







METHOD OF COLLECTING GREEN FROGS FOR SCIENTIFIC AND ENVIRONMENTAL STUDIES BY HAND NET CATCHING



Mihails Pupins, Andris Čeirāns, Oksana Nekrasova, Kathrin Theissinger, Jean-Yves Georges



The material was developed in a cooperation with the project Emys-R, funded through the join 2020-2021 Biodiversa & Water JPI joint call for research proposals, under the BiodivRestore ERA-Net COFUND programme, and with the funding organisations Agence Nationale de la Recherche (ANR, France), VDI/VDE-IT (Germany), State Education Development Agency (VIAA, Latvia), and National Science Center (NSC, Poland) and the project "Ecological and socioeconomic thresholds as a basis for defining adaptive management triggers in Latvian pond aquaculture" (Izp-2021/1-0247). We thank for the cooperation the project "The mobile complex of Daugavpils University pond aquaculture scientific laboratories" (16-00-F02201-000002) for the possibility of using the mobile complex of scientific laboratories for the research purposes.

Recommended citing:

Pupins M., Čeirāns A., Nekrasova O., Theissinger K., Georges J.-Y. (2022): Method of collecting green frogs for scientific and environmental studies by hand net catching. – Latgales ecological society, 11 pp.

Affiliation:

Mihails Pupins¹, Andris Čeirāns¹, Oksana Nekrasova^{1,2}, Kathrin Theissinger³, Jean-Yves Georges⁴

- ¹ Department of Ecology, Institute of Life Sciences and Technologies, Daugavpils University, Daugavpils, Latvia
- ² Schmalhausen Institute of Zoology, NAS of Ukraine, Kyiv, Ukraine
- ³ LOEWE Center for Translational Biodiversity Genomics, TBG Senckenberg Nature Research Institute, Frankfurt, Germany
- ⁴ Université de Strasbourg, CNRS, IPHC UMR 7178, F-67000 Strasbourg, France

Abstract: This material is a brief description of the basic methods of collecting green frogs for scientific and environmental studies by the hand net catching, used by the authors for many years in Latvia. The preparation for catching, the equipment, the basic methods of catching and storage frogs are described. The material can be used for sampling green frogs for various studies in the field of genetics, parasitology, ecology etc. and PhD and Master students' studies of green frogs.

Reviewers:

Prof., Dr.biol. Arturs Skute, Daugavpils University, Latvia Dr.biol. Valērijs Vahruševs, Latgales Zoo, Latvia

Cover photo: Green frog (Pelophylax spp.), Silene Nature Park, Latvia, 2022. Author of the photo: Mihails Pupins

METHOD OF COLLECTING GREEN FROGS FOR SCIENTIFIC AND ENVIRONMENTAL STUDIES BY HAND NET CATCHING

Legislation and nature conservation

Before starting to collect green frogs for scientific and conservation studies, it is necessary to fulfil the requirements of the legislation of the country where the study is held (in Latvia include obtaining permits from the competent state authorities Nature Conservation Agency for catching frogs, the research and capturing in protected areas, also agreements from land owners, acceptance of the research method by the ethical commission and may include other documents and procedures). Collecting of green frogs is relevant for various studies in the field of genetics, parasitology, ecology, etc. (in Latvia: Čeirāns et al. 2020, 2021; Kulikova et al. 2022; Ozolina et al. 2021).

Animal welfare

Capture and storage should comply with legislation and animal welfare regulations, minimizing stress and distress to captured individuals. The number of individuals caught for research should always be kept as low as possible. Research procedures should be as minimally invasive as possible (photography, measurement, sampling, etc.). Depending on the objectives of the study, after veterinarian advices, frogs can be released on site where they were captured.

Necessary equipment and materials

Technical equipment

- a hand net with a handle length of 1.5-3 m and a mesh bag depth of 40 cm or more (the net and its rim are dark in colour, can be purchased at a fishing store; nets with a folding handle and a net bag fixed to the rim are convenient - otherwise it screwed onto the rim when catching (Fig.1.);
- small plastic boxes with ventilation holes (disposable plastic boxes for storing food in the refrigerator are convenient, their bottom corners should be cut off so that the frog can't get out);
- a permanent marker (to record the biotope and animal number, species and coordinates on the box);
- adhesive transparent tape (for fixing the cover of the box and protecting the marker);
- handy GPS device with map (or any device with similar function, e.g. mobile phone applications);
- photo camera or alternatively mobile phone (for taking pictures of the study area and biotope and of every frog caught for post-field detailed observation and if necessary for confirming species identification);

- a rucksack for carrying materiel and a large solid plastic box for carrying samples;
- a real (paper) map of the study area;
- a note book / field sheets for marking every action made in the field: one sheet per pond planned to be visited; alternatively, an app dedicated to field data recording (e.g. Cybertracker).



Fig.1. Net for fishing with a wide dark rim, a deep bag and a folding handle.

Safety and comfort

- rubber boots (boots with a top that tightens from falling leaves are convenient);
- car (sufficient cross-country ability);
- anti-reflective glasses (handy for finding diving frogs, can be bought at a fishing store);
- insect and tick repellents;
- headlight, little knife, pepper spray, rope/string for fixing material when needed all in a handy small belt bag.

Sanitary

- plastic bag for used gloves;
- means and equipment for washing hands;
- rubber disposable gloves (recommended to replace at least after the visit of each pond);
- means and equipment for disinfection (rubber boots disinfection procedure is recommended after the visit of each pond);
- a mobile phone with emergency number.

Training of personnel

- training in field methods for identification of amphibian species;
- training in methods of working with catching equipment and materials;
- training in methods of finding and catching green frogs;
- training in expedition planning and record keeping;
- getting the necessary vaccinations;
- · safety training.

Distribution of green frogs

Green frogs (also referred as to Pelophylax complex: *Pelophylax lessonae*, *Pelophylax ridibundus*, *Pelophylax* kl. *esculentus*) are differently distributed throughout the territory of Latvia (Fig.2.) (Pupina et al. 2019; Čeirāns et al. 2020). During the active season they can be found in 70-90% of all water bodies of the country (ponds, lakes, rivers, warm streams, ditches and beaver dams, etc.), including water bodies and places frequently visited by people (beaches, fishing places, technical reservoirs, etc.).

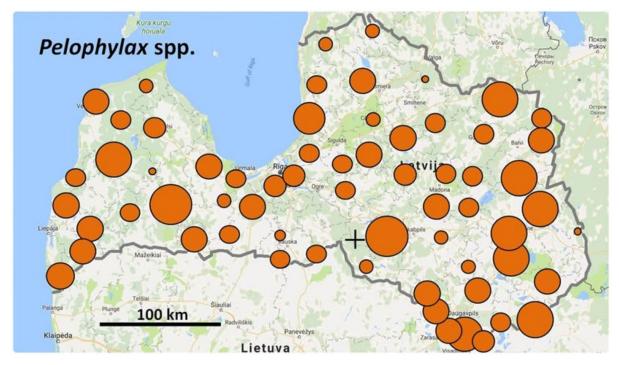


Fig.2. Maps of estimated densities of calling males (Dc) for Pelophylax spp. observed in anuran audial surveys at 65 plots (circles) throughout Latvia in 2016–2018. *The size of a brown circle is proportional to the density of the taxon (circle size corresponds to same density range, from <2 to >40 calling males/km2); Images are based on maps from https://www.mapas-del-mundo.net/ (the study area location) and Google Maps (taxa densities).* (Čeirāns et al. 2020).

Catching season

In Latvia, green frogs can be caught in and near water bodies during their active season from mid-April to September. Dates may be shifted depending on the weather conditions of a particular year.

Catching time

Green frogs are caught with a hand net during daylight hours, usually from 9-10 am, catching ends at dusk.

Weather

Green frogs in Latvia are caught in warm weather, without heavy rain and strong winds. Light rain (sprinkling, periodic moderate rain) and light wind do not interfere with catching frogs, especially in microbiotopes closed from the wind (warmed shallow water between bushes, overgrown pond, etc.).

Planning

Before the expedition ensure all material is functioning, batteries fully charged, and in sufficient quantity (specially field sheets). If you are student / project participant, ensure the person in charge of the project or your supervisor is informed about the place you are and the expected duration of your field visit. The route is planned using maps and services (e.g. Google Earth). It is convenient to mark all ponds on the map in advance, calculate the route

and enter it into the navigator. The route is planned in such a way as to explore all the planned ponds as efficiently as possible, moving between them by car or on foot and minimizing pedestrian crossings longer than 50 m. The car should be parked 10-15 m before the place of catching. When planning catching on large reservoirs, places are selected on the southern coast that are not overgrown with reeds and shrubs.

Features of the behaviour of green frogs

During daylight hours, green frogs spend a lot of time on the shore line, where they are sunbasking on the shore or are feeding on land and in shallow water. Basking frogs often lose their vigilance and are easier to catch. The shadow of the catcher falling on the frog frightens it, so this should be avoided. When threatened, frogs fast dive into the water and hide at the bottom and among plants. In search of food, especially in wet weather, frogs often move inland 5-10 meters from the shore, but in case of danger they quickly return to the water along the shortest route. When the catcher is on the shore for more than 20-30 minutes without movements, the frogs get used to it, the vigilance of the frogs weakens and they can be caught with slow movements.

Hand net catching main methods

1. Catching in the water

1.1. The green frog floats on the surface of the water or basks on/in a layer of mud floating on the water surface (Fig.3a,b).

The catcher slowly and without sudden movements goes along the coast. Seeing a frog in the water, the catcher slowly brings the net under the frog and picks it up. Frogs are more careful in clear water; in muddy or duckweed water frogs do not see the catcher until they emerge and can be caught more easily.





Fig.3.a,b

1.2. The green frog dived into the water (Fig.4).

When catching a diving frog, the hand net is quickly set across the trajectory of its movement and with a quick movement rises along with the frog that has swum into it.



Fig.4.

1.3. The green frog basks on a layer of mud or duckweed floating on the water surface, seeing the catcher, the frog hid under the duckweed (Fig.5).

Usually the frog remains in the same place under the duckweed at a depth of 5-15 cm. The catcher quickly scoops up a layer of duckweed with a net to a depth of 20-30 cm and pulls the net ashore.



Fig.5.

2. Catching on the shore

2.1. A frog is sun-basking on a low bank near the water (Fig.6).

The catcher slowly and without sudden movements goes along the coast. Once close to the sun-basking frog on the shore, the catcher quickly covers it with a hand net, pressing it slightly to the ground. The frog jumps into the water and falls into the bag of the net, where it must be quickly taken by hand through the net. In case of a miss, see methods of catching in the water.



Fig.6.

2.2. A frog is sun-basking on a high bank above the water (Fig.7).

The catcher slowly and without sudden movements goes along the coast. Seeing a sun-basking frog on a high bank, the catcher quickly blocks the probable trajectory of its jump with a hand net. The frog jumps into the water and falls into the bag of the net. In case of a miss, see methods of catching in the water.



Fig.7.

2.3. The green frog feeds on the shore at a distance of 1-10 m from the water (Fig.8).

The method is most effective in wet weather and after rain. The catcher slowly and without sudden movements goes along the coast at a distance of 1-3 m from the water. When a frog moves towards water (usually in grass), the catcher quickly blocks the probable trajectory of the frog movement with a hand net installed vertically. The frog jumps and gets into the net bag. In case of a miss, see methods of catching in the water. The first route is laid at a distance of 1-2 meters from the coast, then, returning, the second and subsequent ones every 1-2 meters. Catching can be repeated, because when disturbed, the frogs that were farther from the coast will move to new places closer to the coast.



Fig.8.

Green frog storage

After being caught, the frog is placed in a disposable plastic box with the bottom 4 corners cut off (Fig.9). The frog is inserted into a box by opening a gap in the thickness of the frog between the wall of the box and its lid. Then the lid can be sealed with a tape to prevent accidental removing of the lid. On the cover, do indicate the date and place of capture, the serial number (of the day), the species and coordinates (can be registered separately). Boxes with the bottom corners cut off can be stored in a stack, one on top of the other. It is recommended to store each frog in a separate box to avoid disease (Chytridiomycosis,

ranavirus etc.) transfer between individuals. If necessary, frogs of the same size can be stored together for a short time. The number of frogs in one box depends on their size: they should occupy no more than a third of the area of the box. When storing small juveniles, wet soft plants from the same pond should be placed in the boxes.



Fig.9. Plastic disposable box (a) and cutting off 4 corners (b) of its bottom (c).

/!\ **Key rules**: Amphibians should be protected from accidental contact with mucus and foam from other species. You can not even briefly put together amphibians of different species!

Boxes with animals during catching are stored in a cool place without access to direct sunlight: a cooler bag, a foam box, a car trunk. Do not store boxes in the car because of the danger of overheating.

When excrement appears in the box, the box, without taking out the animals, is immersed in water 2-4 times and taken out of the water - flowing out through the cut corners, the water washes out the excrement and washes the frogs. If necessary for research purposes, for longer storage, boxes with frogs are placed in the refrigerator. About once a week it is recommended to carry out the above washing procedure.

According to your research goals, after frog removal from its box for scientific study clean this disposable box or utilize it.

9

References

- Čeirāns A., Gravele E., Gavarane I., Pupins M., Mezaraupe L., Rubenina I., Kvach Y., Skute A., Oskyrko O., Nekrasova O., Marushchak O., Kirjusina M. (2021): Helminth communities in amphibians from Latvia with an emphasis on their connection to host ecology. *Journal of Helminthology*. 95, E48. https://doi.org/10.1017/S0022149X2100047X.
- Čeirāns A., Pupins M., Pupina A. (2020): A new method for estimation of the minimum adult frog density from a large-scale audial survey. *Scientific Reports*: 10:8627. https://doi.org/10.1038/s41598-020-65560-6.
- Kulikova A.A., Pupina A., Pupins M., Čeirāns A., Baláž V. (2022): Survey for *Batrachochytrium dendrobatidis* and *B. salamandrivorans* in Latvian water frogs. *Journal of Wildlife Diseases*, 58(2). https://doi.org/10.7589/JWD-D-21-00082.
- Ozoliņa Z., Deksne G., Pupins M., Gravele E., Gavarane I., Kirjušina M. (2021): *Alaria alata* mesocercariae prevalence and predilection sites in amphibians in Latvia. *Parasitology Research*, https://doi.org/10.1007/s00436-020-06951-6.
- Pupina A., Pupins M., Ceirans A., Pupina Ag. (2019): Chapter 65. Decline and Conservation of Amphibians in Latvia. In: Heatwole H. (Editor), Wilkinson J.W. (Editor): Amphibian Biology, Volume 11: Status of Conservation and Decline of Amphibians: Eastern Hemisphere, Part 5: Northern Europe. United Kingdom, Pelagic Publishing: pp. 122-141.
- Rubenina I., Kirjusina M., Ceirans A., Gravele E., Gavarane I., Pupins M., Krasnov B.R. (2021): Environmental, anthropogenic, and spatial factors affecting species composition and species associations in helminth communities of water frogs (*Pelophylax esculentus* complex) in Latvia. Parasitology Research: https://doi.org/10.1007/s00436-021-07303-8.





Pupins M., Čeirāns A., Nekrasova O., Theissinger K., Georges J.-Y. (2022): Method of collecting green frogs for scientific and environmental studies by hand net catching. – Latgales ecological society, 11 pp.