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Trishuli Assessment Tool Field Manual

A Standardized Methodology for Freshwater Aquatic Biodiversity Sampling and Long-Term Monitoring for Hydropower Projects in the Himalayan Region

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Citation:
IFC. 2021. *Trishuli Assessment Tool Field Manual*. International Finance Corporation.
Washington, D.C., USA.

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Foreword

Hydropower projects can be transformational in nature for a variety of reasons. They may produce a step change in electricity supply that supports electrification; they may back the integration of variable renewable energy (VRE); they may bring multipurpose benefits such as flood control or climate change mitigation; or they may support regional integration. It is this transformational nature of hydropower projects that often make them both complex and rewarding to pursue. If planned sustainably, they can provide benefits to local communities.

Hydropower projects have always faced a range of environmental and social problems, but today, it is recognized that the knowledge base and tools are in place to ensure that projects are implemented sustainably and responsibly, following best practices.

In some countries like Nepal, the transformational nature of a project can therefore be in demonstrating this good practice and building capacity to hold future projects to an agreed standard.

Making sure that Nepal's rich biodiversity is conserved while developing large infrastructures such as hydropower projects and dams will be of paramount importance.

Aquatic biodiversity preservation needs even more support. Recent studies found that most hydropower projects are not adequately considering their impacts on the environment, particularly Nepal's important freshwater resources and threatened aquatic species. Basin planning based on strategic environmental and social impacts is often missing. Hydropower EIAs need to more robustly assess aquatic resources and biodiversity to properly assess impacts and develop mitigation to help maintain freshwater resources while developing hydropower in Nepal.

To this end, the Trishuli Assessment Tool provides a standardized approach that will enhance hydropower project EIAs and promote monitoring of aquatic resources, helping in aquatic biodiversity conservation. The World Bank and IFC encourage hydropower projects to consider adopting an approach such as that offered in this field manual to adequately assess and monitor aquatic biodiversity. Robust environmental and social assessment is the first step in ensuring good practice for planning and implementing sustainable hydropower that will benefit Nepal's people while safeguarding its natural environment.

Pravin Karki
Global Lead for Hydropower & Dams
The World Bank Group, USA

Foreword

Wisdom on freshwater resource management of the Himalayan region is crucial for sustainable development in most Trans-Himalayan countries. Developing hydropower in the region faces many challenges, including climate change and the preservation of globally threatened fish species. In recent years, decommissioning of hydropower dams due to safety, law, policy, economy, and ecology has even become a trend in other parts of the world. Yet, hydropower development, if done properly by taking into account a deeper understanding of fish migration patterns and ecosystem services in mid-hill rivers, could facilitate sustainable energy production. Despite a proliferation of hydropower projects in the Himalayas, knowledge of fish behaviors in high-altitude areas remains rudimentary. The water basins of the mid-hills with many endemic fishes are also highly feasible areas for hydropower projects. Thus, caution, along with wisdom, is required to protect endemic and migratory fish species.

It is a matter of great acknowledgment that many international lenders, such as IFC and the World Bank, require hydropower projects to avoid a net loss of biodiversity values for critical habitats. Such wisdom should be adopted by other lenders and institutions for sustainable hydropower development. Recent studies have highlighted the importance of the development of fish sanctuaries as well as declaration of national parks for conserving rare, vulnerable, endemic, and key fish species close to hydropower locations.

The Trishuli Assessment Tool—developed following a workshop held in Nuwakot—shares new information and approaches for conducting proper environmental impact assessments (EIAs) of hydropower projects. Designed to collect and analyze field data, the tool provides a standardized approach to enhance the hydropower EIAs for monitoring aquatic biodiversity focusing on fish, macroinvertebrates, and periphyton. The tool describes sampling and interpretation methods in simple, precise, and clear language, which should be highly useful and practical for those who need to perform such EIAs. I would like to congratulate all associated authors and IFC for bringing this important publication to fruition.

Tek Bahadur Gurung
Adjunct Professor, Fisheries Program, Agriculture and Forestry University
Former Executive Director, Nepal Agricultural Research Council

Acknowledgments

This field manual is based on the Trishuli Assessment Tool, which was developed at a workshop in November 2019 by more than 30 international and Nepalese aquatic biology specialists, government scientists, and hydropower environmental staff, as part of IFC's Nepal Hydropower Environmental and Social Advisory Program in Nepal. The tool was field tested on the Trishuli River in February 2020. The project was led by a team of aquatic biodiversity specialist consultants hired by IFC:

- ***William Beaumont***, Electric Fishing Technical Services Ltd., United Kingdom
- ***Julie Claussen***, Fisheries Conservation Foundation, USA
- ***Dibesh Karmacharya***, Center for Molecular Dynamics Nepal
- ***David Philipp***, Fisheries Conservation Foundation, USA
- ***Adrian Pinder***, Bournemouth University, United Kingdom
- ***Adarsh Man Sherchan***, Center for Molecular Dynamics Nepal
- ***Deep Narayan Shah***, Central Department of Environmental Science, Tribhuvan University, Nepal
- ***Ram Devi Tachamo Shah***, Aquatic Ecology Centre, Kathmandu University, Nepal
- ***Gina Walsh***, Independent Consultant, South Africa

The Trishuli Assessment Tool and preparation of this field manual were initiated and coordinated by IFC Biodiversity Consultant Leeanne E. Alonso as part of IFC's Nepal Hydropower Environmental and Social Advisory Program, led by Senior Asia Environmental, Social, and Governance Advisory Lead Kate Lazarus.

Many thanks to Adrian Pinder for assisting with the data analysis section, Upasana Shrestha for logistical support, Helen Ho Yan Luk for editing, and Martinus Johannes for design. We are grateful to William Beaumont for providing electrofishing guidance in Appendixes F and G as well as numerous reviewers for their helpful comments. We would also like to thank the governments of Australia, Norway, and Japan for their support.

Special thanks to all participants of the Trishuli Assessment Tool workshop including:

- ***Nurendra Aryal***, Department of National Parks and Wildlife Conservation, Ministry of Forests and Environment, Nepal
- ***Ashok Baniya***, Nepal Water and Environment Development Corporation Upper Trishuli – 1 Hydropower Project, Nepal
- ***Auras Bhandari***, Nepal Water and Environment Development Corporation Upper Trishuli – 1 Hydropower Project, Nepal
- ***Tara Datt Bhatt***, Nepal Electricity Authority Training Center
- ***Milan Dhungana***, Ministry of Forests and Environment, Nepal
- ***Prakash Gaudel***, Nepal Electricity Authority
- ***Tek Bahadur Gurung***, Nepal Agricultural Research Council
- ***Bibhuti Ranjan Jha***, Kathmandu University, Nepal
- ***Janak Kumar Jha***, Water and Energy Commission Secretariat, Nepal
- ***Baburaja Maharjan***, Nepal Electricity Authority
- ***Raj Kapur Napit***, Freelance Consultant, Nepal

- ***Umesh Pathak***, Upper Sanjen Hydropower Project, Nepal
- ***Mark Pedersen***, IFC, USA
- ***Nikita Pradhan***, Center for Molecular Dynamics Nepal
- ***Shankar Pyakurel***, Trishuli Hydropower Company, Nepal
- ***Asha Raymajhi***, Fisheries Research Division, Ministry of Agriculture and Livestock Development, Nepal
- ***Subodh Sharma***, Kathmandu University, Nepal
- ***Anjana Shrestha***, Center for Molecular Dynamics Nepal
- ***Rabindra Timilsina***, Swet Ganga Hydropower & Construction Ltd., Nepal
- ***Suresh Wagle***, Program on Aquatic Natural Resources Improvement, United States Agency for International Development, Nepal
- ***Rakesh Yadav***, Freelance Consultant, Nepal Environmental and Scientific Services, Nepal

Participants at the November 2019 workshop where the Trishuli Assessment Tool was developed.

Acronyms and Abbreviations

AC	Alternating current
APHA	American Public Health Association Inc.
BMEP	Biodiversity monitoring and evaluation program/plan
CPR	Cardiopulmonary resuscitation
CMDN	Center for Molecular Dynamics Nepal
CPUE	Catch per unit effort
CSBI	Cross-Sector Biodiversity Initiative
DC	Direct current
eDNA	Environmental DNA
EDTA	Disodium ethylenediaminetetraacetic acid
EFlow	Environmental flow
EIA	Environmental impact assessment
EPT	Ephemeroptera, Plecoptera, and Trichoptera
ESIA	Environmental and social impact assessment
FRTC	Forest Research and Training Center
GBIF	Global Biodiversity Information Facility
GIS	Geographic information system
GPS	Global positioning system
HPP	Hydropower project
IFC	International Finance Corporation
IUCN	International Union for Conservation of Nature
MoFE	Ministry of Forests and Environment
NNL	No net loss
RTU	Recognizable taxonomic unit

Units

cm	centimeter
Hz	hertz
M	molarity (moles per liter)
m	meter
m²	square meters
ml	milliliter
mm	millimeter
mm²	square millimeters
MW	megawatt
µm	micrometer

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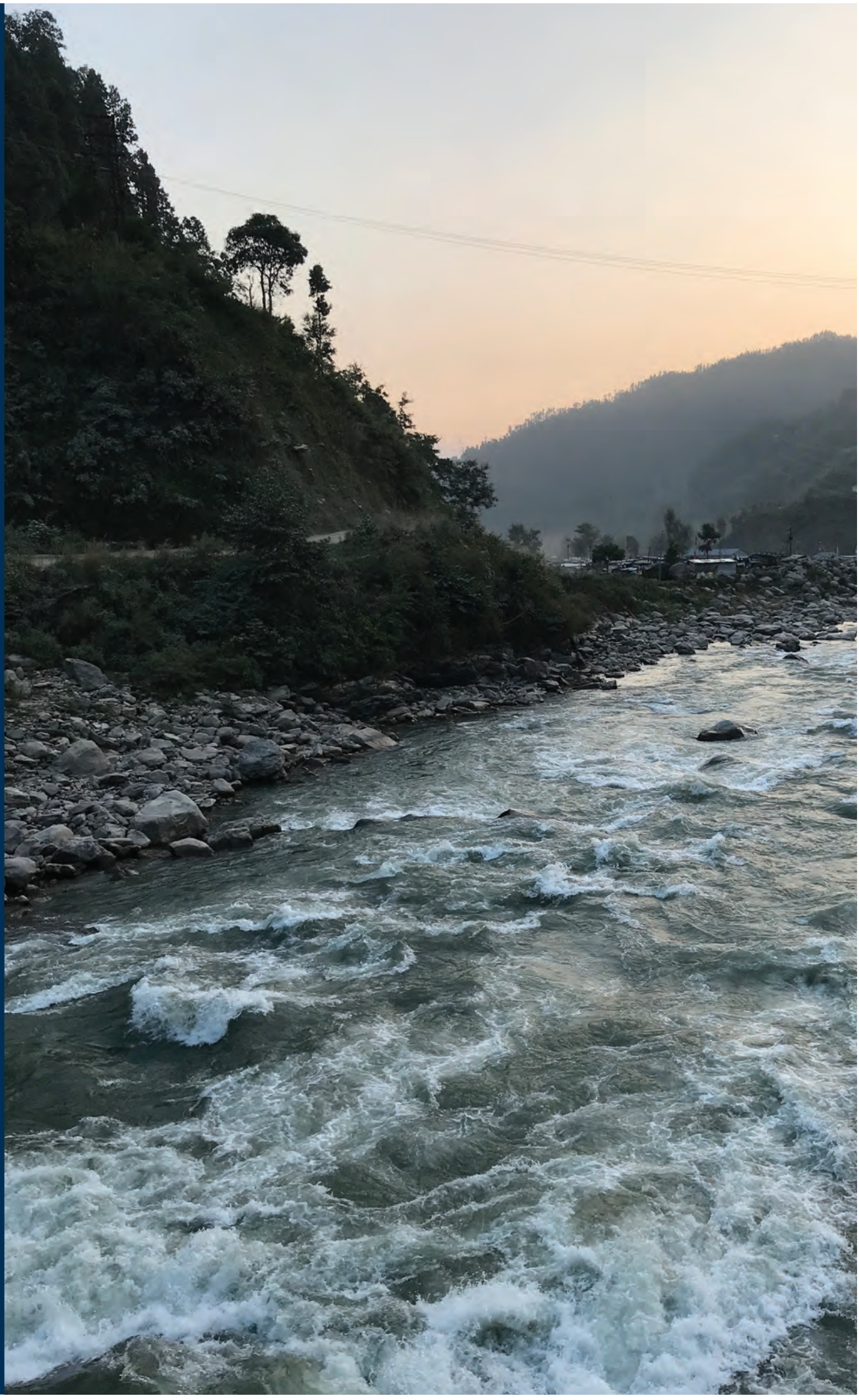


1 Introduction

1.1 Overview and Applications of the Trishuli Assessment Tool

The Trishuli Assessment Tool is a standardized methodology for sampling freshwater aquatic biodiversity in hydropower projects. This tool was developed to: 1) strengthen the collection of aquatic biodiversity data for environmental impact assessments (EIAs)[1] and international-level environmental and social impact assessments (ESIAs) and 2) provide a simple yet standardized method for the long-term monitoring of aquatic biodiversity in relation to hydropower projects.

The Trishuli Assessment Tool project is a follow-up to the cumulative impact assessment of the Trishuli River Basin led by IFC (2020), which identified the need for more robust and standardized sampling of aquatic biodiversity when planning hydropower projects. The tool was developed by a group of 30 international and Nepalese aquatic scientists at a workshop in 2019 and tested during a field survey in 2020 (Philipp et al. 2020; IFC 2021). It provides a field sampling methodology for three focal groups of aquatic biodiversity: fish, macroinvertebrates, and periphyton as indicators of overall aquatic biodiversity. The collected data document species richness and relative abundance of fish and macroinvertebrates as well as provide a measure of the status and health of the aquatic ecosystem. For fish, the group evaluated and field tested many aquatic sampling methods and concluded that the following methods are best for assessing and monitoring fish in the Himalayan region: backpack electrofishing, cast nets, dip nets, underwater video, and environmental DNA (eDNA). This field manual provides guidance for implementing the Trishuli Assessment Tool in the rivers of Nepal and other Himalayan regions.

For fish, the field methods include cast nets, which are typically used to collect freshwater fish for hydropower EIAs in Nepal. Studies have shown that cast nets alone are only moderately effective for catching fish, thus many fish species are missed. The tool adds the method of electrofishing, which is highly effective for sampling fish but requires training and can only be used in low-flow and clear waters, such as tributaries. Additional methods of dip nets and underwater video add data for tributaries. The emerging technology of environmental DNA (eDNA) is also part of the field methodology, as it can be extremely effective at detecting species that are not captured by other field methods.

This field manual is ideal for use by environmental staff, consultants, researchers, academics, and government agencies to collect robust data for EIAs and monitor aquatic habitats and biodiversity to evaluate impacts of hydropower projects and the success of mitigation measures. The data analyses presented in this manual allow hydropower projects to track changes in specific indicators between the pre-construction baseline and the construction and operational stages. This can help demonstrate if a hydropower project's mitigation measures successfully maintain aquatic biodiversity, resulting in no net loss or even a net gain of biodiversity values to comply with government and international lender requirements.[2]

This manual is applicable to all types of hydropower projects (HPPs), from small run-of-river to larger peaking projects because all of them have some impact on the aquatic environment. Evaluating and monitoring aquatic biodiversity before, during, and after construction of an HPP provides essential data to guide the project on how to reduce its impacts on the aquatic environment.

[1] For the purpose of this manual, EIAs cover both national-level and international-level EIAs. Where used, an EIA refers to international lender requirements and Good International Industry Practice.

[2] International lenders include institutions such as the World Bank, IFC, Asian Development Bank, Asian Infrastructure Investment Bank, Korea Exim Bank, CDC Group, and Japan International Cooperation Agency.

1.2 Trishuli Assessment Tool at a Glance

Objectives and application	Standardized field methodology to:
	• Collect robust baseline of aquatic biodiversity for hydropower EIA
	• Monitor a set of aquatic indicators over time to assess:
	  • Changes in target groups during HPP cycle:
	  · Fish · Macroinvertebrates · Periphyton
	  • Success of hydropower project mitigation measures
	  • No net loss or net gain of biodiversity (international lenders)

EIA sampling regions and sites	Three sampling regions, each with multiple sampling sites: • Upstream of dam (including reservoir, main stem, and spawning tributaries) • Diversion reach between dam and powerhouse • Downstream of powerhouse (main stem and tributaries)
EIA sampling seasons	EIA baseline sampling should be done as often as possible. Three seasons listed below should be sampled as minimum: • Fall (post-monsoon): October to November • Winter (post-monsoon): January to February • Spring (pre-monsoon): March to May
Long-term monitoring sampling sites	Long-term monitoring sampling sites should be selected based on the EIA results to track important biodiversity indicators in locations where project impacts are expected and mitigation measures are implemented.
Long-term monitoring sampling seasons	Long-term monitoring should include at least two seasons per year: • Fall (post-monsoon): October to November • Winter (post-monsoon): January to February A third season should be included when possible, especially for fish migrations: • Spring (pre-monsoon): March to May
Target taxa	• Fish (all species and target fish species) • Macroinvertebrates • Periphyton
Field sampling methods	Fish field sampling methods that should be used, where feasible, for each site: • Backpack electrofishing • Dip net • Cast net • Environmental DNA (eDNA) • Underwater video Macroinvertebrates and periphyton standardized field sampling method developed for Nepal by Tachamo Shah et al. (2020a): • Macroinvertebrates: multihabitat sampling using kick net • Periphyton: stone scrubbing
Data analysis metrics	Fish metrics: 1. Species richness 2. Species composition 3. Proportion of species 4. Species distribution 5. Relative abundance of target fish species 6. Recruitment of target fish species 7. Length of target fish species Macroinvertebrate metrics: 1. Taxa richness 2. Ephemeroptera, Plecoptera, and Trichoptera (EPT) index 3. Proportion of functional feeding groups Periphyton metric: 1. Dry biomass
Field sampling team	Sampling team should include (as appropriate): • Fish specialist(s) trained and experienced in electrofishing • Fish specialist(s) with expertise in identification of Himalayan fish species • Macroinvertebrate specialist capable of identification of species • Students or field assistants experienced with sampling and processing of fish or macroinvertebrates • Local fishermen with expertise in cast-netting

1.3 Why Sample and Monitor Freshwater Aquatic Biodiversity

Hydropower projects have significant impacts on the aquatic ecosystem and the organisms living in the river basin. HPPs change the river flow, quantity, timing, water chemistry, and water temperature as well as create blockages to migrating fish and other organisms moving upstream and downstream in the river. Altering flows change the aquatic habitats and often create conditions for predators, invasive fish, plants, and other organisms to flourish in the new flow conditions above the dam, in the diversion reach, and below the powerhouse.

"Biodiversity monitoring is the process of determining the status of and tracking changes in living organisms and the ecological complexes of which they are a part. Biodiversity monitoring is important because it provides a basis for evaluating the integrity of ecosystems, their responses to disturbances, and the success of actions taken to conserve or recover biodiversity. Research addresses questions and tests hypotheses about how these ecosystems function and change and how they interact with stressors," according to the *Canadian Biodiversity Ecosystem Status and Trends 2010* report (Federal, Provincial and Territorial Governments of Canada 2010).

By Nepalese government regulations and laws as well as international lenders' standards, hydropower projects are required to implement mitigation actions to avoid or reduce project impacts on the environment, particularly on aquatic species and habitats, to protect aquatic animals—Aquatic Animal Protection Act, 2017 (1960)— and support biodiversity. Mitigation actions typically include: 1) releasing an environmental flow (EFlow) at all times to ensure sufficient water is available in the river for aquatic species, 2) building a fish ladder to allow migratory fish to pass the dam, and 3) captive breeding of native fish species and stocking. Other mitigations may include aquatic habitat restoration or modifications, regulations on fishing in the reservoir, trapping and trucking of fish upstream, and measures to ensure safe fish passage downstream over or through the dam (Adeva-Bustos et al. 2021).

Government agencies and international lenders require long-term monitoring to demonstrate successful implementation of the mitigation measures and the sustainability of the aquatic ecosystem during the construction and operational phases of an HPP. A 2020 World Bank review of 50 hydropower projects in Nepal (Shah et al. 2020) found that none of them have conducted any monitoring of the aquatic ecosystem and biodiversity to evaluate project impact or the success of their mitigation actions. An Asian Development Bank study (ADB 2018) of the potential impacts of damming of rivers in Nepal on aquatic biodiversity revealed inadequate assessment and monitoring.

While most EIAs of hydropower projects in Nepal include some field sampling of aquatic biodiversity, the geographical coverage, taxonomic groups, sampling effort, field methodology, and data analysis vary greatly across project EIAs and are usually minimal (Shah et al. 2020). There is a clear need for a field methodology that promotes the use of robust and standardized methods to document aquatic biodiversity for Nepal's Himalayan rivers.

As more HPPs are built on Nepal's rivers, monitoring of the impacts and changes in the aquatic ecosystem are essential not only for the survival of the aquatic species but also for ensuring good water quality and healthy aquatic ecosystems for future generations of Nepalese.

1.4 Questions Addressed with the Trishuli Assessment Tool

The Trishuli Assessment Tool focuses on data collection and analysis for fish, and macroinvertebrates, and periphyton to answer the following questions:

For an EIA baseline (pre-construction phase):

1. Which species are there? (richness and composition)
   a. Species lists
   b. Number of species
2. How many individuals are there? (relative abundance)
   a. Number of individuals per species collected
3. Where are the species and individuals located? (distribution)
   a. Map of species distributions
   b. Map of relative abundance
4. Recruitment (reproductive success)
   a. Relative abundance of juveniles
   b. Fish sizes
5. Aquatic ecosystem health and water quality
   a. Macroinvertebrate indexes
   b. Periphyton biomass

For long-term monitoring of a hydropower project during the construction and operational phases, questions that can be addressed with the Trishuli Assessment Tool include:

1. What impact is the hydropower project having on aquatic biodiversity?
2. Are the project mitigation measures working to reduce project impacts?
3. Is no net loss or net gain achievable for the aquatic biodiversity indicators?

To answer these questions, the following are assessed with field data:

1. How do fish and macroinvertebrate species vary between the pre-construction, construction, and operational phases?
   a. Number of species
   b. Community composition (including presence of invasive species)
   c. Relative abundance of all species
   d. Distribution of species
   e. Relative abundance of target fish species such as:
      i. Mahseer species
      ii. Snow trout species
   f. Recruitment of target fish species
2. How do indicators of aquatic ecosystem status and health vary over time?
   a. Macroinvertebrate indexes
   b. Periphyton biomass

2 Field Methodology

2.1 Sampling Design for Environmental Impact Assessment

2.1.1 What to Sample for the EIA—Aquatic Biodiversity Indicators

The Trishuli Assessment Tool focuses on sampling three crucial elements of aquatic biodiversity:

- Fish
- Macroinvertebrates
- Periphyton

These three aquatic biodiversity groups were selected because they are abundant, play key roles in the aquatic ecosystem in Nepal's Himalayan rivers, and provide ecosystem services such as food to local people. They serve as good indicators for monitoring due to their sensitivity to specific changes within the aquatic ecosystem. Selection of target species within these groups is recommended to focus on species of conservation concern or those that may be at higher risk from project impacts. Within the fish group, two target species that are globally threatened and distributed throughout the Himalayan river basins are recommended: the mahseer species, including the golden mahseer (*Tor putitora*) and *Tor tor*, and snow trout species, particularly the common snow trout (*Schizothorax richardsonii*). **Table 2.1** outlines the key aspects of these groups.

Table 2.1 Key Aspects of Monitoring the Three Target Groups

Taxon	Description and importance	Sensitive to changes in	Aspect to monitor

Fish	Prominent aquatic vertebrates and top predators, including threatened species; serve as a commercially important food source	• River flow rate and depth
		• Water temperature
		• Habitat for spawning
		• Connectivity
		• Food availability
			• Species richness
			• Species composition
			• Relative abundance, maturity stage, and distribution of selected fish species:
			  • Mahseer species
			  • Snow trout species

Macroinvertebrates	Aquatic invertebrates larger than 500 micrometer (µm), including insects, crustaceans, mollusks, and annelids that serve as food sources for fish, birds, and other animals; serve important functions within the aquatic ecosystem, such as breaking down organic matter as well as filtering and cleaning the water	• River flow rate and depth
		• Water temperature
		• Sediments
		• Riverbed substrate
		• Organic matter
			• Community composition
			• Relative abundance of key taxa:
			  • Ephemeroptera
			  • Plecoptera
			  • Trichoptera
			• Composition of functional feeding groups

Periphyton	Blue-green algae, fungi, microbes, bacteria, plant detritus, and animals that cling to rocks and other substrates; serve as the basis of the aquatic ecosystem food chain	• River flow rate and depth • Water temperature • Sediments • Rocks	Dry biomass

2.1.2 Where to Sample for the EIA—Sampling Sites

Sampling Regions

An aquatic survey for an EIA of a hydropower project should include sites where impacts from the project may occur. These sites are generally located in three sampling regions:

- Upstream of the HPP, including the reservoir area
- Diversion reach between the dam and the powerhouse (for HPPs with a diversion reach)
- Downstream of the powerhouse, especially for peaking projects

Sampling Sites

Within each of these regions, sampling sites should include:

- The main stem river
- Large tributaries at least 300 meters (m) from the confluence with the main river; also upstream if tributary is not affected by other dams
- Small tributaries at least 300 meters from the confluence with the main river
- Key sites for fish spawning and larval nursing grounds, often upstream in the tributaries and at the end of the tributary just before it meets the main river
- Key aquatic habitats for macroinvertebrates (for example, diverse riverbed habitats including different flow types)

Selecting Sampling Sites

Sampling sites should be selected by first evaluating and mapping all the main aquatic habitats in the project area using available satellite imagery and field reconnaissance. Access and safety are important considerations for site selection. The aquatic habitats (**Figures 2.1** and **2.2**) include:

- Rapids—fast-flowing and turbulent areas where water flows over rocks
- Riffles—similar to rapids but with a less intense and lower flow rate
- Runs—areas where water flows are uninhibited
- Pools—still water areas within the river channel, usually deeper than other areas
- Backwater—still or low-flowing water created by natural channel migration along the site of the river
- Braided channels—a network of river channels separated by small sand bars

Figure 2.1 Aquatic Habitats within a River

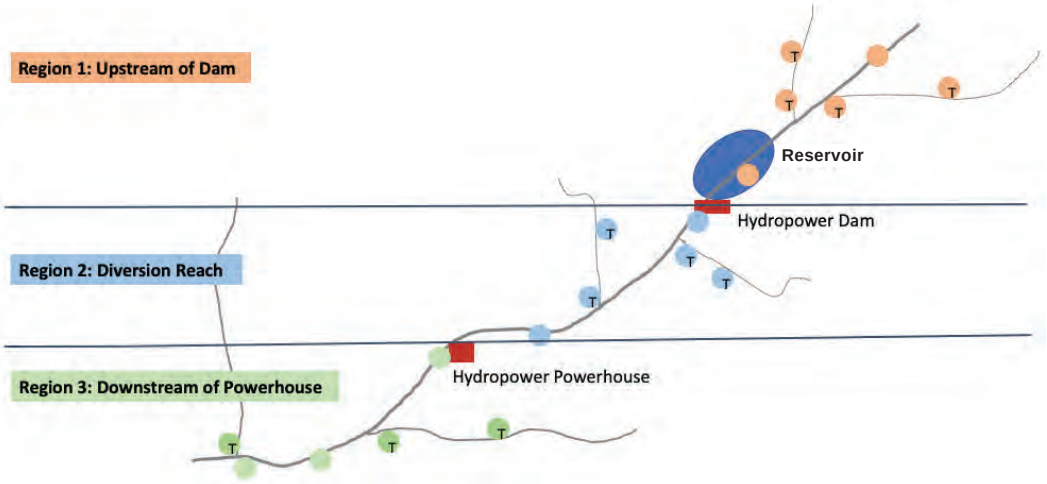
Figure 2.2 Example Satellite Image Showing Different Habitat Types in the Main Stem River and a Tributary within the Area of Impact of an HPP

Source: Google Earth.

Number of Sampling Sites

The aquatic survey should include multiple (replicate) sampling sites in each region to capture the natural variation between sites within the region. This natural variation is high in Himalayan river basins and can vary even within a few meters. For an EIA baseline, as many replicate sites as possible should be sampled in each region. At a minimum, two to six sites should be sampled in each of the major aquatic habitats identified in each region, covering both the main stem river and tributaries. Additional sampling sites should be included to cover more habitats or important sites. Additional sites upstream should be included to effectively cover the movement range of migratory fish species. Likewise, sites further downstream may be needed to assess changes in the river ecology due to alterations in water or sediments flows. **Figure 2.3** illustrates an ideal sampling design.

Figure 2.3 Recommended Sampling Design to Collect Aquatic Data for an EIA Baseline or Long-Term Monitoring of an HPP



Note: Dots signify sampling site replicates within each of the three sampling regions. Dots with "T" are on the tributaries or at the confluence of the main river with a tributary. Other dots are along the main stem river.

2.1.3 When to Sample for the EIA—Seasonality

Field sampling for the EIA baseline of a hydropower project must be conducted in all of the seasons relevant for aquatic biodiversity. In the Nepal Himalaya, the onset of the monsoon season in May or June (pre-monsoon) is the trigger for many migratory fish to start moving upstream to their spawning sites. Similarly, many migratory fish species start moving downstream for overwintering in October or November at the end of the monsoon season (post-monsoon). During the winter season (December to March), fish may reside under rocks as the water level and temperature drop. Many macroinvertebrates that are insects spend only part of their lives in water and complete their life cycle mostly within a year. Sampling in all seasons allows the capture of a wide range of macroinvertebrates at mature larval stages. Similarly, water levels in the river affect the distribution and abundance of fish and macroinvertebrates; therefore, sampling in multiple seasons is essential to establishing a robust baseline.

For an EIA baseline, sampling should be conducted as often as possible to document the variation between seasons and months. Seasonal sampling provides a strong baseline of information about where and when the fish and macroinvertebrates are found in the project area and the watershed prior to construction of an HPP. This information is important for assessing project impacts and developing mitigation actions to maintain aquatic biodiversity. See **Box 2.1** for key elements of sampling design for an EIA.

Ideal Sampling Schedule

A sampling survey should ideally be carried out monthly for at least a year prior to HPP construction to document a yearly cycle for the EIA. Two years of baseline data would provide a robust baseline. A full year of sampling data provides information on the lifecycle of target species that will serve as a solid baseline against which to measure changes and evaluate if no net loss or net gain has been achieved. Each sampling survey should include all of the sampling sites and dedicate sufficient time at each site to fully implement the field methods. This usually requires one to two days per sampling site. Extra care must be taken when sampling during the wet season due to strong river flows.

Minimum Sampling Schedule

Hydropower project budgets and field access often limit the number of pre-construction sampling surveys conducted for an EIA. However, a minimum number of seasonal sampling surveys is essential to obtaining a solid understanding of the aquatic biodiversity. Field sampling for the EIA baseline should be conducted in at least three seasons:

- Fall (post-monsoon): October to November
- Winter (post-monsoon): January to February
- Spring (pre-monsoon): March to May

When possible, sampling during a fourth season—May to June (pre-monsoon)—is also recommended, particularly for migratory fish species. In Nepal, four seasons are often sampled for an EIA.

2.2 Sampling Design for Long-Term Monitoring

The first step in long-term biodiversity monitoring is to clearly define the objectives of the monitoring program and the questions that will be answered

Box 2.1 Key Elements of Sampling Design for the EIA

1. Use satellite imagery and field visits to identify and map all aquatic habitats in three regions:
   - Upstream of HPP project
   - Diversion reach (if applicable)
   - Downstream of powerhouse

2. Select at least two to six sampling sites in each of the three regions to represent all habitat types.

3. Conduct field surveys during three seasons for one to two years prior to HPP construction:
   - Fall (post-monsoon): October to November
   - Winter (post-monsoon): January to February
   - Spring (pre-monsoon): March to May

with the monitoring results. **Section 1.4** outlines some key questions that can be answered using the Trishuli Assessment Tool for long-term aquatic biodiversity monitoring related to hydropower.

The sampling design for long-term monitoring is developed based on information obtained during the EIA field surveys. Sampling sites and field methods will usually be a subset of those used for the EIA baseline, with some exceptions.

2.2.1 What to Sample for Long-Term Monitoring—Aquatic Biodiversity Indicators

Long-term monitoring using the Trishuli Assessment Tool focuses on the same three groups of aquatic biodiversity:

- Fish
- Macroinvertebrates
- Periphyton

2.2.2 Where to Sample for Long-Term Monitoring—Sampling Sites

Selecting Sampling Sites

In contrast to an EIA, for which field sampling must be done at many sites to obtain a robust understanding of the aquatic biodiversity, sampling sites for long-term monitoring should be selected based on the objectives of the monitoring program. Such a program is normally used to evaluate if an HPP's mitigation measures are successful in maintaining aquatic biodiversity during its construction and operational phases.

Thus, long-term monitoring sites should include:

- Sites with predicted impacts from an HPP (derived from the EIA)
- Sites where HPP mitigation measures will be implemented (from the EIA)
- Sites important for aquatic biodiversity (for example, migratory routes, spawning sites, feeding grounds, nursing grounds, areas of high biodiversity, or unique habitats)
- Control sites outside of the HPP's area of impact

Long-term monitoring sites are usually selected from those surveyed for the EIA. However, sometimes the EIA study reveals additional sites that may be important for monitoring, particularly if threatened species or unique habitats are documented, or if there are site-specific project impacts.

Project Impact and Mitigation-Specific Sampling Sites

Long-term monitoring for a hydropower project often focuses on assessing specific measures designed to reduce project impacts on aquatic biodiversity. Sampling sites must be located appropriately in order to evaluate the success of such measures. Some examples of mitigation measures and associated sampling sites are presented in **Figure 2.4**. If the EIA concludes that an HPP has no impacts on a sampling region (for example, downstream of the powerhouse), the number of monitoring sites in that region may be reduced or eliminated.

Control Sites

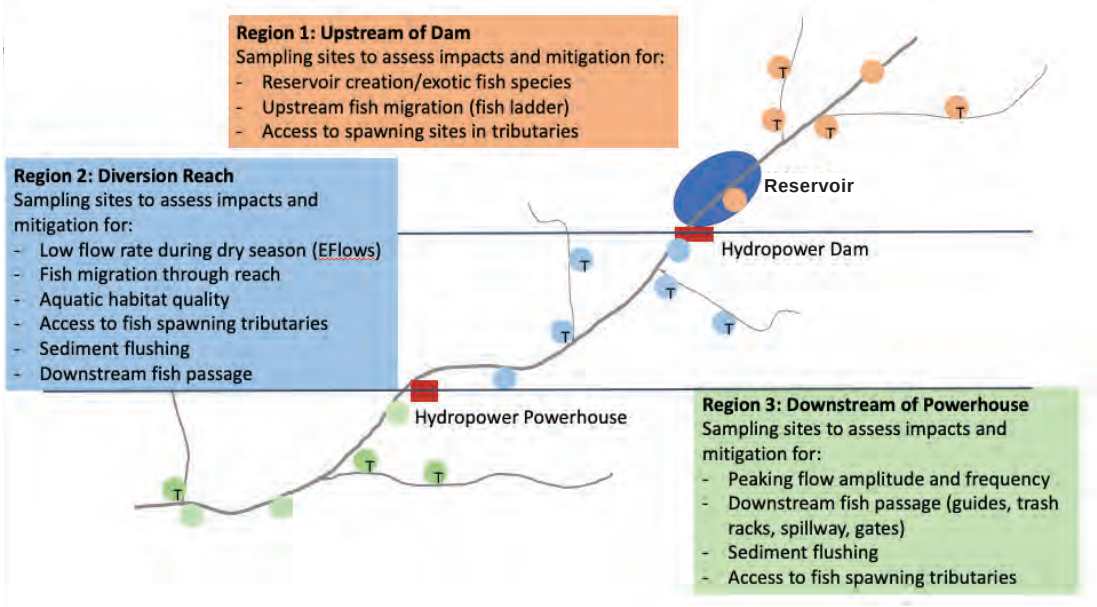
Some biodiversity monitoring programs, such as the "Before-After-Control-Impact" approach (Green 1979), include control sites that are not affected by a hydropower project for comparison to sites within the project impact areas. For HPPs in the Himalayas, it is often challenging to find true control sites that are equivalent to the pre-project conditions of the impact sites. This challenge is due to the cumulative impacts of hydropower projects and other developments, such as road construction, fishing pressures, and water mills. In addition, the natural variation in water flow rate, temperature, and substrate is high within the river basin, resulting in seemingly similar sites with different habitats, conditions, and species. In **Figure 2.4**, control sites may be on tributaries downstream of the powerhouse as an evaluation of fish spawning outside of the project impact areas.

Control sites can be used to compare what is happening in another part of the river over time. In this case, each control site should be analyzed over time independently to evaluate changes at that site. The trajectory of changes at a control site can be compared to that at impact sites. See **Section 3** for suggested analysis using control sites.

Number of Sampling Sites

The number of sampling sites for long-term monitoring will depend on the extent of HPP impacts and the number of species of conservation concern or important habitats documented in the aquatic ecosystem. For long-term monitoring, sampling sites should be surveyed within each of the three regions to evaluate the project impact and success of mitigation measures at different HPPs (**Figure 2.4**). Preferably, two to six sites will be monitored in each region in order to consider natural variations and different habitats.

Figure 2.4 Example of Sampling Design for Monitoring HPP Impacts on Aquatic Biodiversity



In addition, one to two control sites outside of the project impact area should be surveyed for comparison of trends over time.

2.2.3 When to Sample for Long-Term Monitoring—Seasonality

Field sampling for long-term monitoring should be conducted two to three times a year in the same seasons for the EIA sampling:

- Fall (post-monsoon): October to November
- Winter (post-monsoon): January to February

Additional seasons should be included when possible:

- Spring dry season (pre-monsoon): March to May
- Spring (pre-monsoon): May to June

Essential Caveats on Long-Term Monitoring

- Monitoring must be conducted as close to the same date during the same time frame (such as season or month) each year.
- Monitoring should be conducted under the same weather and river conditions each year to minimize changes caused by changing weather or river conditions. Sampling should be avoided in rain or flooding when flow and turbidity are not normal or typical for the season. If rain is reported in the basin or water is turbid, survey must not begin until turbidity normalizes.
- Monitoring should be done with the same field methods and sampling effort for each sampling site and survey period. If sampling effort is not equivalent, it can be standardized using the catch per unit effort in order to make comparisons between survey periods or years (see **Section 3**).
- Field sampling data must always be compared between the same season and not between different seasons (see **Section 3**). For example, data can be compared between spring 2020 and spring 2021 field surveys but not between spring 2020 and fall 2020 field surveys.

How Long to Monitor

The length of the long-term monitoring program should be determined by the program objectives and questions. Monitoring to evaluate the success of mitigation measures and maintenance of aquatic biodiversity indicators usually takes several years before changes become apparent or target thresholds are met (see **Section 3**).

Long-term monitoring should be conducted for at least one year, prefereably two, prior to construction and during all years of construction of an HPP. Monitoring should continue during operation until the data indicate that the project is not having negative impacts on the aquatic environment and all parties (HPP operator, government, and funding agencies) agree that monitoring is no longer needed. See **Box 2.2** for key elements of sampling design for long-term monitoring.

In general, long-term monitoring should be conducted:

- Pre-construction phase: One to two years
- Construction phase: Throughout all years of construction
- Operations phase:
  - Minimum: Three years
  - Robust: 10 years
  - Ideal: Life of project

2.3 How to Sample for the EIA and Long-Term Monitoring

2.3.1 Preparation for Field Sampling

This field manual assumes that users of the Trishuli Assessment Tool are familiar with the basics of field work and sampling in Nepal. Thus, the tool does not cover all information needed to conduct a field survey. Additional information can be found in Nepal's *Hydropower Environmental Impact Assessment Manual* (MoFE 2018) and the *Freshwater Ecosystem Assessment Handbook* (FRTC/MoFE 2022).

Several key points are highlighted below as essential preparation for the field surveys:

- Necessary permissions for sampling from all relevant government departments and authorities must be obtained before leaving for the field. This includes permits to conduct research in national parks or other protected areas, permits to collect fish, macroinvertebrate, and periphyton samples, and permits for electrofishing and eDNA.
- An accurate weather forecast of the study area should be reviewed to identify expected extreme weather conditions that can compromise the ability of an expert or an observer to perform field activities. Surveys should be rescheduled to alternate days if extreme weather conditions, such as cold temperatures, rain, flood, and high wind, are expected.
- The field team must have all the necessary personal protective equipment, including first-aid box, life jackets, working communication devices, and safety boots or shoes. Electrofishing requires additional safety gear.
- All equipment must be in good working condition, which should be checked by the field team leader.

Box 2.2 Key Elements of Sampling Design for Long-Term Monitoring

1. Select sampling sites to include:
   - Sites with predicted HPP impacts (derived from the EIA)
   - Sites where HPP mitigation measures will be implemented (from the EIA)
   - Sites important for aquatic biodiversity (for example, migratory routes, spawning sites, areas of high biodiversity, or unique habitats)
   - Control sites outside of the HPP's area of impact

2. Select two to six sampling sites in each of the three regions to cover all habitat types and one to two control sites outside of the HPP's area of impact.

3. Conduct field surveys during at least two (ideally three) seasons for each year:
   - Fall (post-monsoon): October to November
   - Winter (post-monsoon): January to February
   - Spring (pre-monsoon): March to May (if applicable)

4. Sample fish, macroinvertebrates, and periphyton

5. Field surveys should be conducted:
   - One to two years prior to construction
   - Throughout the HPP's construction
   - Three to 10 years during the HPP's operations (ideally throughout the life of the project)

2.3.2 Field Team

Implementation of the Trishuli Assessment Tool requires a team of qualified biologists who are trained in field methods with field-work experience in Nepal. The team should include:

1. A field team leader with demonstrated proficiency in the field sampling methods and field team management as well as experience or knowledge of the survey areas; experience with report preparation and data analyses is also required for the team leader
2. One to two fish researchers with qualifications and experience or training to identify local and regional fish species in the field and in the laboratory preferably with fish taxonomy training; they must also have experience with the fish sampling methods of the Trishuli Assessment Tool
3. One to two fish researchers trained in the use of electrofishing and its safety measures
4. One to two macroinvertebrate researchers qualified and trained in the field sampling methods with experience in sorting and identifying macroinvertebrates in the field
5. A data recorder trained in the data recording methodology of the Trishuli Assessment Tool
6. Two to four field assistants who may be students, consultants, or trained local community members to assist with fish and macroinvertebrate sampling
7. One to two local fishermen proficient in cast netting for fish

A laboratory or analysis team may also be required. These may include (as appropriate):

8. Laboratory macroinvertebrate researchers (one expert and one assistant) qualified and trained to sort and identify macroinvertebrate samples, preferably an aquatic insect taxonomist
9. An ecological statistics data analyst to assist with data analysis (if needed)
10. A genetics laboratory collaborator to analyze eDNA

The field team must have the following resources and training:

- Training to use and maintain the sampling equipment in the field, data collection, specimen preservation, and data recording and keeping
- Ability to swim in deep water
- Willingness to follow the directions of the field team leader and to wear a life jacket and other personal protective equipment as necessary
- Backup support of geographic information system (GIS) and other data management as well as logistic and emergency management from their home organization (consulting firm, university, or research institute)

2.3.3 Site Sampling Design

The following steps should be followed to set up the sampling design for each sampling site:

1. At each sampling site, select a 400 m section of river that contains a variety of aquatic habitats such as rapids, riffles, runs, pools, backwater, and braided channels.
   - Tributaries: select a section that is more than 300 m above its confluence with the main stem river or larger stream
   - Main stem river: select a section with appropriate shallow, low-flow areas that are safe for sampling, such as near the confluence with tributaries, river bends, and backwaters
2. Mark the midpoint of the 400 m sampling stretch with a permanent mark (such as paint on a rock) or select a landmark like a bridge or other marker. Record the global positioning system (GPS) coordinates of the midpoint.
3. Mark and record GPS coordinates of the boundaries of the sampling site:
   - 200 m downstream of the midpoint
   - 200 m upstream of the midpoint
4. Within the 400 m stretch, identify the best areas for each sampling method so that each method has its specific sampling locations and does not overlap (if possible).
5. Record and describe in detail the specific areas delineated for each method so that sampling during future monitoring surveys will be able to find the exact sampling locations.
6. Start sampling downstream and work upstream to avoid disturbing the riverbed and causing sediments to flow to downstream sites.

2.3.4 Habitat Descriptions

The aquatic habitat should be described and documented in a data sheet (see **Appendix B**) before sampling begins:

1. Describe the stretches of the river or stream in the sampling site to include information on:
   - Description of upstream, midpoint, and downstream boundaries plus length (in meters)

- Wetted width and total width (in meters) in upstream, midpoint, and downstream areas
- Flow conditions (high, medium, or low)
- Percentage of aquatic habitats as below for upstream and downstream areas:

  Rapids = high turbulence, high flow with steep vertical drop over rocks or boulders

  Riffles = less turbulence, high flow over smoother substrate, shallower than 0.5 m

  Runs = low turbulence, high flow over smoother substrate, deeper than 0.5 m

  Pools = low turbulence, low flow, deeper than 1 m

  Shallow slacks = low turbulence, low flow, shallower than 1 m

  Backwater = low turbulence, low flow, connected to but off from the main flow

2. Draw a map of the study site with details of the boundaries, easily identifiable habitats, location of water types (such as pools, riffles, and rapids), and sites where sampling was conducted. Use a field notebook.

2.3.5 Associated Data to Collect

In addition to data on target organisms, data on the location (GPS coordinates), habitat, weather conditions, flow rate, and water depth should be recorded at each sampling site. See data sheet in **Appendix B** for additional data that need to be recorded.

2.4 Fish Field Sampling Methods

The sampling methods for fish aim to collect data for:

- All fish species
- Target fish species, such as mahseer (*Tor* spp.) and snow trout (*Schizothorax* spp.)

2.4.1 Field Method Selection

The Trishuli Assessment Tool comprises the following set of fish field sampling methods:

- Backpack electrofishing
- Cast nets
- Dip nets
- Underwater video
- Environmental DNA (eDNA)

These methods and others were field tested in February 2020 on the Trishuli River. Electrofishing was found to be the most effective method for collecting fish in the tributaries, documenting two to four times as many fish as were collected by cast nets (Philipp et al. 2020; see **Table 2.2**). Gill nets were evaluated but excluded as a recommended method due to its harmful effects on the captured fish (Philipp et al. 2020).

As many of these field methods should be used at each sampling site as possible, **but not all methods are suitable for all sampling sites**. Methods will need to be selected based on the target indicator to be sampled, habitat type, and feasibility (see **Table 2.3**). Feasibility will include access to sampling site; availability of experienced field personnel and necessary equipment; depth of the water and ability of researchers to walk and wade in the river; river flow rate, turbidity, and depth; as well as weather conditions.

Table 2.2 Comparison of Fish Catch Using Cast Nets and Electrofishing in the Trishuli River Tributaries in February 2020

Site code	Site	Cast net				Electrofishing			
		Total no. of fish	Sample time (min.)	CPUE	No. of species	Total no. of fish	Sample time (min.)	CPUE	No. of species
TAD	Tadi Khola	20	57	21.1	4	106	32	199	15
MAI	Mailung Khola	26	445	34.7	1	44	35	75.4	4
LCH	Lower Chilime Khola	22	55	24	1	80	15	320	2
SAK	Salankhu Khola	5	26	11.5	3	99	34	175	7

Source: Philipp et al. 2020

Note: no. = number; min. = minutes; CPUE = catch per unit effort (see **Section 3.1**)

Table 2.3 Field Methods for Each of the Fish Indicators to Be Used in Each Habitat Type

Fish indicators	Tributaries	Main stem channel	Main stem shore*
All fish species	• Backpack electrofishing • Cast nets • Underwater video • Dip nets • eDNA	• Cast nets • eDNA	• Backpack electrofishing • Underwater video • Dip nets
Snow trout and mahseer adults	• Backpack electrofishing • Cast nets • eDNA	• Cast nets • eDNA	• Backpack electrofishing • eDNA
Snow trout and mahseer juveniles	• Backpack electrofishing • Cast nets • Underwater video • Dip nets	• Cast nets	• Backpack electrofishing • Dip nets

Note: *Along the shore of the main stem river in areas with lower flow and low turbidity (clear water) that are suitable for wading

2.4.2 Sampling Effort for Each Field Method

The Trishuli Assessment Tool's recommended sampling effort for each fish sampling method is shown in **Table 2.4**. This standard protocol was tested in the Trishuli River in February 2020 and was found to provide a robust assessment of the fish biodiversity at each site (Philipp et al. 2020).

As with all field sampling, circumstances may arise that prevent the full implementation of the recommended sampling effort. For example, weather conditions may change and halt field sampling, or the river may become turbid as a result of upstream sand mining, thus affecting the effectiveness of electrofishing. The sampling effort for each field method at each site should be carefully recorded, including minutes spent

Table 2.4 - Sampling Effort Per Site for Each of the Fish Field Sampling Methods

Field method	Units of sampling effort	Number of units per site*	Approximate sampling time per site**	Personnel
Core methods				
Backpack electrofishing	Time (minutes) sampling with electrofisher current on	40 minutes sampling: 20 minutes downstream 20 minutes upstream	40 minutes**	3 people
Cast nets	Number of cast-net throws	100 cast-net throws: 50 throws downstream 50 throws upstream	~60 minutes	2 people
Underwater video	Time spent recording per set	12 sets of 5 min. each: 6 sets downstream 6 sets upstream	60 minutes	1 person (plus 1 for safety)
Dip nets	Number of dip-net emersions	10 dip-net samples: 5 samples downstream 5 samples upstream	~30 minutes	1 person
eDNA	Number of 2-liter water samples	Six 2-liter water samples	~120 minutes	2–4 people

Note: *Sampling design at each site includes a 400 m river stretch marked at a midpoint; **record the time spent on actual sampling for every method, subtracting travel or setup time; "downstream" refers to sampling 200 m downstream of the midpoint; "upstream" refers to sampling 200 m upstream of the midpoint.

electrofishing or underwater video recording as well as the number of cast-net throws and dip-net samples (**Table 2.4**). The sampling effort at each site can be standardized using catch-per-unit-effort (CPUE) transformation in order to draw comparisons between sites and sampling periods (see more on CPUE in **Box 3.1** in **Section 3**).

2.4.3 Specifics of Fish Field Sampling Methods

Backpack Electrofishing

Overview

Electrofishing using a backpack electrofisher delivers a low-voltage electrical field into the water, which temporarily incapacitates fish so that they float to the surface of the stream and can be collected with a net. It is the most effective method for sampling and documenting fish; thus, it should be implemented whenever conditions are suitable. The Trishuli Assessment Tool protocol recommends electrofishing for a total of 40 minutes at each sampling site: 20 minutes within the 200 m downstream of the midpoint and another 20 minutes within a second area in the 200 m upstream of the midpoint. During each of these 20-minute periods, the team should sample the full range of representative habitats that can be safely surveyed in each of the six habitats—rapids, runs, riffles, pools, slack water, and backwater—on a percentage of time basis that is representative of the amount of such habitats in the upstream and downstream locations.

Advantages

- Extremely effective in sampling large numbers and high levels of species or size diversity
- Requires little time for actual in-water sampling
- Can sample in shallow water (slow or fast) effectively
- Can sample in complex, rocky habitats very effectively

Challenges

- Specialized and expensive backpack electrofisher (US$3,000–US$10,000)
- Training and practice required
- Heavy equipment
- Requires a three-person team
- Safety concerns and precautions
- Special permits from government needed
- Requires shallow and clear water (tributaries and backwater as well as side channels)

Training and Safety

When done properly, electrofishing can be very safe and effective for capturing fish. However, it can also be highly dangerous if the operator is not familiar with the electrofisher and safety features. All members of the team must wear electrically insulating chest waders and rubber boots and be careful not to touch water during sampling. The electrofisher operator must obtain training from a certified professional prior to using the equipment. The electrofisher must have adequate safety systems, such as immersion cutout and emergency shut-off button.

Target Organisms and Habitat

All fish species of various sizes and ages can be collected with electrofishing. Backpack electrofishing is only possible in shallow areas suitable for wading with low flow and low turbidity (clear water). Thus, this method is best suited for tributaries and at the confluence of the main stem with tributaries (mouth of the tributary, where fish spawning often occurs).

Seasonality

Electrofishing is most effective during the dry season when water has low flow and low turbidity (clear). Electrofishing cannot be used in high-flow (monsoon season) or turbid waters.

Personnel

Three people are needed: 1) an "operator" who will operate the electrofisher and collect the fish with a net, 2) a "bucket" person to carry the bucket for the fish collections and to assist the operator if needed, and 3) a "recorder" to keep track of the time and record data as well as to ensure that safety precautions are observed. Since the electrofisher is heavy (15 kg), the team may choose to rotate the duties if all team members are trained in the use of the equipment. The operator must be trained by a professional in the use of the electrofisher and be able to carry it for an extended period of time in the cold, rocky streams of the Himalayan region.

Time

The Trishuli Assessment Tool recommends a total of 40 minutes of electrofishing at each sampling site: 20 minutes within the 200 m downstream of the midpoint and 20 minutes within a second area in the 200 m upstream of the midpoint for best results. Sometimes, conditions do not allow for the recommended sampling time; for example, changing weather may halt sampling or upstream sand mining may cause turbidity, thus affecting electrofishing effectiveness. The time spent on

electrofishing should be recorded and standardized using CPUE to allow for comparisons between sites with different sampling efforts (see **Section 3**).

Sampling Process

An operator carries a backpack electrofishing unit on his or her back, holding the pole with the electric node in one hand and a long handle dip net in the other hand while walking slowly through the water—ready to catch any fish floating to the surface (**Figure 2.5**). The electric node must be underwater when operating. A second "bucket" person should accompany the operator and carry the bucket (perhaps an additional net) for collecting fish. A third "recorder" will watch the other members carefully to ensure the safety of the team, keep track of the time, and record information from the operator.

Specific details of the procedures for operating the electrofishing equipment and for choosing the correct power settings for the safest and most effective fish collection are described in **Appendix F: Detailed Instructions for Conducting Backpack Electrofishing**. See **Box 2.3** for equipment needed for electrofishing.

In addition, and very importantly, prior to using this equipment, all members of the team should read and understand the information presented in **Appendix G: Best Practice Manual for Backpack Electrofishing.**

Specimen Collection and Processing

The collected fish will be held alive in buckets of fresh water for processing at the end of each 20-minute period (see **Section 2.4.4**). If lots of fish are caught, it is best to process them immediately and keep them together in a large tub until sampling is complete. All fish that are not kept as voucher specimens will be returned to the river alive.

Figure 2.5 Backpack Electrofisher and its use in the Rocky Streams of the Trishuli River Basin

Box 2.3 Electrofishing Equipment

- Backpack electrofisher with battery and electrodes (Smith-Root LR-24 backpack electrofisher recommended)
- Long-handled dip net with electrically insulated handle (for collecting fish)
- Two pairs of chest waders with built-in electrically insulated boots (for the operator and the fish collector)
- One pair of rubber boots (for the recorder)
- Three to four buckets
- GPS
- Data notebook and pencil
- Camera or cellphone to photograph habitats and fish

Data Management

Fish collections from downstream and upstream should be recorded and kept separately. Data will be recorded using a standardized data sheet (**Appendix A**). Locations and durations of sampling efforts as well as records of all fish captured will be documented in detail in field notebooks, including photographs of the fish collected and the areas sampled.

Unit of Sampling Effort for Analysis

Electrofishing sampling effort is measured as the time (number of minutes) spent actively electrofishing when the current is on for each site (minutes per site). Time to move between sections or adjust the equipment should be excluded.

Cast Net

Overview

Cast-net sampling involves a recorder (or bucket person) and a net caster who will throw the cast net 100 times at each sampling site: 50 casts downstream and 50 casts upstream. The number of cast-net throws can be adjusted according to the habitat and environment. At some sites, 25 throws may be sufficient while in other sites with more diverse habitats, 200 throws may be needed. For comparisons over time or between sites, it is best to keep the number of throws the same for each site, but comparisons between different number of throws (different sampling effort) can still be done using CPUE (see **Box 3.1** and **Table 3.7** in **Section 3**).

Advantages

- Moderately effective for catching fish of small and medium size
- Can be used in many different habitats including deep and moderately moving water
- Requires only two people (caster and bucket carrier)
- Cast nets are relatively inexpensive and available in Nepal
- The most used technique in Nepal, therefore most compatible with previous data

Challenges

- Requires skill and experience to cast the net well
- Limited efficacy for sampling small benthic species (for example, *Loach* spp.)
- Less effective in some aquatic habitats, such as rocky substrate
- Inconsistent mesh size between studies limits comparisons

Training and Safety

The caster must be experienced with throwing the cast net (**Figure 2.6**). A local fisher should be hired to use the cast net. The team must be able to swim in case they are pulled or fall into the stream. Care must be taken to avoid falling into the river when sampling in the main stem.

Figure 2.6 Fisher Throwing Cast Net in the Trishuli River

Seasonality

Cast nets can be used in both dry and wet seasons, although high monsoon would likely be too dangerous.

Personnel

A team of two people, including a net caster and another to hold the collecting bucket and record the data, is sufficient.

Equipment Requirements

Cast nets come in many sizes and shapes. For long-term monitoring, cast nets with the exact same size of mesh, length, and diameter must be used during every sampling survey at each sampling site. A cast-net mesh size of about 25 millimeters (mm) is recommended for the Trishuli Assessment Tool in order to capture small fish, including juveniles. Cast nets can range from 2 m to 3 m in length with a 2.5 m to 5 m expanded diameter. See **Box 2.4** for equipment needed for cast-net fish field sampling.

Box 2.4 Cast Net Equipment

- Cast net(s)
- Buckets
- GPS
- Data notebook and pencil
- Camera or cellphone to photograph habitats and fish

Time

The amount of time needed to cast 50 throws per 200 m river stretch depends on the skill of the caster and access to the river. The time spent during the casting of the 50 throws should be recorded so that time can be used in the data analysis if desired.

Specimen Collection and Processing

All netted fish will be held alive and kept in good condition in buckets of fresh water for processing at the end of the 50 cast-netting attempts (see **Section 2.4.6**). All fish that are not kept as voucher specimens will be returned to the river alive.

Data Management

Results of fish numbers captured will be recorded for each cast-net throw to assess variation in success across the site. Locations and durations of sampling efforts as well as records of all fish captured will be documented in detail in field notebooks, including photographs of the areas sampled.

Unit of Sampling Effort for Analysis

Sampling effort is measured by the number of cast-net throws per site.

Dip Net

Overview

For dip-net sampling, a single individual will attempt to collect larval and juvenile fish (less than 30 mm in total length) opportunistically in 10 very shallow areas using a small or micro mesh dip net of appropriate size for the sampling area. Record the total time spent sampling.

Advantages

- Equipment is inexpensive and easy to use
- Requires little time for actual in-water sampling
- A reliable method for capturing larval fish
- Requires only a single operator
- Provides evidence of species recruitment and identifies spawning and nursery areas

Challenges

- Requires spotting larval fish visually in shallow water
- Extremely size selective
- Requires shallow and clear water
- May result in low capture rates

Training and Safety

No special training is required although knowledge of fish habitats is advantageous. The dip-net user must be able to swim in case they are pulled or fall into the stream. See **Box 2.5** for equipment needed for dip-net fish field sampling.

Box 2.5 Dip-Net Equipment

- Dip net—select the appropriate net size based on the depth and extent of the habitat to be sampled. A good option is a 40 centimeter (cm) wide X 46 cm long X 20 cm deep net with 3 mm mesh and a telescopic pole extending up to 3 m (see **Figure 2.7**)
- Buckets
- GPS
- Data notebook and pencil
- Camera/cellphone to photograph habitats and fish

Figure 2.7 Two Types of Dip Nets

Target Organisms and Habitats

Dip nets are ideal for collecting larval and juvenile fish. However, they can only be used in shallow, low- flow areas, mostly in tributaries, where juvenile fish may be present. Areas in which larval fish can be observed swimming should be targeted preferentially, but if none can be found, then the dip netter should sample in areas where larval fish may likely occur.

Seasonality

Dip nets will work best in the dry season when flow is low and water is clear. They should not be used in high water season.

Personnel

One person is sufficient, with a second person nearby for safety and recording data.

Time

Time for dip-net use will depend on the skill of the user and access to adequate sampling sites. Sampling time is estimated to be around 30 minutes. The time spent actively using the dip net should be recorded for each sample and added up for a total time spent dip netting.

Specimen Collection and Processing

Netted fish will be held alive and in good condition in buckets of fresh water for processing at the end of each successful dip-netting trial (see **Section 2.4.6**).

Data Management

Locations and durations of sampling efforts as well as records of all fish captured will be documented in detail in field notebooks, including photographs of the areas sampled.

Unit of Sampling Effort for Analysis

Sampling effort is measured as the number of "dips" or dip-net samples per site.

Underwater Video

Overview

At each sampling site, a researcher will use an underwater video camera, such as a GoPro camera, to record all fish activity for 12 sets of five-minute recording periods (**Figure 2.8**). Video should be taken in all aquatic habitat types available at the site (such as rapids, runs, riffles, pools, slack water, and backwater). Video recording should start at the lower end of the 400 m delineated sampling area, recording six sets of five-minute recording downstream of the midpoint and then six sets upstream of the midpoint.

Figure 2.8 Researcher Holding Video Camera Underwater in a Tributary

Advantages

- Can observe many fish and often species not captured with other gear
- Good for documenting fish in specific habitat types and to record juveniles in spawning sites
- Possible to document migrating fish in particular habitats
- Requires only one operator for recording and a second person for safety
- Minimal training
- Provides permanent record

Challenges

- Equipment is minimally expensive (US$100–300)
- Data analysis requires lab-based viewing to count and identify fish
- Requires very clear water
- Deployment and retrieval of equipment may require swimming
- May be time consuming

Training and Safety

Little training is required to operate the video camera. The operator must observe safety precautions and know how to swim in case they fall into the water while taking video. See **Box 2.6** for equipment needed for fish field sampling using underwater video.

Target Organisms and Habitats

Underwater video can capture any fish species but is particularly effective for documenting juvenile and larval fish, which are often hard to catch with other methods. Habitats should include tributaries and the confluence of the main stem river with tributaries, where spawning occurs for many fish species. The 12 five-minute video segments should be recorded in different target habitats at each site.

Sampling Process

For this field method, the videographer will position himself or herself close to the edge of the water and hold the GoPro video camera underwater. A consistent method should be developed for all sites and surveys, such as holding the camera straight ahead to facilitate comparisons over time. Sampling sites should be selected where juvenile fish may occur. The videographer may sit or lie on rocks near the river's edge to obtain a good position for holding the camera underwater. Each sampling period should be five minutes at a habitat. The videographer and the data recorder (second person) then move to another habitat type at the site and record another five-minute segment. A total of 12 segments should be recorded within the 400 m sampling site.

Seasonality

Underwater video can only be used in clear water, so dry season is best.

Personnel

A team of two people is sufficient: one to do the underwater video and the other to record data and be close by for safety.

Time

Twelve sets of five-minute videos will be recorded for a total of 60 minutes. Additional time will be needed to select sampling locations and move between them.

Video Processing and Data Management

The videos need to be downloaded onto a computer and reviewed by people who can identify Himalayan fish. To collect the data, the reviewer will list the species and number of individuals of each species observed in each five-minute video.

Unit of Sampling Effort for Analysis

Sampling effort is measured as the time (minutes) spent actively recording per site.

Box 2.6 Underwater Video Equipment

- Handheld, waterproof video camera, such as GoPro (***Figure 2.9***)
- Batteries and cables
- Laptop computer (for reviewing the videos)
- GPS
- Data notebook and pencil
- Camera or cellphone to photograph habitats

Figure 2.9 Examples of GoPro Waterproof Video Cameras

Source: gopro.com

Environmental DNA

Overview

Environmental DNA (eDNA) is an emerging technology that documents species through detection of DNA in water or soil samples. The Trishuli Assessment Tool recommends using eDNA when possible because this technique can detect and record species that are not captured with other methods. Species lists can grow with this technique, which is particularly useful for EIA baseline sampling to detect rare or threatened species. Its applicability for long-term monitoring is still in research stages since measurement of abundance is only possible as a relative comparison of the amount of DNA detected in each sample. eDNA sampling requires collaboration with a genetics laboratory to sequence the DNA from the samples.

Field Methodology

Environmental DNA sampling involves taking samples of water from each site and filtering them to collect animal DNA from the water (**Figure 2.10**). There are many approaches to collecting and analyzing the water samples for DNA. Hydropower projects are encouraged to investigate options and decide on the best approach and partner for their eDNA sampling needs. Some eDNA laboratories, such as Nature Metrics (https://www.naturemetrics.co.uk), offer simple field collecting kits and resources for eDNA sampling and analysis.

The following procedure was developed by the Center for Molecular Dynamics Nepal's Fish Biodiversity Project (see http://fish.org.np/background).

At each sampling site, five 2-liter water samples (one each from upstream, downstream, pool, riffle, and sediment habitats) are collected in aseptic glass bottles at locations within the 400 m delineated sampling area. These water samples can then be taken to filtration stations set up on the bankside safe from disturbing other activities. Those five water samples plus a separate control distilled water sample will be filtered to collect cells or DNA on a fine filter membrane (Whatman or Millipore filter with 47 mm diameter and pore size of 0.45 µm) using a hand-pump portable vacuum system. The six filters will then be preserved separately in Longmire's solution to protect the DNA and taken back to the lab where the DNA will be extracted. Specific sequences will be amplified using polymerase chain reaction techniques, with different fish DNA samples amplified and then sequenced; by comparing sequences amplified from the eDNA water samples with known sequences from public databases like

GenBank, species present at or upstream from the sampling sites will be identified. Each location will be assessed for the presence or absence of all species of fish potentially in the river at the site. Locations and durations of sampling efforts will be documented in detail in field notebooks, including photographs of the sites.

Advantages

- Is highly effective in detecting presence of high numbers of species
- Can detect the presence of species that are very difficult to collect with other methods
- Can be employed in almost any water conditions
- DNA samples can be kept long term for future reference studies
- DNA samples can be used to target species other than fish by changing the target genomic code (changing base primer set) from cyprinids to mammals or particular species of interest

Challenges

- The method is still in a developing phase; some anomalies still need scientific validation
- Optimal collection standard in terms of type of water (emerging research suggests shallow sediments), collection buffer (Longmire buffer currently offers the best DNA preservation retention), and optimal lab protocols are constantly evolving to extract the best genomic data
- Specific and bulky field equipment and supplies
- A team of genetic specialists is required in pre- and post-processing, especially in developing the most robust bioinformatics pipelines
- Expensive laboratory analysis (around US$8,000 per set of 18 samples)
- Abundance data questionable but improving (relative abundance by proportion is the currently available standard)
- False positives are possible (unless blocking primers are used to negate particular taxa groups that are least likely to exist in said waters, but that increases bias)
- The DNA reference databases for the Himalayan region do not include all fish species and may include incorrectly identified DNA sequences
- Requires substantial time to get final results
- Machinery sensitivity is high and multifactorial elements (such as temperature, technical handling, and data pipeline robustness) determine the sensitivity and specificity of results

Figure 2.10 Environmental DNA Process

Note: Steps 1 and 2—filtering water samples in the field; step 3—evaluating DNA results with computer software

Target Organisms and Habitats

eDNA can sample all types of organisms that shed DNA in the water. A challenge for eDNA is that DNA travels with the water flow so that the sample may not be from where it was collected. eDNA can sample all types of habitats.

Seasonality

eDNA sampling can be implemented in all seasons. It is recommended not to sample immediately after heavy rainfall as silt and mudflow causes high water turbidity causing clogging of filter papers and blocking the particle of interest (DNA) from remaining on the filter paper.

Personnel

Field sampling requires two to three people to filter the water samples. Simple filtering kits are now available that require only one researcher to collect the samples. A genetic specialist or collaboration with a genetics laboratory is necessary to sequence the DNA. A specialist in the taxonomic group sampled (fish in this case) is needed to verify the species list and interpret the results.

Time

Collection of water samples takes only a few minutes. Filtering the water from each sample can be quick (less than 5 minutes) with new sampling kits or may take 30 minutes to an hour with traditional methods. Genetic analysis of the samples may take several months.

Data Management

Data from the genetic analysis of the DNA will be a list of species with DNA sequences that match those found in the water sample. The species list comes from the international GenBank reference database, which may contain errors. A fish specialist should review the list and evaluate the source of the GenBank samples to verify the identifications. The data produced also include the number of DNA strands or recognizable taxonomic units (RTUs) in the sample from each species. The RTU number may possibly be used as an estimate of the relative abundance of each species. Research is ongoing to verify if RTUs can be used as relative abundance for long-term monitoring.

2.4.4 Monitoring Fish Movement through a Fish Ladder

In 2020, a World Bank study of 50 hydropower projects in Nepal revealed that 13 of them have constructed fish ladders to allow for fish to migrate past the dam (Shah et al. 2020). However, only one of these fish ladders at the Khimti HPP has ever been studied or monitored to evaluate its effectiveness for passing fish over the dam (Kaasa 2008).

International good practice calls for fish ladders to be monitored constantly through project operations to record if fish are using the ladder, which species they are, and how many fish are able to pass through. Monitoring also allows for evaluation of the design of the fish ladder so that modifications can be made if needed.

Monitoring the movement of fish through a fish ladder requires different methods from those included in the Trishuli Assessment Tool and thus will not be addressed in detail in this manual. Fish-ladder monitoring should be continuous during the fish-migration periods, both upstream and downstream. Each fish species has its own migratory periods, so multiple periods may need to be monitored to evaluate all target species. Monitoring is not required when fish are not migrating.

There are many methods hydropower projects can use for the long-term monitoring of fish in their fish ladders. Some of these methods are listed as follows, from simplest to most complicated (see **Table 2.5** for comparison):

1. Manual fish counts
2. Fish traps
3. Camera or video recording
4. Pit-tag telemetry
5. Active telemetry
6. Automated underwater video with fish identification software

Table 2.5 Comparison of Fish-Ladder Automated Monitoring Techniques

Characteristics	PIT Telemetry	Active Telemetry	Underwater Video	Vaki Riverwatcher	ARIS Camera
Start-up Cost	$ to $$	$$ to $$$	$ to $$	$$ to $$$	$$$
Monitor fish behavior	Indirectly	Yes	Yes	Indirectly	Yes
Estimate population size	Yes	Yes	Yes	Yes	Somewhat
Uniquely identify individual fish	Yes	Yes	No	When combined with PIT	No
Requires fish handling	Yes	Yes	No	No	No
Identify to species	Yes	Yes	Yes	Yes	Yes
Can detect and measure fish size	No	No	Somewhat	Yes	Yes
Can monitor smaller fish	Yes	Yes	Yes	Somewhat	Somewhat
Mobile or actively track fish	Yes	Yes	No	No	Yes
Can be solar powered	Yes	Yes	Yes	Yes	Yes
Works in turbid water	Yes	Yes	Limited	Yes	Yes
Requires natural light or lighting	No	No	Yes	Sometimes	No
Works in deep water	No	Sometimes	No	No	Yes
Requires a narrow opening to channel fish	No	No	Yes	Yes	No
Works in brackish or saltwater	Limited	Sometimes	Yes	Yes	Yes

Source: https://fishbio.com/automated_monitoring

2.4.5 How to Record Fish Data

Detailed and consistent data recording is a fundamental part of data management (see **Figure 2.11** for a sample fish field sampling data sheet). Thus, it is important to be extremely diligent in recording the data:

- Fish data should be recorded in a standardized data sheet such as **Appendix A**.
- Habitat and location data should be recorded in a data sheet like **Appendix B**.
- Each specimen sample must be clearly labeled with the sampling-site number, specimen number, and date.
- For data analysis, the data should be entered into an Excel spreadsheet.
- Be sure to include the unit of measurement for every set of data, for example, degree Celcius (°C) for water temperature, gram (g) for weight, and millimeter (mm) for fish length.
- Take photographs of all sampling habitats and selected fish specimens.

Considerations

The following are several important aspects to consider:

- *Completeness*. Prepare data sheets (see **Appendix B**) for recording every detail, including habitat and sampling survey information, names of places and details of locations, methods, dates, times, and names of people involved. Examples would be site description maps, fish-collection data sheets, and sampling-method sheets. Sampling sites should have full names as well as ID codes, GPS locations, and a written description of the location. Another data sheet (**Appendix A**) should be used for fish data: species, number, length, and weight.
- *Organization*. Store the data sheets in an organized manner. Clearly label all samples using easily distinguishable codes and numbering systems before storing them in a safe and organized fashion.
- *Redundancy*. All data should be stored in at least three places or formats. For example, the handwritten data sheets need to be kept in a secure location; photos of every sheet should be taken on a designated cell phone at the end of each day and those photos should be uploaded to the cloud for storage.
- Finally, all data need to be entered into Excel spreadsheets (or a similar data storage system) that are housed in a secure site accessible by all team members who need access (read-only).

2.4.6 How to Process the Fish Collections

Field Processing

All fish captured using the sampling methods of the Trishuli Assessment Tool should be kept alive and healthy in buckets of fresh water until processed. All fish should be handled with care so that they can be released unharmed.

For every fish collected, the following data should be recorded (see **Appendix A**):

1. Identify the species. If the species cannot be identified in the field, a specimen, photograph, and detailed description should be taken. Note if the identification provided is of high, medium, or low confidence. Species identification must include the scientific name (genus and species) or the genus and a species number (such as *Schizothorax* sp. 1). The common name (in Nepalese or English) should also be noted along with the scientific name.
2. Measure total length (mm) of fish from the snout to the end of tail.
3. Measure fork length (mm) of fish from the snout to the fork in tail.
4. Measure weight (grams).
5. Record the maturity stage of sub-sample of target species (for example, reproductive male with seed or female with eggs).
6. Note if a photograph was taken of the specimen.
7. Note if a DNA fin clip was taken.
8. Note if a voucher specimen was taken.
9. Record the fish ID code.
10. Include any notes on the fish collected.

Figure 2.11 Example of Fish Field Sampling Data Sheet

TRISHULI RIVER FISH SAMPLING DATA	
Site Number	1
River Name	Tadi Khola
Location	Panchakanya
Code	TAD
Date	23-Feb-20
Time	25 Casts
Method	Cast Net

Sample #	US/DS	Species	Fin Length (mm)	Total Length (mm)	Weight (grams)	Photo (Y/N)	DNA - Fin clip (Y/N)	Voucher specimen (Y/N)	Fish ID Code
1	US	*Opsarius bendelisis*	65	73	2.5	Y	Y	Y	TAD-CN-US-1
2	US	*Opsarius bendelisis*	73	81	4.0				
3	US	*Opsarius bendelisis*	64	73	2.0				
4	US	*Opsarius bendelisis*	70	79	3.0				
5	US	*Opsarius bendelisis*	74	83	4.0				
6	US	*Opsarius bendelisis*	76	85	4.0				
7	US	*Opsarius bendelisis*	81	91	5.0				
8	US	*Opsarius bendelisis*	65	74	2.0				
9	US	*Opsarius bendelisis*	67	74	3.0				
10	US	*Schizothorax* sp.	43	47	1.0	Y	Y	Y	TAD-CN-US-10
11	US	*Schizothorax* sp.	45	50	1.0				
12	DS	*Garra* sp.	80	89	8.0	Y	Y	Y	TAD-CN-DS-12
13	DS	*Paracanthocobitis botia*	60	61	2.0	Y	Y	Y	TAD-CN-DS-13
14	DS	*Opsarius bendelisis*	62	69	2.0				
15	DS	*Opsarius bendelisis*	63	69	2.0				
16	DS	*Opsarius bendelisis*	80	89	4.0				
17	DS	*Opsarius bendelisis*	67	76	2.0				
18	DS	*Opsarius bendelisis*	65	73	2.0				
19	DS	*Opsarius bendelisis*	77	86	4.0				
20	DS	*Paracanthocobitis botia*	63	63	3.0				

Most fish will be released after each sampling method is completed.

For each new species of fish captured, the following additional steps should be taken:

1. Photograph the fish.
2. One specimen should be preserved in 85 percent ethanol in a sampling bottle for later verification in the laboratory and as a voucher specimen for the reference collection.
3. If possible and of interest, a small (5–10 mm^2) sample of fin tissue can be removed immediately (from live fish or immediately after death) and preserved in a DESS solution containing 20 percent dimethyl sulfoxide, 0.25 molar (M) disodium ethylene diamine tetra acetic acid (EDTA), and saturated sodium chloride (Yoder et al. 2006) for DNA extraction and subsequent genetic analyses.

Laboratory Processing and Deposition

All fish specimens collected in the field will be examined in a laboratory, such as a government or university fish collection, to identify the species using fish-identification resources and the knowledge of fish taxonomy experts. Specimens should be deposited in a recognized fish collection. In Nepal, this would include the National Fisheries Research Centre Godawari of Nepal Agricultural Research Council, Kathmandu University, and Tribhuvan University.

2.5 Field Sampling Method for Macroinvertebrates

Macroinvertebrates are an important component of the freshwater ecosystem, comprising the largest portion of the aquatic food web and forming a vital link between aquatic plants, algae, and leaf litter to the fish species and other animals that depend on the river system, including birds.

Macroinvertebrates are diverse groups of small invertebrates less than 0.5 mm that can be seen with unaided eye, including insects, annelids, arachnids, crustaceans, clams, and gastropods. These organisms inhabit diverse habitats from flowing to still water and feed on a wide range of substrates, depending on their habitat preferences.

Macroinvertebrate communities in a river's downstream reaches are linked to those in the upstream. Headwater streams harbor organisms known as "shredders" that break coarse organic particulate matters; the mid-rivers contain "scrapers" that feed on algae, diatoms, and other aquatic vegetation, while the lower reaches have "collector-gatherers" and "collector-filterers" that consume fine organic particulate matters. "Predators" feed on live animals such as small invertebrates. Maintaining all these types of macroinvertebrates is essential for the aquatic ecosystem as they help break down organic matter and filter the water, providing clean water for humans and aquatic animals (**Table 2.6**).

Table 2.6 Functional Feeding Groups and Food Resources of Benthic Macroinvertebrates

Functional feeding groups	Food resources of the functional group	Example family or order of macroinvertebrates
Shredders	Coarse organic particulate matter, including twigs and leaves	Amphipoda; Limnocentropodidae
Scrapers	Periphyton and diatoms	Brachycentridae; Glossosomatidae; Coleoptera
Collector-gatherers	Diatoms, bacteria, and fine organic particulate matter	Trichoptera; Ephemeroptera
Collector-filterers	Fine organic particulate matter	Simuliidae; Chironomidae
Predators	Zooplankton and small invertebrates	Plecoptera; Megaloptera; Odonata

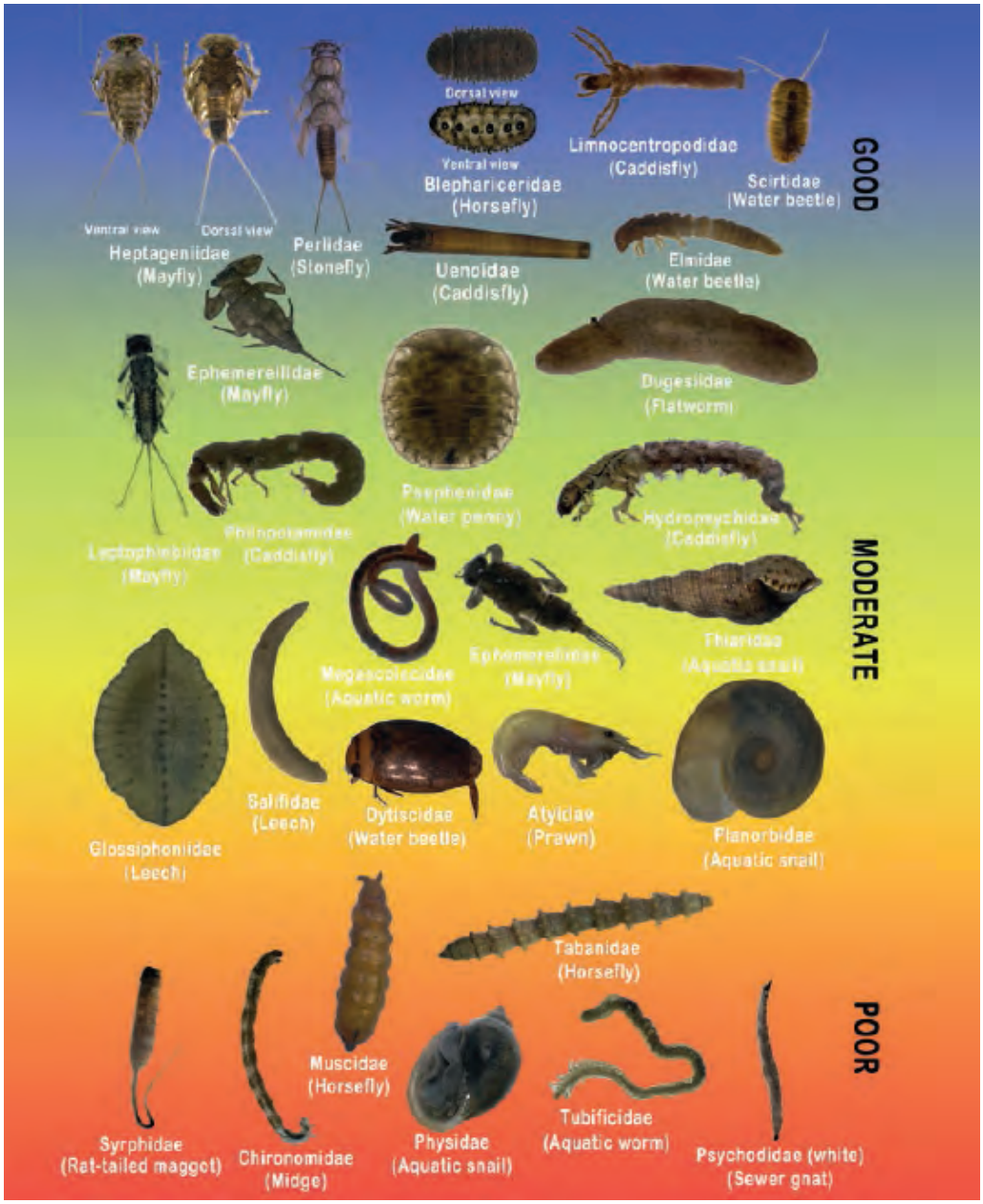
Source: FRTC/MoFE 2022

Some macroinvertebrate taxonomic groups or taxa (species, genera, or families) serve as excellent indicators of river basin health and ecosystem change. Three major orders of aquatic insects—Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies)—make up the EPT index, which uses the species' presence or abundance to measure water quality. Some families of Diptera (flies), such as Chironomidae, are tolerant of poor water quality and may be the only macroinvertebrates found in heavily modified aquatic ecosystems. Macroinvertebrates are good indicators for assessing the health of the aquatic ecosystem because they:

- Live their lives partly or wholly in water
- Are cosmopolitan in nature and highly diverse
- Are abundantly found in river systems
- Remain in a generally small area and habitat

Many macroinvertebrates are sensitive to changes in habitat, water quality, temperature, flow rate, and sediments. **Figure 2.12** illustrates how different macroinvertebrate taxa have varying levels of sensitivity to pollutants in water basin, with some taxa tolerant of poor water quality, some moderately tolerant, and some that can only live in good quality water.

Figure 2.12 Macroinvertebrate Orders and Sensitivity to Pollutants in River Basin



Source: Tachamo Shah et al. 2020a

2.5.1 Multihabitat Sampling Using Kick Net

The macroinvertebrate sampling method for the Trishuli Assessment Tool follows the standardized methodology of multihabitat sampling using kick net (Tachamo Shah et al. 2020a).

Macroinvertebrate sampling can only be done in relatively shallow and low-flow waters, such as in tributaries, at the confluence of tributaries with the main stem river (at the mouth of the tributary), and along the banks of the main stem.

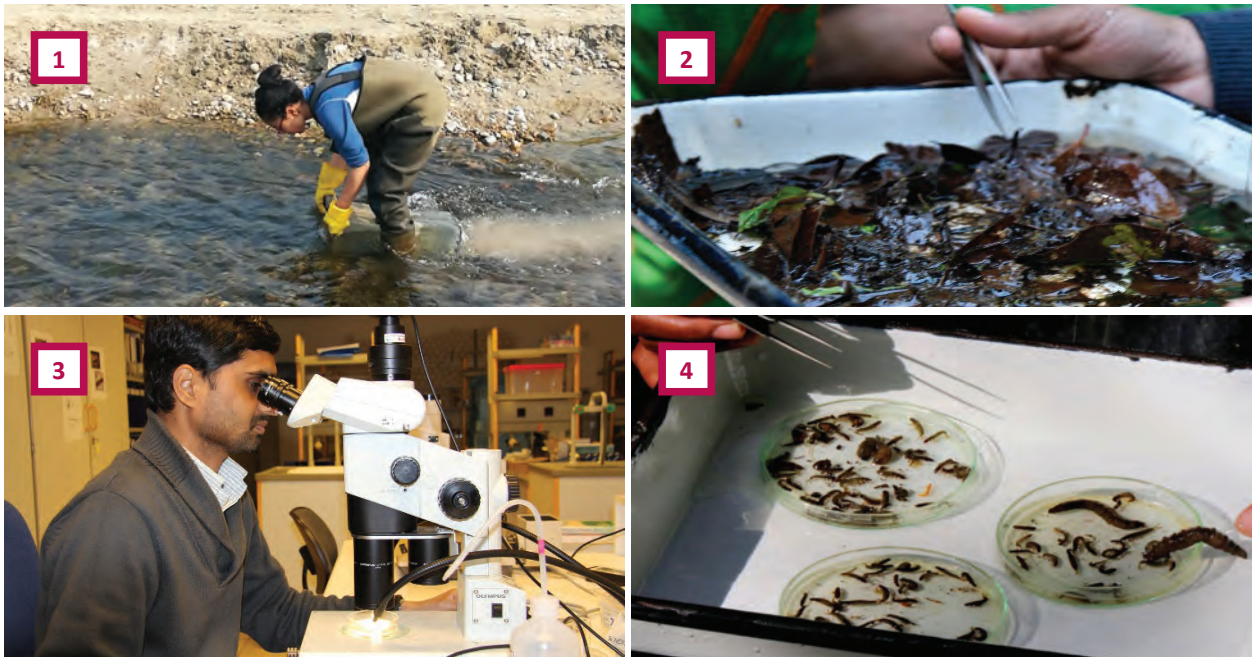
Overview of the Sampling Process

At each sampling site, 20 macroinvertebrate samples are collected within a 100 m river stretch that contains a variety of aquatic habitats. The samples cover a total area of around 1.25 m² of stream bottom. Sampling is done using a standard kick net with a square metallic frame (25 cm × 25 cm) and mesh size of 0.5 mm.

The field process for the multihabitat sampling using a kick net is as follows (see also **Figure 2.13**):

1. Start sampling from downstream to upstream at each sampling site.
2. Place the kick net at the river bottom against the flow of the river.
3. Move, mix, or rub the river-bottom substrates manually for a minute to dislodge organisms and substrates so that they flow into the kick net.
4. Rub and wash rocks and other substrates for a minute to collect additional macroinvertebrates.
5. Keep and store each sample separately.
6. Transfer each sample into a white tray and inspect it for macroinvertebrates of rare or high conservation value, such as the threatened Himalayan relict dragonfly (*Epiophlebia laidlawi*).
7. Remove large organic debris and stones from the sample.
8. Transfer the rest of the remaining samples into a plastic bucket filled halfway with water.
9. Stir the sample and pass it through a hand net of mesh size 500 µm.
10. Repeat this rinsing process until only mineral substrates remain in the bucket.
11. Visually inspect the sample to pick out any remaining macroinvertebrates.
12. Transfer the collected macroinvertebrates (from the hand net) to a sample container or bottle with 95 percent ethanol for later identification in the laboratory.

Figure 2.13 Sampling Process for Benthic Macroinvertebrates



Non–IFC photographs: ©R.D. Tachamo Shah. Used with the permission of R.D. Tachamo Shah. Further permission required for reuse.

Note: Step 1 = using a standard kick net in a sampling site; step 2 = sorting macroinvertebrates in the field; step 3 = sorting and identifying specimens in the laboratory; step 4 = macroinvertebrate specimens in petri dishes

Selecting the Sampling Sites

Before sampling at each sampling site, the diversity of aquatic habitats should be assessed within the selected 100 m stretch of river (within the 400 m Trishuli Assessment Tool sampling area). The percentage coverage of each habitat type within the 50 m downstream stretch below the midpoint and the 50 m upstream stretch should be estimated and recorded on the Habitat Data Sheet (**Appendix C**). Macroinvertebrate samples should be selected from this information to ensure that all microhabitats, substrates, water depths, and flow velocities are included in the sample (**Figure 2.14**).

Advantages of Macroinvertebrates for Sampling and Monitoring

- Occur in high abundance and relatively easy to sample
- Relatively larger body size, easier to identify
- Highly diverse taxonomically and ecologically
- Live from a few months to years so they integrate short- and long-term pollution and disturbance exposures
- Limited mobility preventing them from escaping from occasional pollutions
- Many taxa are highly sensitive to changes in water quality, flow regimes, water-level fluctuations, and habitat changes

Challenges

- Samples can only be taken from relatively shallow and low-flowing waters
- Expertise in identification of macroinvertebrate groups required

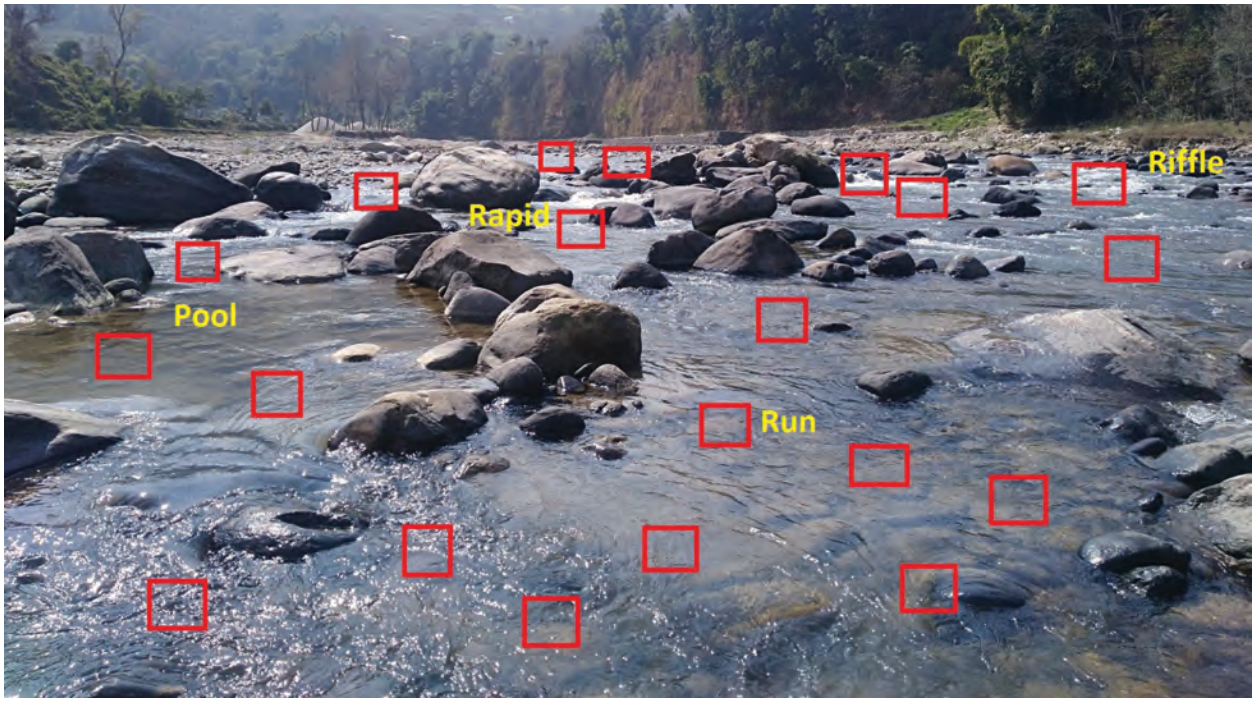
Training and Safety

Field sampling does not require much training. Safety precautions must be taken when sampling in the water: life jacket is recommended, particularly in sites with high river discharge and large rivers. Training in macroinvertebrate identification is required for sorting and identifying the specimens in the laboratory. See **Box 2.7** for a list of equipment needed for macroinvertebrate sampling.

Target Organisms and Habitats

Macroinvertebrates can serve as indicators of water quality and health of the river basin. Larva and nymph stages of benthic macroinvertebrates are included in the assessment as they spend their entire lives in water. All representative riverbed habitats including flow types—rapid, riffle, run, and pool—should be sampled for macroinvertebrates.

Figure 2.14 Categorization of Aquatic Habitat Types for Multihabitat Field Sampling Using a Kick Net



Note: Red squares are locations of the selected 20 sampling subsites that represent the diversity of aquatic habitats.

Box 2.7 Macroinvertebrate Sampling Equipment

IN FIELD

- **Nonconsumables**
  - GPS or topographic map
  - Camera or cellphone to photograph habitats and specimens collected
  - Magnifying glasses
  - Kick net (25 cm × 25 cm] with a square-shape metallic frame and mesh size of 0.5 mm
  - Hand net (circular-shaped metallic frame with mesh size of 0.5 mm)
  - One pair of chest waders
  - One pair pf half boots
  - One pair of rubber gloves
  - White trays
  - Wide forceps
  - Plastic buckets
- **Consumables**
  - 99.9% ethanol
  - Printed methodology, pencil and sharpener, cardboard, permanent marker, cellotape, and scissors
  - Sample box
  - White transparent plastic vials (8 ml)

IN LABORATORY

- Hand net (circular-shaped metallic frame with mesh size of 0.5 mm)
- White trays
- Fine forceps
- Petri dishes
- Stereomicroscope
- 99.9% ethanol
- White transparent plastic vials (8 ml)

Seasonality

Macroinvertebrate sampling can be done throughout the year except in heavy monsoon season. Sampling must be done in both dry and post-wet seasons with low and high flows to capture a diverse range of macroinvertebrates in the site.

Personnel

A team of two to three people is needed to collect the samples and sort the specimens. One to two researchers are needed to identify the specimens in the laboratory.

Time

It takes one to two hours to sample each field site. Laboratory work to sort and identify the specimens depends on the diversity and number of individuals in the sample. Usually, 10 to 12 hours per sample are required to completely sort and identify the specimens as well as count the number of individuals per taxon.

Specimen Collection and Processing

In the laboratory, each benthic sample is rinsed in clean water and transferred onto white trays. All specimens are picked out of the sediments and sorted into groups based on their taxonomic order. They are then identified to the highest possible taxonomic level (species, genus, and family) using available reference sources and museum collections. After sorting, the specimens are stored in transparent plastic vials containing 95 percent ethanol. Each vial is labeled with a paper slip containing a sample code before being sealed and stored in a recognized invertebrate museum or collection. Use of a high-powered halogen lamp and sharp forceps are advised.

Data Management

Data should be collected on the standardized data sheets in **Appendix C**.

Unit of Sampling Effort for Analysis

Sampling effort for macroinvertebrates is measured as 20 kick-net samples per site.

2.6 Field Sampling for Periphyton

Periphyton are small aquatic plants, such as algae, that cling to rocks in the river. The dry biomass of periphyton is a good indicator of the primary productivity within the aquatic ecosystem, which forms the base of the food chain that sustains all aquatic life in the river basin. The biomass of periphyton supports diversity and abundance of macroinvertebrates in a river.

Overview of the Sampling Process

1. Periphyton sampling should proceed from downstream to upstream at each sampling site. Periphyton samples are to be collected across various substrates, water depths, and flow velocities.
2. At each sampling site, five hand-sized stones (with a maximum diameter or long axis of 10–15 cm) are removed from the stream at a depth of 20–40 cm in slow-flowing areas perennially under water and from locations undisturbed by benthic sampling. The stones should be picked from the depth at random without the collector looking into the water at the stones.
3. The stones are to be separately scrubbed in a rinsed tray with a brush to scrape off all of the periphyton and then rinsed with 100 ml distilled water.
4. The tray, the brush, and the funnel used are rinsed thoroughly with water after each stone is sampled, and the wash is added to the sample.
5. The periphyton collection in the tray is transferred to a 100 ml sample bottle and then 2 ml Lugol's iodine solution is added for preservation.
6. The bottles are then labeled and stored in a dark bag for transportation.

See **Box 2.8** for a list of equipment needed for periphyton sampling.

Selecting the Stones

The dimensions of each stone are measured with a measuring tape and noted in the field data sheet in accordance with the periphyton sample labels. The longest axis or length (X), the longest horizontal axis perpendicular to X or width (Y), the longest vertical axis of the stone or thickness (Z), and circumference (C) are measured for calculating the surface area of the stone. To enhance standardized comparisons, the same person should sample the stones and process the periphyton samples for the entire length of the field trip.

Data Management

Locations and durations of sampling efforts should be documented in detail in field notebooks, including photographs of the sites. Periphyton data should be recorded on the Periphyton Data Sheet (**Appendix D**).

Specimen Processing in the Laboratory

The biomass of periphyton is determined by the standard ash-free dry mass method (APHA 1995). In the laboratory, the following steps should be undertaken to dry the periphyton sample:

1. Weigh a clean glass-fiber filter paper.
2. Filter 100 mL of water with periphyton sample through the glass-fiber filter paper.
3. Dry the collected periphyton residue on the filter paper at 105°C for one hour in a laboratory oven.
4. Dry the sample at 500°C for three to four hours in a muffle furnace.
5. Weigh the filter paper with the dried periphyton sample (known as ash).
6. Calculate the biomass of periphyton using the following formula:

   Biomass of periphyton = [(weight after drying at 105°C – initial weight of filter paper) – weight after drying at 500°C]/area of periphyton sample collection.

Unit of Sampling Effort for Analysis

Sampling effort for periphyton is measured as scraping five stones per site.

Box 2.8 Periphyton Sampling Equipment

IN FIELD

- **Nonconsumables**
  - GPS or topographic map
  - Camera or cellphone to photograph habitats and specimens collected
  - Scrub brush
  - White trays
  - 100 ml sample bottles (five per site)
  - Funnel (for transferring sample to sample bottle)
- **Consumables**
  - 99.9% ethanol
  - Distilled water
  - Lugol's iodine solution
  - Paper labels for samples

IN LABORATORY

- Glass-fiber filter paper (with pore size of 0.45 µm)
- Filter bottle
- Drying oven
- Muffle oven
- Digital scale for fine measurements (four digits)

3 Data Analysis and Presentation

3.1 Introduction

Presentation, analysis, and reporting of the data collected in the field is an extremely important but often overlooked part of the EIA and monitoring process. It is essential that the data are interpreted and conveyed in a way that can be readily understood and used by a hydropower project so that it can implement changes to mitigate any negative impacts from the project. Similarly, data analysis is needed to clearly show that the project has resulted in no net loss or even a net gain of biodiversity values.

There are many ways to present and analyze data for the EIA and for long-term monitoring, with many statistical tests that can be run. *Ecological Diversity and Its Measurement* (Magurran 1988), Rosenzweig (1995), and Feinsinger (2001) are excellent references for field-study design and statistical comparisons; there are also many recent papers on data-analysis methods (Magurran et al. 2010; Sreekanth et. al 2015; Tachamo Shah et al. 2020b) and studies of aquatic biodiversity monitoring (Tachamo Shah and Shah 2012; Birindelli et al. 2016). Graphs, figures, charts, and tables are excellent means of presenting the data, but be sure to label them well (for example, label X and Y axes) and give each a title.

Raw Field Data

The raw field data should be included in the EIA and monitoring reports, either in the text or the appendixes, to allow readers to properly understand the data analysis and metrics. The raw data presented should include all the data categories recorded in **Appendix A**, as outlined in **Section 2.4.6**.

Metrics

The data collected using the Trishuli Assessment Tool should be analyzed using a set of metrics. Metrics are a quantitative means of measuring, comparing, and tracking target indicators over time. This manual includes a recommended set of metrics for analyzing the fish data and a recommended set of metrics for macroinvertebrates and periphyton. All analyses and graphs recommended here can be done using Excel. The metrics should be selected and analyzed to evaluate specific project impacts and/or the success of mitigation measures.

3.2 Fish Metrics

Fish should be included in a hydropower project's EIA and long-term monitoring program because they are prominent organisms in the aquatic ecosystem, with many globally or regionally threatened and rare species that warrant protection. Fish can be identified and analyzed at the species level.

Below are seven recommended metrics that provide informative analyses for the EIA and long-term monitoring of a hydropower project as well as help fulfil national and international biodiversity requirements (**Table 3.1**):

1. Species richness
2. Species composition
3. Proportion of species
4. Species distribution
5. Relative abundance of target fish species
6. Recruitment of target fish species
7. Length of target fish species

All of these metrics are calculated and analyzed for each site separately. Hydropower impacts need to be site specific because there are many other disturbances within a watershed, such as sand mining, fishing, and road construction, which may cause general changes.

Thus, data combined for the entire project area do not show where the impacts are happening or where and how the metrics are changing. In some cases, data for a region may be combined and analyzed, such as when there are multiple sampling sites within a small area, or when impacts on an entire region warrant an investigation.

Field Data

The EIA or monitoring report should include the raw data in the text or in appendixes. **Table 3.2** presents a hypothetical example of fish data from electrofishing for presentation in EIA and monitoring reports. These data are used for some of the metrics examples below.

Table 3.1 Recommended Metrics for Fish Data Analysis

Metric no.	Indicator	Field method	Metric calculated for each site	Significance
1	Species richness	All combined	No. of species/site	Documents the number of fish species
2	Species composition	All combined	List of species; presence or absence of species/site	Identifies fish species and selects target species for monitoring
3	Proportion of species	All combined	No. of individuals of each species/ no. of individuals of all species combined/site	Shows the percentage of individuals for each species
4	Distribution of species	All combined	Map of species locations for all sites	Maps fish distribution to identify important sites and document locations
5	Relative abundance of target species	Each method—electrofishing and cash nets—separately	CPUE = No. of individuals for each target species/sampling effort/site	Documents changes in relative abundance of key fish species
6	Recruitment of target species	Each method—electrofishing and cash nets—separately	CPUE for juveniles = No. of juvenile fish individuals/sampling effort/ site Density of juveniles = No. of juvenile fish individuals/100 m^2/site	Documents continued recruitment and breeding of key fish species to sustain population
7	Length of target species	All combined	Mean length +/– standard deviation = total of fork length for all fish/no. of fish/site	Assesses size and evaluates if a fish is a juvenile or an adult

Note: CPUE = catch per unit effort

Table 3.2 Sample Field Data Presentation for EIA and Monitoring Reports

FIELD DATA—ELECTROFISHING, SPRING 2021

	REGION 1: UPSTREAM OF DAM					
	Number of fish individuals captured					
Fish species	**Site 1 Main stem**	**Site 2 Tributary**	**Site 3 Tributary**	**Site 4 Tributary**	**Site 5 Tributary**	**Site 6 Main stem**
Schizothorax richardsonii	16	40	15	24	55	20
Schizothorax progastus	2	0	3	0	0	3
Garra annandalei	0	5	0	0	1	0
Opsarius bendelisis	1	0	2	1	0	0
Neolissochilus hexagonolepis	1	3	1	2	0	2
Paracanthocobitis botia	0	0	0	0	0	0
Psilorhynchus pseudecheneis	0	0	0	0	0	0
Total	20	48	21	27	56	25

	REGION 2: DIVERSION REACH					
	Number of fish individuals captured					
Fish species	**Site 7 Tributary**	**Site 8 Main stem**	**Site 9 Tributary**	**Site 10 Tributary**	**Site 11 Main stem**	**Site 12 Main stem**
Schizothorax richardsonii	25	13	15	32	25	25
Schizothorax progastus	1	1	0	0	3	0
Garra annandalei	2	5	7	2	1	8
Opsarius bendelisis	1	0	9	1	0	2
Neolissochilus hexagonolepis	0	1	2	4	1	2
Paracanthocobitis botia	0	4	0	1	2	0
Psilorhynchus pseudecheneis	0	0	2	0	0	3
Total	29	24	35	40	32	40

	REGION 3: DOWNSTREAM OF POWERHOUSE					
	Number of fish individuals captured					
Fish species	**Site 13 Tributary**	**Site 14 Main stem**	**Site 15 Tributary**	**Site 16 Tributary**	**Site 17 Main stem**	**Site 18 Tributary**
Schizothorax richardsonii	30	22	30	40	10	21
Schizothorax progastus	10	4	0	3	10	7
Garra annandalei	5	7	15	6	8	4
Opsarius bendelisis	2	3	5	7	0	2
Neolissochilus hexagonolepis	2	5	9	4	1	0
Paracanthocobitis botia	0	5	3	2	4	1
Psilorhynchus pseudecheneis	3	1	0	2	1	0
Total	52	47	62	64	34	35

Metric 1: Fish Species Richness

Definition: Species richness is the number of species recorded

Calculation: Species richness = number of species per site

Field Methods: Combine data from all sampling methods used at the site

Scale of Analysis: Analyze each site separately

Presentation of Data in EIA Report

- Number of species per site, by region, and overall
- Bar chart (**Figure 3.1**)

Monitoring

- Visually compare the bar charts over time to look for general trends.
- Species richness is not recommended for long-term monitoring comparisons. Changes over time in the number of species are challenging to interpret since the number of species is relatively small and natural variation may be large.

Interpretation

- This number should be contrasted with the number of species in similar rivers in the region. Is it high, low, or typical for a Himalayan river? This number can highlight if there are only a few species to focus on, or if many species need to be considered for impacts.
- Species richness data can be compared between regions to investigate if there are baseline differences between the regions that could be attributed to other factors such as elevation, water temperature, number of tributaries, sand mining, and other HPPs.

Example

Table 3.3 shows an example of summary data of the number of fish species recorded per site based on the hypothetical data presented in **Table 3.2**, which only covers electrofishing. Additional data from other methods need to be added to these data for a full picture of species richness.

Table 3.3 Example Summary Data: Number of Fish Species Recorded per Site

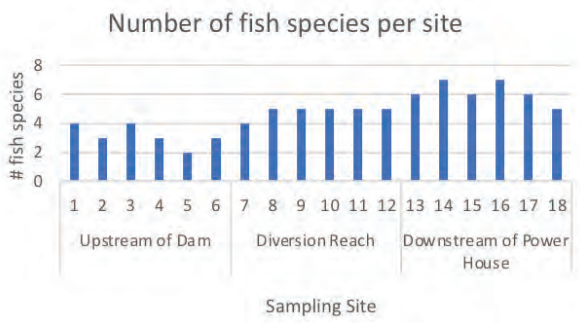
Region	Site	Number of fish species
Upstream of dam	1	4
	2	3
	3	4
	4	3
	5	2
	6	3
Diversion reach	7	4
	8	5
	9	5
	10	5
	11	5
	12	5
Downstream of powerhouse	13	6
	14	7
	15	6
	16	7
	17	6
	18	5

The total number of fish species for the project area with 18 sites is seven. The total number of species per region are:

- Upstream of dam: five species
- Diversion reach: seven species
- Downstream of powerhouse: seven species

Visual Presentation

Figure 3.1 Number of Fish Species Recorded per Site



Example Interpretation

EIA

- The number of species per site generally increases toward downstream. Discuss possible reasons for this, such as water temperature, nutrient availability, food abundance for fish, nursing and recruiting grounds, and other possible disturbances in the river. General characteristics of each region should be presented and discussed.
- Is the number of species typical of a Himalayan river of this altitude? Why or why not? Provide comparative data and reference to other scientific studies of the area.

Monitoring

- Changes in species richness may not be very informative for long-term monitoring due to the high natural variation and low numbers, but such changes can be compared visually over time to detect major trends that could indicate an impact.

Metric 2: Species Composition

Definition: Species composition is the identity of all species in the project area

Calculation: Identify all species recorded using valid references, collections, and experts

Field Methods: Combine data from all sampling methods

Scale of Analysis: By site, region, and project area as appropriate for the impacts

Presentation of Data in EIA Report

- Include a list of species recorded overall.
- Identify species of conservation concern using the International Union for Conservation of Nature (IUCN)'s Red List of Threatened Species and any national threatened species categories.
- Identify any other species of interest, such as migratory species, range-restricted (endemic) species, non-native species, and rare species.
- Select target species for long-term monitoring, which may be highly threatened species, or other species that could be affected by the HPP.
- For taxa with a long list of species, also include a list of the 25 most common across sampling sites (number of sampling sites) or most abundant (number of individuals).
- Note relevant information on the ecology, lifecycle, range, and biology of the species of interest.

Analysis of Long-Term Monitoring Data

- Compare the lists to see if there are any changes in species composition.
- Note any new species of conservation concern or non-native species.
- Compare species lists of impact regions to control sites (if applicable).
- Compare presence or absence of species over time between surveys.

Reporting and Interpretation Suggestions

- Changes in species composition can indicate if species drop out or are introduced into the project area over time.
- Note whether a species disappears through time consistently across sites and surveys. This will warrant further investigation into the cause.

- New species that appear over time may be introduced by people, such as fish species being released into the reservoir or species arriving through new access to the area. Non-native species are of particular concern and should be noted and monitored closely, with possible adaptive management to remove them. Correctly identifying the species in each survey is very important.
- This metric is important for identifying species of conservation concern and of interest to the project as "target or indicator species." These species should be monitored over time to assess any project impacts on them and as an umbrella species representing other species. Monitoring is less effective for species with few data points, such as rare or uncommon species. Thus, even though these species may be of interest, target species should have sufficient data points for analysis over time.
- Comparison between impact sites and control sites (if applicable) can indicate if a species is dropping out of the HPP impact zone but is still present in the control sites. This will indicate the need for further investigation.

Example

Table 3.4 shows an example of fish species recorded by all sampling methods in spring 2021 based on hypothetical fish data in **Table 3.2**, which only covers electrofishing. Additional data from other methods need to be added to these data for a full picture of species richness.

Example Interpretation

EIA

- Discuss the biology of the species recorded and include references, such as the migratory behaviors of the two snow trout species and their spawning sites. Since there are only a few species, a paragraph or two and a photo should be included for each species. If there are more species, select those of most relevance to the project impacts.
- Identify species of biodiversity importance for the project (see **Table 3.5**):
  - o Two species on the list are classified by IUCN Red List (https://www.iucnredlist.org) as globally threatened: *Schizothorax richardsonii* (vulnerable) and *Neolissochilus hexagonolepis* (near threatened). Both species are migratory and have declining populations across their range.
  - o *Schizothorax progastus* is a mid-range migratory species whose access to spawning sites may be blocked by the HPP dam.
  - o *Psilorhynchus pseudecheneis* is only found in Nepal and northern India, so any impacts on its populations may be detrimental to the global population.
- Looking at the abundance data of the fish in the data set above, some of the species have low numbers of individuals recorded and thus would not likely provide sufficient data for monitoring analysis.

Table 3.4 Species Recorded by All Sampling Methods in Spring 2021

Species no.	Fish species	Global common name	Nepal common name	IUCN Red List category	National status	Migratory	Range-restricted
1	*Schizothorax richardsonii*	Common snow trout	Buche Asala	VU (decreasing)		Mid-range	No
2	*Schizothorax progastus*	Dinnawah snow trout	Chuche Asala	LC (unknown)		Mid-range	No
3	*Garra annandalei*	Annandale garra		LC (unknown)		No	No
4	*Opsarius* (formerly *Barilius*) *bendelisis*			LC (stable)		No	No
5	*Neolissochilus hexagonolepis*	Chocolate mahseer	Katli	NT (decreasing)		Long-range	No
6	*Paracanthocobitis botia*			LC (decreasing)		No	No
7	*Psilorhynchus pseudecheneis*	Stone carp		LC (unknown)			Nepal and northern India only

Note: IUCN Red List categories: CR = Critically Endangered; E = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; population trend is shown in parentheses.

- *Schizothorax richardsonii* should be selected as a target species as it is the most common, has sufficient data for analysis, and is an important threatened and migratory species. *S. richardsonii* may serve as an umbrella species for species with similar biology (such as *S. progastus* and *Neolissochilus hexagonolepis*) but should be confirmed with information on their biology and references.
- *Psilorhynchus pseudecheneis* may be an important species but is found in such low numbers that it may be difficult to compare over time. However, its presence or absence should be monitored over time.
- Photos of important species can be included to help readers visualize the species (**Figure 3.2**).

Figure 3.2 Important Fish Species

Schizothorax richardsonii

Opsarias bendelisis

Tor Putitora

Non-IFC photograph of *Tor putitora*; ©A. Pinder. Used with the permission of A. Pinder. Further permission required for reuse.

Monitoring

Table 3.5 Presence or Absence of Fish Species Upstream of Dam with All Methods Combined

Species	Spring 2021 pre-construction baseline	Spring 2022 construction	Spring 2023 construction
Schizothorax richardsonii	X	X	X
Schizothorax progastus	X		
Garra annandalei	X	X	X
Opsarius bendelisis	X	X	X
Neolissochilus hexagonolepis	X		X
Paracanthocobitis botia		X	
Psilorhynchus pseudecheneis			X
Oncorhynchus mykiss		X	X

Example Interpretation

- *Schizothorax progastus* has not been collected upstream since the pre-construction baseline. This may indicate that project construction is impeding its migration or has affected its upstream populations. Further investigation is needed on this species and the cause of its disappearance. Mapping of its distribution is needed (see **Metric 4**).
- A new species has been added to the list. *Oncorhynchus mykiss* is the non-native rainbow trout commonly bred in hatcheries for food. It can cause declines in native fish species where it is introduced into the natural river system. This species may have been present before construction but has not been recorded; it may also have been introduced or its population has increased during construction. Further investigation is needed to determine the cause of this species' introduction or increase with adaptive management to prevent further growth.

Metric 3: Proportion of Individuals of Each Fish Species

Definition: Percentage of all individuals of each fish species out of all species combined (**Table 3.6**)

Calculation: Number of individuals of species/ number of individuals of all species combined

Field Methods: Combine data from all sampling methods (except eDNA)

Scale of Analysis: By site, region, and project area as appropriate for the impacts

Presentation of Data in EIA Report

- Calculate percentage of individuals of each species for each site, region, and project area, depending on where project impacts may occur.
- Compare percentages between sites or regions.
- Present the data in pie charts (**Figure 3.3**).

Monitoring

- Visually compare pie charts from the same site or region between years to observe changes in percentages of each species.
- Note whether any species has become dominant or has significantly reduced.
- Compare between regions to document any natural variation and potential causes.
- Changes in percentages of species over time may indicate that ecosystem conditions have changed to favor the populations of some species over others (for example, reservoirs will favor lake species rather than river species). Investigate these changes to determine if they are due to hydropower project impacts, which would require adaptive management.

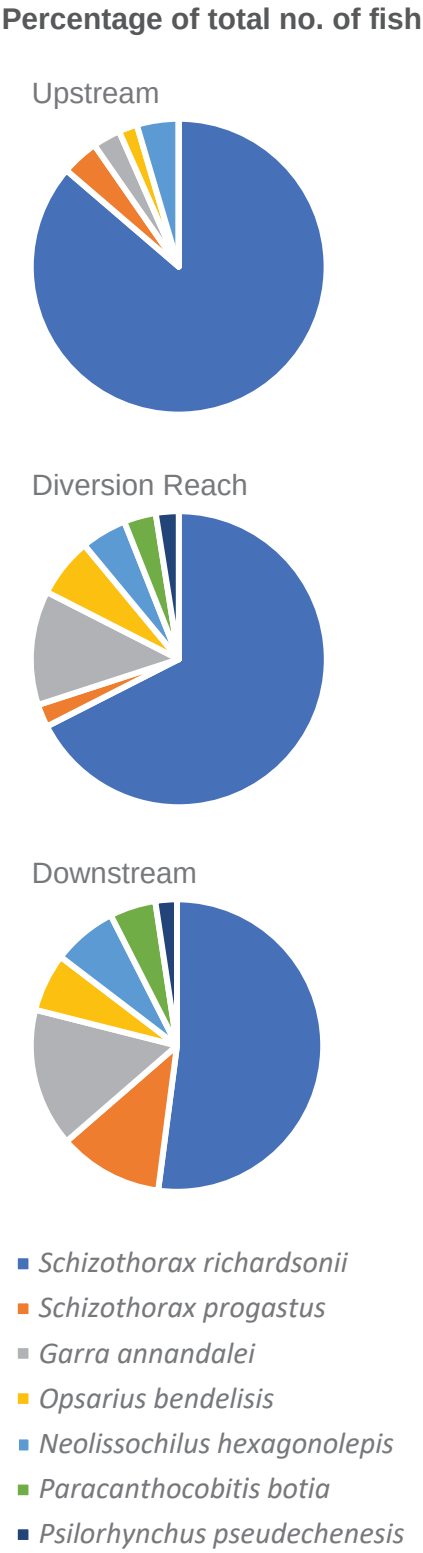
Example

Table 3.6 Number of Fish Recorded Upstream of Dam (Six Sites)—All Methods Combined

Fish species	No. of fish	% of total
Schizothorax richardsonii	170	86.29
Schizothorax progastus	8	4.06
Garra annandalei	6	3.05
Opsarius bendelisis	4	2.03
Neolissochilus hexagonolepis	9	4.57
Paracanthocobitis botia	0	0.00
Psilorhynchus pseudecheneis	0	0.00
Total no. of fish, all species	197	

These data can be visualized using pie charts for each site, region, or overall:

Figure 3.3 Pie Charts Showing Species and Percentages of Fish Recorded in Various Sampling Regions



Example Interpretation

EIA

- The proportion of individuals per species differed across the three regions, with *Schizothorax richardsonii* being the most abundant in each region but with decreasing proportion going downstream. In the upstream region, over 86 percent of the individuals were *S. richardsonii*, with four other species each making up less than 5 percent; *Paracanthocobitis botia* and *Psilorhynchus pseudecheneis* were not recorded upstream at all. *S. richardsonii* also made up 67.5 percent of individuals in the diversion reach captures.
- All other species increased in proportion in the diversion reach and downstream regions, with *Garra annandalei* increasing the most in the diversion reach (12.5 percent) and downstream of the powerhouse (15.3 percent).
- Discuss possible reasons for these trends.

Monitoring

- Pie charts can be compared over time, as is done above between regions, to assess changes in the proportion of individuals of each species.
- Changes may need to be investigated to determine location and cause.

Metric 4: Distribution of Target Fish Species

Definition: This metric maps the location where target fish species were recorded

Calculation: Map of target fish species records

Field Methods: Combine data from all sampling methods

Scale of Analysis: Analyze by site

Presentation of Data in EIA Report

- Maps of sampling sites where each target fish species was located, preferably a separate map for each target species
- Maps to include information on the relative abundance (number of individuals) of the fish species at each sampling site
- Show on the maps whether each site is on a tributary or main river and note any characteristics of the site, such as the confluence of tributary and river, backwater, deep-flowing river, and downstream disturbances (such as sand mining)

Monitoring

- Maps can be compared to see highlight changes in distribution for any of the target fish species.
- Maps can show changes in relative abundance at each site and highlight where the changes are occurring so that causes can be investigated and adaptive management implemented. If a fish species drops out consistently at a particular site or its relative abundance declines at the site, further study is needed.
- Compare the locations of each species and the habitat. Protection of particular sites and type of habitat may be essential for the long-term sustainability of the species.

Example

See **Figure 3.4** and **Figure 3.5**.

Example Interpretation

- The distribution map of *Schizothorax richardsonii* across the sampling sites shows that the species is found at all sites across all surveys (**Figure 3.4**). Adding abundance data to the map would provide additional data on changes in abundance at each site.
- The distribution map of *Schizothorax progastus* reveals changes in its distribution over time (**Figure 3.5**). The species is

present upstream of the dam before but not after construction. This would warrant further investigation and possible adaptive management.

- Similar distribution maps could be drawn for other target fish species, abundant fish species as well as migratory and endemic fish species.

Figure 3.4 ***Schizothorax richardsonii*** **Distribution Map across the Sampling Sites**

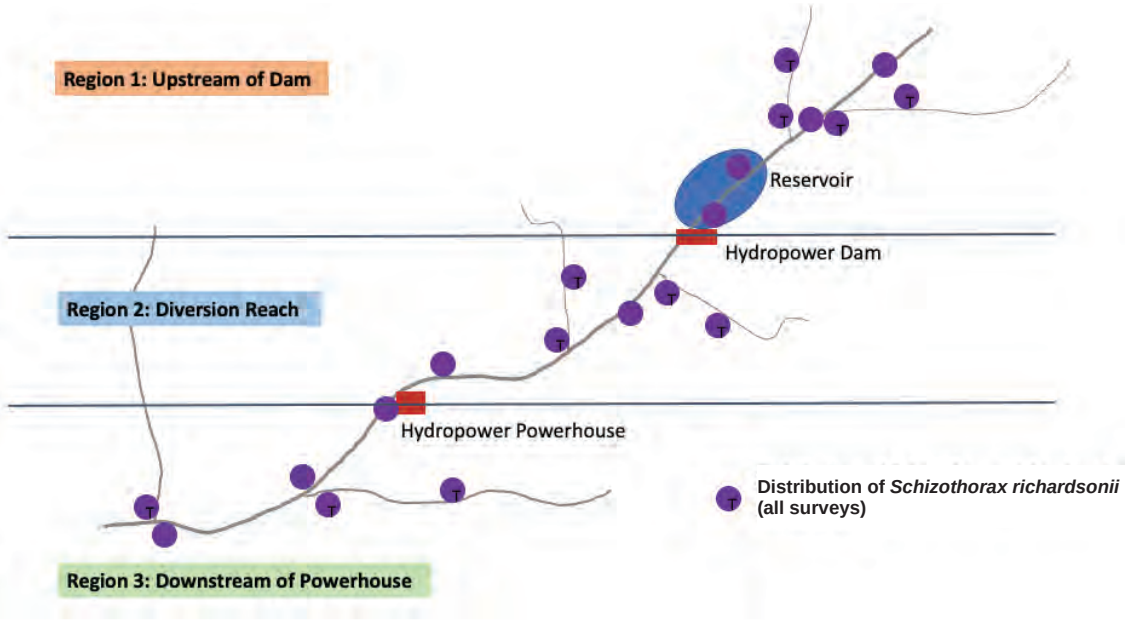
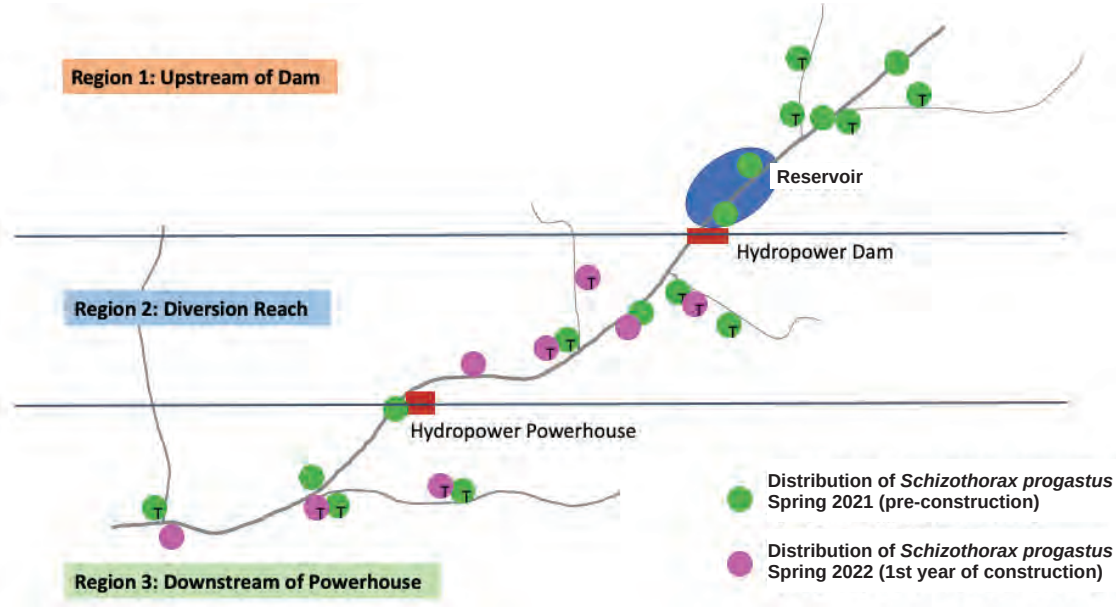


Figure 3.5 ***Schizothorax progastus*** **Distribution Map across the Sampling Sites**



Metric 5: Relative Abundance of Target Fish Species

Definition: Number of individuals (relative abundance) for target fish species, such as the threatened snow trout (*Schizothorax richardsonii*) or golden mahseer (*Tor putitora*), species that may serve as umbrella species, migratory species, or other species important to monitor due to their role in the ecosystem

Calculation: Number of individuals (and/or CPUE) of a target fish species (**Table 3.7**)

Field Methods: Analyze each method separately: electrofishing and cast nets

Scale of Analysis: Analyze each site separately

Target Fish Species: *Schizothorax richardsonii*

Presentation of Data in EIA Report

- Number of individuals and CPUE for each target fish species for each site
- Bar plot, box and whiskers plot, and line graph

Monitoring

- Compare the CPUE per site over time between sampling periods (always compare the same season).
- Examine the trends in CPUE changes over time (statistical trends analysis can be used if desired).
- Compare using bar charts or other graphs to assess trends over time.
- Compare control sites to project impact sites to assess if the trends stay similar over time.

Box 3.1 CPUE Definition

What Is CPUE?

CPUE, or "catch per unit effort," is a way to standardize the data between samples collected by the same method that have not been collected with the same effort. For example, if 25 casts of the cast net were used at sites A and B, and 100 casts were done at sites C and D, the data collected will not be comparable since more effort was put in at sites C and D. In order to make them comparable (see example in **Table 3.7**), divide the number of individuals recorded by the number of sampling effort units: CPUE = number of fish collected/ sampling effort.

It is important to define the sampling effort unit as it is different for each sampling method. CPUE should not be used for comparisons between sampling methods; it can only be used to compare different sampling efforts for the same method. CPUE is suitable for data collected by electrofishing and cast nets. Data from dip nets, underwater video, and eDNA should be used to complement the CPUE analysis for tributaries and juveniles.

Interpretation

- This metric focuses on a few important target fish species that have sufficient data and can serve as an umbrella indicator for other species.
- Each site should be analyzed separately to locate where the changes are occurring and possibly requiring adaptive management.
- It may take several years before changes are observed. Thus, monitoring is done throughout the construction of a hydropower project and for several years during operations.

Table 3.7 Sample CPUE Conversion

Method	Sampling effort units	Sampling unit	No. of fish collected	CPUE (no. of fish/sampling effort)
Cast net	25 casts	1 cast	15	15÷25=0.60 per cast
Cast net	100 casts	1 cast	24	24÷100=0.24 per cast
Electrofishing	20 min (0.34 hour)	Hour of electrofishing	36	36÷0.34=105.88 per hour of electrofishing
Electrofishing	40 min (0.67 hour)	Hour of electrofishing	65	65÷0.67=59.70 per hour of electrofishing

- Comparisons of the trend or slope between seasons can reveal if there is an upward (increasing number of individuals), flat line (stable number), or downward (declining number) trend. Statistical tests such as linear regression can be used to test if the changes are statistically significant when sufficient sample size is reached.
- Statistical analyses can be used to compare the mean CPUE between sites or over time if there are sufficient (more than five) data points, such as sampling sites within a region or multiple sampling surveys at a site over time. Before combining sites and data, consider if the analysis will provide meaningful information to assess changes as a result of HPP impacts.

Example

From **Metric 2**, *Schizothorax richardsonii* was selected as a target fish species, so the analysis here focuses only on this species (**Table 3.8** and **Figure 3.6**). Additional analyses can be done for other target fish species.

Visual Presentation

Figure 3.6 CPUE of *S. richardsonii* by Electrofishing Upstream of Dam, Spring 2021

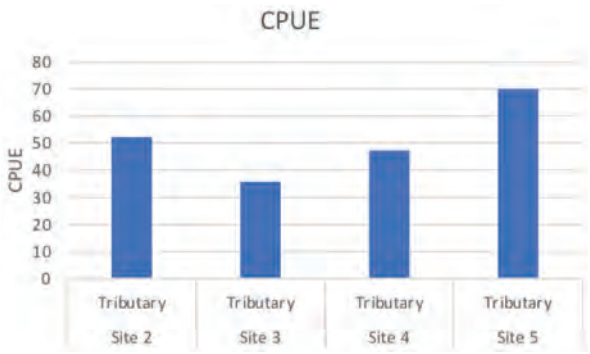


Table 3.8 Electrofishing Field Data, Spring 2021

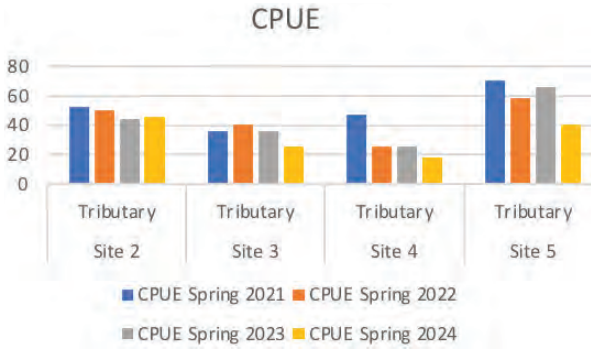
	REGION 1: UPSTREAM OF DAM					
	Number of fish individuals captured					
Fish species	**Site 1 Main stem**	**Site 2 Tributary**	**Site 3 Tributary**	**Site 4 Tributary**	**Site 5 Tributary**	**Site 6 Main stem**
No. of *Schizothorax richardsonii* recorded	--	28	24	24	18	--
Effort (minutes)	--	40	40	23	34	--
Effort (hours)	--	0.67	0.67	0.38	0.57	--
CPUE		**41.8**	**35.8**	**63.16**	**31.58**	

Monitoring

Table 3.9 Summary Data for Spring Survey Field Data (Electrofishing)

	Site 2 Tributary	Site 3 Tributary	Site 4 Tributary	Site 5 Tributary	Control site Tributary
CPUE Spring 2021	52.2	35.8	47.4	70.2	65.3
CPUE Spring 2022	50.3	40.6	25.7	58.8	61
CPUE Spring 2023	43.9	36.2	25.8	66.2	75
CPUE Spring 2024	45.8	25.2	18.2	40.5	82

Figure 3.7 Bar Charts Presenting Spring Survey Field Data (Electrofishing)



Example Interpretation

- The data are presented and analyzed at the site level because we want to see where changes are occurring in order to determine if they are caused by HPP impacts. Upstream sites are important because the HPP dam may block migration of *S. richardsonii* adults to spawning sites in the tributaries upstream. Thus, changes in specific tributaries should be studied.
- In **Figure 3.7**, each of the upstream tributaries (sites 2 to 5) has a downward trend, with Tributary 4 showing the largest drop in spring 2022. This site should be investigated for potential project impacts or other disturbances to the fish population.
- The control site shows an upward trend (**Table 3.9**). This site is outside of the project area and is not directly affected by any HPP or other disturbance, such as sand mining. The trends of this site may indicate that *S. richardsonii* populations are maintained in other sites so that any declines within the project site should be further investigated.
- In this example, only tributary data are provided because electrofishing was not able to be conducted in the main stem. Sites 1 and 6 were on the main stem. Comparisons should always be done between similar types of sites and under similar weather conditions.
- The data from cast netting could show different trends. The same type of analysis should also be done for data from cast nets and compared with the electrofishing data as well as data from dip nets and underwater video if implemented.
- Data from dip netting and underwater video from tributaries can be reviewed separately to provide further information and details for understanding the biology of the fish species.

Metric 6: Recruitment of Target Fish Species (Relative Abundance of Juveniles)

Definition: Number of individuals of juvenile fish, focusing on "young of the year" fish (zero to one-year-old) and young juveniles in their second year of growth (one to two-year-old)

Juveniles should be identified by total length (length of fish from snout to end of tail) or fork length (length of fish from snout to tail fork). This would be determined separately for each species based on the size ranges caught or from literature. For *S. richardsonii*, individuals with less than 10 cm of fork length would be considered juveniles.

This metric can be analyzed as: 1) CPUE or 2) density of individuals per 100 m². Density is a possible metric for juveniles in tributaries since they often congregate in "shoals" and can be unevenly distributed across the sampling site. Focusing on a smaller area where juveniles are congregating can provide more detailed data that can be used to evaluate no net loss of juveniles (recruitment) over time.

Calculation: CPUE and/or density of "young of the year" individuals and/or young juveniles per site

Field Methods: Analyze each method separately, including electrofishing, cast nets, dip nets, and underwater video

Scale of Analysis: Analyze each site separately

Target Fish Species: *Schizothorax richardsonii* juveniles (fish with fork length of less than 10 cm)

Presentation of Data in EIA Report

- CPUE and/or density of individuals (number of individuals/100 m²) for juveniles of *S. richardsonii* and other target fish species for each site
- Bar plot, box and whiskers plot, and line graph

Monitoring

- Compare the CPUE and/or density per site over time between sampling periods (always comparing the same season).
- Analyze the trends of changes in CPUE and/or density over time.
- Compare using bar charts or other graphs to assess trends over time.
- Compare control sites to impact sites to assess if the trends are similar over time.

Interpretation

Same as "Example Interpretation" in **Metric 4** plus the following. This metric is especially important and effective due to several aspects of fish biology (A. Pinder, pers. comm.):

- The presence of juvenile snow trout in tributaries provides definitive evidence that adult fish have successfully migrated into the tributary during the spawning migration.
- Due to the high numbers of eggs deposited by many fish species, particularly cyprinids, and the cumulative mortality throughout life, the numbers of juvenile fish and their availability for capture are considerably higher than that of older life stages.
- The presence of snow trout in early life stages provides evidence beyond the successful immigration of adults from the main river. They also qualify the functionality of habitats to support egg incubation and provide nursery support during the most critical phase of a fish's life.
- Fish in early life stages are present in the tributaries throughout the year and are easy to sample using electrofishing. Adult fish are much more mobile, so they are only present at certain times of the year and are more challenging to catch by surveyors.
- Fish in early life stages are not subject to harvest depletion by local fishers.
- The continued presence of juvenile fish in the tributaries, particularly upstream of the HPP, would indicate that fish passage is being facilitated by the project (for example, if a fish ladder is present) or the river upstream of the HPP provides all necessary critical habitats to support a fragmented fish population.

Example

CPUE is calculated in a similar manner to **Metric 5** but focuses only on juvenile fish. A second metric, density (number of individuals per 100 m^2), is valuable for documenting large groups (shoals) of juveniles, particularly in microhabitats within tributaries. For *S. richardsonii*, juveniles can be identified as fish with fork length of less than 10 cm (100 mm).

Table 3.10 *Schizothorax richardsonii* Juveniles (Electrofishing)

Spring 2021	Site 2 Tributary	Site 3 Tributary	Site 4 Tributary	Site 5 Tributary	Control site Tributary
Number of fish recorded	35	24	18	40	65
Effort (minutes)	40	40	23	34	60
Effort (hours)	0.67	0.67	0.38	0.57	1
CPUE	**52.2**	**35.8**	**47.4**	**70.2**	**65**

Table 3.11 *S. richardsonii* Juveniles (Cast Nets)

Spring 2021	Site 2 Tributary	Site 3 Tributary	Site 4 Tributary	Site 5 Tributary	Control site Tributary
Number of fish recorded	10	6	9	5	15
Effort (no. of casts)	50	100	100	50	100
CPUE	**0.2**	**0.06**	**0.09**	**0.1**	**0.15**

Table 3.12 *S. richardsonii* Juveniles (Targeted Electrofishing in Selected 100 m² Area in Spring Sampling)

Spring 2021	Site 2 Tributary	Site 3 Tributary	Site 4 Tributary	Site 5 Tributary	Control site Tributary
No. of juvenile fish recorded in spring 2021	100	10	22	50	34
No. of juvenile fish recorded in spring 2022	75	56	25	68	55
No. of juvenile fish recorded in spring 2023	20	45	32	59	48
Area	100 m²	100 m²	100 m²	100 m²	100 m²
Density (No. of individuals/100 m²) 2021	100	10	22	50	34
Density 2022	75	56	25	68	55
Density 2023	20	45	32	59	48

Figure 3.8a Bar Charts of CPUE and Density for Five Tributary Sites

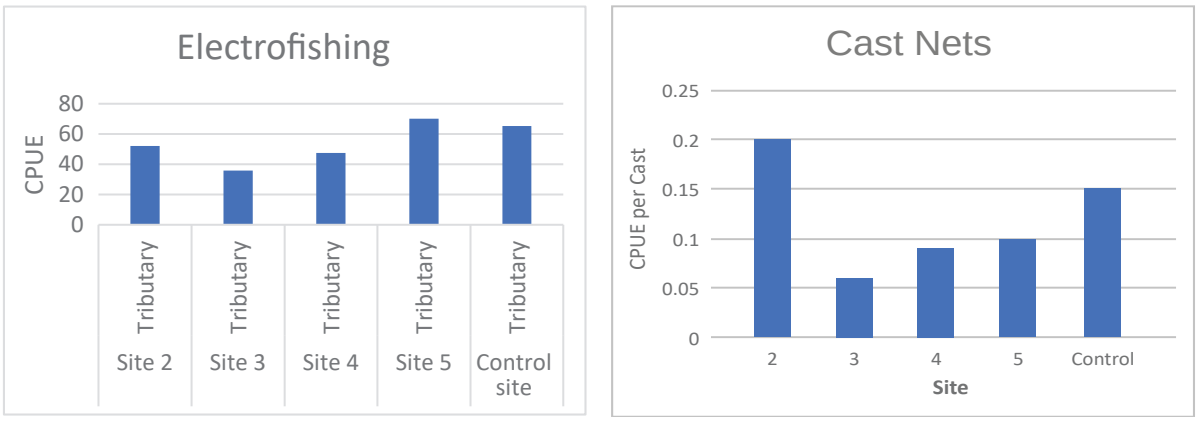
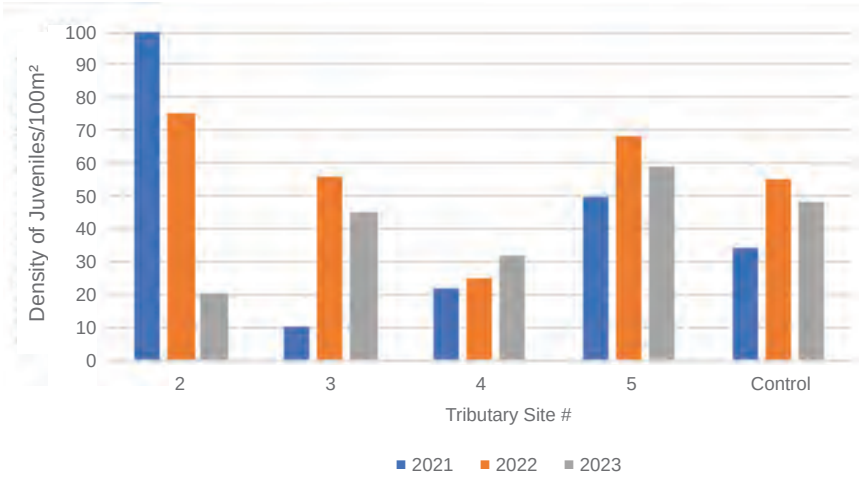


Figure 3.8b Density of Juveniles per 100 m² for Five Tributary Sites in Spring Surveys over Three Years



Example Interpretation

- This metric provides information on the recruitment and spawning success of fish. For *Schizothorax richardsonii*, it happens mainly in tributaries and at the confluence of tributaries with the main river. Thus, in this example, only tributary data are analyzed.
- Of the sampling methods, electrofishing and cast-net data can be analyzed using CPUE and density (example data in **Table 3.10**, **Table 3.11**, **Table 3.12**, and **Figure 3.8**).
- Additional data from dip nets and underwater video are also effective for documenting juvenile fish in tributaries. Data from these methods should be reviewed and analyzed more qualitatively to add details to the analysis. The number of fish documented by underwater video can be analyzed per minute or hour within a specific area to estimate density. The number of juveniles collected by dip nets should be evaluated for each microhabitat sampled to provide more details on the habitats of the juvenile fish.
- The data from these methods are analyzed separately since the efforts and sampling approaches are different and each provides a different perspective to measuring fish abundance. Note that the scales for CPUE are different in the graphs of **Figure 3.8**, so they are not comparable and should be assessed separately.
- Compare the data over time as in **Metric 5**. In **Figure 3.8b**, the density of juveniles is relatively consistent over the three years at all sites except for Tributary Site 2, which dropped in the third year. This could indicate some type of impact by the project or other causes and should be investigated.
- Trends over five years or more should be evaluated to assess if the project is aligned with no-net-loss goals.

Metric 7: Length of Target Fish Species

Definition: Length of each target fish species, measured as fork length and total length, using data from all sampling methods combined, including electrofishing, cast nets, dip nets, and underwater video

Calculation: Mean length plus or minus standard deviation = total of fork length for all fish/number of fish per site

Field Methods: All methods combined (except eDNA)

Scale of Analysis: Analyze each site separately

Target Fish Species: *Schizothorax richardsonii* juveniles (fish with fork length of less than 10 cm)

Presentation of Data in EIA Report

- Present a table of the length data of target fish species per site for all fish from all methods combined (**Table 3.13**). If there are many individuals, this data should be put in an appendix.
- Calculate the mean (average) length plus or minus standard deviation (SD) per site. These can be calculated in an Excel spreadsheet using =AVERAGE (range of values) and =STDEV (range of values).
- Visualize using bar graphs (**Figure 3.9**).

Monitoring

- Compare mean length plus or minus standard deviation for a site across several survey years, for example, spring 2021, 2022, and 2023 (**Table 3.14**).
- Visualize with bar graphs (**Figure 3.10**).
- Compare statistically by using a Student's T-Test to compare two samples, analysis of variance (ANOVA) for multiple samples, and nonparametric tests (Mann-Whitney-Wilcoxon Test and Kruskal-Wallis ANOVA), linear regression, or other statistical tests.

Interpretation

- The mean fish length provides an indication of the population structure in terms of size and age for each sampled site.
- The mean length can show whether the population at a site is primarily of juveniles or adults. Tributaries with many juveniles can indicate that spawning is occurring.

- Fish length is correlated with age, so the age structure of the population can be assessed and compared over time.
- Changes in the mean length can indicate that certain age groups are decreasing or increasing.
- The mean length can indicate if adult fish are reaching particular sites, especially tributaries where they spawn.
- The standard deviation illustrates the amount of variation within the fish sizes at each site.

Example

Table 3.13 Fork Length Measurements for *S. richardsonii* at Four Tributary Sites

	Tributary upstream fork length (cm)	Tributary in diversion reach fork length (cm)	Tributary downstream fork length (cm)	Tributary control fork length (cm)
	9.2	6.5	7.5	10.6
	6.2	12.5	4.3	4.3
	8.5	3.3	8.4	5.7
	15.8	7.7	13.5	7.4
	10.5	15.8	5.6	5.5
	5.2	20.4	9	8.2
	7.7	5.5	8.5	4.6
	6	5.8		12.5
	8	6.9		6.7
	12	10.5		8.2
	6.5			10
Mean	8.69	9.49	8.11	7.61
Standard Deviation (SD)	3.11	5.33	2.92	2.61

Visual Presentation

Figure 3.9 Mean Fish Length at Four Tributary Sites, Spring 2021

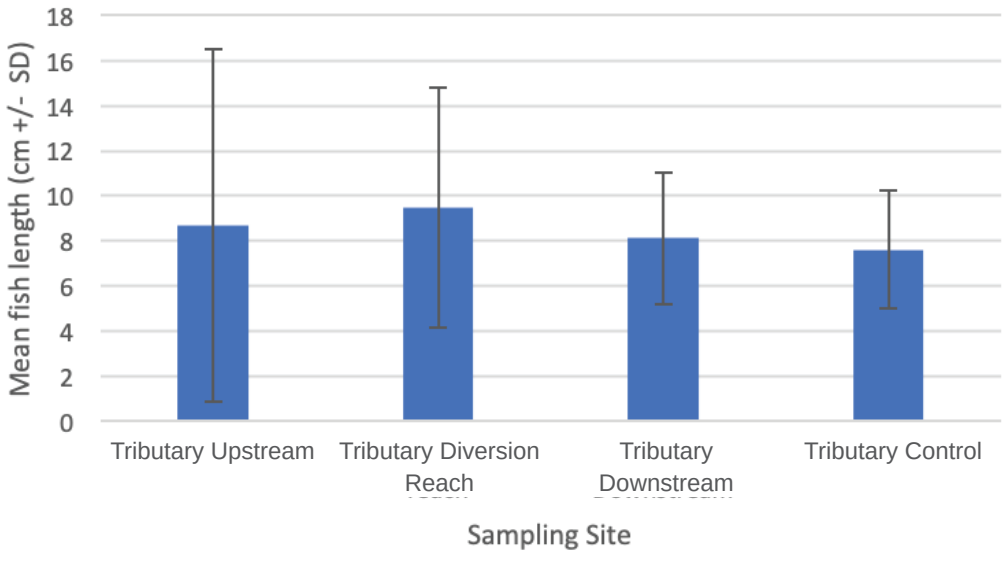
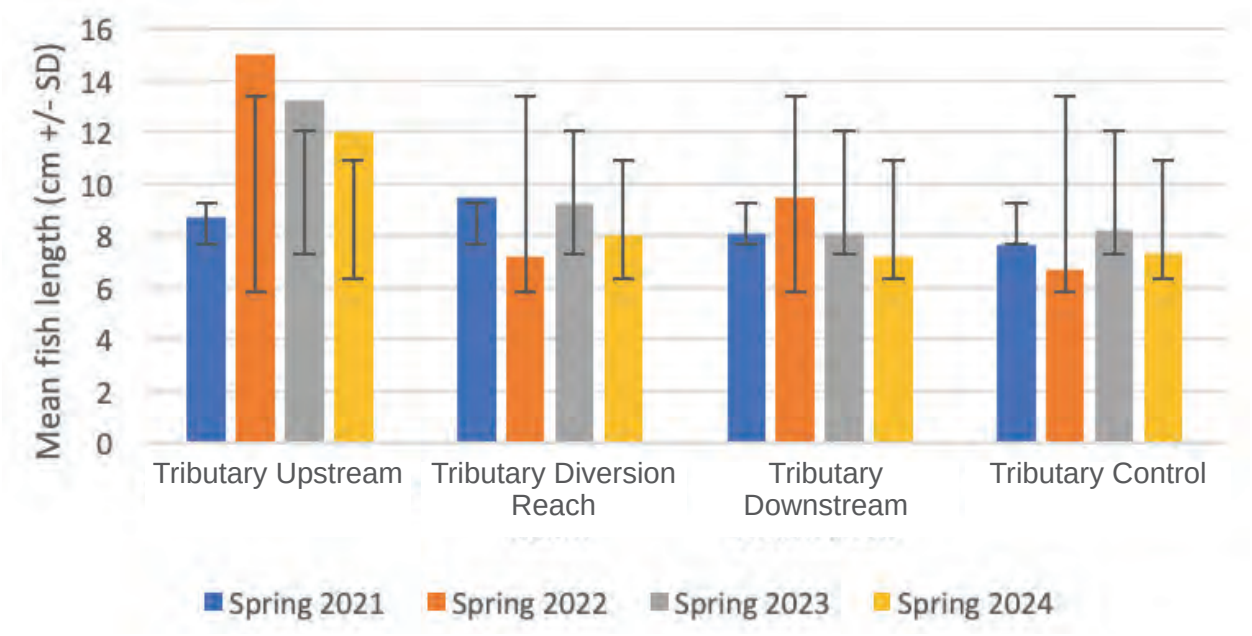


Table 3.14 Mean Fish Length at Four Tributaries over Four Years

	Tributary upstream	Tributary diversion reach	Tributary downstream	Tributary control
Spring 2021	8.69	9.49	8.11	7.61
Spring 2022	15	7.2	9.5	6.7
Spring 2023	13.2	9.2	8.1	8.2
Spring 2024	12	8	7.2	7.3

Figure 3.10 Mean Fish Length at Four Tributaries over Four Years



Example Interpretation

- **Figures 3.9** and **3.10** present the mean length of fish plus or minus the standard deviation for four tributary sites over four years of spring-season sampling from 2021 to 2024.
- The figures show trends in the data over time.
- The fish length data can also be compared using statistical tests such as ANOVA, linear regression, and trends analysis to evaluate if the data are statistically different over time.
- The impact sites, such as the upstream tributary, can be compared with the control site to evaluate differences. If the mean fish size in the control site is relatively constant over time, such as in the graph above, but a tributary site within the impact zone has a change in mean size, this may indicate a change in the age and size structure due to the HPP. These sites warrant further investigation.
- In this example, the mean size of fish upstream is increasing, while the control site is constant. Fish may not be spawning upstream because of barriers to migration or a change in spawning-site conditions. This warrants further investigation to see if it is related to HPP impacts.

3.3 Macroinvertebrate Metrics

Macroinvertebrates are important to include in the EIA and long-term monitoring of a hydropower project because they form the basis of the aquatic ecosystem. Changes to their composition and populations affect the aquatic food chain, resulting in a knock-on effect on fish and other aquatic organisms. Macroinvertebrates are also sensitive to changes and thus can serve as indicators of aquatic ecosystem health.

In contrast to fish, it is difficult to identify macroinvertebrates to species level. Thus, specimens are usually identified to the order, family, and genus taxonomic level where possible. Since the level of identification can vary, macroinvertebrates are characterized and referred to as a "taxon" or as multiple "taxa," which indicate a distinct taxonomic unit.

In addition to taxonomic challenges, very few macroinvertebrate taxa in the Himalayan region have been evaluated to determine their status as threatened species. Therefore, macroinvertebrate data analysis focuses on their use as indicators of the status of, and changes in, aquatic ecosystem health. There are many ways to analyze macroinvertebrate data. See Tachamo Shah et al. (2012; 2020a; 2020b) and FRTC/MoFE (2021) for additional analyses and details as well as examples of macroinvertebrate monitoring in Nepal.

This manual recommends the following three metrics (**Table 3.15**) for analysis of macroinvertebrate data that are useful for assessing and monitoring the impacts of HPPs on the aquatic ecosystem:

1. Macroinvertebrate taxa richness
2. Ephemeroptera, Plecoptera, Trichoptera (EPT) index
3. Relative abundance of functional feeding groups

Field Data

To allow readers to properly understand the metrics and data analysis, the raw field data should be included in the EIA and monitoring reports. The data should include at minimum:

- List of taxa, arranged by order, family, and genus
- Number of organisms captured for each taxon at each site
- Description of each sampling site, including weather conditions and habitat notes

See **Appendix E** for macroinvertebrate field data from the Trishuli River collected in February 2020 (Tachamo Shah et al., unpublished data). The **Appendix E** table illustrates the preferred format for presentation in EIA and monitoring reports. The organisms were identified to family or genus level and data represent the number of individuals recorded for each taxon. These data are used for some of the metric examples below.

Table 3.15 Summary of Recommended Macroinvertebrate Metrics

Metric no.	Indicator	Field method	Metric for each site	Significance
1	Macroinvertebrate taxa richness	Multihabitat assessment using 25 kick-net samples per site	No. of macroinvertebrate taxa per site	Diversity of macroinvertebrate community
2	EPT index	Multihabitat assessment using 25 kick-net samples per site	EPT index = no. of taxa from EPT orders; proportion of EPT taxa per site	Indicator groups assess and detect changes in water quality and aquatic ecosystem health
3	Relative abundance of functional feeding groups	Multihabitat assessment using 25 kick-net samples per site	No. of individuals from each functional feeding group/ total no. of individuals/site	Functional feeding groups represent the condition of aquatic ecosystem health

Metric 1: Macroinvertebrate Taxa Richness and Proportion

Unlike fish, macroinvertebrates in the Himalayas are difficult to identify to species level. Thus, they are usually identified to the genus or family level.

Definition: Taxa richness is the number of taxa (species, genera, or families) recorded

Calculation: Number of taxa per sampling effort

Field Methods: Multihabitat sampling using kick nets

Scale of Analysis: Analyze each site separately

Presentation of Data in EIA Report

- Number of taxa per site, by region, and overall (**Table 3.16**)
- List of taxa by genus, family, and order
- Calculate the proportion of taxa for each order
- Bar charts and pie charts (**Figure 3.11**)

Monitoring

- Visually compare the bar charts and pie charts over time to look for general trends.
- Taxa richness is less useful for long-term monitoring comparisons.

Interpretation

- Taxa richness and proportions should be compared with the number of taxa in similar rivers in the region. Is it high, low, or typical for a Himalayan river? This number can highlight whether there are only a few or many species that need to be considered for impacts.
- Taxa richness data can be compared between regions to see if there are regional baseline differences that could be attributed to other factors, such as elevation, water temperature, number of tributaries, sand mining, and other HPPs.
- Changes in the number of taxa over time are challenging to interpret since the number of species is relatively small and natural variation may be large. Thus, taxa richness is not recommended for quantitative comparisons over time.

Example

Based on the data in **Appendix E**, the survey sampled 78 taxa from 46 families and 11 orders.

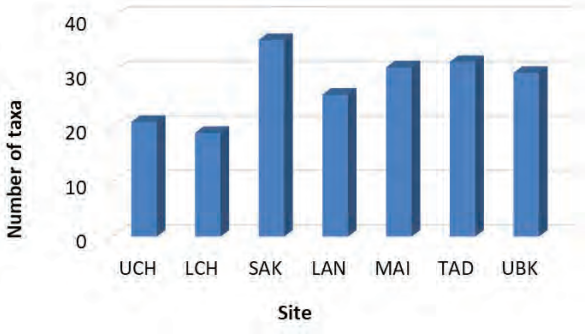
Table 3.16 Number of Macroinvertebrate Genera per Site

Site codes	Number of taxa
UCH	21
LCH	19
SAK	36
LAN	26
MAI	31
TAD	32
UBK	30

Note:
UCH = Upper Chilime Khola; LCH = Lower Chilime Khola;
SAK = Salankhu Khola; LAN = Langtang Khola;
MAI = Mailung Khola; TAD = Tadi Khola;
UBK = Upper Bode Khosi

Visual Presentation

Figure 3.11 Number of Macroinvertebrate Genera per Site



Note:
UCH = Upper Chilime Khola; LCH = Lower Chilime Khola;
SAK = Salankhu Khola; LAN = Langtang Khola;
MAI = Mailung Khola; TAD = Tadi Khola;
UBK = Upper Bode Khosi

Example Interpretation

EIA

- Adding information on the type (tributary or main stem) and locale (upstream, diversion reach, or downstream) of sites would make this graph more informative for showing initial differences between sites and regions.
- The number of taxa per site ranges from 19 to 36. Discuss possible reasons for this, such as habitat type, tributary or main stem river,

water temperature, and other disturbances in the river. General characteristics of each region should be presented and discussed.

- Is the number of taxa per site typical of a Himalayan river of this altitude? Why or why not? Provide comparative data and reference to scientific studies.
- The bar charts can be compared over time for each site to note any major changes in taxa richness.

Metric 2: EPT Index

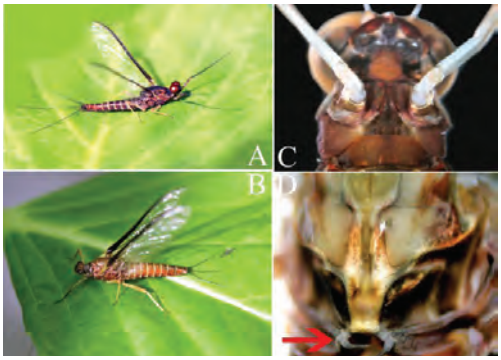
Several aquatic macroinvertebrate taxa are used to evaluate water quality and aquatic ecosystem health. Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies), known as the EPT orders, require clean water quality to survive (see **Figure 3.12**). The EPT index was developed to use these three orders to assess and detect changes in water quality and aquatic ecosystem health.

Figure 3.12 Images of EPT Taxa

Ephemeroptera (mayflies)

Nymph

Adult



Plecoptera (stoneflies)

Nymph

Adult

Trichoptera (caddisflies)

Adult

These three orders have aquatic immature stages that live in rivers and streams. Ephemeroptera and Plecoptera have incomplete metamorphosis, meaning that their immature stage is a nymph that molts and grows larger until it is ready to change into an adult, while Trichoptera have complete metamorphosis consisting of four life-stages, namely egg, larva, pupa, and adult. The nymph leaves the river to molt on land into an adult, which is a flying and terrestrial stage. The adults mate and females lay eggs in the water, starting the cycle again. The nymphs and adults of all three orders are prey items for many fish species. Ephemeroptera and Trichoptera nymphs are collector-gatherers, feeding on organic matter in the water. Odonata nymphs are predators and prey on other macroinvertebrates and small fish. Trichoptera larvae often build themselves a house out of gravel, sand, and organic materials.

Definition: EPT index is a measure of the richness of Ephemeroptera, Plecoptera, and Trichoptera in the sample as an indicator of water quality

Calculation: Number of Ephemeroptera, Plecoptera, and Trichoptera taxa per site; this can also be shown as a proportion (number of EPT taxa/number of total taxa x 100 = % per site)

Field Methods: Multihabitat sampling using kick nets

Scale of Analysis: Analyze each site separately

Presentation of Data in EIA Report

- Total number of macroinvertebrate taxa per site
- Number of Ephemeroptera, Plecoptera, and Trichoptera taxa per site
- EPT index for each site
- List of EPT taxa per site
- Compare the EPT index to scores from other sites in the Himalayas
- Description of each order and its importance in the aquatic ecosystem
- Pie charts

Monitoring

- Compare the EPT index for each site over time to observe trends and compare the index to rating charts.
- Compare the proportion of EPT taxa over time to evaluate changes in water quality.
- Compare pie charts of the proportion of taxa to determine if there are changes over time.

Interpretation

- The EPT Index is used as an indicator of water quality and aquatic ecosystem health. In general:
  - More than 50 percent indicates high water-quality status
  - From 20 to 50 percent indicates moderate water-quality status
  - Less than 20 percent indicates poor water-quality status

Example

Table 3.17 shows the number of genera for different macroinvertebrates collected from the Trishuli River based on field data in **Appendix E**, followed by an example demonstrating how the EPT index is calculated. **Table 3.18** shows the number of taxa of the EPT orders at three different sites and how the EPT index for each site is calculated.

Table 3.17 Number of Macroinvertebrate Genera for All Sites at the Trishuli River

Order	Number of genera	% Total
Coleoptera	4	0.05
Diptera	18	0.23
Ephemeroptera	18	0.23
Hemiptera	1	0.01
Lepidoptera	1	0.01
Megaloptera	1	0.01
Odonata	3	0.04
Opisthopora	1	0.01
Plecoptera	8	0.10
Trichoptera	22	0.28
Trombidiformes	1	0.01
Total	**78**	

EPT taxa = Ephemeroptera (18) + Plecoptera (8) + Trichoptera (22) = 48 taxa

EPT index = Ephemeroptera (18) + Plecoptera (8) + Trichoptera (22)/total taxa x 100 = 48/ 78 x 100 = 61.54% of taxa

Table 3.18 Number of EPT Taxa per Site

Number of taxa	Site		
Order	UCH	LCH	SAK
Ephemeroptera	7	6	10
Plecoptera	2	0	3
Trichoptera	6	7	5
Total number of taxa	21	19	36

Note: UCH = Upper Chilime Khola;
LCH = Lower Chilime Khola; SAK = Salankhu Khola

EPT index per site (see Figure 3.13 and Figure 3.14):

Upper Chilime Khola (UCH) = 7+2+6 = 15;
15/21 x 100 = 71.43% of all taxa

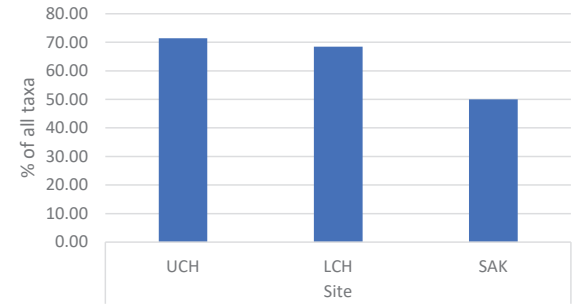
Lower Chilime Khola (LCH) = 6+0+7 = 13;
13/19 x 100 = 68.42% of all taxa

Salankhu Khola (SAK) = 10+3+5 = 18;
18/36 x 100 = 50% of all taxa

Visual Presentation

Figure 3.13 EPT Index at Three Different Sites

Figure 3.14 EPT Taxa as Percentage of All Taxa at Three Different Sites



Example Interpretation

- EPT index ranges from 50 to 71 percent of all taxa in the sampling sites, indicating that the sites have good water quality and that the hydro-morphology of the sites is relatively undisturbed.
- The EPT index can be compared to assess changes in water quality over time.

Metric 3: Relative Abundance of Functional Feeding Groups

Macroinvertebrates play many important roles in the aquatic ecosystem—as shredders, scrapers, collector-gatherers, collector-filterers, and predators. See more details of these functional feeding groups and their food sources in **Table 2.6**. Information on the relative abundance of each functional feeding group at a sampling site provides a picture of the natural balance of these groups in the aquatic ecosystem. Monitoring changes in these groups can highlight if the aquatic ecosystem is becoming out of balance.

Definition: Relative abundance of individuals from each functional group

Calculation: Number of individuals per functional group/number of total individuals/site

Field Methods: Multihabitat sampling using using a kick net

Scale of Analysis: Analyze each site separately

Presentation of Data in EIA Report

- Sort the raw data by functional group
- Total number of taxa for each functional group for each site
- Bar chart

Monitoring

- Compare relative abundance of each group over time and assess trends.
- Compare bar charts over time and assess changes and trends.
- Compare pie charts of the proportion of each functional group over time.

Example

Table 3.19 uses hypothetical data of the number of individuals for each functional group at three sites and **Figure 3.15** is a visual presentation of the same data.

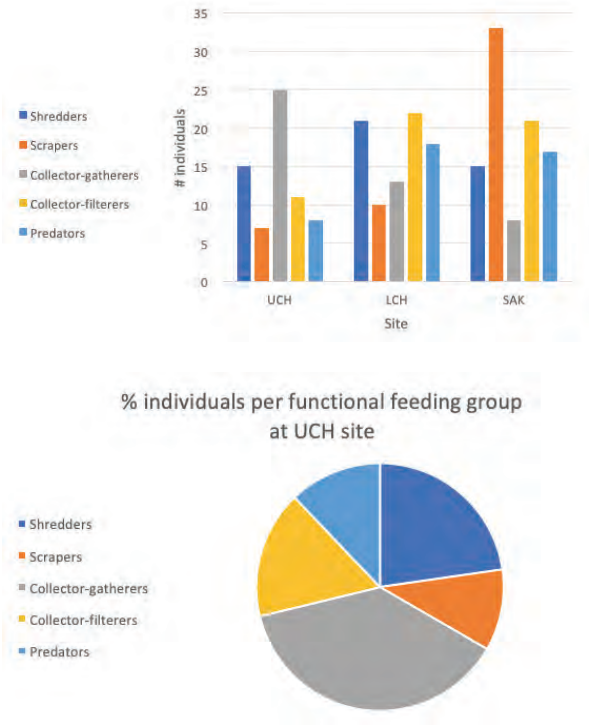
Table 3.19 Number of Individuals for Each Functional Feeding Group at Three Sites, Spring 2021

	Site		
Functional feeding groups	UCH	LCH	SAK
Shredders	15	21	15
Scrapers	7	10	33
Collector-gatherers	25	13	8
Collector-filterers	11	22	21
Predators	8	18	17
Total number of individuals	66	84	94

Note: UCH = Upper Chilime Khola; LCH = Lower Chilime Khola; SAK = Salankhu Khola

Visual Presentation

Figure 3.15 Relative Abundance of Each Functional Feeding Group at Three Sites, Spring 2021



Note: UCH = Upper Chilime Khola

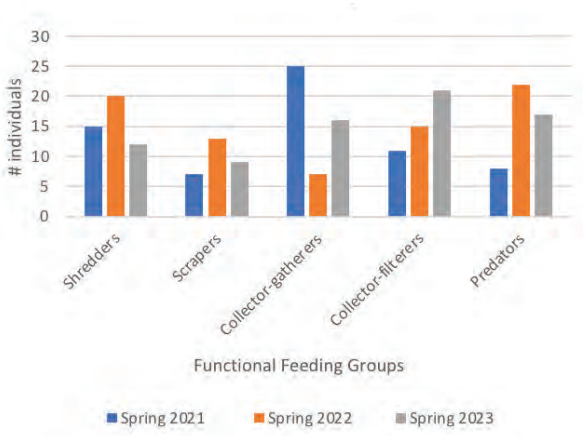
Monitoring

Table 3.20 Number of Individuals for Each Functional Feeding Group at Site UCH over Three Years

	Site		
Functional feeding groups	Spring 2021	Spring 2022	Spring 2023
Shredders	15	20	12
Scrapers	7	13	9
Collector-gatherers	25	7	16
Collector-filterers	11	15	21
Predators	8	22	17
Total number of individuals	66	77	75

Note: UCH = Upper Chilime Khola

Figure 3.16 Relative Abundance of Each Functional Feeding Group at Site UCH over Three Years



Note: UCH = Upper Chilime Khola

Example Interpretation

- The relative abundance and proportion of each functional feeding group is different at each site. Scrapers are most variable between sites during this survey.
- The relative abundance of each functional group varied over time at site UCH (**Table 3.20**). It depends on the availability and quality of allochthonous[3] or autochthonous[4]

[3] Allochthonous inputs are organic particulate matters that come from outside of the river, such as fallen plant leaves, branches, or twigs from surrounding or upstream reaches as well as trees that topple into the river. Allochthonous inputs are a source of carbon, nitrogen, and phosphorus.

[4] Autochthonous inputs are the large plants, attached algae, and phytoplankton that are present within the river system where energy is provided by the photosynthesis of the plants and algae growing in the system.

inputs in the study sites. This is particularly important as any reductions in flow variability downstream of an HPP dam increases the relative proportion of scrapers due to the growth of algae.

- Upstream site and tributaries are important because they receive allochthonous inputs from adjacent riparian vegetation, so the relative abundance of shredders and collector-gatherers are usually high. During construction of a hydropower dam, the riparian vegetation may be removed upstream and downstream of the dam, which will likely change the proportion of shredders and collector-gatherers in the sites as indicated in **Figure 3.16** for spring 2022.
- The results should be discussed in relation to how each functional feeding group contributes to the aquatic ecosystem and what the changes mean for the health of the ecosystem. See more in Tachamo Shah and Shah (2012) and Tachamo Shah et. al (2020a).

3.4 Periphyton Metrics

Similar to aquatic macroinvertebrates, periphyton are important as the foundation of the aquatic ecosystem, serving as food for many organisms and breaking down organic matter.

Table 3.21 shows the principal metric for monitoring periphyton—ash-free dry weight, which is the weight of the periphyton sample after it is dried and oxidized (ashed).

Definition: Biomass of periphyton per site

Calculation: Ash-free dry weight (micrograms) of periphyton/site

Field Methods: Scraping five rocks

Scale of Analysis: Analyze each site separately

Presentation of Data in EIA Report

- Ash-free dry weight of periphyton per site
- Bar charts

Monitoring

- Compare dry weight per site to assess trends over time.
- Changes in periphyton over time may indicate that the food base is declining.

Interpretation

- Ash-free dry weight can be an indicator for monitoring river ecosystem health because its increase may indicate an increase in the river system's productivity.
- Ash-free dry weight should be compared to the pre-construction samples and to control sites, as well as over time, to evaluate if the values are within the normal range for the river system.
- Higher than normal productivity may lead to eutrophic[5] or hypereutrophic state, which may indicate poor quality of the aquatic system. Zero to little flow discharge downstream of an HPP dam elevates the water temperature and the nutrient concentration, which can cause periphyton and algae to increase rapidly.

Table 3.21 Periphyton Metric

Metric	Indicator	Field method	Metric	Significance
1	Periphyton biomass	Scraping five rocks per site	Ash-free dry weight (AFDW) of periphyton per site	Biomass of periphyton indicates health of aquatic ecosystem and shows changes due to river flow and water quality

[5] A eutrophic river is rich in nutrients and may have a dense plant population, the decomposition of which can kill aquatic animal life by depriving it of oxygen.

3.5 Preliminary Assessment of No Net Loss or Net Gain for International Lenders

Many international lenders, such as IFC and the World Bank, require hydropower projects that are operating in natural or critical habitats to show that the project achieves no net loss of biodiversity values and possibly even a net gain of biodiversity values for critical habitats (IFC 2012). It should be noted, however, that natural habitats should not be interpreted as untouched or pristine habitats. It is likely that the majority of habitats designated as natural would have undergone some degree of historical or recent anthropogenic impact, such as the presence of invasive alien species, secondary forest, human habitation, or other human-induced alteration.

With respect to HPPs, where the watershed has been degraded but assemblages of largely native species are present in the water body itself, then IFC's or World Bank's no-net-loss requirements should be applied to the species regardless of the degradation of the surrounding riverine or watershed habitat.

There is no standard calculation for determining metrics or targets for no-net-loss or net-gain status. Given the longer time frames required to achieve no net loss or net gain, one or more interim targets may be needed to track progress toward the final targets. A few examples of ways to achieve no net loss or net gain include IUCN's net-positive-impact approach,[6] the World Bank's recommendations for biodiversity offsets (WBG 2016), and Forest Trends' Business and Biodiversity Offsets Program.[7]

Based on these and other interim targets, thresholds should be set to produce a value that will indicate if the metrics are within or beyond acceptable levels and if adaptive management is warranted. This involves reviewing current management practices that have led to the breach of the threshold and proposing a change to address the failure. Thresholds for no net loss and net gain should be calculated from the baseline data collected in the field and models of predicted changes to determine the natural levels before the project is constructed. Preliminary thresholds can be set as a percentage increase or decrease from the baseline.

This manual does not cover how to calculate thresholds and assess no net loss or net gain. However, the following steps are recommended to begin an assessment:

1. Use the metrics recommended in this manual to track changes over time during pre-construction, construction, and operation of the HPP.
2. Set preliminary thresholds for no net loss for each site based on the baseline data, such as the lowest, mean, or highest baseline value. These thresholds are only preliminary and should be reviewed and updated based on analysis of the monitoring data.
3. Set key color-coded thresholds (red and orange) to alert the HPP to potentially significant deviations from its anticipated trajectory toward no net loss (**Table 3.22**). The green target indicates the project is on track. The orange zone indicates that the project is below the target threshold and the cause of which should be investigated. The red zone indicates that the project is not on target and requires immediate investigation and adaptive management.
4. Make line graphs with interval lines to indicate the maximum, mean, and minimum baseline values for each metric. Changes in the data between the baseline and monitoring surveys should be easily visualized in these line graphs.
5. Preliminary targets can also be set to indicate the desired or predicted net gain in the metrics. This could be set at 1–10 percent and adjusted later based on actual monitoring data.
6. Metrics within the orange and red thresholds alert the project that further investigation and possible adaptive management is required.

[6] https://www.iucn.org/theme/business-and-biodiversity/resources/business-approaches-and-tools/business-and-biodiversity-net-gain

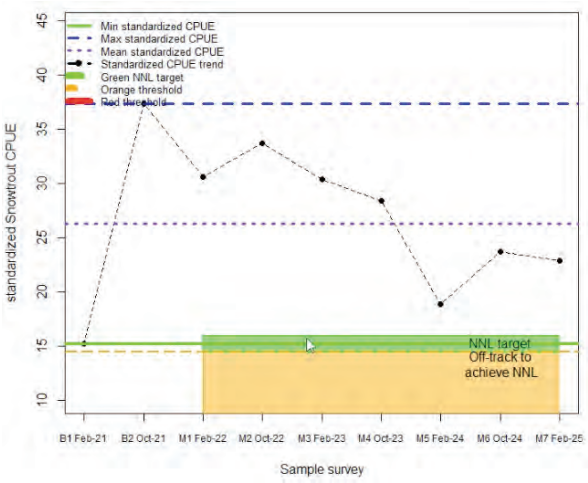
[7] https://www.forest-trends.org/bbop/bbop-key-concepts/biodiversity-offsets/

Table 3.22 Adaptive Management Thresholds

Adaptive management threshold	Definition
No-net-loss target	Metric values within the green block indicate that mitigation measures are effective and supporting progress toward achieving no net loss. The target falls within a user-defined percentage range above the orange threshold.
Orange adaptive management threshold	Metric data points within the orange range indicate that mitigation is off track. The project should check if mitigation measures are being implemented and if they are successful in achieving no net loss. This threshold has a lower and upper percentage change (below and above) the minimum baseline metric values.
Red adaptive management threshold	Metric values within the red range indicate that the project is severely off track. The functioning and effectiveness of mitigation measures should be reviewed immediately, including the operation of EFlows of the hydropower project, to determine what urgent corrective action can be taken to put the project back on track to achieve no net loss. This is a user-defined threshold and extends a percentage (lower limit) below the bottom end of the orange threshold.

Example 1

Figure 3.17 CPUE of *Schizothorax richardsonii* for One Site over Nine Annual Surveys

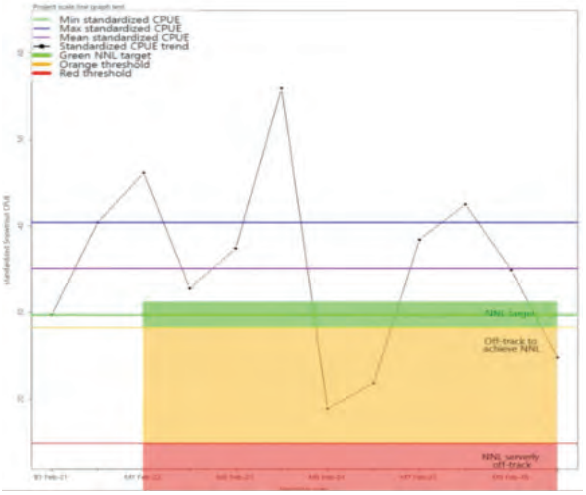


Note: CPUE = catch per unit effort; NNL = no net loss

Note that all values in **Figure 3.17** are above the no-net-loss (green) target that was set as the minimum baseline value. This indicates that the project is on track to achieve no net loss for this metric. Minimum, mean, and maximum baseline values are marked with the dotted lines. Net-gain thresholds should exceed the maximum recorded baseline value by a pre-determined percentage.

Example 2

Figure 3.18 CPUE of *Schizothorax richardsonii* for One Site over 12 Annual Surveys



Note: CPUE = catch per unit effort; NNL = no net loss

CPUE values in **Figure 3.18** are above the no-net-loss threshold until the 7th survey when values drop into the orange zone. Values rise but then fall again into the orange zone. These values alert the project to investigate what is going on at this particular site and to correct or enhance mitigation as needed. Minimum, mean, and maximum baseline values are marked with colored lines.

To achieve a net gain, the metric values should exceed the maximum recorded baseline value by a pre-determined percentage. In this example, the values exceed the maximum value in the 3rd and 6th annual survey (data points) before dropping. This shows the importance of long-term monitoring since changes may greatly vary from year to year.

4 Reporting

4.1 Overview

Nepal's Environment Protection Rules (Nepal Law Commission 1999) and *Hydropower Environmental Impact Assessment Manual* (MoFE 2018) outline requirements and provide guidance for the structure of initial environment examination (Schedule 11) and EIA (Schedule 12) reports. **Table 15** of the *Hydropower Environmental Impact Assessment Manual* (MoFE 2018) also outlines the required structure for government EIAs. Other countries have their own requirements for what an EIA should include. International lenders often have additional requirements and expect a higher level of field assessment and detail in an environmental and social impact assessment (ESIA) report.

The Trishuli Assessment Tool is designed to collect and analyze field data on aquatic biodiversity for EIAs and the long-term monitoring of hydropower projects. The data produced from the tool are suitable for international-level ESIAs and biodiversity evaluation and monitoring programs (BMEPs).

Below are sample outlines of the aquatic biodiversity baseline chapter of an international-level ESIA (**Section 4.2**) and of an international-level BMEP (**Section 4.3**). These examples can be used as a guide to fulfil the requirements of most international lenders.

In addition to presenting the EIA and monitoring data to the hydropower project and the authorities, such data can also be shared with the broader scientific community through scientific publications and the Global Biodiversity Information Facility (GBIF; www.gbif.org). By making data accessible through the international GBIF or similar information platforms, the data can be used by other scientists to advance understanding of the species, which will increase global knowledge of biodiversity and contribute to finding conservation solutions.

4.2 Sample ESIA Report—Aquatic Biodiversity Baseline Chapter

1. Overview of the taxonomic group in the region
   - Summary of information collected from literature with references
2. Sampling sites
   - Rationale and justification for site selection and strategy
   - Map of the sampling sites
   - GPS coordinates of all sampling sites
3. Field methods
   - Dates of surveys at each sampling site
   - Seasonality of the sampling dates, such as wet, dry, and transitional seasons
   - Map and GPS coordinates of all sampling points
   - Detailed field methods
     - Description of each sampling method
     - Sampling effort (such as time, number of samples, and time spent sampling per site)
     - Details of equipment used
   - Names of field workers performing the surveys (including qualifications and affiliations)
   - Names of people identifying the species (including qualifications and affiliations)
4. Data for all species recorded
   - Total number of species recorded, including the number of genera and of families
   - Number of species recorded at each sampling site
   - Number of species recorded in each region (upstream of dam, diversion reach between dam and powerhouse, and downstream of powerhouse)

- List of all species by sampling site and date, with number of individuals of each species
- For taxa with a long list of species, include a list of the 25 most common (by number of sampling sites or number of individuals)

5. Lists and discussion of target species of conservation importance, including:
   - Threatened species (IUCN Global Red List and regionally or nationally threatened species)
   - Range restricted species (endemic)
   - Rare species
   - Migratory and congregatory species
   - Species of cultural and religious importance
   - Species of medicinal importance
   - Species that may cause or promote disease in humans
   - Species of livelihood importance
   - Invasive species
6. Compilation of the data with data previously collected for the area for comparisons
7. Analysis of data metrics for target species and establish baseline for long-term monitoring; metrics should include species richness, species composition, relative abundance of target species, relative abundance of juveniles, and aquatic ecosystem health indexes (see **Section 3**)
8. Identification of important habitat types for species such as fish spawning sites, migration routes, habitats, or other areas of high importance for biodiversity; for example, analyze high macroinvertebrate species richness and concentrations of threatened species
   - Map(s) of important areas for biodiversity
   - Discussion of the importance of the areas
9. Discussion of species of conservation importance listed in the literature, which could be in the project area but were not documented during the survey
10. Photos and video of sampling sites, field methods, and species of interest
11. Appendixes with raw data

The Impact Assessment Chapter of the EIA should include an evaluation of the direct, indirect, residual, and cumulative impacts that the project may have on the biodiversity documented, particularly on species of conservation importance, important habitats, and areas of high or unique biodiversity. Impacts may be negative, neutral, or positive depending on the species or habitats' adaptability to new conditions, such as reservoirs and altering flow regimes. A cumulative impact assessment should follow international guidelines such as those prepared by IFC (2020). For the aquatic ecosystem, a high resolution, holistic environmental-flow (EFlow) assessment should be used to evaluate the project impacts upstream and downstream of the proposed project location. IFC's *Good Practice Handbook on Environmental Flows for Hydropower Projects* (IFC 2018) provides guidance on selecting and implementing the appropriate EFlow assessment for HPPs.

The Mitigation Chapter of the EIA should include actions to minimize project impacts on the overall aquatic environment and on species of conservation importance according to the mitigation hierarchy, that is, avoid, reduce, restore, and offset impacts (CSBI 2015). For many international lenders, including the World Bank, IFC, and the Asian Development Bank, the mitigation measures must demonstrate that the project achieves no net loss of biodiversity values for natural habitats and a net gain for critical habitat values (IFC 2012).

4.3 Sample BMEP Report for Monitoring Aquatic Biodiversity for a Hydropower Project

1. Background on the project and biodiversity of the area
2. Monitoring results and analysis

Fish

- Objectives of monitoring fish
- HPP mitigation measures assessed with the monitoring metrics
- Long-term monitoring field sampling methodology
  - Dates of surveys at each sampling site
  - Seasonality of the sampling dates, such as wet, dry, and transitional seasons
  - Map and GPS coordinates of all sampling points
  - Detailed field methods
    - Description of each sampling method
    - Sampling effort (such as time, number of samples, and time spent sampling per site)
    - Details of equipment used

- Names of field workers performing the surveys (including qualifications and affiliations)
- Names of people identifying the species (including qualifications and affiliations)
- All raw data from the field surveys presented in appendixes, including habitat descriptions, species, number of individuals, and measurements

- Summary of overall monitoring results with graphs and figures
- Analysis and discussion of fish metrics (see **Section 3.2**)
  1. Species richness
  2. Species composition
  3. Proportion of species
  4. Species distribution
  5. Relative abundance of target fish species
  6. Recruitment of target fish species
  7. Length of target fish species
- Interpretation of analysis results in relation to the mitigation measures and impacts
- Recommendations for changes to monitoring methodology
- Recommendations for adaptive management based on monitoring metrics

Macroinvertebrates

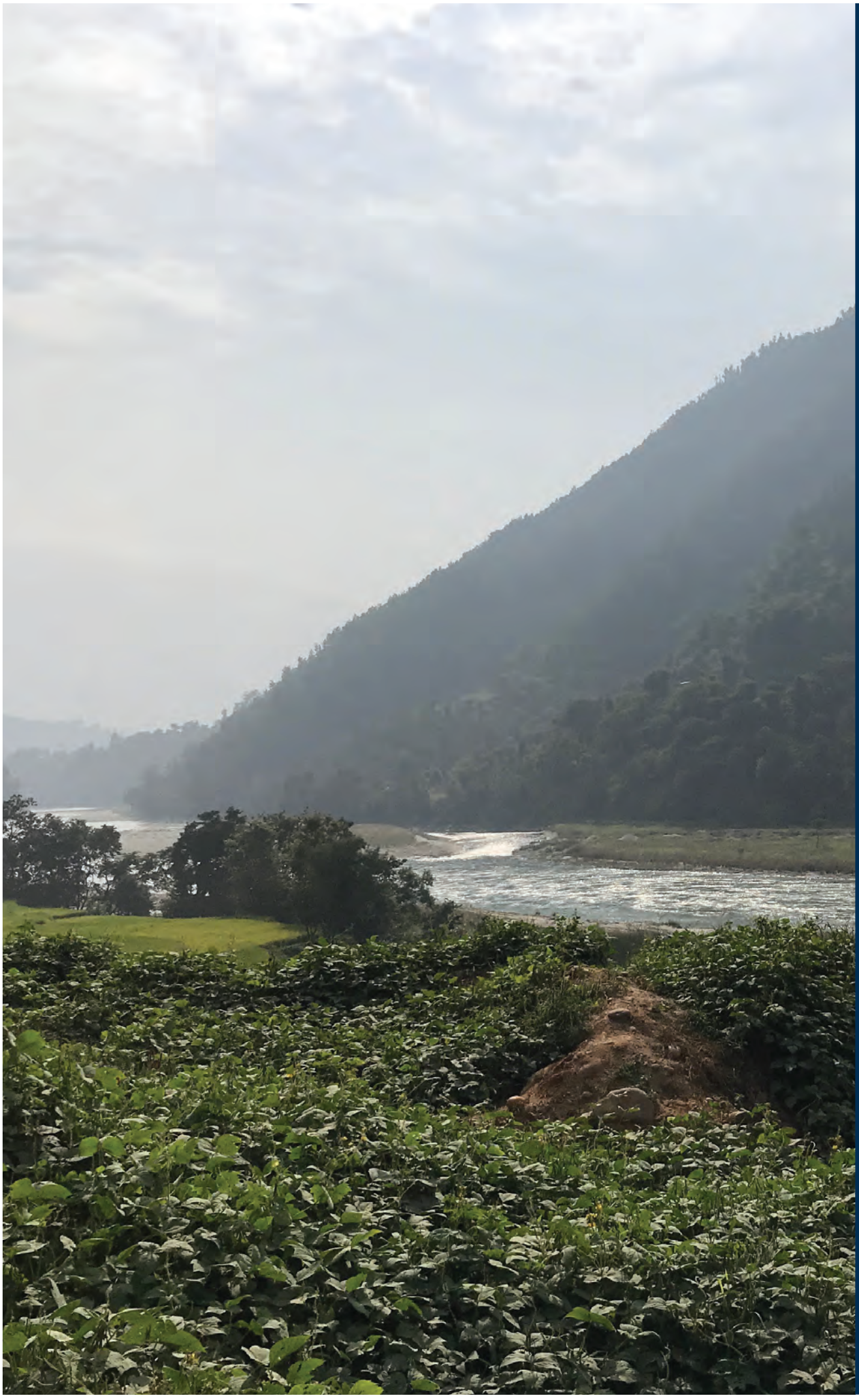
- Objectives of monitoring macroinvertebrates
- HPP project mitigation measures assessed with the monitoring metrics
- Long-term monitoring field sampling methodology
  - Dates of surveys at each sampling site
  - Seasonality of the sampling dates, such as wet, dry, and transitional seasons
  - Map and GPS coordinates of all sampling points
  - Detailed field methods
    - Description of each sampling method
    - Sampling effort (such as time, number of samples, and time spent sampling per site)
    - Details of equipment used
  - Names of field workers performing the surveys (including qualifications and affiliations)
  - Names of people identifying the species (including qualifications and affiliations)
  - All raw data from the field surveys presented in appendixes, including habitat descriptions, species, number of individuals, and measurements
- Summary of overall monitoring results with graphs and figures
- Analysis and discussion of macroinvertebrate metrics (see **Section 3.3**)
  1. Macroinvertebrate taxa richness
  2. EPT index
  3. Relative abundance of functional feeding groups
- Interpretation of analysis results in relation to the mitigation measures and impacts
- Recommendations for changes to monitoring methodology
- Recommendations for adaptive management based on monitoring metrics

Periphyton

- Objectives of monitoring periphyton
- HPP project mitigation measures assessed with the monitoring metrics
- Long-term monitoring field sampling methodology
  - Dates of surveys at each sampling site
  - Seasonality of the sampling dates, such as wet, dry, and transitional seasons
  - Map and GPS coordinates of all sampling points
  - Detailed field methods
    - Description of each sampling method
    - Sampling effort (such as time, number of samples, and time spent sampling per site)
    - Details of equipment used
  - Names of field workers performing the surveys (including qualifications and affiliations)
  - Names of people identifying the species (including qualifications and affiliations)
  - All raw data from the field surveys presented in appendixes, including habitat descriptions, species, number of individuals, and measurements
- Summary of overall monitoring results with graphs and figures
- Analysis and discussion of periphyton metrics (see **Section 3.4**)
  - Biomass, measured as ash-free dry weight
- Interpretation of analysis results in relation to the mitigation measures and impacts
- Recommendations for changes to monitoring methodology
- Recommendations for adaptive management based on monitoring metrics

3. Summary of all monitoring results

4. Recommendations for monitoring and adaptive management



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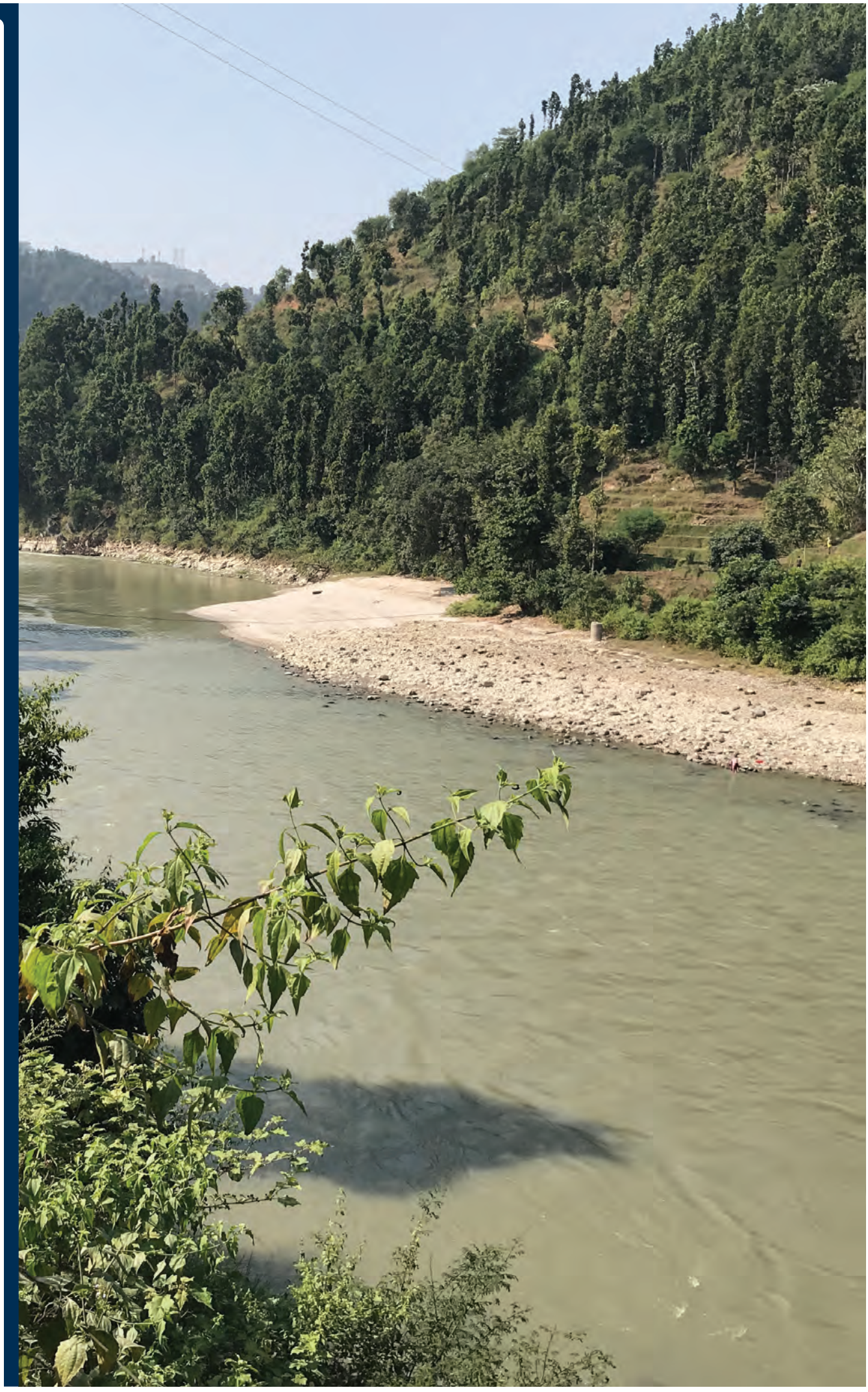


Appendixes

Appendix A
Field Data Sheet for Fish Data Recording

FISH SAMPLING DATA SHEET										
Site Number										
River Name										
Location										
Location Code										
Date										
Time										
Method										
Sampling Effort (time or #)										
Upstream										
Dpwnstream										
Total # fish										
Total # fish										
Upstream/ Downstream Sample #	Fish #	Species	Fork Length (mm)	Total Lenght (mm)	Weight (grams)	Photo (Y/N)	DNA - Fin Clip (Y/N)	Voucher Specimen (Y/N)	Fish ID Code	Notes

Appendix B
Field Data Sheet for Recording Site and Habitat Characteristics

Field data sheet for recording site and habitat characteristics, with a set of example field data from the February 2020 Trishuli River field sampling (DS = downstream, US = upstream of sampling center point)

		Example Field Data	Field Data
REGION		**Upstream of HPP**	
SITE CATEGORY	**SITE #**	**3**	
Location	**RIVER**	**Lower Chilime Khola**	
Location	**SITE CODE**	**LCH**	
Location	**LOCATION**	Shyrapru Besi	
Location	**GPS LATITUDE (N)**	28.1816	
Location	**GPS LONGITUDE (E)**	85.3423	
Location	**DATE SAMPLED**	FEBRUARY 27, 2020	
Location	**ELEVATION (m)**	1495	
Water Data	**WATER TEMP (°C)**	12.4	
Water Data	**CONDUCTIVITY (µmhos/cm)**	377	
Water Data	**FLOW**	Moderate	
Water Data	**TURBIDITY**	Low/Mod	
Site Total Area	**SITE LENGTH TOTAL (m)**	400	
Site Upstream Area	**SITE LENGTH US (m)**	200	
Site Downstream Area	**SITE LENGTH DS (m)**	200	
Upstream Area	**UPSTREAM WET WIDTH (m)**	8	
Upstream Area	**UPSTREAM TOTAL WIDTH (m)**	30	
Upstream Area	**US % RAPID**	30	
Upstream Area	**US % RIFFLE**	20	
Upstream Area	**US % RUN**	10	
Upstream Area	**US % POOL**	40	
Upstream Area	**US % SLACK**	0	
Upstream Area	**US % BACKWATER**	0	
Center Area	**CENTERPOINT**	Suspension bridge	
Center Area	**CENTERPOINT WET WIDTH (m)**	5	
Center Area	**CENTERPOINT TOTAL WIDTH (m)**	40	
Downstream Area	**DOWNSTREAM WET WIDTH (m)**	12	
Downstream Area	**DOWNSTREAM TOTAL WIDTH (m)**	20	
Downstream Area	**DS % RAPID**	30	
Downstream Area	**DS % RIFFLE**	20	
Downstream Area	**DS % RUN**	10	
Downstream Area	**DS % POOL**	40	
Downstream Area	**DS % SLACK**	0	
Downstream Area	**DS % BACKWATER**	0	
Method	**ELECTROFISHING**	Yes	
Method	**CAST NET**	Yes	
Method	**DIP NET**	No	
Method	**GO PRO VIDEO**	Yes	
Method	**E-DNA**	Yes	
Method	**MACRO-PERIPHYTON**	Yes	
NOTES			

Appendix C
Data Sheets for Macroinvertebrate Field Data Recording

Appendix C.1
Site Information Sheet

1. Site Information

1.1 **River/Stream**	1.2 **River system**	1.3 **Place, district, province**
1.4 **Site code**	1.5 **Coordinates** **N:** **E:** **Altitude:**	1.6 **Date/Time**
1.7 **Surveyor**	1.8 **Investigator**	

2. Catchment Characteristics

2.1 **Predominant surrounding land-use:** Indicate at 10% intervals for 1 km river stretch (taken upstream of site) ☐ Forest .....% ☐ Field/Pasture .....% ☐ Agricultural .....% ☐ Residential .....% ☐ Commercial .....% ☐ Industrial .....% ☐ Other (Specify) .....%	2.2 **Riparian vegetation (within 18 m buffer in sampling):** 2.2.1 **Dominant vegetation type:** Trees ☐ Shrubs ☐ Grasses ☐ Herbaceous ☐ 2.3 **Canopy cover at zenith:** Open ☐ Partly open ☐ Partly shaded ☐ Shaded ☐ 2.4 **Local watershed erosion:** None ☐ Moderate ☐ Heavy ☐

3. Hydro-Morphological Parameters (Instream Features)

3.1 **River depth:**	3.2 **Wetted river width:** (Avg. of 4 measurements within 100 m stretch)	3.3 **Discharge (m^3/s)**	3.4 **Proportion of reach represented by flow types:**
Min.: ..... **Max.: .....** **Avg.: .....**	**i) .....** **ii) .....** **iii) .....** **iv) .....** **Avg.: .....**	**Min.: .....** **Max.: .....** **Avg.: .....**	Rapid .....% Riffle .....% Run .....% Pool .....%

4. Water-Quality Parameters

4.1 **Temperature** **.....˚C**	4.2 **pH** **.....**	4.3 **Turbidity** **..... NTU**	4.4 **DO, DO Saturation** **..... mg/L, ...%**
4.5 **Electrical conductivity** **..... µS/cm**	4.6 **TDS** **..... (mg/L)**	4.7 **Nitrate** **.....(mg/L)**	4.8 **Phosphate** **.....(mg/L)**

Source: FRTC/MoFE 2022

Appendix C.2
Habitat Estimation Sheet

Site code:	Date/time:	Investigator:					
Mineral substrate	Coverage (5% steps)	Sampling units (no.)	Flow types				
			Run	Pool	Riffle	Glide	Rapid
Boulders, bedrock (> 40 cm)							
Cobbles (> 20 cm – 40 cm)							
Stones (> 6 cm – 20 cm)							
Pebbles (> 2 cm – 6 cm)							
Gravel (>0.2 cm – 2 cm)							
Sand and mud (>6µm – 2 mm)							
Silt loam, clay (inorganic) (< 6 µm)							
Artificial substrates							
Sum	100	20					
Biotic substrate							
Algae							
Macrophytes – Emergent							
Macrophytes – Submerged							
Macrophytes – Floating							
Living parts of terrestrial plants							
Wood – tree trunks, branches, roots							
Coarse particulate organic matter deposits							
Fine particulate organic matter deposits							
Debris – organic and inorganic matter deposits							

Source: FRTC/MoFE 2022

Appendix D
Data Sheet for Periphyton Sampling Field Data

Periphyton sampling data

Site number: River Name:

Location: Site code: Date: Time:

Stone measurement

Stone	Dimensions (cm)			Circumference (cm)	Water depth (cm)
	X	Y	Z		
1					
2					
3					
4					
5					

Sketch of sampling site from where the stones were picked up

Appendix E
Sample Macroinvertebrate Field Data for the Trishuli River

Order	Family	Subfamily/ Genus	Functional feeding groups	UCH	LCH	SAK	LAN	MAI	TAD	UBK
Coleoptera	Elmidae		Collector-gatherers					1	8	2
Coleoptera	Gyrinidae		Predator			3				
Coleoptera	Psephenoidinae	Psephenoidinae	Scrapers			12			5	
Coleoptera	Scirtidae		Collector-gatherers			1				
Diptera	Athericidae		Predator	2	2		6	3		4
Diptera	Blepharicera	Blepharicera	Scrapers							6
Diptera	Blepharicera	Horaia	Scrapers			9		20	1	
Diptera	Ceratopogonidae		Predator		1					
Diptera	Chironominae	Tanytarsini	Collector-filterers			1				
Diptera	Chironominae	Tanypodinae	Predator		1	1			1	
Diptera	Chironominae	Diamesinae	Collector-gatherers	10						3
Diptera	Chironominae	Orthocladiinae	Scrapers	2		21	5	2		10
Diptera	Chironominae	Chironominae	Collector-gatherers							
Diptera	Deuterophlebiidae		Scrapers							
Diptera	Dolichopodidae		Predator			1				
Diptera	Empididae		Predator			1				
Diptera	Limoniidae	Hexatoma	Predator	10	1		5		6	2
Diptera	Limoniidae		Predator		1	5	19	13	7	9
Diptera	Pediciidae	Dicranota	Predator							1
Diptera	Simuliidae		Collector-filterers			27	3		5	
Diptera	Tabanidae		Predator			4		1	4	
Diptera	Tipulidae		Predator	36		1	2			2
Ephemeroptera	Ameletidae	Ameletus	Scrapers, Collector/ Gatherers				2			
Ephemeroptera	Baetidae	Platybaetis	Collector-gatherers			1		1		
Ephemeroptera	Baetidae	Baetiella	Collector-gatherers	5	53	33		48	21	
Ephemeroptera	Baetidae	Acentrella	Collector-gatherers	4	26	18	20		15	1127

Order	Family	Subfamily/ Genus	Functional feeding groups	UCH	LCH	SAK	LAN	MAI	TAD	UBK
Ephemeroptera	Baetidae	Baetis	Collector-gatherers	1444	420	26	87	32	92	303
Ephemeroptera	Baetidae	Caenis	Collector-gatherers		2				8	
Ephemeroptera	Ephemereliidae	Uracanthella	Collector-gatherers						3	
Ephemeroptera	Ephemereliidae	Torleya coheri	Collector-gatherers			8			26	
Ephemeroptera	Ephemereliidae	Torleya	Collector-gatherers						34	
Ephemeroptera	Ephemereliidae	Cincticostella	Collector-gatherers	9	1	2	44	5	4	376
Ephemeroptera	Heptageniidae	Eletrogena	Scrapers					2		
Ephemeroptera	Heptageniidae	Ecdyonurus	Scrapers					4		
Ephemeroptera	Heptageniidae	Cinygmina	Scrapers	7		1			10	
Ephemeroptera	Heptageniidae	Rhithrogena	Scrapers			2	14		26	1
Ephemeroptera	Heptageniidae	Epeorus	Scrapers	20		11	8	21	2	3
Ephemeroptera	Heptageniidae	Iron	Scrapers	4	4	23	5	7		81
Ephemeroptera	Leptophlebiidae	Choroterpides	Collector-gatherers						9	
Heteroptera	Aphelocheiridae		Predator						1	
Lepidoptera	Pyralidae	Eoophyla	Scrapers			1				
Megaloptera	Corydalidae		Predator	1		3	1	1		2
Odonata	Euphaeidae		Predator			4				
Odonata	Gomphidae		Predator		1	17	1		2	1
Odonata	Platystictidae		Predator			1				
Opisthopora	Megascolecidae	Perionyx exavatus	Collector-filterers							1
Plecoptera	Nemouridae	Indonemoura	Shredders	350			6			74
Plecoptera	Perlidae	Kiotina	Predator							1
Plecoptera	Perlidae	Janoneuria	Predator				2			
Plecoptera	Perlidae	Acroneurinae	Predator	3						
Plecoptera	Perlidae	Calineuria	Predator					1		3
Plecoptera	Perlinae	Perlinae	Predator			6				
Plecoptera	Perlidae	Neoperla	Predator			12	1			
Plecoptera	Perlidae	Paragnetina	Predator			11	37	4	2	7
Trichoptera	Brachycentridae	Brachycentrus	Scrapers							

Order	Family	Subfamily/ Genus	Functional feeding groups	UCH	LCH	SAK	LAN	MAI	TAD	UBK
Trichoptera	Calamoceratidae	Anisocentropus	Shredders							1
Trichoptera	Uenoidae	Uenoa	Scrapers					1		
Trichoptera	Glossosomatidae	Agapetinae	Scrapers						1	
Trichoptera	Glossosomatidae	Glossosomatinae	Scrapers		5			28	10	4
Trichoptera	Goeridae	Goera	Scrapers					2		
Trichoptera	Hydrobiosidae	Apsilochorema	Predator		1			1		
Trichoptera	Hydropsychidae	Paratopsyche	Collector-filterers				1	12		1
Trichoptera	Hydropsychidae	Cheumatopsyche	Collector-filterers						27	
Trichoptera	Hydropsychidae	Hydropsyche	Collector-filterers			71	1	32	4	15
Trichoptera	Hydropsychidae	Ceratopsyche	Collector-filterers	4	2	20	1	3	58	
Trichoptera	Lepidostomatidae	Lepidostoma	Shredders	1		1			1	
Trichoptera	Limnephilidae	Limnephilinae	shredders, Collector-gatherers	6				1		1
Trichoptera	Limnocentropo-didae	Limnocentropus	Predator					1		
Trichoptera	Philopotamidae	Chimarra	Collector-filterers						3	
Trichoptera	Philopotamidae	Dolophilodes	Collector-filterers					5		
Trichoptera	Philopotamidae	Wormaldia	Collector-filterers							
Trichoptera	Polycentropodidae	Poycentropus	Collector-filterers					3		
Trichoptera	Psychomyiidae	Psychomyia	Collector-gatherers					1		
Trichoptera	Rhyacophilidae	Hypo-rhyacophila	Predator		2	1	2	1		1
Trichoptera	Rhyacophilidae	Himalopsyche	Predator	2	2		8			
Trichoptera	Rhyacophilidae	Para-rhyacophila	Predator	2	1		4			11
Trichoptera	Stenopsychidae	Stenopsyche	Collector-filterers	6	1	4	58	4	1	3
Trombidiformes- Hydracarina			Predator					1		

Source: Tachamo Shah, unpublished data

Appendix F
Detailed Instructions for Conducting Backpack Electrofishing

This appendix (Beaumont 2021a) outlines the steps for conducting electrofishing using the Smith-Root LR24 backpack electrofishing equipment, as recommended by William R.C. Beaumont of Electric Fishing Technical Services (EFTS) Ltd. Other types of backpack electrofishing equipment may have different operational methods and controls, so users must read the user manual carefully.

Important Considerations

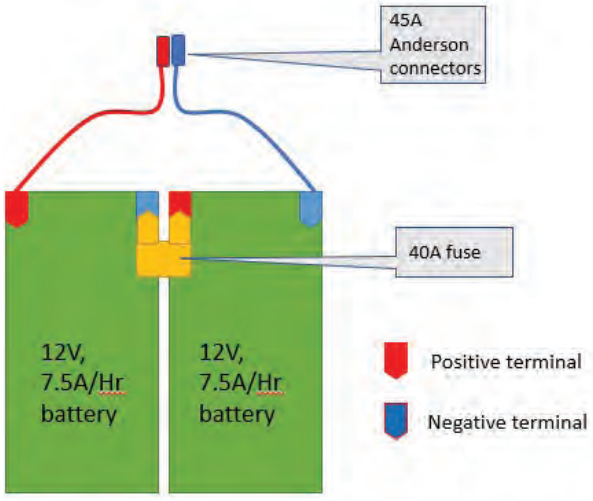
- All users of the equipment must be suitably trained.
- A person trained in resuscitation should be included in the team using the equipment.
- The use of the anode-out-of-water detection system is recommended to improve safety.
- To prolong battery duration, a pulsed-direct-current (PDC) waveform is recommended to be used for fishing.

Steps for Electrofishing

1. Examine all equipment for damage. Do not use damaged equipment, which may pose safety risks.
2. Ensure the red "stop switch" is in the depressed (off) position.
3. Install the battery in the lower section of the backpack. **Do not connect the battery leads**.
4. Connect the cathode and the anode to appropriate sockets on the backpack. Ensure the plug "collars" are tightened. Electrode leads should either be routed out of the side of the battery compartment or routed in the pre-formed channels under the battery (in which case the electrodes will need connecting before the battery is installed). Whichever route is used for the cables, make sure that the electrode lead strain relief systems are correctly positioned.
5. When ready to begin fishing, connect the battery leads and secure the cover over the battery.
6. Measure the ambient conductivity of water to be fished and set the output voltage and pulse width appropriately (see **Appendix G** for more information on setting the output voltage).
7. Set pulse frequency to settings appropriate for fish species, size, and river conditions.
8. Ensure all members of the fishing team know their roles and responsibilities.
9. Switch the red "stop switch" to the ON position. Test the safety systems are operating correctly (lean forward to test tilt switch and put wet tissue or material on the immersion sensor).
10. Test the equipment in an area outside the survey reach. Make a note of the output settings displayed on the status screen of the equipment, including power (watts), amperes (A), and voltage.
11. Begin fishing the survey section.
12. When the survey is completed, depress the red "stop switch" to the OFF position and disconnect the battery leads.
13. Remove the electrodes and the battery.
14. Clean and disinfect the equipment.

Maintenance

- Before assembling the equipment, make sure that the battery pack/s are fully charged. Batteries should always be charged after use. If the equipment is not used for long periods, the batteries should be charged every three months to maintain capacity.
- Be sure to use the correct type and voltage of battery for the electrofisher.
- If additional/replacement battery packs are required, a competent electrician should be able to assemble new ones. Batteries should be 12 volts (V), 7.5 ampere hour (Ah) lead-acid gel. They should be wired in series to produce an output of about 24 V. The battery packs are connected to the electrofisher using 45 A Anderson Powerpole connectors. A 40 A fuse should be wired between the connected positive and negative terminals (see diagram below).



Appendix G
Best Practice Manual for Backpack Electrofishing

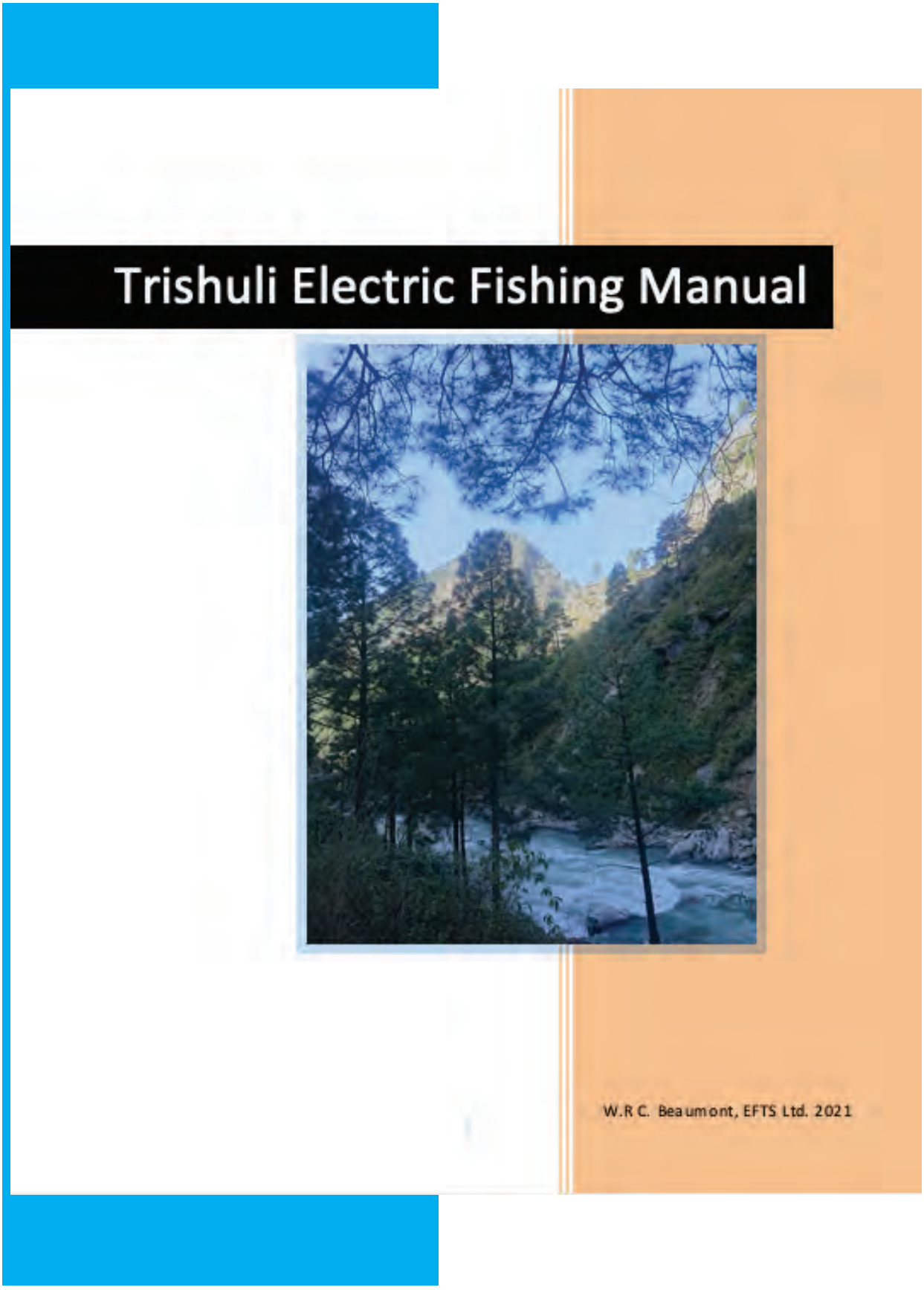


Table of Contents

1. INTRODUCTION

Electric fishing is an effective way to sample freshwater fish populations. However, electric fishing may also cause fish injury or mortality to both fish and humans. The purpose of this overview is to provide specific, concise, guidance on health and safety, and correct adjustment of electrical output settings. This will enable the safe optimization of efficient fish capture, under a range of environmental conditions, ensure safety for operators and minimize injury to fish. The manual is intended as supplementary information to accompany the electric fishing courses run by EFTS Ltd. For more detailed information, a brief biography of research papers is included and purchase of *Electricity in Fisheries Research and Management: Theory and Practice* by W.R.C. Beaumont is recommended.

It should be remembered that electric fishing is not the only method for fish population evaluation or removal and these other methods (netting, trapping etc.) should be considered when deciding on the most appropriate method to be used.

2. HEALTH AND SAFETY

Electric fishing is potentially hazardous. No one should be in close proximity to energized electrodes if they have a history of cardiac problems or stress induced respiratory problems. Severe electric shocks can cause distortion of the heart's rhythm and/or respiratory arrest. It is recommended that at least two persons in each electric team be trained in cardiopulmonary resuscitation (CPR) techniques (in case the trained person is the patient).

All equipment used must be in good condition and should be suitable for the purpose of electric fishing. It should be regularly checked by a competent person and visually checked after each use. Faults must be reported, and faulty equipment must not be used.

There are three principal hazards associated with electric fishing: electric shock, drowning, and tripping/falling; however, other dangers are present when carrying out fieldwork.

2.1. Electric Shock

The severity of electric shock is related to the magnitude of the current, the duration of the shock and the current waveform. For example, direct current (DC) causes a severe shock only when the current is made or broken, not when the current is steady. By contrast, a 50 or 60 Hz alternating current (AC) will produce a continuous painful shock. It also requires three times more DC than AC for a lethal shock.

First aider safety. **In all cases of electrocution, the source of the electricity should be shut off or removed BEFORE HELPING THE CAUALTY. DO NOT** touch the casualty until there is no live electrical contact between them and the equipment. This will ensure that all people helping will be kept safe.

2.1.1. Major Symptoms of Electric Shock

Atrial and/or ventricular fibrillation is the uncoordinated, asynchronous contraction of the atrial or ventricular muscle fibres of the heart. The risk of fibrillation is high if an electric shock is received with the path of the current through the chest (e.g., between two arms). The heart's natural rhythm is replaced by an asynchronous quivering with no effective pumping of blood. This is extremely dangerous, and death can occur in minutes unless correctional steps are taken immediately. **When safe**, CPR must be used to maintain the patient, but defibrillation will normally be required as the pulse is extremely unlikely to be restored by itself. Medical assistance must be sought.

Respiratory arrest. Electric shock can cause this. The control centre for respiration is contained at the base of the skull and can be deactivated by an electric shock. CPR or artificial respiration should be commenced, **when safe** and medical assistance sought immediately. It can also be linked to fibrillation (above).

Asphyxia. This is caused by the chest muscles contracting and not releasing. Current from an electric shock which is above a certain level (i.e., 0.005 A at 60 Hz) can cause a person (if they are holding a live wire) to be unable to let go. This can also be enough to cause the chest muscles to contract and, in turn, asphyxiate the victim. **When safe**, CPR should be commenced, and medical assistance sought immediately.

2.1.2. Possible Sources of Contact with Electricity

Source Contact

The danger of contact with the electrical generator or battery should be minimized by only plugging in pulse boxes and electrodes when the generator is off, or, for battery equipment, the

main emergency stop button should be in the off position or the battery disconnected.

Dry Contact

The greatest danger in electric fishing is when a live electrode is out of the water and makes direct contact with a person. **For this reason, electrodes must only be energized when in the water.** In addition, it is **strongly recommended** that the anode is never touched or brought into close proximity with operators while the generator is running, or the battery connected.

Wet Contact

Shocks from in-water contact with the electric field are less severe than dry contact due to the electric field dissipating in the water. None-the-less, **operators should not put their hands in the water in the vicinity of any energized electrode (anode or cathode)**. If necessary, electrically insulating gloves may be worn.

Fault contact

Faulty equipment can also give rise to electric shocks, so **all electrical equipment should be checked before use and regularly maintained**.

2.2. Other Dangers

2.2.1. Drowning

Although injury resulting directly from the electric current is considered the most likely aspect of electric fishing surveys, drowning is also a significant danger. Electrical shocks, or just falling into cold water, can impair the swimming ability of operators. In addition, some workers have drowned because they are either unable to swim or have failed to wear life jackets or buoyancy aids.

2.2.2. Tripping/Falling

Movement on riverbeds and boats can be made difficult by slippery surfaces. Always try to ensure that non-slip footwear is worn whenever possible. Move at a pace that is consistent with conditions underfoot. Be aware of trip hazards such as cables and ropes on the ground or branches and rocks on the riverbed and communicate these dangers to other team members.

2.2.3. Other Hazards

Working in rivers and lakes and carrying heavy equipment can give rise to many potential dangers. Slippery rocks can lead to strains or even broken bones. Long-term use of anodes and/or nets can also lead to repetitive strain injuries.

Rainfall will potentially make equipment unsafe by allowing electricity to track through the wet equipment. Fishing in thunder and lightning storms should be avoided as the live electrodes could act as “lightning rods.”

Several diseases can be caught from river and lake environments, (e.g., hepatitis, Weil’s disease, etc.). All team members should be aware of the symptoms of potentially severe diseases and the risk of disease should be guarded against.

Wild or aggressive domestic animals can be a problem both to staff and equipment and even driving to the site can be a significant danger.

3. EQUIPMENT AND SETTINGS

Equipment set up will vary with make of pulse/control box and environmental conditions. The following are variables that will need to be determined for the environmental conditions encountered.

- 3.1. Output type DC/PDC
- 3.2. Voltage
- 3.3. Electrode dimensions
- 3.4. Frequency
- 3.5. Pulse width

Environmental variables that influence choice of the above include:

- ambient water conductivity
- temperature
- target species / sizes
- presence of sensitive/rare/valuable fish
- size of water body being fished

Knowledge of ambient water conductivity is vital for successful and safe electric fishing. It is recommended that reliable portable conductivity meters are included as an integral part of the electric fishing survey equipment.

3.1. Personal Protective Equipment

Suitable waders should be used when electric fishing. Thigh waders can be used in shallow waters, but chest waders will be needed in deeper water. Operators should not wade in water deeper than thigh depth due to risk of drowning or river flow sweeping them away. Life jackets are advised when working in deep and/or fast flowing streams.

Caution should be used if wearing "breathable" waders (particularly if operators are wearing shorts) as cases have been reported of electric shocks being experienced by operators using these.

Suitable clothing should be worn for hot or cold conditions and sunscreen used as appropriate.

Polaroid glasses can improve visibility in bright conditions and improve fish capture. Light or yellow-coloured lenses help when operating under tree cover. Safety rated polaroid glasses are also available and have the advantage protecting against eye injury (e.g., from net poles, tree branches swinging back, etc); this makes their use applicable to all staff.

3.2. Power Source

The power source for electric fishing can be either from generators or batteries; domestic mains power should not be used unless routed through an opto-isolated power supply. Generators used for electric fishing are typically AC units producing 230-240 volts output, but DC units can also be used. It is important that the earth on the generator is disconnected in equipment used for electric fishing (due to earth/neutral bonding). Such generators should not, therefore, be used for any other purpose.

The output from AC generators is modified by the control box to produce the waveform and output type (DC or PDC) chosen by the user. Never use AC to fish as it has been shown to be highly damaging to fish.

Power can also come from batteries, particularly when using small portable "backpack" equipment. Batteries should be "non-spill" to avoid the possibility of acid leakage. The most common batteries are lead based but increasingly Lithium Iron Phosphate (LiFePO) batteries are being used, which are much lighter and have a higher Amp/hour (A/Hr) capacity.

3.3. Output Waveform

Summary:

Alternating current (AC) should never be used for fishing as it is very harmful to fish. AC generators, however, are used to supply electricity to pulse/control boxes where it is converted into direct current or pulsed direct current.

Direct current (DC) causes less injury to fish than pulsed DC (PDC), therefore, wherever possible it should be used in preference to PDC. However, in medium to high conductivity waters the high-power requirements of DC make its use impractical and thus, in those situations, PDC is the only possible waveform that can be used.

3.3.1. Direct Current (DC)

The use of smooth direct current for electric fishing potentially offers several advantages over other waveforms, notably in respect of attraction properties and fish welfare, therefore **DC should be used wherever and whenever it is practicable (see above)**. However, its disadvantages are that it is a "power-hungry" waveform, and its effectiveness is more prone to disruption by local variations in the conductivity of the riverbed. It also needs higher voltage gradients to immobilize fish compared with PDC.

When using backpack gear and single anode, it is possible to fish effectively with smooth DC in ambient conductivities less than 150–200 mS.cm^{-1}. At higher conductivities, it may be necessary to use generator-based systems or switch to PDC since output may exceed the rating of the control box or, if using battery equipment, depletion of the batteries before sampling is finished.

Portable generator-based systems can be used to fish with smooth DC in waters where ambient conductivity is up to about 350–450 mS.cm^{-1}. Note that generators larger than 3 kVA (depending on model) are not considered portable, and hence power output from such a machine imposes an upper limit on the use of DC. The generator power requirements can be estimated from the **ElectroCalc** spreadsheet (available from author).

Ensure that the control box you are using is adequately rated for the electrical current (Amps) and/or power (Watts) expected. Reading from the left-hand charts in the single-and double anode spreadsheets in **ElectroCalc**, gives the estimated current demand in Amps. For instance, fishing DC with a single anode of 400-mm diameter and 3000-mm x 25-mm braid cathode, in water of ambient conductivity 300 mS.cm^{-1} with a voltage set at 250V, current drawn will be 4.6 Amps and power requirement will be about 1800VA.

3.3.2. Pulsed Direct Current (PDC)

When conductivities exceed the values at which DC fishing can take place, PDC is the recommended option. Its fish attraction properties are not as good as smooth DC but

it is better at immobilizing fish and has (for the same output voltage) a larger capture field radius. Some equipment gives options for novel waveforms, however, the capture efficiency and fish welfare characteristics of these have not yet been fully evaluated and so their use cannot be recommended for routine electric fishing at this stage. A possible exception to this is "Gated Burst" where power savings can be made from using this waveform without badly compromising fish capture or welfare.

Most backpack systems can be used to fish using PDC mode in waters with ambient conductivity of around 500 $mS.cm^{-1}$ and, exceptionally, up to 3000 $mS.cm^{-1}$ when using low voltage and gated burst waveforms.

Average power requirements for PDC are much lower than for DC. However, it is still important to ensure you have sufficient power for the combination of water conductivity, applied voltage and electrode configuration you are using. Always consult **ElectroCalc** to make sure you have sufficient generator capacity to deal with the water you intend to fish. For instance, fishing square-wave PDC with a single anode of 400-mm diameter and 3000-mm x 25-mm braid cathode, in water of ambient conductivity 300 $mS.cm^{-1}$ with a voltage set at 200V and 25% duty cycle, average current drawn will be 0.9 Amps and mean power requirement will be about 300VA.

3.4. Voltage

The circuit voltage required to be applied at the electrodes in order to attract and immobilize fish will vary according to

- the output type used (direct current or pulsed direct current)
- ambient water conductivity
- the anode size used
- the cathode size used (and the anode/cathode resistance ratio)
- size of effective capture field required.

Note: When measuring voltage, amps and power the value can either be measured as peak values or as average values. DC output will always be peak values but when using PDC output the average value will be lower than the peak due to the zero output between the voltage pulses. It is important to know what your equipment measures because some equipment is limited by peak values, and some average values.

The control box circuitry in the more modern electric fishing systems enables higher and lower voltages than the generator output voltage to be selected and controlled systematically. In some equipment, voltage and duty cycle cannot be varied independently, and the equipment is usually fitted with an input voltmeter which only measures the voltage produced by the generator, not that applied at the electrodes: the main concern of the operator.

3.4.1. Voltage Gradient and Output Type

Attraction of fish toward the anode can be achieved at voltage gradients of as little as 0.1 volt/cm when using PDC. When using DC, gradients of 0.2–0.3 volt/cm are needed. Immobilization of fish using DC can be achieved at voltage gradients of 1.0 volt/cm while with PDC this can occur at gradients as low as 0.5–0.6 volt/cm. You should make every attempt to prevent the fish coming closer to the anode than the distance at which voltage gradient is sufficient for immobilization and you should endeavour not to touch a fish with an energized anode.

Larger fish are generally susceptible to lower voltage gradients than smaller fish in any given situation; hence when larger fish are expected or targeted, circuit voltage can be reduced to below the values suggested above.

3.5. Choice of Frequency When Using PDC

Choice of PDC frequency will be influenced primarily by the species being sought, bearing in mind that under normal circumstances we wish to maximize the attractive properties of the electric field while reducing the harmful zone to a minimum. Research has shown that while medium to high frequencies are more effective in immobilizing and tetanizing fish of some species groups, particularly salmonids, these are also more harmful.

Paradoxically, very high frequencies, >400 Hz, have been shown to be both effective and relatively benign for small fish species, and point abundance sampling of cyprinid fry has been successfully carried out using 400–600 Hz. However, standard electric fishing control boxes do not include such high settings as an option.

As a very general rule, injury rates in larger fish will reduce if lower frequencies are used.

Frequency guidelines for European species:

Salmonids: For large adult fish, 20–40 Hz will attract and immobilize well. For juveniles, 50 or 60 Hz is effective and causes (at the correct voltage setting) very little mortality. The use of 100 Hz settings on older control boxes should be avoided.

Cyprinids: Optimum frequencies may vary, but for roach (*Rutilus* spp.), 40 Hz has been shown to give both good attraction and good immobilization. Switching to 10 Hz reduces the zone of immobilization while increasing attraction properties, however there may be difficulties in capturing cyprinids in some circumstances if they are only immobilized in a very small zone around the anode.

Perch (*Percid* spp.) are more similar to salmonids in their response to electric fields and 100 Hz has the best attraction and immobilization properties. However, as fish damage (to perch and other species) is more likely at this frequency 30–40 Hz is recommended.

Pike (*Essox* spp.): – no specific references have yet been found in the literature, but personal experience has found that fishing at 40 Hz has proved effective.

Eels (*Anguillid* spp.): most frequencies investigated were effective in both attracting and immobilizing eels, so bearing in mind the potentially more harmful effects of higher frequencies on some other species, frequencies of 10 – 40 Hz should be employed as standard. In depletion fishing it is common for the second pass fishing to catch more eels than the first, this is likely due to both targeting by operators but also displacement of fish in the first pass making them more catchable in the second pass. Obviously, this anomaly negates the ability to calculate a population estimate.

The attributes of other intermediate frequencies e.g., 5 Hz, 20 Hz, have not been reported to any extent in the literature examined but could prove more favourable than the frequencies quoted. Lamprey juveniles have been successfully caught using low (2 Hz) frequencies to draw them from the riverbed sediments.

3.6. Pulse Width/Duty Cycle

Pulse width refers to the duration of each individual pulse of electricity and can be expressed in milliseconds (ms) or in percentage duty cycle. Percentage duty cycle is the percentage time (during one electrical cycle) that the electricity actually flows. It is important to note that at different frequencies the same duty cycle will result in different time duration of pulse i.e., at 50 Hz a 10% duty cycle will result in a 2 ms pulse but at 30 Hz, a 3 ms pulse (see **ElectroCalc** for a conversion chart between pulse width and duty cycle). The greater the duty cycle or pulse width selected, the higher will be the current drawn and power required: 100% duty cycle is the same as DC (i.e., the power is on all the time).

When fishing with PDC, duty cycle should be kept to about 20–30%, increasing duty cycle above 30% has little effect on attraction properties of the field, though often improves immobilization strength. A short pulse width reduces the possibility of fish damage and conserves average power.

In more conductive water, it may be necessary to increase pulse width if fish are seen to be escaping the expected capture field. Values in excess of 35% however are unlikely to improve capture and different frequency or voltage outputs should be considered. In the case of some pulse boxes it may not always be possible to adhere to this because voltage and duty cycle cannot be varied independently and if high voltage is required then high duty cycle is selected simultaneously.

We recommend that when using PDC, fishing should start with a pulse width of 5 ms (25% duty cycle at 50 Hz) but for medium to high conductivity waters it may be necessary to increase this. Some control boxes do not have independently variable voltage and duty cycle control but nevertheless fishing should start with the "select power" dial turned down to perhaps a quarter of its range ("nine-o'-clock" position").

3.7. Water Conductivity

The conductivity of a substance will vary with temperature. For that reason water conductivity is either measured as "specific conductivity" where the value is adjusted to what it would be at 25°C, or "ambient conductivity" where the value is not adjusted. In electric fishing it is the ambient conductivity that will determine the equipment set up – unless the water is at 25°C! In the following all conductivities are ambient.

3.7.1. Low Conductivity Waters

When the water being fished has low conductivity (conductivity less than 150 $\mu S.cm^{-1}$), a higher

voltage gradient is required to incapacitate the fish than in high conductivity water. A higher applied voltage is therefore required.

Even when using PDC very high voltage outputs (in excess of 500 Volts) are needed at low conductivities.

Use of higher voltage systems, that generate voltages up to 1200 V, have been used in some ultra-low conductivity waters (15 $\mu S.cm^{-1}$), however, users should be aware of the dangers of using such high, and potentially dangerous, output voltages.

3.7.2. Medium and High Conductivities

At medium and high conductivities, progressively lower circuit voltages will be effective in fish capture because a lower voltage gradient is needed to elicit a response from fish at a given point in the electric field in higher conductivity waters.

Conductivity ($\mu S.cm^{-1}$)	Applied voltage – PDC	Applied voltage – DC
10–100	300–900+	400–900++
100–200	250–300	300–400
200–500	150–250	250–300
500–1000	120–180	Not applicable
> 1000	100–150	Not applicable

These guidelines assume the use of a typical, recommended electrode configuration such as a 400-mm diameter anode and single cathode consisting of 3 m braided copper or stainless-steel strap. Different anode and cathode dimensions may require more or less circuit voltage to be effective. Note that waveform type (DC or PDC) will also affect immobilization thresholds.

At higher conductivities, it may be necessary to increase pulse width (duty cycle) to impart sufficient power into the water to capture the fish.

Note that when fishing in areas where fish have laid their eggs, high voltage gradients may affect the eggs or embryo within the egg.

The overall aim during any electric fishing operation should be to maximize the effective field of fish capture while minimizing the zone of high voltage gradient around the anode/s in which fish can be damaged. Where very sensitive or valuable species are present, operators should consider further reducing the risk of damage to fish by reducing applied voltage even if this means some compromise of fishing efficiency.

The right-hand diagrams in the **ElectroCalc** spreadsheet give an indication the effective size (vertical axis) of electric fields, based on nominal voltage gradients required to catch fish, at different anode voltages for a given anode and cathode configuration and circuit voltage.

As a general approach, electric fishing under any field conditions should be started at the lower end of the range of voltages recommended for those conditions.

3.8. Temperature

At low temperatures, fish may be less responsive to the electrical field due to their lower reaction capability at low temperatures. Reducing the pulse frequency can mitigate against this.

At higher temperatures, the fish are likely to be very reactive and this may cause muscle or skeletal damage to the fish due to excessive reaction.

Welfare of fish post capture will also be an issue at high temperatures due to higher respiration rates and lower oxygen holding capacity of the water.

3.9. Electrode Dimensions

3.9.1. Anode

Anodes should generally be circular and should not have sharp corners, as these will produce areas of high voltage gradient. **Capture nets should not be attached to anodes** as this will increase the time fish are held in the area of maximum voltage gradient. Nets on anodes also present a very high risk to operators.

The use of very small anodes (<25 cm diameter ring) is not recommended under normal circumstances as they result in a small but intense electric field. The aim should be to use as large a diameter anode as is practicable. The constraints on anode size in narrow streams will be the physical limitations imposed by the nature of the site and ease of handling by the operator. If the physical nature of the stream necessitates the use of a very small anode (for instance fishing for bullhead or 0+ salmonids in a boulder-strewn stream) then the applied voltage can be reduced accordingly, since in such a case size of capture field is not an issue.

In larger streams, maximum usable anode size may be limited by power available. Graphs showing the size of effective capture fields using different applied voltages and different-sized anodes, using

both DC and PDC, and the power requirements of these different configurations are shown in the **ElectroCalc** spreadsheet.

It is recommended that the standard anode size for normal use is a 10 mm gauge stainless steel ring, diameter 35–50 cm. Thicker gauge steel is stronger and creates a larger surface area but is heavier and has relatively little effect on electrode resistance and overall field characteristics, and, although it may reduce voltage gradients in close proximity to the anode edge, 10 mm gauge is a good compromise.

In flowing water rings of 60 cm or larger are very difficult to hold against the flow of the water are likely to cause excessive arm strain on the operators. Supports for the anodes can be used but it is probably better to switch to "boom" or fixed anodes. Teams should carry a range of ring diameters (50 cm, 40 cm, 30 cm and 25 cm) to cope with situations where very high conductivity places excessive demands on power available, or where the physical nature of the stream renders a large diameter anode impractical.

Never keep fish in the electric field for longer than necessary, avoid getting too close to fish with an energized anode and never touch a fish with an energized anode.

As a general rule, **no more than one anode (40-50 cm diameter) is required per 5 m width or river channel being fished.** If more than one anode of this size is used in a channel narrower than this, the size of effective electric field around each one will be reduced, and the operation will be less cost effective and wasteful of power.

In wider channels where it is desirable to increase the number of anodes, the surface area of cathodes must also be increased *pro rata* (see below) in order to gain maximum benefit from the increased anode size. Note, however, that power requirements will increase (see left-hand figures in **ElectroCalc**). When fishing with multiple anodes it is not good practice to hold the anodes heads closer than about 3m because the size of effective electric field around each one will be reduced, and thus capture efficiency will be reduced. In addition, the electrical current is increased and may overload the control box. Notwithstanding this, users should be aware that if too great a gap is left between a pair of anodes for too long during a fishing operation fish may pass between the two anodes and not be caught.

When fishing PDC multiple electric fishing pulse boxes should not be used. This is because overlapping out-of-phase pulses will, in effect, increase the pulse frequency, possibly leading to harmful frequencies. Multiple independent units also negate the safety system of "one-off, all-off."

3.9.2. Cathode Size and Shape

The system should always comprise a cathode surface area that is larger than the surface area of the anode(s). Cathode: anode surface area ratios of as large as 30:1 have been quoted in the literature but there is a limit to the practicality of such configurations. Cathodes such as copper braids are more ergonomic than metal plates or mesh grids, however, braids produce more intense cathode fields in their immediate proximity which can be harmful in situations where fish may come close to the cathode. In such cases a cathode grid or grids are preferable to braid.

It is recommended that the standard cathode should be at least a 3–metre length of 25 mm wide copper or stainless-steel braid or a sheet of perforated metal of at least 75 cm x 75 cm square or other shape of equivalent surface area.

If the surface area (or number) of the anodes is doubled then the cathode surface area should also be doubled; separating the cathodes will improve even further the resistance characteristics of the cathode array. Control boxes for use with more than one anode should if possible be fitted with extra cathode sockets. If extra cathode sockets are not fitted, then multiple cathode braids or grids can be fed from a single control box socket using a trouser-joint and a spacing device made from non-conducting material.

Very long cathodes may be impractical for backpack electric fishing where the cathode is dragged behind the fisher, nevertheless the cathode should comprise **at least 1.5 metres** of braid or 4–6 mm diameter steel wire.

As a general rule for anodes and cathodes, bigger is better, but there is a law of diminishing returns and little advantage will be gained by using much larger sizes than those recommended.

Where possible cathodes should be placed in fast flowing water so fish cannot remain in close proximity to the cathode for long periods and be harmed.

3.9.3. Effective Size of Capture Field Required

In most electric fishing situations, it is desirable to create as large an effective capture field as

possible. However, in shallow, narrow streams, there is no need to create a field that will attract fish from many metres away since any fish present will never be far from the fisher. In very turbid water there is equally no point in immobilizing fish at a depth/distance from which they cannot be seen and retrieved.

Size of capture field required also depends to some extent the species and sizes of fish being targeted. Small fish species with limited mobility such as bullheads can be captured using small effective electric fields employing relatively low voltages – even in larger rivers.

Hence, when fishing in very small streams of whatever conductivity the operator should consider using lower voltages than those indicated in 3.7.2.

3.10 Standardizing Capture Probability

When comparing fish population assessments taken with semi-quantitative methods (e.g., 5-minute surveys, etc.), it is **vital** to standardize fish capture probability between survey sites.

Firstly, the time element should be the anode energization time, not the total time fishing. In low density sites a greater area can be fished in a total time of 5 minutes compared to high density sites due to less time being taken to remove captured fish from nets etc. Using anode energization time negates this bias.

In addition, to standardize capture probability, it is important that the equipment used has the same anode and cathode dimensions, uses the same voltage waveform and that the circuit voltage is adjusted to give similar capture probability at the differing conductivities.

For example, if two sites, with significantly different water conductivity, are surveyed the voltage output must be altered between the sites to standardize the capture probability. This is due to the different voltage gradient needed to catch fish in different water conductivities noted earlier.

Two methods have been published to calculate this standardized output voltage value; Power Transfer Theory (Kolz 1989), and standardized output Wattage (Meyer et al. 2020).

Conductivity: voltage output graphs using information specific to the equipment being used (see appendix) should be carried by teams to maintain this standardization.

4. FISH WELFARE

Proper handling of the fish once caught is essential; bad handling of fish that are already under some stress due to capture can exacerbate problems and cause injury. Good handling will help to prevent injury and to reduce stress.

In the past, considerations about a fish's ability to "suffer" have been somewhat overlooked. Present research is inconclusive, but some has shown that fish can react to stressing actions and some researchers' surmise that fish can not only feel pain but also experience fear. While the debate continues regarding this issue, fishery workers must be aware of the fact that they are dealing with sentient organisms and act appropriately. If killing fish is required, then cerebral maceration should be carried out. Fishery workers should be aware of the regulations within their country regarding working on animals (including fish). In many countries experimental research (as opposed to husbandry) can only be carried out if licensed by government or regulatory organizations. In the UK licenses are controlled by the Home Office under the Animals (Scientific Procedures) Act 1986 (ASPA). If the work is classified as research or involves pain, stress or the use of anaesthetic then the work can only be carried out under ASPA project licence. Persons working under ASPA need to have been trained and hold a Personal Licence for all the procedures that they are carrying out.

The following general rules should be observed to improve fish welfare:

- Avoid fishing in high water temperatures (greater than 16-18°C for salmonids, 22-24°C for coarse fish especially when pike and perch are present).
- Use separate bins to separate large and small fish and to separate eel and common bream (due to the amount of slime they produce).
- Provide aeration (oxygen diffuser plus compressed air is best) in both catch bins and fish storage bins – this is essential in warmer weather and when large numbers of fish are expected.
- Keep-cages and keep-nets are a good alternative to fish storage bins but ensure there is adequate depth of clean, gently flowing, and well-aerated water. If these conditions are not available at the survey site, then aerated storage bins are preferable.

Measures that Can Be Taken to Reduce Stress during Holding, Handling, and Transportation of Fish
(Adapted from Pickering 1993 and Ross & Ross 1999)

Problem	Suggested Action	Comments
Duration of the stress response is usually proportional to duration of exposure.	Shorten the duration of stress.	Some effects may result in long recovery times.
Stress-induced mortality increases with water temperature.	Work at lower temperatures (e.g., use ice to cool water).	Not always practical under field conditions.
Stressors may be additive or synergistic.	Prevent simultaneous stress.	Possibly allow time between processes.
Abrasion between fish causes damage.	Reduce numbers handled per batch.	May conflict with time pressures.
Stress increases O_2 consumption.	Use O_2 or air bubbled through holding tank.	Safety and O_2 use may make air better option.
Stress increases O_2 consumption, and ammonia and CO_2 output.	Use mild anaesthesia or sedation.	Note, some anaesthetics can act as stressors.

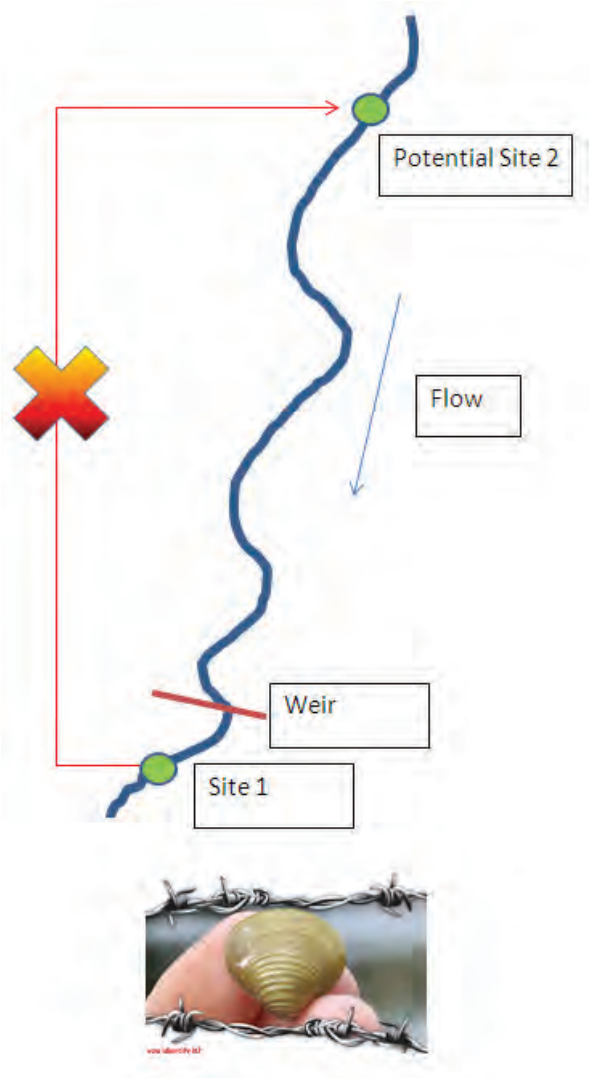
5. BIOSECURITY

Every effort should be made not to transfer pathogens or alien plants and animals between sites and particularly catchments. On completion of any field operation (particularly when moving between catchments), all equipment used must be treated with appropriate disinfecting agent (e.g., Virkon Aquatic). Equipment needs to be clean for the agent to disinfect properly so any obvious material or dirt should be removed.

All gear that has been in contact with the water should be cleaned i.e., boats, trailers, outboard motors, anchors and rope, weights, tanks, buckets, hand and stop nets; all PPE (including boots, wellingtons, waders, wetsuits, dry suits, etc.) plus all technical or sampling apparatus used as part of the survey/operation. For difficult or large gear (e.g., stop nets) freezing will successfully kill most organisms, however, seeds and some bacteria may still be viable after freezing.

Recommended concentration of disinfectant (usually a 1% solution) can be applied by a small garden sprayer onto equipment. Suitable PPE should be worn when using the disinfectant, i.e., safety glasses, gloves & dust mask. Note that the disinfectant will degrade over time so fresh batches should be regularly made up.

When planning sampling always try to have sites sampled in a downstream direction. This is particularly important where there are weirs etc. that make obstructions to the potential upstream passage of alien species.



6. ELECTRIC FISHING "BEST" PRACTICE

In general terms there are two choices regarding equipment set-up for electric fishing. The equipment can be set-up to cause the least possible damage to the fish, or the equipment can be set-up to capture the highest proportion or number of fish. Rarely do these two set-ups correspond. Knowledge of the theory behind electric fishing can help bring together the two options.

The following deals predominantly with the options and techniques to use in order to minimize damage.

Where *possible* fishing should be carried out using direct current (DC) voltage fields. This is because DC has good attraction of fish to the anode (increasing capture probability), induces harmful tetanus only in the near vicinity of the electrode and has the lowest recorded rate of injury for any waveform type. **However, there will be *many* cases where it is not possible to use DC** (high conductivity water, variable electrical characteristics of stream topography, poor fish response to DC field for unspecified causes, low endurance of battery powered equipment in high conductivity water, etc.). **In these cases, pulsed direct current (PDC) voltage fields should be used**. However, PDC has poorer anodic electrotaxis and tetanizes further from the electrode; possibly preventing some fish from reaching the capture zone. **Pulse frequencies should be kept as low as possible** (Snyder 1992 suggests 30-40 Hz or lower) note however, that frequencies below 20 Hz may not be good for attracting the fish to the anode. There is also some evidence that high frequencies may be more efficient for capturing small fry.

Evidence shows that alternating current (AC) causes more injuries than DC and PDC and therefore **AC fields should not be used for fishing unless warranted by specific circumstances** (use of fishing frames, PPAS or fish to be killed).

All fields should be adjusted to the minimum voltage gradient and current density concomitant with efficient fish capture. Pulse box settings should be adjusted to optimize recovery, capture efficiency should be a secondary consideration and can often be offset by carrying out more runs (if depletion fishing). This is an area where some measure exists for some trade-off between fish capture and fishing efficiency. It should be noted that **it is INCORRECT to increase pulse width (and thus amperage) at deeper sites**. For the same conductivity water this will not increase the field area of the anode but simply increase the power transfer to the fish within the field and thus lead to higher injury. The practice of "turning up" the output setting comes from old style boxes where this also increased voltage output; and thus increased the range of the anode. Increasing the voltage at the anode will increase the size of the voltage field but will also lead to high gradients near the anode with associated risk to both fish and operators. It will also markedly increase the power demand of the equipment.

Most users of electric fishing equipment use a "standard" setup when fishing. If this "standard" has been determined on the basis of past fishing success and lack of fish injury these standards are probably satisfactory. Personnel using DC for the first time will need to adjust or modify their fishing technique to account for the much smaller effective field found with DC (Snyder 1992). Calculated field intensity data are good for planning, but on-site, in-water measurements are better for confirming actual intensity and distribution of the electrical field, especially given the importance and potential variation in substrate conductivity. Given that, adjustment should initially be carried out based on theoretical considerations and then adjusted based on values actually measured in the stream or river (e.g., by use of "penny probe" etc.). Voltage field measurements should be made using either a custom-made peak voltage meter or a portable oscilloscope. Part of this set-up process will be the decision regarding what voltage to use. In the past, few pulse boxes in use in the UK have had this option but it is a powerful tool in tailoring the field gradient to ambient conditions. Voltages can be reduced when having to use small anodes in small high conductivity streams or increased in low conductivity streams (if larger anode diameters are impractical). Note that there is no physiological reason for 200 volts to be the default voltage used, often lower voltages will be equally effective in producing adequate field intensities.

The anode head size should be as large as possible. If using DC, available power may influence the size of anode that can be used, but if using PDC available power is rarely an issue. The practicalities of handling large anode heads and the physical size of the stream are more likely to be an issue. In small low conductivity streams, if small physical anode size is required, voltage levels can be increased to increase the capture area (although it is not likely you will need a large field). Adding metal mesh to the anode can reduce the consequential high voltage gradient that will then exist in the vicinity of the anode. The mesh should not be used for actually capturing the fish.

The cathode should be as large as possible. The commonly used "braid" design of cathode is both efficient and ergonomic in use. Braid should be approximately 3000 mm long although shorter lengths are more practical for backpack gear. Expanded mesh design of cathode can also be used but are more difficult to transport and can be affected by water flow. If multiple anodes are used, cathode area will need to be further increased. Knowledge of the electrode resistance of both anode and cathode will allow intelligent assessment of requirements. If copper cathodes are used, they should be kept clean of an oxide layer, as it will reduce their effectiveness. Soaking cathodes in vinegar is an effective way of removing the oxide.

Fishing technique using DC and PDC. When using DC, fishing should be conducted in a discontinuous fashion, in order to use the element of surprise, to improve capture efficiency and in order not to herd or drive the fish. In preference the operators should switch on when near, rather than in, areas such as clumps of weed, tree roots or other likely refuges. Fish will be in the attraction zone and this will have the effect of pulling the fish out from their refuge to where they can be captured. If the anode is too close, or actually in, refuge areas when switching on, the fish may be in the immobilization field and will not be drawn from cover. Sweeping the anode when in areas of open water may encourage fish to seek out areas such as weed beds etc. where again the above technique can be used. When using twin anodes however this discontinuous method becomes difficult due to the requirement for both anodes to be powered simultaneously. This problem can lead to the practice of keeping the anode live while lifting it from the water; **this should not be done** (due to the danger of dry contact with the anode). It should be noted that the effective fishing radius of the anode will vary dependent upon the localized changes in the physical attributes of the stream. For this reason, it may be difficult to obtain good depletion sampling population estimates (or more fishing runs may be required to get adequate confidence in the results).

Unlike DC, the tetanizing zone of PDC extends some way out from the anode. Thus, when using PDC care needs to be taken that the anode is not so close to the fish that the fish is instantly in the tetanizing zone of the field or that the fish is tetanized while still outside the catching zone. This aspect can however be minimized by using an anode radius and voltage output suitable for the conditions being fished.

Actual techniques used will vary between running and still waters. In still waters the fish are far more likely to be able to escape the voltage field. This can be reduced by either fishing next to the bank (to trap the fish against the bank) or by enclosing sections of still water with nets. Discontinuous fishing should also be carried out when using PDC.

Generally electric fishing teams work in an upstream direction. This reduces the problem associated with stirred-up silt impeding visibility. In fast running clear streams, however, downstream fishing, especially when using "Banner Nets", has been shown to be very effective.

When fishing wide sites, multiple anodes can be used. Zig-zagging upstream when fishing allows random or target habitat types across the width to be sampled. Moving anodes when fishing side to side and up and down to "draw" fish will also help. When using twin anodes in wide rivers when only part of the width is being covered, it is sometimes advantageous for the mid-river anode to move slightly ahead of the bank-side anode. This technique will tend to scare the fish into the bank and make capture by the bank-side anode more effective. **In general, one anode for every 5 metres of river width has been found to be effective for quantitative electric fishing surveys of whole rivers**.

Fish should be removed from the electrical field as quickly as possible. While length of exposure to the electric field does not appear to increase rate of trauma, length of exposure does increase stress levels. Repeated immersion of fish into an electric field has been shown to increase blood lactate levels (and thus will increase post-exposure muscle acidosis). Holding fish in the net is poor practice as it also considerably increases oxygen debt and should be avoided.

Regarding the non-electric considerations when fishing, five major issues arise, water depth, water temperature, water visibility, fish welfare and communication.

Electric fishing by wading is limited to the depth in which wading can be safely carried out. The U.K. Environment Agency Code of Practice states that an overall depth of thigh deep with a hip depth maximum should be used as the criteria. These measurements should be taken from the shortest person in the fishing team.

Extreme temperatures should be avoided when fishing is carried out. Fishing in the hottest months should be avoided, but it is also important to avoid the coldest months as well. In general, there is a trade-off between efficiency (poor at low temperatures) and welfare (poor at high temperatures). A temperature range of 10–20°C is preferred for coarse fish and 10–15°C for salmonid species. If fishing is carried out at low temperatures, due to logistics (e.g., low growth in winter so better between-site growth comparisons), increasing pulse width or voltage gradient may improve capture efficiency.

The rule regarding the visibility required for electric fishing is simply "**do not put the anode head deeper than you can see**." The electrode should be visible and should be near enough to the riverbed for its field to encompass the riverbed. The visibility required will vary for different species (e.g., small benthic fish requiring higher visibility than if surveying larger mid-water fish). In poor visibility more runs may be required to achieve adequate population estimates.

A wide variety of techniques can be used to ensure good fish welfare while they are being held prior to processing. The temperature of water is an important issue in maximizing welfare, with greater care regarding maintaining oxygen needed in hot weather. **The use of floating mesh cages is considered to be a particularly effective way of keeping the fish in good condition**. It is also a good idea to separate eels and bream from the catch as the large quantities of mucous these fish produce lowers the water quality (especially if the fish are held in bins) and "clog-up" other fishes gills. Note that eels are adept escape artists and holding bins should have a large amount of "freeboard" between the water surface and the lip of the bin.

Oxygen levels in bins can decline rapidly. With an approximately 50% stocking density (45 litres of water: 20 kg [equivalent to ~20 litres] of fish) oxygen levels can decline to 50% of their starting level in 7 minutes. This stocking level in bins should therefore be regarded as maximal. **Remember that the water needs to be agitated to remove CO_2**. It is possible to supply adequate O_2 with a fine diffuser and still build up toxic levels of CO_2.

Good communication systems need to be in place between anode operators (especially due to the one-off, all-off safety system) and/or anode operators and bank personnel. This system can be plain speech but in wide or noisy sites some system of either hand signals (difficult if anode in one hand and net in the other), whistles or radio communication is preferable. Modern voice activated radios fitted to headsets are ideal.

7. SUMMARY

- Ambient water conductivity should be known (to within 100 $\mu S.cm^{-1}$)
- Always ensure that you have enough power (generator/control box combination, or batteries) to supply the configuration you have chosen for the field conditions
- Do not survey in extreme water temperatures, especially high temperatures (>16-18°C for salmonids, >22 –24°C for coarse fish.)
- Provide adequate processing, recovery and storage facilities for the catch.

Applied Circuit Voltages (assuming maximum effective capture field is desired and recommended anode and cathode combinations are used)

Ambient conductivity ($\mu S.cm^{-1}$)	Applied voltage – PDC	Applied voltage – DC
10–100	300–900+	400–900++
100–200	250–300	300–400
200–500	150–250	250–300
500–1000	120–180	Not applicable
> 1000	100–150	Not applicable

Frequencies (for optimum combination of attraction, immobilization and welfare)

Species	Pulsed DC frequency (Hz)
Salmonids	40–60
Cyprinids	30–50
Percids	10–40
Pike	30–50
Eel	10–40

NB: for all species, use smooth DC whenever it is practicable.

Pulse width / Duty cycle (at 50 Hz)

Conductivity ($\mu S.cm^{-1}$)	Pulse width (ms)	Duty cycle (%)
<150	2–5	10–15
150–800	3–8	15–25
800–1000	5–10	25–40
> 1000	7–15	25–40

NB: always start fishing with duty cycle/pulse width set at the minimum.

Anodes and Cathodes

- Always use largest anode that is practicable. If using very small anodes (due to site configuration) reduce applied voltage.
- 40-50 cm diameter anode 10 mm gauge recommended size.
- Do not fish with twin anodes held close together.
- Always use a cathode that has larger surface area than anode. At least 3 metre x 25 mm braid; or 75 cm x 75 cm expanded mesh or plate is recommended size.
- If surface area of anodes is increased, cathode surface area should be increased by at least the same factor. Use of multiple cathodes is preferable.

Always disinfect gear after sampling.

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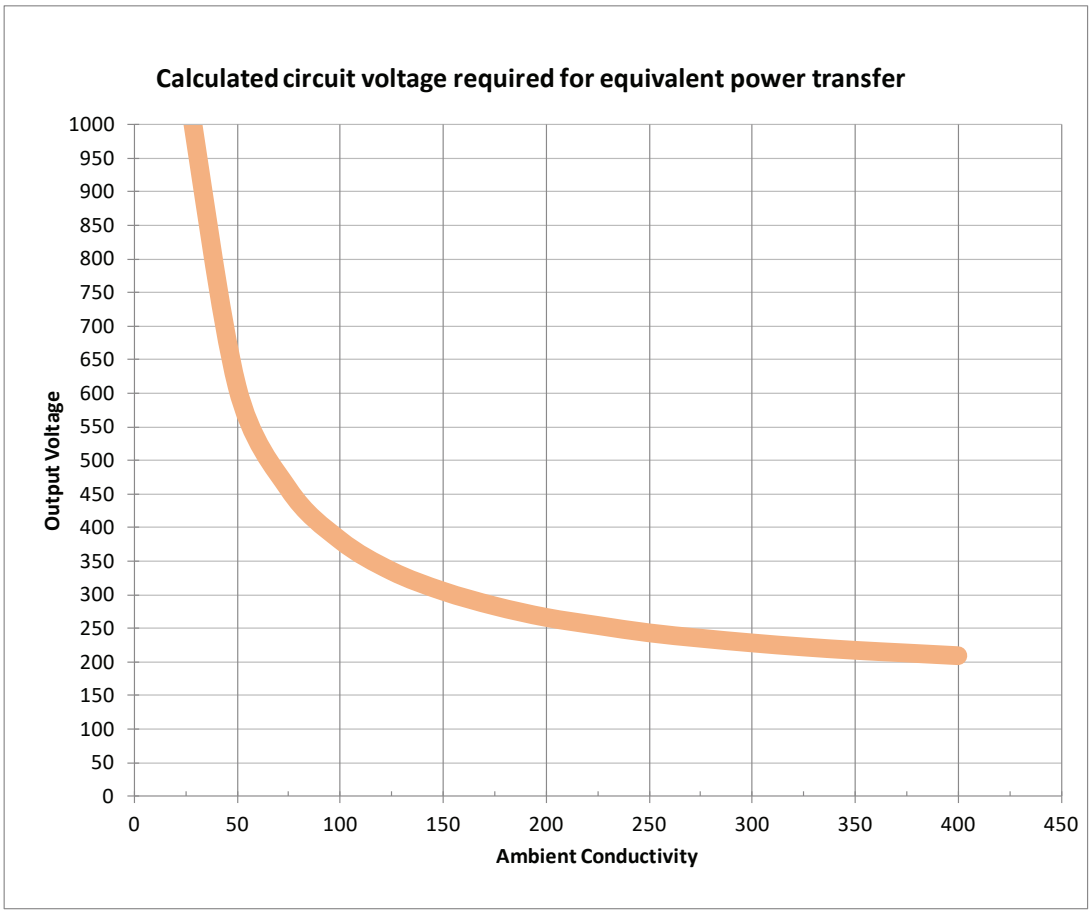
9. APPENDIX

The following graph shows predicted output voltages, calculated by Power Transfer Theory, for a range of ambient water conductivities, when using Smith-Root LR24 backpack electric fishing equipment. These settings proved successful in the trial fishing exercise in the Trishuli catchment in March 2020.



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