Standardized Rapid Biodiversity Protocols: Freshwater Fishes

4 authors:

Jose Birindelli
Universidade Estadual de Londrina

Vanessa Meza-Vargas
Pontifícia Universidade Católica do Rio Grande …

Leandro M. Sousa
Federal University of Pará

Max H. Hidalgo
National University of San Marcos

FRESHWATER FISHES

Photo © Luciano Candisani/iLCP

STANDARDIZED RAPID BIODIVERSITY PROTOCOLS: FRESHWATER FISHES

José L. O. Birindelli[1], Vanessa Meza-Vargas[2], Leandro M. Sousa[3] and Max Hidalgo[2]

Introduction

Definition of taxon – With over 32 thousand species (Nelson, 2006), fishes are more diverse than all other vertebrates combined. Fishes comprise a variety of distinct lineages including lampreys and hagfishes, sharks, rays and chimaeras, ray-finned and lobe-finned fishes. All of these species live in or are associated with aquatic habitats and breathe through gills, even if only in a supplementary way. Fishes exhibit enormous diversity in their morphology, physiology, reproduction and genetics. Similarly, fishes occupy a wide range of habitats, from small streams at five thousand meters elevation in the mountains of Tibet to sea valleys deeper than seven thousand meters below sea level (Helfman *et al*., 2009). However, fishes are not homogeneously distributed on earth, as approximately half of all species occur in continental waters, representing no more than 3% of the water available on the planet.

What criteria make the taxon suitable for rapid baseline surveys and for guiding conservation decisions in general? Freshwater fishes are the most dominant group of vertebrates in inland aquatic ecosystems, including rivers, streams, lakes, floodplains, intermittent pools, and subterranean aquifers. Aquatic habitats occupied by fishes are extremely diverse in terms of their physicochemical characteristics (e.g., white, black and clear water), geological origins (e.g., uplift of mountains and plateaus, rifting of continents, and erosion of ancient crystaline shields), and vegetational associations (e.g., piedmont forest, floodplains, rainforest, savanna). All these attributes influence fish diversity and community structure.

Fish species and assemblages respond quickly to environmental disturbances, including anthropogenic activities that alter hydrology (e.g. construction of dams) and water quality (e.g. mining and agriculture activities). Due to recent human activities, many freshwater fish populations have undergone drastic reductions and many species are now considered to be threatened or extinct. Globally, 1,670 freshwater fish species (Actinopterygii) are considered threatened at some level, a number nearly four times greater than that of marine fishes. The most important threats to freshwater fishes are dams and soil erosion (sedimentation), which are respectively responsible for impacting 494 and 489 species on the IUCN red list (data available at http://iucnredlist.org).

[1] Museu de Zoologia, Departamento de Biologia Animal e Vegetal, Universidade Estadual de Londrina, Londrina, PR, Brazil. Email: josebirindelli@yahoo.com

[2] Departamento de Ictiología, Museo de Historia Natural UNMSM, Av. Arenales 1256, Jesús María, Lima, Perú. meza.sv@gmail.com; Email: mhidalgod@unmsm.edu.pe

[3] Laboratório de Ictiologia de Altamira, Faculdade de Ciências Biológicas Universidade Federal do Pará, Campus Altamira, Altamira, PA, Brazil. Email: leandro.m.sousa@gmail.com

Fishes are often conspicuous, abundant and relatively easy to collect and store, either as living or preserved specimens. Distinct methodologies and efforts can be applied according to the taxon, habitat, and ultimate objectives of the study (e.g., diversity, ecology, genetic).

Because fishes occupy water that drains off or through the land, and are sensitive to changes in this water, they are excellent, integrative indicators of overall ecosystem health and can be a cost-effective means of conducting biomonitoring assays. Moreover, fishes often have high sociocultural and economic value as sources of food, recreation, and income (e.g., when collected and sold to the ornamental aquarium trade). Their sustainable management as a renewable resource can therefore provide a primary motivation for conserving natural ecosystems.

Core Method

Methods for collecting fishes vary greatly according to habitats, as all types of equipment cannot be applied in all situations. The following three main and most often-found habitat types are used as a guide:

1. small-scale lotic habitats (e.g., headwaters, forest streams),
2. medium and large-scale lotic habitats (e.g. rivers),
3. lentic and slow-water habitats (e.g., lakes, floodplains).

Small lotic habitats are those with flowing water and classified as first, second or third order streams according to the Horton method, modified by Strahler (1957). Medium and large-scale lotic habitats are those with flowing water and classified as fourth or larger order streams. Lentic and slow-water habitats consist of any still water body including lakes, swamps and floodplains. Distinct standardized protocols are suggested for surveying fishes in each of these habitat types. More specialized habitats, such as caves and temporary isolated ponds, for example, should utilize other protocols (see supplementary methods below).

Core standardized sampling protocols for rapid survey – Sampling methods are described below for each habitat type. Information regarding gear specifications, sample site selection, and sampling effort, are provided below.

Small-scale lotic habitats (headwaters and small streams):
Each sample should be carried out by at least three people working together in a 50 meter long stretch of waterway (i.e., reach) for one hour of effort using the following equipment: dip nets, sieves and seine nets. Prior to sampling, each end of the reach should be blocked by seine nets to prevent fish from escaping. If samples are made in the same stream, these should be separated by at least 500 meters to maintain independence among samples. Samples should always be made during daytime, for comparisons.

Medium and large-scale lotic habitats (large streams and rivers):
Each sample in a medium or large-scale lotic habitat should be made using combinations of seine nets, cast nets and gill nets. A seine net should be applied 10 times along the shore, preferably keeping a distance of approximately five meters between each sampling event. Cast nets should be applied 10 times along shallow stretches, usually found near the shore, preferably keeping a distance of at least five meters between each sampling event. Five gill nets should be placed in the water for at least 8 hours, preferably from sunset to sunrise, ideally at a safe distance from each other (e.g., at least 10 meters apart), to avoid influencing among them. Samples should be made during both day and night, emphasizing transitional periods between day and night when most fishes are more active.

Lentic and slow-water habitats (lakes, swamps and floodplains):
Each sample in a lentic or slow-water habitat should be made using gill nets and seine nets. A seine net should be applied 10 times along the shore, preferably keeping a distance of at least 5 meters between each sample. Five gill nets (net specifications below) should be placed in the water for at least 8 hours, preferably from sunset to sunrise, ideally at a safe distance from each other (e.g., at least 10 meters apart), to avoid influencing among them. Samples should always be made during day and night, emphasizing transitional periods between day and night when most fishes are more active.

Supplies/equipment needed in the field and after fieldwork

List of supplies and equipment:
Field work:

- 2 dip nets (mesh 1 to 3 mm, diameter of approximately 30 cm)
- 2 sieves (mesh 3 to 5 mm, diameter of approximately 60 cm)
- 2 seines (mesh 3 to 5 mm, approximately 5 meters long and 1.5 to 2 meters high)
- 2 cast nets (mesh 12 to 20 mm and 2 to 3 meters high)
- 5 gill nets (nylon mesh size ranging from 12 to 70 mm bar (vs. stretched), approximately 10 meters long and 2 meters high)
- 40% formalin solution (amount depending on the estimated number of samples and target place)
- 96% alcohol solution (amount depending on the estimated number of samples)
- Large gauge syringe and needles, and gloves
- Vials of 5 mL, dissection kit with scalpel, razor blades, and microscissors
- Field book and waterproof and alcohol-proof pen and paper
- Photographic camera and batteries
- Aquarium for photographs (including a supply of clear, bottled water specifically for photo tank)
- Anesthetic solution (e.g., clove oil)
- Plastic bags of different sizes (including rubber bands)
- Handheld GPS

Lab work:

- Trays and forceps
- 96° alcohol solution
- Jars of distinct sizes (ranging from 250 ml to 3 liters)
- Heavyweight label paper and printer
- Computer
- References for species identification (including internet access)

Selecting sampling sites – In order to apply each of the three suggested protocols for a selected site, it is necessary to previously determine if that water body is a small-scale lotic habitat, a medium or large-scale lotic habitat or a lentic or slow-water habitat. Thus, maps that allow determining the stream order according to the Horton method, modified by Strahler (1957), should be examined prior to going to the field, and also available during the field collection. Selection of sites should favor heterogeneity of habitat structure, in order to have the best opportunity to focus collecting and achieve the most complete species list possible.

Typical sampling effort required – For small-scale lotic habitats, the minimum effort needed for standardized sampling is a team of three people working together in the same site for one hour. For medium and large-scale lotic habitats and lentic and slow-water habitats, the minimum amount of effort needed for standardized sampling is a team of four people working together in the same site for one hour.

The minimum number of samples for a rapid assessment should be 30. Based on available data (Chernoff *et al*., 1999a; Chernoff *et al*., 1999b; Mol *et al*., 2006; Anjos & Zuanon, 2007), it is likely that up to 90% of the species of fishes in small-scale lotic habitats are collected after approximately 30 samples in relatively small and homogeneous areas, using the protocols suggested herein (one sample corresponding to one collection event at one site).

However, if a more complete inventory is the objective of the survey, especially focusing on increasing the number of species sampled in larger areas, it is strongly suggested that more effort is made using additional sampling equipment and techniques, described below, as well as more samples.

As rapid assessments are intended to be fast and to last a short period of time, the sampling effort will depend ultimately on the amount of time available for field trips. Using the methods and protocols described herein, it is possible to make up to five samples in small-scale lotic habitats, or up to one or two samples in medium and large-scale lotic habitats or, lentic or slow-water habitats per day. In a field trip that lasts 15 days, it is possible to make up to 50 samples, usually less however due to difficulties in reaching each collection site.

Medium and large-scale habitats and lentic and slow-water habitats have usually more diverse fish fauna and more microhabitats to sample, which diminishes the percentage of species sampled during a rapid survey. Shotgun-style inventories based on qualitative surveys may detect more species, as more distinct sampling equipment is used and more effort is employed. However, shotgun approaches ignore standardized protocols, making the resulting data not suitable for later comparison in terms of species relative abundance. Species accumulation and rarefaction curves should be calculated based exclusively on data obtained through standardized sampling. Species accumulation and rarefaction curves can be obtained through distinct methods including Chao and Jacknife, for example, or a combination of these (Gotelli & Colwell, 2010; Ortega *et al*., 2014).

Context Dependent Sampling Considerations

Sampling considerations for assessing different types of environmental change/disturbances – If one of the goals of a rapid survey is to measure environmental disturbances, collections should be made in the same site prior to those changes, possibly more than one time in order to have more data that can be compared to the data obtained in the same site after disturbance. Alternatively, a reference site can be selected with similar overall characteristics (width, depth, water volume and velocity, type of substrate, abundance of vegetation, etc) but lacking the environmental disturbance. Data obtained in a reference site could replace those obtained in the studied area prior to disturbances, if the latter are not available. Some environmental disturbances change the type of the habitat, for example, from lotic to lentic, making comparisons of fish diversity much more complex, as the sampling methods applied in those habitats are distinct. In those cases, inventories should use a greater variety of equipment, techniques and more extensive effort.

Habitat considerations – The standard rapid assessment protocols herein suggested are not appropriate for every aquatic environment. There are several particular habitats that need special consideration. Details and suggestions on additional sampling methods are found below. Creeks and small streams with high elevation and steep slope (e.g., those found in the Andes of South America) cannot be efficiently sampled using only the protocols herein suggested for small lotic water bodies. Strong water flows prevent the suggested protocol from being implemented effectively (e.g., it may not be possible to block the stream with a net). For high gradient mountain streams, for example, electrofishing is often a requirement for efficient sampling (Ortega *et al*., 2014).

Other unique and difficult to access habitats include the main channels of large rivers, most of which have highly specialized fishes occupying the bottoms of the main channel. Large predatory fishes of deep river channels can be collected by drifting large gill nets or through other local fishing techniques (e.g. fishtraps with certain kind of bait depending on the species), whereas the majority of small bottom fishes can only be collected using bottom trawl nets. This technique can yield many types of fishes that are not collected otherwise (Barthem & Goulding, 1997).

Temporary isolated ponds are generally shallow and ephemeral habitats. The rapid survey techniques proposed in this chapter do not apply to them, as it is impossible to use the same sampling equipment (e.g., gill nets in some cases). The most common method to inventory these habitats is to use sieves and dip nets. Similarly, caves and subterranean waters are complex and highly variable habitats. Sampling methods applied in these types of environments are mainly traps, sieves and dip nets (Bichuette & Trajano, 2003).

Biogeographic or regional considerations – Fishes are excellent subjects for biogeographic studies, as many fish species are often restricted to particular watersheds. It is common to find distinct species separated by relatively short distances, and distinct faunas in far away places (distinct continents, for example). Nevertheless, the basic methods proposed here for collecting fishes apply equally to sites globally.

Seasonality – The most significant change in the fish fauna in tropic environments is related to changes in the water level, turbidity, and chemistry. Those changes occur seasonally in many tropical environments, in which species composition vary in abundance and diversity of fishes tend to be greater in the rainy season, when food resources are more available as water floods into riparian forests (Matthews, 1998). Therefore, the minimum protocol must include collections in periods when the water is highest and also in periods when the water is lowest, to obtain a realistic representation of a region. On shorter timescales, rain events can often cause spates in which water levels rise rapidly. It is during these periods that many large fish move from their shelters, making such events ideal opportunities to sample using baited hook and line. On the other hand, it is easier to sample fishes, particularly in most water bodies in the dry season, when there are fewer places for fishes to hide and is easier for researchers to explore the water body and manage nets.

Supplemental Methods

Many other fishing methods could be used to maximize the number of species sampled during a rapid inventory. If the aim of the survey is to sample as many species as possible, the minimum protocol herein suggested should be supplemented by additional effort, using the methods and sampling equipment described above, and also other fishing methods detailed below.

Underwater observation – this method is only feasible in places where visibility is of 50 cm or (preferably) more. This method depends also on the target species, for example, to observe fast swimming fishes high visibility is needed, whereas slow benthic fishes can be observed in places with approximately 50 cm of visibility. There are many different techniques for collecting data based on underwater observation, most of them developed in studies of fishes in marine habitats where water clarity is much higher. In addition, some benthic fishes can be hand-caught (or caught with help of a dip net) once they are located underwater, a technique that works well for loricariid armored catfishes in South America, for example, and is known as hogging or noodling.

Electrofishing: this method is only feasible in water bodies with medium to high conductivity (i.e., 50 uS/cm or higher). This method is most effective in Andean rivers of South America, in addition to creeks and lakes (Ortega *et al*., 2014). The standardized protocol for electrofishing includes the delimitation of a stretch of the water body (100 meters, for example) and time for determination of collecting effort.

Trawling: this method is only feasible in large rivers with bottoms that are free of large rocks and trunks (which can be determined by sonar or local fishermen). In addition, this technique requires the use of a motorboat and a fully equipped trawling net (with otter boards and cable). This technique is especially interesting for species that live deep in river channels (for example, catfishes and electric fishes in South America).

Hook and line (including trotline): methods including hook and line are among the oldest and most diverse fishing technique available. These methods can be used in a great variety of environments focusing especially on medium to large fishes, usually predatory species at low density in fast flowing rivers and deep lakes. However, even small fish can often be collected effectively using small hooks at the water's surface.

Trapping: most traps work on a "funnel" or "maze" principle, with fishes being attracted by bait, passing through an opening and being unable to find their way out. Traps are especially good in deep pools, lakes and places with difficult access such as caves.

Electric detectors: this method is used for detecting electric fishes. This method requires a small battery-powered audio amplifier and duplex cable with terminal on one end bare and is extremely effective at locating electric fishes (e.g., Gymnotiformes in South America, Mormyridae (Osteoglossiformes) in Africa). Once the fishes are located they can be caught by sieves and dip or seine nets.

Removal of structure: In medium to large size rivers, there are often zones near the bank where dead, partially rotten wood and/or lateritic rocks with many holes accumulate. In these habitats, it can be very effective to remove whole pieces of wood and/or lateritic boulders to the shore where they can be broken and their holes explored more thoroughly. Many types of fishes use these structures as hiding places and are often only collected by this method. Additionally, scooping submerged sand from the river bottom into a bucket and dumping it on dry land can sometimes yield specialized sand-dwelling fishes.

Data Management

Species identification, specimen processing and management – Rapid inventories must try to maximize the information collected in the field during a short time period. In fish inventories, fish specimens are traditionally collected and preserved in formaline solution in the field and kept in alcohol solution in the lab. For details of methods for processing and preserving specimens see Motomura & Ishikawa (2013). In more modern days, two additional sources of information collected in fish inventories, tissue samples and photograph of live specimens, should be encouraged in order to help species identification and to increase the current knowledge of fish diversity, evolution, and biogeography through future studies. Tissue should be taken primarily in the field and tagged by sequential numbers linked to individual specimens, which should be photographed for subsequent identification (more details in Motomura & Ishikawa, 2013). Other informative data include photographs of live specimens, which can be taken in the field with specimens anesthetized (details in Lucena *et al*., 2013).

At least one specimen of each species per site should be photographed live, and possibly more than one when specimens of different sizes or dimorphically distinct genders are collected (details on methods in Sabaj Pérez, 2009; Motomura & Ishikawa, 2013). Photographs of each sampling site should also be taken and uniquely identified. Field forms should be filled for each site, including a field number associated to each collection event. Important information that should be included in the field forms are:

- locality,
- municipality/state,
- country,
- geographical coordinates (datum),
- collectors,
- date of collection, and
- general information on the site (e.g., type of substrate, water velocity, amount of underwater and marginal vegetation).

All fishes collected should be deposited in fish collections that are available to the scientific community. If possible, samples should be deposited in more than one fish collection, minimising the risk of losing all vouchers due to eventual disasters, and making data more easily available for the global scientific community. Many countries require, in order to issue permits, that at least 50% of the collected specimens are deposited in fish collections of that country.

Data collection – All data collected during a rapid survey through the herein suggested protocols consist of fish specimens. Additional collecting methods might include other types of data such as observations of fishes in the environment (using dive techniques, for example).

The data collected during a survey should include a species list per site, with number of specimens per species per site, and a species accumulation curve to demonstrate the effectiveness of the sampling. In addition, a survey should identify those species that are endangered species based on IUCN criteria, on national or regional red lists, endemic species, migratory species, potentially new species, invasive species, ornamental and commercial species, and new distribution records. The data obtained thorough standardized sampling should also be used to calculate Diversity and Richness of each site, indices that could be used to make comparisons among areas or in the same collection site at different times. Details on how to analyze data are summarized in Ortega *et al*. (2014).

Conservation Implications and Limitations

Conservation implications – When rapid surveys are effectively executed, the collected data provide information that can be used to recognize areas with high endemism, are well-preserved, or to identify target species for conservation actions. Also, data collected in this way facilitates the understanding of how ecosystems change due to disturbances, including those related to human activities. Diversity and abundance of fish communities can be used to understand the integrity of a studied area, as well as changes over time (Bozzetti & Schulz, 2004). In addition, the presence of indicator species, such as threatened, exotic, or species with particular needs (i.e., forested riparian zones) may help to evaluate the current status of a selected area (Cambray, 2003).

Rapid surveys can provide the chance to collect specimens of poorly known or new species, and improve the knowledge of a particular taxon or an area of interest. In addition, surveys are often performed by multi-institutional teams, strengthening local scientific communities.

Constraints and limitations for rapid survey – The most obvious limitation of rapid surveys is the short amount of time applied towards inventory efforts, which can result in incomplete species lists necessary to devise complete species richness and diversity. This is especially true for large and heterogeneous sampling areas. As a consequence, comparisons among distinct habitats and environments might be complicated by biased collections. However, the core standardized sampling methods proposed here strives to avoid these issues. Also of note, rapid surveys are more effective at sampling common and abundant species, whereas rare species are often missed. Rapid surveys can be supplemented by desktop searches via online fish collection databases (e.g., SpeciesLink, http://www.splink.org.br/index; VertNet: http://vertnet.org), and other regional studies in peer-reviewed published references.

Literature Cited

Anjos, M. B. and J. Zuanon. 2007. Sampling effort and fish species richness in small terra firme forest streams of central Amazonia, Brazil. Neotropical Ichthyology 5(1): 45-52.

Barthem, R. & M. Goulding. 1997. Os bagres balizadores. ecologia, migração e conservação de peixes amazônicos. Tefé: IPAAM, 130p.

Bichuette, M. E. &. L. Trajano. 2003. Epigean and subterranean ichthyofauna from the São Domingos karst area, Upper Tocantins River basin, Central Brazil. Journal of Fish Biology, 63: 110-1121.

Bozzetti, M. & Schulz, U. H. 2004. An index of biotic integrity based on fish assemblages for subtropical streams in southern Brazil. Hydrobiologia, 529(1-3): 133-144.

Cambray, J. A. 2003. Impact on indigenous species biodiversity caused by the globalization of alien recreational freshwater fisheries. Hydrobiologia, 500 (1-3): 217-230.

Chernoff, B., Willink, P. W., *et al*. 1999. Fishes of the Rios Tahuamanu, Manupiri and Nareuda, Depto. Pando, Bolivia: Diversity, Distribution, Critical habitat and Economic Value. Bulletin of Biological Assessment 15: 39-46.

Chernoff, B., Willink, P. W., *et al*. 1999. Geographic and Macrohabitat Partitioning of Fishes in the Tahuamanu-Manuripi Region, Upper Orthon Basin, Bolivia. Bulletin of Biological Assessment 15: 51-67.

Gotelli, N. J. & R. K. Colwell. 2010. Estimating species richness. Pp. 39-54. In: A. E. Magurran & B. J. McGill (eds.). Biological Diversity: Frontiers In Measurement And Assessment. University Press, Oxford, 345p.

Helfman, G. S., B. B. Collette, D. E. Facey & B. W. Bowen. 2009. The diversity of fishes: Biology, Evolution, and Ecology. West Sussex, Wiley-Blackwell, 720p.

Lucena, C. A. S., B. B. Caleagari, E. H. L. Pereira, and E. Dallegrave. 2013. O uso de oleo de cravo na eutanásia de peixes. Boletim Sociedade Brasileira de Ictiologia 105: 20-24.

Matthews, W. J. 1998. Patterns in freshwater ecology. New York: Chapman & Hall, 756p.

Mol, J. H., P. Willink, B. Chernoff, and M. Cooperman. 2006. Fishes of the Coppename River, Central Suriname Nature Reserve, Suriname. Bulletin of Biological Assessment 39: 67-79.

Motomura, H. and S. Ishikawa (eds.). 2013. Fish Collection Buildings and Procedures Manual. The Kagoshima University Museum, Kagoshima and the Research Institute for Humanity and Nature, Kyoto, 70p.

Nelson, J. S. 2006. Fishes of the World. 4th edition. John Wiley & Sons, Hoboken, New Jersey, 601p.

Ortega, H. et. al. 2014. Necton (Peces). In: Universidad Mayor de San Marcos. Museo de Historia Natural. Métodos de colecta, identificación y análisis de comunidades biológicas: plancton, perifiton, bentos (macroinvertebrados) y necton (peces) en aguas continentales en Perú. Lima. Zona Comunicaciones, 1st edition, pp. 46-58.

Sabaj Pérez, M. H. 2009. Photographic atlas of fishes of the Guiana Shield. In: Vari, R. P., C. J. Ferraris, Jr., A. Radosavljevic, and V. A. Funk (eds). Checklist of the freshwater fishes of the Guiana Shield. Bulletin of the Biological Society of Washington, 17: 53-93.

Strahler, A. N. 1957. Quantitative analysis of watershed geomorphology. Transactions, American Geophysical Union, 33(6): 913-920.


Photo © Pete Oxford