* 7: 67-115.
- RIDDLE, G. (1971): The breeding season in a rural colony of feral pigeons. *Scot. Birds* 6: 321-329.
- SIEGFRIED, W.R.; FROST, P.G.H. & REDELINGHUYS, E.P. (1972): Lead concentrations in the bones of city and country doves. *South African Journal of Science* 68: 229-230.
- SIMMS, E. (1979): The Public Life of the Street Pigeon. Hutchinson, London.
- TANSY, M.F. & ROTH, R.P. (1970): Pigeons: a new role in air pollution. Journal of the Air Pollution Control Association 20(5): 307-309.
- VOGEL, K. (1980): Die Taube Biologie, Haltung, Fütterung. VEB Deutscher Landwirtschaftsverlag, Berlin.
- WOHLFARTH, J. (1993): Rechtsprobleme um die Stadttaube. Die öffentliche Verwaltung (4): 152-157.

# Checklist to Prepare and Conduct the Sampling

Specimen Type:	Feral pigeon (Columba livia f. domestica)					
Target Compartments:	egg contents					
Individual Specimens:	undamaged eggs, (incubation stage a-d acc. to HAYS & LECROY 1971)					
Random Sample Number:	at least 25 Eggs per sampling site					
Sample Quantity for the ESB:	150-180 eggs are required per sampling site to gain the needed quantity of 2.200 g					
Sampling Period:	main incubation period (March until September)					
Sampling Frequency:	3-4-weekly rhythm until the required number of eggs is reached					
Equipment Required for Field Work:	<ul> <li>specimen data sheets for documentation during the sampling, for description of the sampling site and nesting sites</li> <li>protective face mask (A2P3), disposable gloves</li> <li>protective clothing (overall, long boots)</li> <li>disinfectant tissues</li> <li>if necessary, extendable ladder (5 to 8 m working-height)</li> <li>torches</li> <li>polyethylene bags (i.e. 12x17 cm)</li> <li>waterproof pen to label the polyethylene bags</li> </ul>					
Sample Packing until Further Processing:	<ul> <li>egg cartons for the eggs</li> <li>stainless steel containers (vessels, 5.5 I) with lids and fasteners for the eggs contents</li> </ul>					
Transport and Interim Storage:	<ul> <li>cooling unit (5 °C (+/-3°))for egg transportation</li> <li>cooling unit (dewar) for the rapid deep-freezing and storage of the samples in the gas phase above liquid nitrogen (LIN)</li> </ul>					
Required Equipment for Laboratory Work:	<ul> <li>beaker with water for the determination of the incubation stage</li> <li>disposable gloves</li> <li>tissues to clean the eggshells</li> <li>clean bench with particles- and activated carbon filtration</li> <li>liquid nitrogen</li> <li>stainless steel scalpel to open the eggs</li> <li>stainless steel sieve</li> <li>Petri-dishes to dry the eggshells</li> <li>pen to label the Petri-dishes and the polyethylene bags</li> <li>precision scale (reading accuracy 0.1 mg) to determine the fresh weight of the egg (FW) and the dry weight of the shell (DW)</li> <li>slide caliper to determine the size of the egg</li> <li>micrometer caliper to measure the thickness of the egg shell</li> <li>specimen data sheets for the documentation of the sample treatment and for the biometric sample characterization</li> </ul>					
Biometric Sample Characterization:	<ul> <li>for 25 Eggs:</li> <li>length of the egg (reading accuracy 0.1 mm)</li> <li>diameter of the egg (reading accuracy 0.1 mm)</li> <li>fresh weight of the egg (reading accuracy 0.1 g)</li> <li>dry weight of the egg shell (reading accuracy 0.001 g)</li> <li>thickness of the egg shell (reading accuracy 1 μm)</li> <li>Ratcliffe-Index</li> </ul>					

GERMAN ENVIRONMENTAL SPECIMEN BANK									
Specimen Data Sheet 1: Sampling Location									
Feral pigeon ( <i>Columba livia</i> f. <i>domestica</i> )									
Identification:									
	/X/	//		/					
				Specimen Type					
				Specimen Condition					
				Collection Date (MM/YY)					
				Sampling Area (SA)					
				Sampling Region (SR)					
				Sampling Site (SS)					
				Additional information					
Sampling Location:									
Gauß-Krüger-Coord	inates:								
Easting:		Northir	ıg:						
Datum:		Ellipso	d:						
Size of the Sampling	Size of the Sampling Location:km <sup>2</sup> haam <sup>2</sup>								
Kind of Use:									
Remarks:									
-									
Person(s) in Charge:									

GERM	GERMAN ENVIRONMENTAL SPECIMEN BANK								
Specimen Data Sheet 2: Description of the Sampling Location									
Feral pigeon ( <i>Columba livia</i> f. <i>domestica</i> )									
Identification:									
	/X//	/							
from	_ Sampling Date	to							
Kind of Use:		Bridge							
(1 naming only)	Public buildings	Storehouse/mill							
		Residential house							
	Tower	Other Usage							
Exposure towards the Er	vironment:								
(1 naming only)	$\Box$ nesting site, shielded from all side	des							
	$\Box$ nesting site, at least partial shie	lded							
	$\Box$ nesting site, completely unshield	ded							
Incidence of Light:	Completely dark dark in	the corners only, otherwise bright							
(1 naming only)									
Height above Ground:	🗌 less than 3 m	□ > 5 to 10 m							
(1 naming only)	🗌 3 to 5 m	☐ more than 10 m							
Description of the Soil St	(up to 6 namings)								
□ concrete	$\Box$ accumulated soils	□ tarmac							
☐ horticultural bed	☐ paving stones	🗌 meadow, green space							
Food Abundance:	$\Box$ natural food not available								
(1 naming only)	$\Box$ natural food available								
Available Water:	□ streams	□ wells							
(up to 5 namings)		$\Box$ stagnant waters							
	☐ puddle								
Dominating Nest Type:	well constructed platform	☐ bare ground							
(1 naming only)	☐ thin layer	☐ mud nest							
Material mainly used for	Nest-building:	(1 naming only)							
$\Box$ thin branches	□ leaves	□ haulms							
☐ feathers	🗌 roots	□ other material							
Material Alternatively Use	ed for Nest-building:	(up to 2 namings)							
☐ pieces of paper	$\Box$ pieces of metal / sheet metal	$\Box$ pieces of synthetic materials							
$\Box$ cloth pieces	other material								
☐ threads									
Nest Hygiene:	great many feces	☐ dead nestlings							
(up to 2 namings)	□ dust	$\Box$ parasites (fleas, ticks)							
☐ dead or unfertilized eggs, or eggshells									

GERMAN ENVIRONMENTAL SPECIMEN BANK										
Specimen Data Sheet 3: Sampling Dates and Storage										
Feral pigeon (Columba livia f. domestica)										
Identification:										
	/X///									
Sampling Location:	Sampling Location:									
Sampling Dates:	1	2	3	4		5	6			
Date of the sampling [dd.mm]										
Date of the sample preparation in the laboratory [dd.mm]										
Duration of the interim storage [dd]										
Number of eggs discarded										
Number of eggs stored										
Sampling Dates:	7	8	8 9		10	11	12			
Date of the sampling [dd.mm]										
Date of the sample preparation in the laboratory [dd.mm]										
Duration of the interim storage [dd]										
Number of eggs discarded										
Number of eggs stored										
Storage			-		-	-				
Number of Stainless Steel Container	Weight Empty [g]	-	Weight Filled [g]		eighted mple [g]	Remarks				
Remarks:										

GERMAN ENVIRONMENTAL SPECIMEN BANK											
Specimen Data Sheet 4.1: Biometric Parameters – 25 Feral pigeon eggs											
Feral pigeon (Columba livia f. domestica)         Identification:       Identification:											
lacita			/ X /	/							
Samp						d					
No.	Date [dd.mm]	Length of the egg , _ mm	Diameter of the egg , _ mm	Fresh weight ot the egg	Dry weight of the shell	Thickness of the shell	Incubation stage a, b, c, d				
1				-							
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											
21											
22											
23											
24											
25											

Specimen Data Sheet 4.2.1: Biometric Parameters – 25 Feral pigeon eggs									
Identification: / X / _			/_		/	Sampl. L	ocation:		
Egg-no.: 1		X (20) =		Eg	gg-no.:	2	X (20) =		
edgeless pole [µm]	pointed pole [µm]	equator 1 [µm	equator 2 [µm]		edgeless pole [µm]	pointed pole	equator 1 [µm	equator 2 [µm]	
1				1					
2				2					
3				3					
4				4					
5				5					
Egg-no.:	3	X (20) =		Eg	gg-no.:	4	X (20) =		
edgeless pole [µm]	pointed pole [µm]	equator 1 [µm	equator 2 [µm]		edgeless pole [µm]	pointed pole [µm]	equator 1 [µm	equator 2 [µm]	
1				1					
2				2					
3				3					
4				4					
5				5					
Egg-no.:	5	X (20) =		Eg	gg-no.:	6	X (20) =		
edgeless pole [µm]	pointed pole	equator 1 [µm	equator 2 [µm]		edgeless pole [µm]	pointed pole	equator 1 [µm	equator 2 [µm]	
1				1					
2				2					
3				3					
4				4					
5				5					
Egg-no.:	7	X (20) =		Eg	gg-no.:	8	X (20) =		
edgeless pole [µm]	pointed pole [µm]	equator 1 [µm	equator 2 [µm]		edgeless pole [µm]	pointed pole	equator 1 [µm	equator 2 [µm]	
1				1					
2				2					
3				3					
4				4					
5				5					
Egg-no.:	9	X (20) =		Eg	gg-no.:	10	X (20) =		
edgeless pole [µm]	pointed pole [µm]	equator 1 [µm	equator 2 [µm]		edgeless pole [µm]	pointed pole	equator 1 [µm	equator 2 [µm]	
1				1					
2				2					
3				3					
4				4					
5				5					

Specimen Data Sheet 4.2.2: Biometric Parameters – 25 Feral pigeon eggs									
Identification: / X /			/_	/Sampl. Location:					
Egg-no.:	Egg-no.: 11		X (20) =		gg-no.:	12	X (20) =		
edgeless pole [µm]	pointed pole [µm]	equator 1 [µm	equator 2 [µm]		edgeless pole [µm]	pointed pole	equator 1 [µm	equator 2 [µm]	
1				1					
2				2					
3				3					
4				4					
5				5					
Egg-no.:	13	X (20) =		Eç	gg-no.:	14	X (20) =	<u>.</u>	
edgeless pole [µm]	pointed pole [µm]	equator 1 [µm	equator 2 [µm]		edgeless pole [µm]	pointed pole [µm]	equator 1 [µm	equator 2 [µm]	
1				1					
2				2					
3				3					
4				4					
5				5					
Egg-no.:	15	X (20) =		Eg	gg-no.:	16	X (20) =		
edgeless pole	pointed pole	equator 1 [µm	equator 2		edgeless pole [µm]	pointed pole	equator 1 [µm	equator 2 [µm]	
1	[hiii]	[µm	[b]	1	[h]	[pm]	[pm	[pm]	
2				2					
3				3					
4				4					
5				5					
Egg-no.:	17	X (20) =		E	gg-no.:	18	X (20) =		
	pointed pole	equator 1	equator 2		edgeless pole [µm]	pointed pole	equator 1 [µm	equator 2 [µm]	
1				1					
2				2					
3				3					
4				4					
5				5					
Egg-no.:	19	X (20) =		Eg	gg-no.:	20	X (20) =		
edgeless pole [µm]	pointed pole [µm]	equator 1 [µm	equator 2 [µm]		edgeless pole [µm]	pointed pole	equator 1 [µm	equator 2 [µm]	
1				1					
2				2					
3				3					
4				4					
5				5					

	Specimen Data Sheet 4.2.3: Biometric Parameters – 25 Feral pigeon eggs									
ld	entification:		/X/	/_	/Sampl. Location:					
Eg	gg-no.:	25	X (20) =		Eg	gg-no.:	26	X (20) =		
	edgeless pole [µm]	pointed pole [µm]	equator 1 [µm	equator 2 [µm]		edgeless pole [µm]	pointed pole	equator 1 [µm	equator 2 [µm]	
1					1					
2					2					
3					3					
4					4					
5					5					
Eg	gg-no.:	23	X (20) =		Eg	gg-no.:	24	X (20) =		
	edgeless pole [µm]	pointed pole	equator 1 [µm	equator 2 [µm]		edgeless pole [µm]	pointed pole	equator 1 [µm	equator 2 [µm]	
1					1					
2					2					
3					3					
4					4					
5					5					
Eg	gg-no.:	25	X (20) =							
	edgeless pole [µm]	pointed pole [µm]	equator 1 [µm	equator 2 [µm]						
1										
2										
3										
4										
5										

GERMAN ENVIRONMENTAL SPECIMEN BANK											
Sampling Record											
Feral pigeon (Columba livia f. domestica)											
Sampling Area: Identification:											
Underlying Version of the Sampling Guideline:											
Underlying Version of the Sampling Scheme:											
1. Objective of											
2. Actual T	imeframe	e of the Sa	mpling:								
Date	Т	ime	Samp	le No.		Remarks					
	from	to	from	to							
3. Participa		onductor/R	ecorder:								
	Ot	her:									
_			oling Sche	me and Sa	ampl	ing Guideline: X as prescribed					
	npling Perio	od and Sampling	Location			4.6 Sampling Technique/Method of Capture					
	(Selectio	on/Definition)				4.7 Sample Amount					
_		e Individual S	pecimens			4.8 Data Collection					
_	chnical Prep		Dookogoo			4.9. Transport and Interim Storage					
	-	edure for the	-	Mariatiana							
Number, Kir	nd of, and	Reason fo	or Possible	variations	s (Cie	ar lext):					
Remarks::											
F	Recorder			Date		Signature					