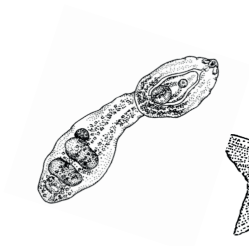
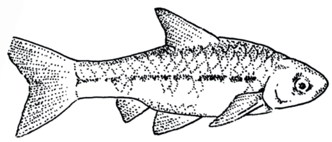
PART 2

FISH AS HOSTS OF PARASITES, THEIR ECOLOGY AND SAMPLING

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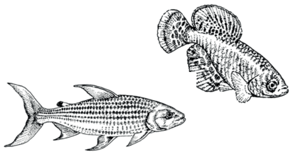


Chapter 2.1.

FISH DIVERSITY AND ECOLOGY

Martin REICHARD

Diversity of fishes in Africa

Fishes are the most taxonomically diverse group of vertebrates and Africa shares a large portion of this diversity. This is due to its rich geological history – being a part of Gondwana, it shares taxa with the Neotropical region, whereas recent close geographical affinity to Eurasia permitted faunal exchange with European and Asian taxa. At the same time, relative isolation and the complex climatic and geological history of Africa enabled major diversification within the continent. The taxonomic diversity of African freshwater fishes is associated with functional and ecological diversity. While freshwater habitats form a tiny fraction of the total surface of aquatic habitats compared with the marine environment, most teleost fish diversity occurs in fresh waters. There are over 3,200 freshwater fish species in Africa and it is likely several hundreds of species remain undescribed (Snoeks et *al.* 2011). This high diversity and endemism is likely mirrored in diversity and endemism of their parasites.

African fish diversity includes an ancient group of air-breathing lungfishes (*Protopterus* spp.). Other taxa are capable of breathing air and tolerate poor water quality, including several clariid catfishes (*e.g.*, *Clarias* spp.; Fig. 2.1.1D) and anabantids (*Ctenopoma* spp.). Africa is also home to several bichir species (*Polypterus* spp.; Fig. 2.1.1A), an ancient fish group endemic to Africa, and bonytongue *Heterotis niloticus* (Cuvier, 1829) (Osteoglossidae), a basal actinopterygian fish. Special adaptations of particular fishes are expected to affect parasite communities.

Functional diversity of African freshwater fishes includes specialised predatory tigerfishes (*Hydrocynus* spp.; Fig. 2.1.1K), weakly electric elephantfishes (Mormyridae; Fig. 2.1.1C), electric catfishes (*Malapterurus* spp.; Fig. 2.1.1B), pufferfishes (*Tetraodon* spp.; Fig. 2.1.1I) and many other specialised forms. Among other unique fishes, Africa has its blind cave fish (*Caecobarbus geertsii* Boulenger, 1921), miniature fishes from rainforest streams (*e.g.*, *Barboides britzi* Conway et Moritz, 2006), small annual killifishes (*Nothobranchius* spp.; Fig. 2.1.1E) that survive annual desiccation of their habitat as dormant embryos encased in dry substrate, or brood parasites that parasitise mouth brooding cichlids and use them as foster parents for their offspring (cuckoo catfish, *Synodontis multipunctatus* Boulenger, 1898; Fig. 2.1.1F). Large functional diversity can evolve even at small temporal and spatial scales, such as in haplochromine cichlids in Lakes Victoria, Malawi and Tanganyika and species of *Labeobarbus* Rüppell, 1835 (Cyprinidae) in

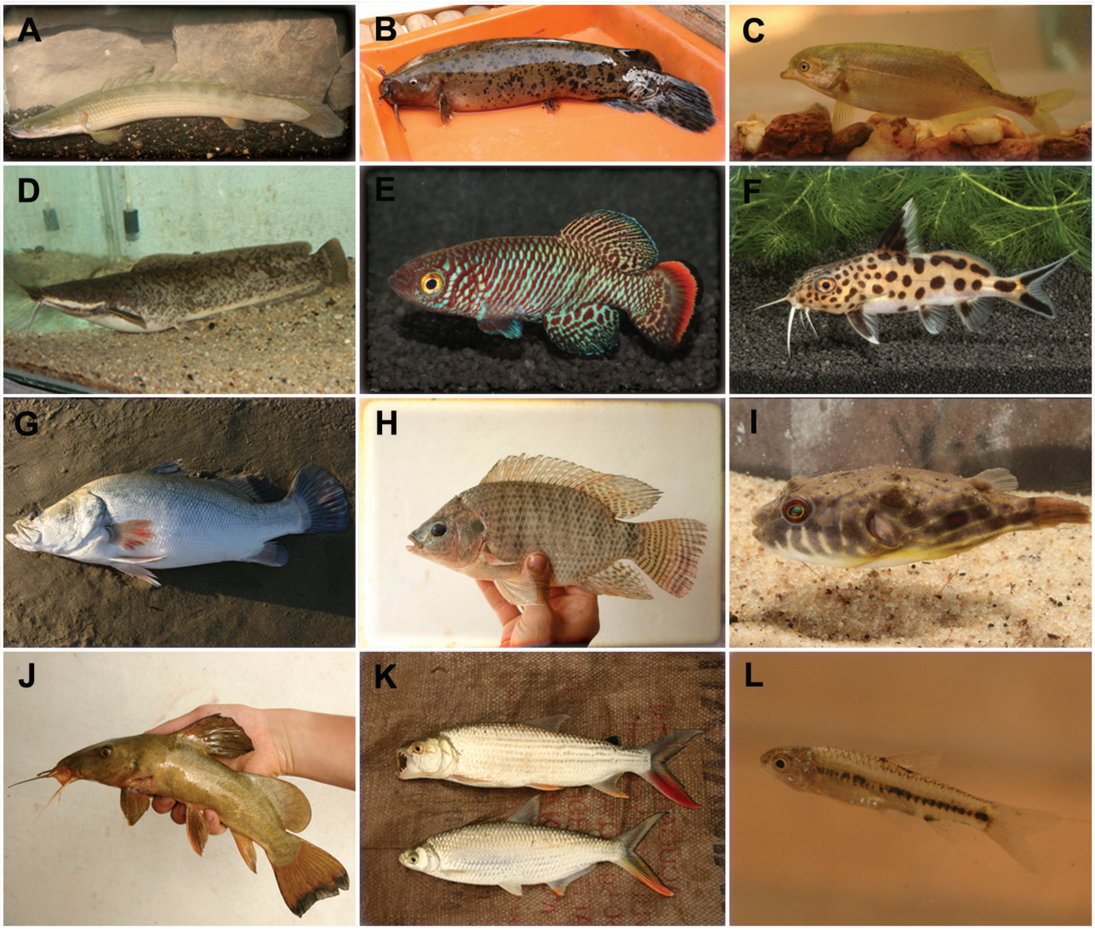


Fig. 2.1.1. Examples of African freshwater fish diversity. **A.** *Polypterus bichir* (Polypteridae); **B.** *Malapterurus occidentalis* (Malapteruridae); **C.** *Marcusenius senegalensis* (Mormyridae); **D.** *Clarias gariepinus* (Clariidae); **E.** *Nothobranchius pienaari* (Nothobranchiidae); **F.** *Synodontis multipunctatus* (Mochokidae); **G.** *Lates niloticus* (Latidae); **H.** *Oreochromis niloticus* (Cichlidae); **I.** *Tetraodon lineatus* (Tetraodontidae); **J.** *Auchenoglanis occidentalis* (Claroteidae); **K.** *Hydrocynus brevis* and *Alestes baremoze* (Alestidae); **L.** *Enteromius niokoloensis* (Cyprinidae). (Photographs by R. Blažek and M. Reichard.)

Fig. 2.1.2. Map of Africa with identification of 10 main ichthyofaunal regions. **1**. Maghreb Province; **2.** Nilo-Sudanian Province; **3.** Congo Province; **4.** Upper Guinea; **5.** Lower Guinea; **6.** Zambezi Province; **7.** East Coast Province; **8.** Southern (Cape) Province; **9.** Quanza Province; **10.** Abyssian Highlands Province (according to Thieme *et al.* 2005). The base map is from Wikimedia Commons: Bamse (self-made) using GMT, CC BY-SA 3.0.

Lake Tana. How such small-scale diversification rates are translated into parasite diversification remains largely unexplored.

The fish diversity in Africa is subject to intense scientific interest, with special attention to understanding their evolution, biology and adaptations, and to explore fish as a resource for local small-scale fisheries and larger scale commercial activities. A better understanding of the diversity and importance of the fish parasite fauna should be based on solid background knowledge of African fish biology and taxonomy.

Zoogeography

The major ichthyofaunal provinces are separated into 10 main continental regions (Roberts 1975; Snoeks *et al.* 2011; Fig. 2.1.2) and Madagascar, though a finer scale resolution to 93 freshwater ecoregions is also available (Thieme *et al.* 2005). The Maghreb Province is the most distinct African ichthyofaunal province. As part of the Palearctic realm, its ichthyofauna displays a high similarity with the European fish fauna (*e.g.*, *Barbus* spp., *Salmo trutta* Linnaeus, 1758, *Cobitis* sp.). The largest province is the Nilo-Sudanian Province, spanning from the River Gambia in the West to the Kenyan coastal drainage in the East. It includes major rivers such as the Nile, the Niger and the Volta, as well as the Lake Chad Basin in its centre. The Congo Province includes the entire drainage of the Congo River, the second largest river basin in the world, with a very high species richness and diversity. It also includes Lake Tanganyika. Two other West African provinces are the Lower and Upper Guinea, separated by the Dahomey Gap and the Volta River. The Upper Guinea includes the coastal rivers of the West African forest region, whereas the Lower Guinea is adjacent to the Congo Province. These regions have been well researched and their ichthyofauna is relatively well known. The Zambezi Province includes rivers flowing eastward to the Indian Ocean from the Zambezi Basin in the North to the Limpopo Basin in the South. It also includes the Okavango Basin. Geographically, Lake Malawi is part of this system, though it has a unique lacustrine ichthyofauna. The East Coast Province includes smaller rivers flowing eastward along the coast of northern Mozambique, Tanzania and southern Kenya, and includes Lake Victoria, with its unique haplochromine cichlid fauna and other lakes in the region. The Southern (Cape) Province includes many temperate rivers south of the Zambezi Province. It has a small number of native (autochthonous) species compared to other provinces (42) and higher-order taxa, but species in the province are often endemic (36 endemic species). The Quanza Province includes a small region of coastal Angolan rivers, with their ichthyofauna being largely unexplored. Finally, the small Abyssian Highlands Province is composed of Lake Tana (with its intra-lacustrine radiation of *Labeobarbus*) and adjacent parts of the effluent rivers.

Main families of fishes

Almost all African freshwater species are continent-endemic and over 40% of the 76 families are restricted to the African continent, which is a relatively high level of endemism at family level. Cichlidae is the most species-rich family (at least 900 species), with the main species diversity in the lakes of East Africa (Tanganyika, Malawi, Victoria), though the number of riverine cichlid species is also high. Cyprinidae (almost 500 species) are typically riverine fishes whose species diversity outnumbers that of the ecologically similar Alestidae (African tetras) by a factor of four (approximately 120 species). Distichodontidae is an endemic family to Africa containing 101 described species. The catfishes are dominated by squeaker catfishes (Mochokidae, 209 species), Claroteidae (86 species) and air-breathing Clariidae (approximately 75 species in Africa). Killifishes are separated into Nothobranchiidae (262 species) and Poeciliidae (about 65 egg-laying species in Africa). The endemic and weakly electric elephantfishes (Mormyridae) include 221 described species (Froese & Pauly 2017). There are also several species from widespread families such as Gobiidae, non-endemic families such as Galaxiidae (in the Southern Province) and many small families endemic to Africa (*e.g.*, Hepsetidae, Pantodontidae). An overview of main freshwater fish families in Africa with estimates of their species richness, general distribution and abundance is shown in Table 2.1.

Ecological guilds

African fishes inhabit all available ecological niches, with examples of species adapted to pelagic and benthic habitats, to strong rapids, swamps, temporary habitats, river margins and deep lacustrine habitats repeated in numerous taxa. Africa harbours native catadromous and anadromous migratory fishes (*e.g.*, Anguillidae) and species with a largely nocturnal lifestyle (*e.g.*, Mormyridae). Several species possess weakly poisonous glands in proximity to sharp fin rays (Mochokidae) and appropriate care should be taken when handling them. Ecological guild largely dictates fish lifestyle and this should aid in the choice of appropriate sampling techniques.

Commercially important fish

Several native African species are commercially exploited in aquaculture in Africa itself and in other continents. Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) (Fig. 2.1.1H) and a few related species and hybrids are the most popular species in tropical aquaculture, and Nile tilapia is successfully cultured across Africa. Information on their parasite fauna and its dynamics may be critical for the success of aquaculture at high population densities. Some species became important for larger-scale fisheries, including many lacustrine cichlids and the Nile perch *Lates niloticus* (Linnaeus, 1758) (Fig. 2.1.1G) in East Africa, West African pygmy herring *Sierrathrissa leonensis* Thys van den Audenaerde, 1969 in Lake Volta and Tanganyikan kapenta sardine *Limnothrissa miodon* (Boulenger, 1906) in Kariba and Cahora Bassa reservoirs and in Lakes Kivu and Tanganyika. Many other fishes are important for local sustainable fisheries, such as *Clarias* spp. (Fig. 2.1.1D) or riverine migratory species. Particularly, the larvae of digeneans with a complex life cycle may opportunistically infect commercial species as intermediate hosts and may reduce the commercial value of these species. Their final hosts are predatory fish or birds.

Table 2.1. An overview of main African freshwater fish families with estimates ot their species richness, general distribution and abundance

Order	Family	Species richness	Distribution
Lepidosireniformes	Protopteridae	1 genus, 4 species	widespread
Polypteriformes	Polypteridae	2 genera, 10 species	widespread
Anguilliformes	Anguillidae	5+ species	Indian and Mediterranean drainages
Clupeiformes	Clupeidae	13 genera	widespread, abundant
	Denticipitidae	1 species	restricted: West Africa
Osteoglossiformes	Arapaimidae	1 species	widespread, introductions
	Pantodontidae	1 species	relatively widespread: West Africa
	Notopteridae	2 genera, 3 species	widespread: West Africa
	Mormyridae	221 species	widespread, abundant
	Gymnarchidae	1 species	widespread: West Africa + Soudanian
Salmoniformes	Salmonidae	several introduced species	native to Magreb (1 species), introduced to East and Southern Africa
Osmeriformes	Galaxiidae	1 species	endemic to South Africa
Gonorynchiformes	Kneriidae	4 genera, 10 species	geographically widespread but locally uncommon
	Phractolaemidae	1 species	endemic to West Africa
Hepsetidae	Hepsetidae	1 genus, 6 species	West, Middle and South Africa
Characiformes	Alestidae	19 genera, 119 species	widespread, abundant
	Distichodontidae	17 genera, 101 species	widespread, abundant
	Citharinidae	3 genera, 8 species	widespread, abundant
Cypriniformes	Cyprinidae	500+ species	widespread, abundant
	Nemacheilidae	1 species	endemic to Ethiopia
	Cobitidae	1 species	endemic to Maghreb
Siluriformes	Bagridae	1 genus, 11 species	widespread
	Claroteidae	13 genera, 86 species	widespread, abundant
	Schilbeidae	5 genera, 33 species	widespread, abundant
	Amphiliidae	13 genera, 68 species	widespread
	Clariidae	13 genera, 75 species	widespread, abundant
	Malapteruridae	2 genera, 21 species	widespread
	Mochokidae	9 genera, 200+ species	widespread, abundant
Perciformes	Poecilidae	7 genera, 65+ species	widespread, abundant
	Nothobranchiidae	12 genera, 250+ species	widespread, abundant
	Channidae	1 genus, 3 species	West and Central Africa
	Latidae	1 genus, 9 species	widespread
	Centrarchidae	2 genera, 4 species	introduced in South Africa

	Percidae	1 species	introduced in South Africa
	Nandidae	2 genera, 2 species	West Africa
	Cichlidae	900+ species	widespread, abundant
	Anabantidae	3 genera, 32 species	widespread
	Gobiidae	30+ genera, 90+ species	widespread
	Eleotridae	approx. 25 species	widespread
Synbranchiformes	Mastacembelidae	1 genus, 45 species	widespread
Tetraodontiformes	Tetraodontidae	6 species	geographically widespread

Estimates on taxonomic richness, distribution and abundance are based from Skelton (1988), Darwall *at al.* (2005) and Froese & Pauly (2017).

Non-native species and other threats to local fish fauna

Many fish species were translocated within Africa, especially for commercial use in aquaculture. The best-known examples include Nile tilapia (Fig. 2.1.1H) and Nile perch (Fig. 2.1.1G). Nile tilapia is more aggressive and competitively superior to other tilapias (and many other cichlids that share its ecological niche) and has displaced them from many habitats. In addition, Nile tilapia hybridises with native species of *Oreochromis* Günther, 1889, further threatening their existence. Predictably, transport of Nile tilapia includes transport of their parasites, with potential spill-over and spill-back effects on local fish fauna. Nile perch, native to West African rivers and Lake Turkana, has been introduced to other places to supplement local fisheries, sometimes with catastrophic consequences for the local fish fauna (exemplified by the Lake Victoria case). Other fish species being translocated worldwide can be found in Africa, including Eurasian cyprinids such as common carp *Cyprinus carpio* Linnaeus, 1958, Chinese silver carp *Hypophthalmichthys molitrix* (Valenciennes, 1844) or grass carp (*Ctenopharyngodon idella* Valenciennes, 1844) and several North American centrarchids, including largemouth bass *Micropterus salmoides* (Lacépède, 1802). These species, however, are mainly constrained to relatively colder parts of Africa such as the Southern Province. Research on the parasite fauna of non-native species is interesting as missing parasites are often linked to the success of introductions.

The African fish fauna is also threatened by water pollution (especially inorganic pollution near mining sites and sedimentation from soil erosion), river regulation (dams preventing upstream migration), conversion of wetlands to rice paddies and overfishing in particular habitats. Especially water pollution is expected to have a major impact on the parasite fauna. Notably, heavy metals tend to accumulate in parasites and fish parasites might be used as potential biomarkers for mining-related water pollution (Sures *et al.* 1999).

References

DARWALL, W., SMITH, K., LOWE, T. & VIÉ, J.C. 2005. *The Status and Distribution of Freshwater Biodiversity in Eastern Africa*. Occasional Paper of the IUCN Species Survival Commission, no. 31. IUCN, Gland, 118 pp.

FROESE, R. & PAULY, D. (Eds). 2017. FishBase. Online publication. http://www.fishbase.org

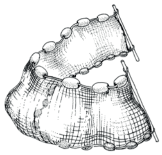
ROBERTS, T.R. 1975. Geographical distribution of African freshwater fishes. *Zoological Journal of the Linnean Society* 57: 249-319.

SKELTON, P.H. 1988. The distribution of African freshwater fishes. *In*: LÉVÊQUE, C., BRUTON, M.N. & SSENTONGO, G.W. (Eds). *Biology and Ecology of African Freshwater Fishes*. Institut français de Recherche scientique pour le Développement en Coopération, Paris, coll. « Travaux et Documents » n° 216, pp. 65-83.

SNOEKS, J., HARRISON, I. & STIASSNY, M. 2011. The status and distribution of freshwater fishes. *In:* DARWALL, W., ALLEN, D., HOLLAND, R., HARRISON, I. & BROOKS, E. (Eds). *The Diversity of Life in African Fresh waters: Under Water, Under Threat. An Analysis of the Status and Distribution of Freshwater Species Throughout Mainland Africa.* International Union for Conservation of Nature, Gland, pp. 42-91.

SURES, B., SIDDALL, R. & TARASCHEWSKI, H. 1999. Parasites as accumulation indicators of heavy metal pollution. *Parasitology Today* 15: 16-21.

THIEME, M.L., ABELL, R., BURGESS, N., LEHNER, B., DINERSTEIN, E. & OLSON, D. 2005. *Freshwater Ecoregions of Africa and Madagascar: A Conservation Assessment*. Island Press, Washington: 431 pp.



Chapter 2.2.

SAMPLING OF FISH FOR PARASITOLOGICAL INVESTIGATION

Pavel JURAJDA

Freshwater habitats

Freshwater ecosystems are highly diverse habitats that vary in size, depth, bottom substrate (rocks, pebbles, mud, sand), chemistry (acidic, neutral, alkaline, oxygen content, productivity, etc.), availability of cover (woody debris, macrophytes, rocks) and character (flowing, standing). Flowing waters include creeks, streams and rivers, whereas standing waterbodies (stagnant or fresh) may be natural (swamps, pools, backwaters and lakes) or man-made (ponds and reservoirs). Canals are a special case of a man-made, standing or slow flowing, sometimes stagnant, waterbody that may have been stocked with fish.

The combination of these geomorphological and chemical factors will dictate not only which species can exist in the waterbodies, but also which fish sampling methods can be applied. Boats are needed for deeper waters, as sampling by wading can be dangerous and inefficient in waters > 1 m with a strong current. Similarly, obstacles such as rocks and dense aquatic vegetation limit the efficiency of methods such as seine netting. Physico-chemical parameters may also limit sampling efficiency, with fish reacting to sampling activity earlier and escaping in clear waters and visibility severely limited in turbid waters. Electrofishing is also ineffective in waters with very low conductivity (low salt content), which is typical for tropical countries.

Sampling methods

Choosing the most appropriate sampling method (Fig. 2.2.1) is critical for the effectiveness of sampling in a given locality and habitat (Figs 2.2.2, 2.2.3). The sampling methods available may be passive or active (Table 2.2). Passive methods generally involve simple and relatively cheap sampling equipment that is placed in the habitat being sampled and left for a defined period. Fish are generally caught as a result of their own movement. Active methods require an operator or team that actively attempts to catch the target fish. Such methods tend to be costlier than passive methods, both in terms of equipment and manpower, but active methods are generally more efficient and less time-consuming than passive methods.

Gill nets (passive; Fig. 2.2.1) are vertical panels of (usually) monofilament nylon netting that are set in a straight line and held in position by weights and floats. Gill nets are very good at intercepting fish that naturally migrate or move frequently;

they are far less effective in catching sedentary or territorial species. Gill nets may have a single mesh size, in which case they are size-specific, or can consist of several panels with different mesh sizes (Nordic gill nets) in order to reduce size selectivity. Gill nets may be placed near the bottom to sample benthic fish, or can be installed nearer the surface to sample mainly pelagic fishes. Gill nets can be used in a wide range of habitats, though their use is generally limited to areas free of obstructions, snags and floating debris, and to localities with little or no current. As monofilament nylon is very hard to see underwater, even in clear waters, animals other than fish may also get caught in the nets. In their efforts to escape, they may become entangled and, if air breathing, may drown. While observers may be able to release smaller animals or unwanted fish, it may be too dangerous to approach larger species such as crocodiles or hippos.

Traps (passive; Fig. 2.2.1) include various types of fyke net, wicker cages and pots, all of which are constructed with a small funnel opening which fish can enter easily but find very difficult to exit. Traps may be baited to increase the probability of success. They are generally used in shallow regions of lakes and reservoirs but can also be used to sample fish in slow-flowing streams, rivers and backwaters. Unlike most other methods, they may also be used in habitats with relatively dense vegetation such as marshes or swamps. Pot traps tend to be most effective in capturing bottom-dwelling species seeking food or shelter. Traps should be checked periodically (at least every 4 to 6 hours) to prevent a build-up of fish, which could lead to fish damage or predation. High densities of trapped fish can

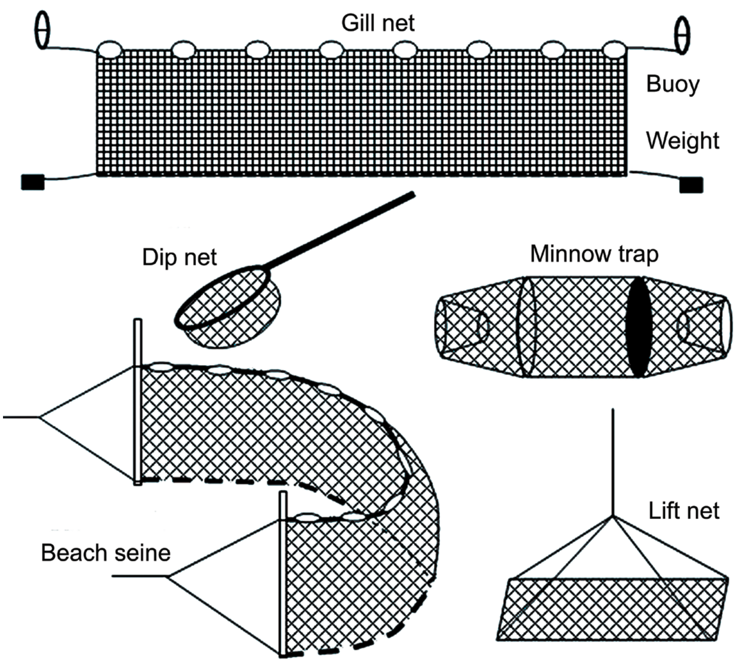


Fig. 2.2.1. Illustrations of some basic active and passive methods of sampling fish.

Fig. 2.2.2. A. A shallow, fast flowing turbid stream, River Nieri Ko, Senegal; **B.** A large slow flowing deep lowland River Gambia, Senegal. (Photographs by R. Blažek.)

Fig. 2.2.3. A. A large deep lake (Lake Tanganyika, Burundi); **B.** A shallow lake with aquatic vegetation, Mozambique. (Photographs by R. Blažek.)

Fig. 2.2.4. A. Beach seining in Lake Turkana, Kenya; **B.** Dip netting in marshes, Mozambique. (Photographs by R. Blažek.)

Fig. 2.2.5. A. Cast net sampling in Lake Turkana, Kenya; **B.** Sampling fish from Uvira fish market, Democratic Republic of the Congo. (Photographs by R. Blažek.)

also lead to the transfer of ectoparasites such as monogeneans between hosts, which may bias subsequent ecological studies.

Rod and line (passive) is a simple angling method that can potentially be used for sampling fish for scientific purposes. Note, however, that use of rod and line tends to be highly selective for particular species (especially predators) and/or sizes of fish, depending on the gear or bait used, and it may take a long time to catch sufficient numbers. On the other hand, the material needed is relatively small and light, making it easy to transport. Efficiency will largely depend on the experience of the user and knowledge of local conditions.

Beach seining (active; Figs 2.2.3, 2.2.4) utilises a large net of uniform mesh size consisting of two ‘wings’ and a ‘purse-like’ central section that holds the catch. As its name implies, a beach seine is typically used in shallow waters, the net generally being set in a semicircle around the target area, either by boat or by wading, and dragging the net back to shore. The net is kept open while deployed by floats on its upper line, while weights on the lower line ensure the net stays close to the bottom substrate. A larger area may be fished by attaching long towing lines to each end of the net. Seine nets are most effective for catching near-shore species or fish that concentrate near-shore periodically. Beaches should preferably be free of obstacles (*e.g.*, rocks, tree stumps) or heavy vegetation.

Push net, dip nets, lift nets and cast nets (active; Figs 2.2.3-2.2.5) are all simple tools that can be used by a single person. Push and dip nets, which are made of netting attached to a round or triangular frame fixed on the end of a pole, are used to collect small fishes along the bank or in places with dense vegetation where other methods may be impractical. Fish are generally pursued by the user. Lift nets or cast nets are used to catch small schooling fish in open waters. Lift nets are left on the bottom (or lowered in deep lakes) by the user who retains hold of the net by a line. Bait (or a light at night) can be used to concentrate fish over the lift net, which are then caught by quickly lifting the net out of the water. Cast nets are circular nets with weighted edges that are thrown so that they cover the fish. The net is closed and retrieved by pulling on a retaining line. The use of cast nets is restricted to areas free of obstacles or plants. Experience is needed to cast the net successfully, allowing it to hit the water completely open over the target fish.

Electrofishing (active) works on the principal of galvanotaxis, whereby direct current (DC, sometimes pulsed) electricity flowing between a submerged cathode and anode causes a muscular convulsion in the fish, causing it to swim toward the anode where it can be caught with a dip net. The cathode, a long, braided steel or copper cable, trails behind the operator and the anode is operated by a switch on a long pole, the operator directing the anode toward the target fish or site. At least two people are required for effective electrofishing, one to operate the anode and the other to catch the fish. The electrical current is produced either by a battery-powered backpack or a petrol-powered generator that remains stationary on the bank or is placed in a boat. The effectiveness of electrofishing will be influenced by a range of biological, technical, logistical and environmental factors. The pulse rate and intensity of the electric field produced can strongly influence the size and nature of catch, whereas conductivity of the water will influence the shape and extent of the electric field, and thus the field's ability to induce galvanotaxis. Electrofishing is limited to sites with clear water, conductivity of 100-600 μS/cm and a depth of ≤ 1 m. Electrofishing is much less effective in waters with low conductivity (low dissolved salt concentration), whereas a stronger generator will be required in waters with high conductivity. Electrofishing is particularly efficient at sites with obstacles (*e.g.*, vegetation, woody debris, rocks) and in running waters, where other methods may be inefficient or impossible to use. In some cases, electrofishing may harm fish by causing muscle spasms that damage the fish's backbone; a problem more common and severe in longer fish. Used correctly, however, electrofishing causes no permanent harm to the fish, which will recover minutes after being caught. Note that the use of any electrical equipment in and around water is dangerous. For operator safety and for efficient and successful sampling, all equipment should be designed specifically for electrofishing and all personnel adequately trained in its use. Many countries also require that the user is licensed. In some countries, electrofishing is illegal.

Table 2.2. A simplified comparison of passive and active methods of sampling fish

	Method	Advantages	Disadvantages
Passive methods	Gill nets, traps, rod and line	Simple manipulation, cheap, low man power, light, easy to transport	Non-selective for species and size, may damage fish, time-consuming, fish may die in gill nets
Active methods (simple)	Dip nets, scoop nets, hand nets, cast nets	Simple manipulation, cheap, low man power, easy to transport, can target specific species/ sizes	Practice needed, less efficient, time consuming
Active methods (technical)	Beach seine, electrofishing	Mobile, faster, can target specific species/ sizes, greater numbers caught	Expensive, practice and/or training needed, transport difficult, higher man power

For more information about fish sampling methods see Bohlin *et al.* (1989), Murphy & Willis (1996), Lapointe & Corkum (2006) and Pierce *et al.* (1990).

Fish sampling strategy for parasite community studies

In comparison with fish population or community studies, different criteria apply when sampling fish for parasitological surveys. Instead of obtaining a general description of fish assemblage structure (*e.g.*, species richness, density, population structure) for the sample site, the operator aims to obtain a representative sample (number) of specific target species and size categories. Sampling area and timing of sampling should be adapted and aimed specifically at where and when the target fish are most likely to occur.

For ectoparasites found on the skin and/or fins, sampling methods should involve minimal handling of the fish as contact could damage or remove the parasites. Appropriate methods include electrofishing, angling, pot traps or small beach seine nets. For endoparasites and gill parasites, more robust sampling methods (*e.g.*, large seine nets) can be used as they are unlikely to affect parasite numbers. It may even be possible to obtain fresh fish from local fishermen or markets.

Fish transport and treatment

Whatever the method used to sample the fish, they should always be handled carefully prior to dissection to prevent loss or transfer of parasites. The fish should be maintained and transported in water taken from the sampling site and only wet hands or dip nets used for manipulating the fish. During transport and storage, oxygen levels should be maintained with an aerator or oxygen cylinder. Fish should be transported to the laboratory as soon as possible and kept alive until examination. Parasitological examination should be carried out no more than three days after capture; any later and parasites may die or reproduce, biasing infection parameters (Kvach *et al.* 2016). During storage, the fish should not be fed and the density kept relatively low to prevent host mortality and transfer of parasites. The tank should be placed in shade to maintain a stable temperature. In the case of untimely mortality, complete freezing should be avoided if possible as it can affect morphological and ultrastructural observations (for transportation or temporary storage, fish may be kept for a short while in crushed ice).

Fish should always be handled with full regard to the animal's welfare (in line with local regulations) and euthanised using the most 'humane' methods available.

References

Bohlin, T., Hamrin, S., Heggberget, T.G., Rasmussen, G. & Saltveit, S.J. 1989. Electrofishing – theory and practice with special emphasis on salmonids. *Hydrobiologia* 173: 9-43.

Kvach, Y., Ondračková, M., Janáč, M. & Jurajda, P. 2016. Methodological issues affecting the study of fish parasites. I. Duration of live fish storage prior to dissection. *Diseases of Aquatic Organisms* 119: 107-115.

LAPOINTE, N.W.R. & CORKUM, L.D. 2006. A comparison of methods for sampling fish diversity in shallow offshore waters of large rivers. *North American Journal of Fisheries Management* 26: 503-513.

MURPHY, B.R. & WILLIS, D.W. 1996. *Fisheries Techniques*. American Fisheries Society, Bethesda, Maryland: 732 pp.

PIERCE, C.L., RASMUSSEN, J.B. & LEGGETT, W.C. 1990. Sampling littoral fish with a seine: corrections for variable capture efficiency. *Canadian Journal of Fisheries and Aquatic Sciences* 47: 1004-1010.