UMWELTPROBENBANK
DES BUNDES

# Guideline for Sampling and Sample Treatment

## Herring gull (*Larus argentatus*)

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**Contents**



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

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## German Environmental Specimen Bank

The German Environmental Specimen Bank (ESB) is an instrument of environmental 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 BACKHAUS et al. (1992) 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

The herring gull (*Larus argentatus*) proved to be a good accumulation indicator for marine habitats as representative of the omnivorous trophic level (e.g. MINEAU et al. 1984; NORDSTROM & WON 1985; FURNESS 1987; GILBERTSON et al. 1987; WESELOH et al. 1988; BECKER 1989; BECKER et al. 1989, 1991; ELLIOT et al. 1989; LEWIS et al. 1993; BURGER & GOCHFELD 1995; KOSTER et al. 1996; KAHLE & BECKER 2000; WESELOH et al. 2002).

Primarily the pollutant content of the eggs is analyzed, which mirror the environmental burden situation of the area to be assessed.

The following criteria underline the use of the sea gull as an accumulation indictor in the scope of the ESB.

- it is widely spread,
- as a sedentary bird respectively short-distance migrant it is relatively resident and stays near by the colony before and after the breeding season,
- it is 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,
- the feeding habits of the herring gull is thoroughly investigated (e.g. GARTHE et al. 1999). Feeding habits are of primary concern as contamination path, because the substance intake of terrestrial animals results primarily from the intake of food and water. In marine habitats it feeds primarily on fish, crustaceans and mussels,
- the eggs pollutant content reveals sufficient spatial relation. The highest food intake of the female herring gull is directly consumed adjacent to the colony, preceding and during nesting.
- the sampling is relatively easy to perform. The breeding colonies normally have high population densities, so the eggs (chap.6.2) can be collected in large numbers within a short time,
- there are no general conservation regulations that would constrain the use of this species eggs for scientific research,

- 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 when 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 more constant than of the 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 in the eggs.

# 5 Predefinitions for the Sampling

## 5.1 Species Determination

Adult herring gulls through their characteristic features are relatively easy to identify (e.g. GRANT 1986). The unambiguous identification of their eggs is much more difficult. They are easily mistaken for the eggs of other gull species due to their broad color variability. Hence, a reliable species determination is often only guaranteed in combination with the nesting birds.

The base shell color of the fusiform, circa 70x49 cm sized egg of the herring gull is usually light olive green, green or auburn, but can vary from whitish blue to deep rubiginous (Fig. 1). Most of the often black, dark brown or olive drab spots or dots are developed. Instead an irregular scribble is unusual. Moreover dense markings and scanty mottling occur. Eggs without markings are uncommon (e.g. HARRISON 1975).

**Fig. 1: Colour varieties of herring gull eggs (Optimedia 1998).**

## 5.2 Selection and Definition of Sampling Sites

The selection of sampling sites is primarily determined through the occurrence of breeding colonies in the sampling areas. Since the sampling sites have to be representative for the marine ecosystem, the vicinity to local sources of emissions must be avoided. This particularly includes areas which gulls like for foraging (e.g. waste disposal sites).First of all the pollutant content of the available eggs is analyzed during a screening. The distance to the nearest source of emission must be defined individually for each

sampling site and documented in the area related sampling scheme.
The breeding colony should be so large that an adequate statistical fixed number of eggs (chap. 5.3) can be removed without endangering the population through the sampling.

### 5.3 Selection of Individuals and Sample Size

The herring gull clutch normally consists of two to three eggs. Since fresh eggs (Fig. 2, chap 6.2) should be sampled, from each clutch only the second egg is gathered. This sampling approach also allows for the classification of the date of lay.

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 70 g multiply to a total sample quantity of at least 1.700 g egg contents. The needed sample quantity of 2.200 g egg contents for ESB storage requires sampling of 30-35 eggs. 75 eggs should be collected per sampling site to reject incubated or damaged eggs (chap. 6.2).

### 5.4 Sampling Period and Frequency

In long term programs as that of the ESB sampling should be carried out annually.

The sampling of herring gull eggs is carried out during the main nesting period (April/March). The removal of the eggs is by preference restricted to a limited time span of 3-5 days.

### 5.5 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,
- addresses of the appropriate authorities,
- supporting ornithological groups,
- cooling devices for interim storage of the eggs,
- 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

**Field work:**

- specimen data sheets for documentation during the sampling, for description of the sampling site and nesting sites
- dowels to mark the clutches
- pencil (soft) for numbering the eggs (no felt pen because of possible contaminations with its contents)
- egg carton for safe keeping the eggs during sampling and the interim storage

- cooling device (5 °C (+/–3 °)) for egg transportation

**Laboratory:**

- specimen data sheets for the biometric sample description,
- cooling device (5°C (+/– 3°)) for the storage of the eggs until further processing
- glass beaker with water for the determination of the incubation stage
- disposable gloves
- 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°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

During the first inspection of the breeding colony a sufficient clutches with one egg are marked by pegging a dowel. At the same time eggs in the clutches are also marked, to distinguish them from the subsequently laid second egg. During a second inspection, two to three days later, the second egg in the marked clutches is removed. The eggs are numbered in the sequence of their removal using a soft lead pencil and are laid in egg cartons to prevent breakage. Immediately after the removal, the eggs are temporarily stored in a cooling device (refrigerator) at 5°C (+/– 3°). An interim storage until further sample processing should not exceed 2 weeks. To prevent the shells from creaking, eggs should not be frozen.

Sample preparation and biometric sample description are carried out in the laboratory. First the incubation stage is determined after the method of HAYS & LECROY (1971) by inserting the eggs in a glass beaker filled with deionized water (approx. 2.5 l). This guarantees that only fresh eggs are used. Only those eggs which comply with the situation a) to d) (Fig. 2) 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 shock-freezing 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 nitrogen. Afterwards the contents of these containers are used for further sample processing (creation of the homogenate in the cryo-mill).

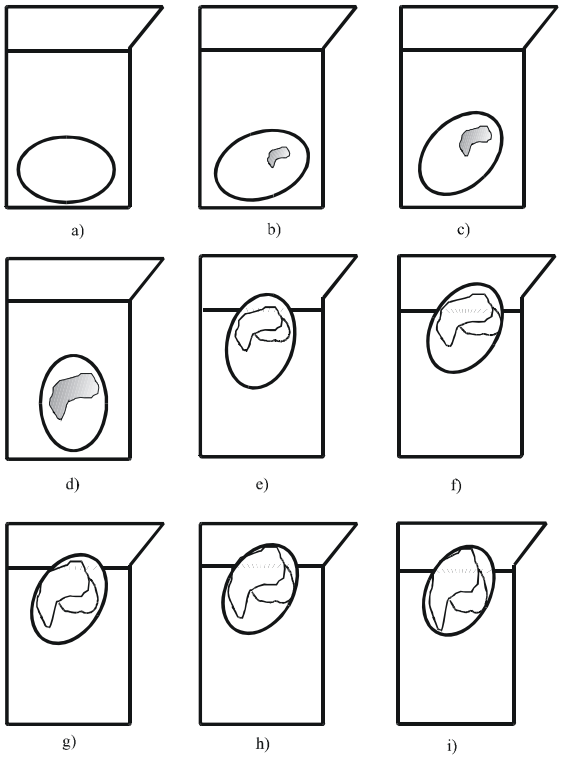



**Fig. 2: Incubation stages of bird's eggs (HAYS & LECROY 1971)**

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.6.2).

After weighing of the shells dried in the Petri-dishes (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 effect indicator of bird's eggs. It is calculated by:

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

# 8 References


ALTMEYER, M. (1995): Standards für ausgewählte Probenarten: 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.

BACKHAUS, F.; BECKER, P.H. & SCHLADOT, J.D. (1992): Richtlinie zur Probenahme und Probenbeabeitung: Silbermöwe (*Larus argentatus*). 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.

BECKER, P.H.; CONRAD, B. & SPERVESLAGE, H. (1989): Chlororganische Verbindungen und Schwermetalle in weiblichen Silbermöwen (*Larus argentatus*) und ihren Eiern mit bekannter Legefolge. *Vogelwarte* 35(1): 1-10.

BECKER, P.H.; KOEPFF, CH. & HEIDMANN, W.A. (1991): Schadstoffmonitoring mit Seevögeln. Forschungsbericht 1160 8070. Im Auftrag des Umweltbundesamtes.

Bundesministerium für Umwelt, Naturschutz und reaktorsicherheit, (Bmu) (Hrsg.) (2008): Umweltprobenbank des Bundes – Konzeption (Stand: Oktober 2008); www.umweltprobenbank.de

BURGER, J. & GOCHFELD, M. (1995): Heavy metal and selenium concentrations in eggs of herring gull (*Larus argentatus*): Temporal differences from 1989 to 1994. Arch.Environ. Contam.Toxicol. 29: 192-197.

ELLIOT, J.E.; NOBLE, D.G.; NORDSTROM, R.J. & WHITEHEAD, P.E. (1989): Organochlorine contaminants in seabird eggs from the pacific coast of Canada 1971 – 1986. *Environ Monit Assessm* 12: 67-82.

FURNESS, R.W. (1987): Seabirds as monitors of the marine environment*. ICBP Techn Publ* 6: 217-230.

GARTHE, S.; KUBETZKI, U.; HÜPPOP, O. & FREYER, T. (1999): Zur Ernährungsökologie von Herings-, Silber- und Sturmmöwe (*Larus fuscus, L. argentatus* und *L. canus*) auf der Nordseeinsel Amrum während der Brutzeit. *Seevögel* 20(2): 52-58.

GILBERTSON, M.; ELLIOTT, J.E. & PEAKALL, D.B. (1987): Seabirds as indicators of marine pollution*. ICBP Techn Publ* 6: 231-248.

GRANT, P.J. (1986): Gulls. A Guide to Identification. T & D Poyser, Calton.

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.

HARRISON, C.O.J. (1975): Jungvögel, Eier und Nester aller Vögel Europas, Nordafrikas und des mittleren Ostens: ein Naturführer zur Fortpflanzungsbiologie. Paul Parey, Hamburg, Berlin.

HAYS, H. & LECROY, M. (1971): Field criteria for determining incubation stage in eggs of the common term. *Wilson Bull* 83: 425-429.

KAHLE, S. & BECKER, P.H. (2000): Die Belastung von Möwen mit Umweltchemikalien an der Deutschen Nord- und Ostseeküste in den Jahren 1995 und 1996. *Seevögel* 21(2): 47-53.

KOSTER, M.D.; RYCKMANN, D.P.: WESELOH, D.V.C. & STRUGER, J. (1996): Mercury levels in great lakes herring gull (*Larus argentatus*) eggs 1972-1992. Environmental Pollution 93(3): 261-270.

LEWIS, S.A.; BECKER, P.H. & FURNESS, R.W. (1993): Mercury levels in eggs, tissues, and feathers of herring gull *Larus argentatus* from the german wadden sea cost. *Environmental Pollution* 80: 293-299.

MINEAU, P.; FOX, G.A.; NORDSTROM, R.J.; WESELOH, D.V.; HALLETT, D.J. & ELLENTON, J.A. (1984): Using the herring gull to monitor levels and effects of organochlorine contamination in the canadian great lakes. In: NRIAGU, J.O. & SIMMONS, M.S. (Hrgs.): Toxic Contaminants in the Great Lakes. Wiley. S. 425-455.

NORDSTROM, R.J. & WON, H. (1985): Long-term preservation of egg and tissue homogenates for retrospective determination of organochlorine compounds: Freezing versus freeze drying. *J Assoc off Anal Chem* 68: 129-135.

Optimedia (1998): Birds of the west palaearctic. Software Optimedia. Oxford University Press.

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.

WESELOH, D.V.; ELLIOTT, J.E. & OLIVE, J.H. (1988): Herring Gull Surveillance Program. The Surveillance Work Group of the Great Lakes Water Quality Board. International Joint Commission Windsor, Ontario.

WESELOH, D.V.; HUGHES, K.D.; EWINS, P.J.; BEST, D.; KUBIAK, T. & SHIELDCASZLE, M.C. (2002): Herring gulls and great black-backed gulls as indicators of contaminants in bald eagles in Lake Ontario, Canada. *Environmental Toxicology and Chemistry* 21(5): 1015-1025

## Checklist to Prepare and Conduct the Sampling

| **Specimen Type:** | **Herring gull (*Larus argentatus*)** |
|---|---|
| Target Compartments: | egg contents |
| Individual Specimens: | undamaged eggs, laid as second egg (incubation stage a-d according to Hays & LeCroy 1971) |
| Random Sample Number: | at least 25 Eggs per sampling site |
| Sample Quantity for the ESB: | 75 eggs are required per sampling site to gain the needed quantity of 2.200 g |
| Sampling Period: | main incubation period (April/May) |
| Sampling Frequency: | 1 sampling per annum |
| Equipment Required for Field Work: | • specimen data sheets for documentation during the sampling, for description of the sampling site and nesting sites<br>• dowels as marker for the clutches<br>• pencil (soft) to number the eggs<br>• egg carton for the safe storage of the eggs during the sampling and the interim storage |
| Sample Packing until Further Processing: | • egg cartons for the eggs<br>• stainless steel containers (5.5 l) with lids and fasteners for the eggs contents |
| Transport and Interim Storage: | • cooling device (5 °C +/–3 °C)for egg transportation.<br>• cooling device (dewar) for the rapid deep-freezing and storage of the samples in the gas phase above liquid nitrogen (LIN) |
| Required Equipment for Laboratory Work: | • specimen data sheets for the documentation of the sample treatment and for the biometric sample characterization<br>• beaker with water for the determination of the incubation stage<br>• disposable gloves<br>• tissues to clean the eggshells<br>• clean bench with particles- and activated carbon filtration<br>• stainless steel containers (5.5 l) with lids and fasteners<br>• liquid nitrogen<br>• stainless steel scalpel to open the eggs<br>• stainless steel sieve<br>• Petri-dishes to dry the eggshells<br>• polyethylene bags and pen to label them<br>• precision scale (reading accuracy 0.1 mg) to determine the fresh weight of the egg (FW) and the dry weight of the shell (DW)<br>• slide caliper to determine the size of the egg<br>• micrometer caliper to measure the thickness of the egg shell |
| Biometric Sample Characterization: | for 25 Eggs:<br>• length of the egg (reading accuracy 0.1 mm)<br>• diameter of the egg (reading accuracy 0.1 mm)<br>• fresh weight of the egg (reading accuracy 0.1 g)<br>• dry weight of the egg shell (reading accuracy 0.001 g)<br>• thickness of the egg shell (reading accuracy 1 µm)<br>• Ratcliffe-Index |

## GERMAN ENVIRONMENTAL SPECIMEN BANK

### Specimen Data Sheet 1: Sampling Location

### Herring gull (*Larus argentatus*)

**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-Coordinates:**

Easting: __ __ __ __ __ __ __ Northing: __ __ __ __ __ __ __

Datum: _____ Ellipsoid: _____

**Size of the Sampling Location:** __ __ km² __ __ ha __ __ a __ __ m²

**Kind of Use:** _____

**Remarks:** _____

_____

**Person(s) in Charge:**

## GERMAN ENVIRONMENTAL SPECIMEN BANK

### Specimen Data Sheet 2: Sampling Dates and Storage

### Herring gull (*Larus argentatus*)

**Identification:**

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

**Food surplus at the clutch:**

**Nest material:**

**Remarks:**

| **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 | | | | | | |

**Storage**

| **Number of Stainless Steel Container** | Weight Empty [g] | Weight Filled[g] | Weighted Sample [g] | Remarks |
|---|---|---|---|---|
| __ __ __ __ __ __ __ | __ __ __ __ | __ __ __ __ | __ __ __ __ | |
| __ __ __ __ __ __ __ | __ __ __ __ | __ __ __ __ | __ __ __ __ | |
| __ __ __ __ __ __ __ | __ __ __ __ | __ __ __ __ | __ __ __ __ | |
| __ __ __ __ __ __ __ | __ __ __ __ | __ __ __ __ | __ __ __ __ | |

**Remarks:** _____

_____

## GERMAN ENVIRONMENTAL SPECIMEN BANK

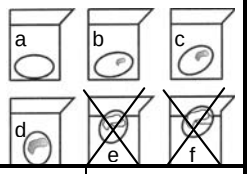
### Specimen Data Sheet 3.1: Biometric Parameters – 25 Herring gull eggs

### Herring gull (*Larus argentatus*)

**Identification:**

__ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __

**Sampling Location::** __ __ __




| No. | Date [dd.mm] | Length of the egg _ _ , _ mm | Diameter of the egg _ _ , _ mm | Fresh weight ot the egg _ _ _ , _ g | Dry weight of the shell _ , _ _ _ g | Thickness of the shell _ _ _ µm | Incubation stage a, b, c, d |
|---|---|---|---|---|---|---|---|
| 1 | | | | | | | |
| 2 | | | | | | | |
| 3 | | | | | | | |
| 4 | | | | | | | |
| 5 | | | | | | | |
| 6 | | | | | | | |
| 7 | | | | | | | |
| 8 | | | | | | | |
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| 10 | | | | | | | |
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| 12 | | | | | | | |
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| 21 | | | | | | | |
| 22 | | | | | | | |
| 23 | | | | | | | |
| 24 | | | | | | | |
| 25 | | | | | | | |

## Specimen Data Sheet 3.2.1: Biometric Parameters – 25 Herring gull eggs

**Identification:** __ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __**Sampl. Location:** __ __ __

| **Egg-no.:** | **1** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **2** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **3** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **4** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **5** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **6** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **7** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **8** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **9** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **10** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

## Specimen Data Sheet 3.2.2: Biometric Parameters – 25 Herring gull eggs

**Identification:** __ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __**Sampl. Location:** __ __ __

| **Egg-no.:** | **11** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **12** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **13** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **14** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **15** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **16** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **17** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **18** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **19** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **20** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

## Specimen Data Sheet 3.2.3: Biometric Parameters – 25 Herring gull eggs

**Identification:** __ __ __ __ **/ X /** __ __ __ __ **/** __ __ __ __ __ **/** __**Sampl. Location:** __ __ __

| **Egg-no.:** | **25** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **26** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **23** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-no.:** | **24** | | X (20) = | |
|---|---|---|---|---|
| | edgeless pole _ _ _ [µm] | pointed pole _ _ _ [µm] | equator 1 _ _ _ [µm | equator 2 _ _ _ [µm] |
| 1 | | | | |
| 2 | | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |

| **Egg-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

### Herring gull (*Larus argentatus*)

Sampling Area: _____ Identification: __ __ __ __ __

Underlying Version of the Sampling Guideline: __ __ . __ __ . __ __

Underlying Version of the Sampling Scheme: __ __ . __ __ . __ __

**1. Objective of the Sampling:** _____

_____

**2. Actual Timeframe of the Sampling:**

| Date | Time | | Sample No. | | Remarks |
|---|---|---|---|---|---|
| | from | to | from | to | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

**3. Participants:** Conductor/Recorder: _____

Other: _____

_____

**4. Checklist referring to Sampling Scheme and Sampling Guideline:** ☒ as prescribed

- ☐ 4.1 Sampling Period
- ☐ 4.2 Sampling Site and Sampling Location (Selection/Definition)
- ☐ 4.3 Selection of the Individual Specimens
- ☐ 4.4 Technical Preparations
- ☐ 4.5 Cleaning Procedure for the Packages
- ☐ 4.6 Sampling Technique/Method of Capture
- ☐ 4.7 Sample Amount
- ☐ 4.8 Data Collection
- ☐ 4.9. Transport and Interim Storage

Number, Kind of, and Reason for Possible Variations (Clear Text):

_____

_____

_____

_____

Remarks:: _____

_____

_____ __ __ . __ __ . __ __ _____

Recorder Date Signature