UMWELTPROBENBANK
DES BUNDES

Guideline for Sampling and Sample Treatment

European Roe Deer (*Capreolus capreolus*)

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Contents

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 (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 KLEIN & NENTWICH (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

The European roe deer (*Capreolus capreolus)* has been investigated as a subject for environmental monitoring since the beginning of the 1970s and has been emphasized as a qualified bioindicator (DRESCHER-KADEN 1976; KLEIMINGER & HOLM 1985; MÜLLER 1985a; Holm 1986, HOLM et al. 1987, 1990, HECHT 1987; TATARUCH 1993a, 2001; MARKERT et al. 1999, KIERDORF et al.2008, POKORNY et al. 2009).

As selective herbivores roe deer take the position of first order consumers in terrestrial ecosystems. Sufficient knowledge concerning food consumption and nutrition of roe deer is available due to several investigations (ESSER 1958; JUON 1963; KLÖTZLI 1965; DROZDZ & OSIECKI 1973; AL-KITTANI 1975; ELLENBERG 1978; BUBINEK 1984; EBERLE 1989; GUTHÖRL 1990; PETRAK et al. 1991; PETRAK 1993; STUBBE 1997; HESPELER 1999).

The following criteria constitute the particular suitability of roe deer for monitoring programs:

- wide geographic distribution. It is spread from South-Western Europe through Central- and Northern Europe to Russia (Ural Mountains) and Asia Minor (Turkey, Caucasian region, Iran) (STUBBE 1997; LISTER ET AL. 1998). Other subspecies substitute the species in Siberia and Eastern Asia (E.G. STUBBE 1997),
- most commonly distributed, free-living, tall herbivore in Europe (STUBBE 1997). In the Federal Republic of Germany approx. 1.000.000 roe deer per year are shot (Deutscher Jagdschutzverband 2010 – German association for hunting regulations),
- distributed throughout almost all terrestrial ecosystems in Central Europe (e.g. STUBBE 1997; HESPELER 1999),
- considerable location-loyalty (STUBBE 1997). The territory range normally varies among 10-40 ha, depending on game density, age, sex, gender ratio, regional habitat structure, food supply and season (STUBBE 1997). Therefore the activity range is appropriately limited.

- physiologically and eco-physiologically well-investigated species (ELLENBERG 1974; EISFELD 1976; HOFMANN 1983; STUBBE 1997; BEHREND 1999; FRÖLICH et al. 2001; GEHRKE 2001; WISSER et al. 2001),
- comprehensive experience concerning the accumulation behavior of wild roe deer for elements and organic substances (TATARUCH 1984, 1993b; LUTZ 1985; MÜLLER 1985b; HECHT 1993, 2001; GUSE & JÄGER 1994; GUFLER et al. 1997; ONDERSCHEKA et al. 1997) as well as radionuclides (MOLZAHN et al. 1987; TATARUCH 1996; HECHT & HONIKEL 1997),
- popular for human nourishment.

Within the ESB program, the roe deer represents the level of consumers in terrestrial ecosystems.

4 Target Compartments

ESB sampling aims at the liver of the roe deer as target compartment. Numerous authors revealed the suitability of the liver for bio-monitoring (e.g. HOLM et al. 1987, 1990).

- most substances are best traceable in the liver. This applies to all hitherto investigated Chlorinated Hydrocarbons, presumably for all lipophilic substances, more or less persistent, organic compounds and for elements like Cr, Mo, Mn, Cu and Fe. The elements Cd, Pb, V, Zn and Ca are stronger accumulated in the kidneys than in the liver. Hg, Al and Mg presumably present equal concentrations in both organs,
- substances are very homogeneously distributed,
- with approx. 300-500 g, the liver provides a sufficiently high sample amount, approx. four- to fivefold higher than the amount from kidneys,
- liver fat contents are subject to less seasonal variations than those in the kidneys,
- removal of the liver causes no decline in value to the venison (TATARUCH 1992),
- the liver is located in the abdomen of the roe deer which is not damaged by a precise heart shot. The risk of unwanted contamination by the bullet can be drastically counteracted by exclusively using roe deer with undamaged abdomens for pollutant studies (HECHT 1984).

5 Predefinitions for the Sampling

5.1 Selection and Definition of Sampling Sites

As a consequence of the deer's mobility (10 - 40 ha activity range), sampling sites are relatively large. Roe deer are shot during a usual hunt by authorized hunters. In Germany, hunting is organized in a district system. Each hunting district tenant holds the right to hunt in his own district, therefore hunting districts are the most important organizational subdivision. Roe deer density determines the number of districts per sampling region. These are included into the Area Related Sampling Scheme, in order to assure long-term sample safety

In case of an extremely low roe deer density and/ or in small-sized sampling regions, it may become necessary to define the whole investigated area as sampling site.

5.2 Selection of Individuals and Sample Size

For statistical reasons the storage of the livers of at least 10 animals (yearlings, of both sexes) is required per sampling site and sampling period.

Yearlings are closely territorially bound by the doe. At the sampling date all of them are older than 12 months and have been exposed to the pollution of an entire year.

Exclusively livers of healthy animals are collected. These must not exhibit any variations of the normal condition of venison and organs. Sicknesses, depending on their kind and peculiarity, alter the physiology of the organism

(STUBBE 1997). Sick animals are able to be identified prior to shooting, by conspicuous appearance (shaggy coat, body mass losses) and/or abnormal behavior.
Therefore, sampling is carried out by experienced hunters, which are able to distinguish these criteria and guarantee for precise consideration of the sampling guideline. Subsequent to shooting and gutting, other manifestations of sickness are detectable, e.g. deviation from the normal size, shape, and color of organs, deposits at organs, increased body fluids, abnormal smell, or extreme ecto- or endoparasite infestation. Livers from non-healthy animals are not used for ESB storage.

5.3 Sampling Period and Frequency

The yearlings are shot from early May until the start of the rutting season (depending on weather conditions, about mid-July). Sampling can be conducted yearly, without serious consequences for the natural 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 resolved and documented in an area related sampling scheme. Among other definitions this includes:

- location and demarcation of the sampling sites,
- required sample size,
- time frame for sampling,
- addresses of the appropriate authorities.

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.

6 Sampling Procedure

All data collected in the course of sampling and throughout the biometric sample description are documented in the respective specimen data sheets (see appendix).

For each roe deer a separate record (specimen data sheet 2) containing the following information is created by the hunter:

- date and time of the culling,
- name of the hunter,
- number of the proof of origin to identify the shot animal,
- chronological sampling procedure,
- position of the bullet hole, exit wound and bullet type,
- description of the health status of the animal.

Thereby a deviation from the SOP can be identified for each individual.

Preparation for roe deer sampling requires comprehensive organization. First of all, with the help of local contact persons, reliable hunters are selected. Preceding initial sampling, these particular hunters must be briefed about all steps in the course of sampling. Instruction thereby focuses on the selection of individuals complying with the guidelines, a contamination free kill and proper sample packaging. At the start of each sampling interval the necessary packaging materials must be compiled and handed over to the huntsmen/-women.

6.1 Required Equipment and Cleaning Procedures

Field work:

- 15 specimen data sheets 2,
- 15 instructions for sampling procedure,
- 15 plastic bags,
- 15 linen bags,
- deep freezing devices for liver transport

For the sampling of a shot roe deer the hunter needs to obtain the following materials/documents in advance:

- 1 specimen data sheet 2,
- 1 instruction for sampling procedure,
- 1 plastic bag.

For packaging the roe deer livers only plastic materials must be used, which are suitable for

cryogenic storage conditions (up to -200°C). They must not contain any additives and should be non-reactive to lipophilic substances in the sample (applicable plastic bags are e.g. made of fluorinated ethylene propylene [FEP]).

Subsequent to the kill a timeframe of 30 minutes is not to be exceeded for liver removal and immediate transfer into the designated bag. Together with the specimen data sheet the bags are put into a linen bag and transferred to a deep-freezer (≤ -15° C) as soon as possible but in-between 24 hours. The interim storage of the samples in the deep-freezer must not exceed 4 weeks.

If necessary, sample collectors are equipped with a deep-freezer.

Transport from the sample collection point to the laboratory is conducted via portable deep freezers at a maximum temperature of -15° C.

Laboratory:

- specimen data sheet 3 for storage condition,
- clean bench with particle- and activated carbon filtration,
- stainless steel containers with lid and fastener
- laboratory scales (reading accuracy 1 g) to determine liver weight,
- waterproof pen to label the stainless steel containers,
- laboratory clothing and disposable gloves
- cooling device (Dewar vessel) with liquid nitrogen,
- tools and protective clothing for liquid nitrogen handling.

The frozen liver is weighed inside of its plastic bag, taken out of it and transferred into a stainless steel container. Thereafter the container is placed in the gas phase above liquid nitrogen.

Cleaning Procedures:

Sample containers and all equipment are 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 (+/– 10°) for a minimum of one hour (sterilization). The containers remain in the closed cabinet while they are left to cool. Sterilization is not applied to synthetic materials

6.2 Sampling Technique

The roe deer are executed by means of an aimed bullet shot during hunting from a raised hide or stalk. Organs of roe deer shot in the course of a battue are not suitable as sample specimen. Pre-mortal stress caused by hounding or not directly lethal shots may influence the pollution concentration within the organs (SCHINNER 1981). The abdomen of the animal should not be injured by the shot. Neck shots and precise chamber shots imply the lowest contamination hazards caused by the bullet, and are therefore the preferable manner of shooting. In the specimen data sheet for sample description, the exact position of the bullets point of entry and exit must be recorded.

During the sampling process extreme attention must be paid to avoiding contact of the samples with hair, plants, soil particles, etc. Sampling should be carried out as follows:

- the roe deer must be gutted within a timeframe of 30 minutes following the kill.
- the uninjured liver must be placed in the plastic bag and transferred within the portable linen bag as soon as possible to the central sample collection point. Alternatively, the whole corpse can be immediately transported to the collection point, where the liver is removed and placed inside the plastic bag. In this case the Order on meat hygiene (VO (EG) 583/2004) must be followed.
- the specimen data sheet for sample description must be filled out,
- livers need to be deep-frozen within the portable bag together with the respective specimen data sheet 2 after 24 hours at the latest (max. -15° C).

The transferal into labeled (container ID, sample ID) steel containers will take place under clean air conditions in the laboratory as follows:

- the ID of the container will be registered together with the weight of the liver and the storage date and time on the specimen datasheet 3,
- the liver samples will be weighed inside their plastic bags, taken out and transferred into pre-cooled stainless steel containers,
- subsequently, the containers will be stored in the gas phase above liquid nitrogen.

7 Biometric Characterization

During sampling, data concerning weight, state of heath, and infestation with parasites of roe deer are collected. All data gained in course of the sampling are to be documented in the respective specimen data sheet. The determination of the liver weight is performed as described in Chap. 5.2. For a better interpretation of the values of the chemical analysis, additional data of the roe deer's browsing behavior is collected. So every 5 years a browsing survey is performed in the sampling areas. The respective instruction can be found in the appendix ('browsing survey').

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Checklist to Prepare and Conduct the Sampling

Specimen Type:	**European Roe deer (*Capreolus capreolus*)**
Target Compartments:	liver
Individual Specimens:	yearlings (bucks and barren does)
Random Sample Number:	at least 10 animals
Sample Quantity for the ESB:	2.200 g liver
Sampling Period:	early May, to the beginning of the rutting season (ca. mid-July).
Sampling Frequency:	annually
Equipment Required for Field Work:	• specimen data sheet for sample description • lists for description of the grazing offer • instruction for the sampling procedure • plastic bags and portable linen bags
Sample Packing until Further Processing:	• plastic bags for interim deep freeze storage • stainless steel containers with lids and fasteners
Transport and Interim Storage:	• deep freezing device (at least -15° C) at the collection point • deep freezing device (at least -15° C) for transport from the collection point to the laboratory • cryogenic (Dewar) vessel for sampling storage in the gas phase above liquid nitrogen
Required Equipment for Laboratory Work:	• specimen data sheet for storage condition, • clean bench with particle- and activated carbon filtration, • stainless steel containers with lid and fastener • laboratory scales (reading accuracy 1 g) for liver weight determination, • waterproof pen to label the stainless steel containers, • laboratory clothing and disposable gloves • cooling device (Dewar vessel) with liquid nitrogen, • tools and protective clothing for liquid nitrogen handling
Biometric Sample Characterization:	• sex • body weight [kg] • state of health • infestation with parasites • liver weight [1 g reading accuracy]

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 1: Sampling Location

European Roe Deer (*Capreolus capreolus*)

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: Sample Description

European Roe Deer (*Capreolus capreolus*)

Identification: **PNG:**

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

No. of Proof of Origin __ __ __ __ District/Devision: _____

Name of the Hunter: _____

Date of Kill: __ __ . __ __ . __ __

Time: shot: __ __ : __ __ perished: __ __ : __ __

gutting: __ __ : __ __ storage: (deep freezer) __ __ : __ __

Bullet Type: _____ Shooting Distance: __ __ __ m

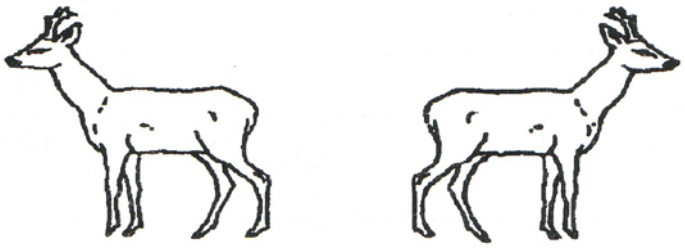
Injured Organs: ☐ lung ☐ spleen ☐ gut

☐ none ☐ heart ☐ rumen

☐ liver ☐ other: _____

Bullet Hole: ☐ heart shot ☐ chamber shot ☐ neck shot ☐ mortally wounded

Entry-/Exit Entry-/Exit



(please mark the bullets point of entry and exit)

Sex: ☐ male ☐ female

Weight: (gutted, with skin) __ __ kg ☐ with head ☐ without head

State of Health: ☐ healthy ☐ sick ☐ emaciated

Injuries: _____

Parasites Infestation:

Ectoparasites: ☐ none ☐ scarce ☐ average ☐ extreme

Endoparasites: ☐ none ☐ scarce ☐ average ☐ extreme

Organs infected: _____

Signature of the Hunter ……

date, signature

GERMAN ENVIRONMENTAL SPECIMEN BANK

Specimen Data Sheet 3: Storage

European Roe Deer (*Capreolus capreolus*)

Identification:

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

Sample: __ __ __ **District/Division:** _____

Routinely Conducted Sampling	**Screening**
Liver Weight: __ __ __ **g**	**Liver Weight:** __ __ __ **g**
Number of Stainless Steel Container:	**Number of Stainless Steel Container:**
Liver: _____	Liver: _____

Date of Storage: __ __ . __ __ . __ __

Time of Storage: __ __ : __ __

Browsing Survey

In order to describe the roe deer´s habitat quality, every five years a mapping of deer browsing activity needs to be carried out within the perimeters of the sampling areas. Browsing intensity determination should be scheduled / undertaken almost at the end of the vegetation period. At this point of time it is possible to record the herbaceous and woody plants, which have been browsed during summer, as well as the woody plants used as food ressource in the course of winter. Mast-years of forest species and the composition of additional winter feed must also be recorded.

Among the cited methods for characterizing roe deer browsing behaviour (ZAI 1964; KLÖTZLI 1965; AL-KITTANI 1973; VOSER-HUBER & NIERVERGELT 1975; PETRAK 1982, 1987; GUTHÖRL 1990), primarily the methods suggested by KLÖTZLI (1965) and GUTHÖRL (1990) were considered. The Investigation should be carried out by a skilled person who ideally supervises all sampling areas.

The browsing survey, whose results are documented in the respective specimen data sheets, should be carried out as follows:

Forest Territories:

In a first step partial habitats important for roe deer within the sampling area and potential browsing grounds are selected. Potential browsing grounds are all sites where forest floor flora and rejuvenation develop due to a sufficient incidence of light (e.g. clear felled areas, seedling brushes until thicket stage, natural regeneration sites, wind throw sites, pipeline alignments, forest edges and waysides and the like). Information on preferential browsing grounds should be gathered by local hunters, foresters or other persons familiar with the area. Thereafter, a representative number of mapping area plots within the sampling region is selected, on which the actual browsing intensity should be estimated. The number and size of mapping plots selected, depend on the respective forest structure.
In case of browsing mappings on larger sites, the area will be divided into five units. The first and fifth unit cover the marginal zones, the third one, the centre. Minimum size of each recorded site is 25m² each. To determine the grazing offer (occurrence of plants) on each recorded site, a vegetation analysis is performed according to the method by SCHMIDT (1974) which presents a refinement of the method by BRAUN-BLANQUET (1964).
Smaller mapping plots will not be subdivided. It must be considered, that the estimations of the cover values (species: area ratio) in case of phytosociological methods do not represent harvest estimations. The cover value is defined as the area which would be covered if all above-ground parts of the recorded plants are projected vertically onto the ground. The estimation of the plant species cover value (Tab. 1) is performed for a stratum of up to 1.5 m height (grazing height of the roe deer).

Tab. 1: Scale for the estimation of cover values (SCHMIDT 1974)

< 1	up to 1	up to 2	up to 3	up to 8	up to 10
up to 15	up to 20	up to 25	up to 30	up to 40	up to 50
up to 60	up to 70	up to 80	up to 90	up to 100	

In contrary to the browsing investigations conducted for forest concerns, which aim at gathering information about of the state of forest rejuvenation and therefore consider the young forest trees only (SCHWAB 1999), investigations concerning the grazing behavior include all occurring plants. For each

plant species on the recorded site, the browsing intensity is determined. Hereby, it must be differentiated by the causative animal species (REIMOSER & REIMOSER 1998). While it is easy to differentiate between the feeding marks of ruminants and rodents (e.g. jack rabbits), it is normally not possible to distinguish between the different ruminant species by their feeding marks (PETRAK 1982). The bite patterns caused by ruminants are uneven and fibered, but not smooth as produced by the jack rabbit (BANG & DAHLSTRÖM 2000). The specific grazing species can be identified by the different browsing height, which complies with body height, the appearance of browsing marcs and unambiguous characteristics, such as droppings and footprints. However, it must be kept in mind that individuals can considerably enlarge their upwards grasp by standing on their hind legs. Additionally a closed snow cover, provided that it bears the burden of the animals, can also increase upwards browsing range (PETRAK 1982).

Tab. 2: Browsing intensity classes for gramineae, herbs, and young trees (KLÖTZLI 1965)

Browsing intensity class	Browsing intensity	Gramineae and herbs	Shrubs or young trees
0	none		
1	low	1-5% of the plant bitten to a minor degree	only about 1-5 browsing hints per plant
2	moderate	6-20% of the plant bitten to a minor degree, plant inhibited in growth	6-20 browsing hints per plant, no inhibition of growth
3	severe	20-50% bitten in a striking degree, e.g. sprouts decapitated, growth often stopped	> 20 browsing hints per plant, plant inhibited in growth (browsing of the top shoot will be especially noted)
4	extreme	> 50% bitten in a striking degree, plants often ± completely destroyed	> 20 browsing hints per plant, plant without noteworthy sprout accrescence in the present vegetation period

A restriction of the browsing survey to a vegetation height of 1.5 m may lead to an underestimation of the browsing degree caused by the roe deer.

For one particular site the browsing degree for each plant species is assessed according to a five-class scale of browsing intensity (Tab. 2) developed by KLÖTZLI (1965). By averaging the values of the browsing intensity ascertained from the recorded sites / grazing grounds, the browsing intensity (= browsing rate) of the sampling area is calculated.
Depending on frequency and intensity of browsing, the plant species are divided into popularity groups (Tab. 3). The five-class popularity scale developed by KLÖTZLI (1965), has been adapted to the situation found in the investigated ESB areas and expanded with four further classes. The measure of browsing frequency is the continuity of browsing.

Tab. 3: Definition of the popularity groups (modified according to KLÖTZLI 1965)

Popularity rate	Definition of the popularity rate	Browsing continuity	Average browsing intensity
0	± Never browsed	< 1%	Little
1	Occasionally slightly browsed	1-40%	Little
2	Often slightly browsed	41-70%	Little
3	Regular slightly browsed	71-100%	Little
	Occasionally moderately browsed	1-40%	Little to moderate

4	Often moderately browsed	41-70%	Little to moderate
	Occasionally moderately to severely browsed	1-40%	Moderate to severe
5	Regularly moderately browsed	71-100%	Little to moderate
6	Often moderately to severely browsed	41-70%	Moderate to severe
	Occasionally severely browsed	1-40%	Severe to extreme
7	Regularly moderately to severely browsed	71-100%	Moderate to severe
8	Often severely browsed	41-70%	Severe to extreme
	Regularly severely browsed	71-100%	Severe to extreme

The browsing continuity for each species is calculated as follows:

$$\frac{\text{No. of surveys in which the species was found browsed}}{\text{No. of surveys in which the species occurred}} \times 100$$

In order to derive the popularity rate, three browsing continuity categories (browsing continuity 1–40% = occasionally browsed, browsing continuity 41–70% = often browsed, browsing continuity 71–100% = regularly browsed) are combined with the average browsing intensity. An estimation for the proportion of feed grazed from an individual plant species is based on the browsing intensity class determined for the respective plant species, multiplied by the cover values of the recorded sites. This provides an indication for browsing stress on the recorded sites / grazing grounds. By averaging the values of browsing stress ascertained from the recorded sites / grazing grounds, the browsing stress for sampling is calculated.

Field districts:

In field districts the grazing offer on the utilized agricultural area must be registered and recorded in the respective specimen data sheet. It is distinguished between fallow land, grassland, and the main field crop species. Groves and adjacent edges must also be recorded. On fallow lands phytosociological surveys as well as browsing surveys should be performed according to the method described above.