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Handbook on Research Ethics

WorldFish

CGIAR

Handbook on Research Ethics

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Standard Operating Procedures (SOP) for Obtaining Ethical Approval of Research

Purpose of the Standard Operating Procedure

This Standard Operating Procedure (SOP) support implementation of the WorldFish policies titled WorldFish Policy On Ethics Of Research Involving People and the policy titled WorldFish Animal Care and Welfare Policy, which were approved by the Board of Trustees in February 2017 and November 2019, respectively. This SOP is also to support implementation of the CGIAR research ethics policies.

This SOP outlines the procedures for:
1. Obtaining human or animal ethical approval
2. Submission of the protocol to the Research Ethics Panel
3. Review of the research protocol by the Research Ethics Panel

Definitions

Institutional Review Board (IRB): An official ethics review board whose purpose is to approve (or reject) research protocols to ensure the safety, dignity and welfare of human and animal participants in research. IRBs are often housed at academic institutions or at Ministries of Health. IRBs exist externally from WorldFish.

IACUC: Institutional animal care and use committee. This is a body that exists externally from WorldFish who are able to assess animal ethics submissions. These bodies are administered in specific universities or research institutions. Only institutions that have been approved by WorldFish will be used.

Ethical approval: Approval obtained from an official human or animal ethics board so that the research can take place. This approval usually has an expiration date and will likely need to be renewed (usually yearly).

Informed consent: A process to ensure that a research participant is aware of all the reasonably foreseeable risks and costs involved in participation in research and enable persons to voluntarily decide whether to participate in the experiment or research project.

Open Access (OA): This indicates that the research products produced will be accessible (and not behind a paywall of a journal, for instance) to the general public.

Protocol: A document outlining the research to be conducted. This document includes the explicitly-stated research question(s), the methods for conducting the research, the statistical analysis plan, the timeline, the informed consent statement, etc. The protocol is the main document that will provide the details for an ethics application.

The Research Protocol

Overview

If you are a scientist planning an experiment you will need to develop a research protocol (see appendices A and B or click here for human and animal templates, respectively). The research protocol is an important document outlining the detailed plans for your research project. The protocol will help you to think through all of the details you will need to report when you ultimately publish your findings. It might also be helpful to think about what details those might be using study reporting checklists. You may find some checklists for different types of human trials in this folder. While some of the details may change over the course of the study or experiment, it is important to plan as many details ahead of time as possible.

A research protocol collates the details needed to seek official ethical approval for the research to be carried out. Ethical approval is required as per WorldFish and CGIAR policies, and also required if the study is published in a peer-reviewed journal. Journals require you to reference your ethics approval letter or number when submitting your paper for review. It is the responsibility of all project leaders within WorldFish to ensure that ethical approval processes are followed as per the policy.

Getting the protocol reviewed to improve research quality

Research quality guidelines have been developed and will be reviewed and improved yearly. Once the protocol is drafted by the PI with the team of co-PIs, it is important that the protocol is reviewed by experts/colleagues/partners **who are not listed on the protocol** for quality and feasibility. You should attempt to get the protocol approved by at least two others. This can include a scientist at WorldFish which a particular subject area of expertise, or perhaps has knowledge on a certain geographical area (such as a scientist in the country where the research will be carried out), or an external partner (such as a university partner, etc). The review rubric (appendix C) is there in case you would like to provide it as a guide for the review. If you use it for review, please upload the document as a supporting document when you submit your protocol to the Research Ethics Panel (as described in section 4.3)

These reviews should be performed prior to submitting to the Research Ethics Panel.

Submission of protocol to the Research Ethics Panel

Composition of the Research Ethics Panel

The constituents of the Research Ethics Panel have been chosen to be a broad representation of scientists and experts, to give adequate feedback to WorldFish protocols, in order to continuously improve the quality of research that is published through the organization and with our partners. The panel will be comprised of:

- Chair (Research Director or senior/principal scientist)[a]
- One animal ethics specialist
- One human ethics specialist
- 2 other WorldFish scientists on a rotating roster[a, b]
- A member of the WorldFish Research Support Unit

[a] *Scientists will be appointed to the roster for a one-year period.*
[b] *The roster will be appointed by the Director of Aquaculture and Fisheries Sciences, in collaboration with the Research Leads.*

The roster will contain at least one scientist from one of the major WorldFish research programs. For example, the roster will comprise of at least one scientist each who specializes in Aquaculture, Value Chains & Nutrition, Small-Scale Fisheries, Gender, Climate Change, and M&E across the country offices. The research support unit will make sure there is representation across expertise and geography for each panel meeting and support the panel in ensuring quality research and adherence to ethical standards.

Title of person in panel	Tenure	Duties
Chair - Principal or senior scientist	Permanent	Chairs meetings, drive agenda for meetings, accept motions, amendments and ruling on points of order The chair will also oversee the process, leading the Research Ethics Panel discussion in regards to what is working and what is not working with the panel.
Animal Ethics Specialist	Permanent	Review protocols and provide input on ethical aspects

Title of person in panel	Tenure	Duties
Human Ethics Specialist	Permanent	Review protocols and provide input on ethical aspects
Scientist or Post-doc (2 called depending on need)	Present on roster for one year, called to meetings sporadically	Review protocols and provide input on ethical and quality aspects. Scientists may be selected based on experience or geography.
Research Support Unit	Permanent	Receive protocol submissions, schedule the monthly meeting calling appropriate subject area and geographic area scientists, distribute submitted protocols to Research Support Unit ahead of meeting, document feedback provided by panel, send to PI, document PI rebuttal, archive all documents in Sharepoint.
External consultant	Ad hoc	Supports ethics processes within the organization

Role of the Research Ethics Panel

The purpose of the Research Ethics Panel is also to ensure that all WorldFish studies are held to the highest research quality standard possible and adhere to the universally acknowledged ethical standards of research. The Research Ethics Panel will also determine if human or animal ethics approval will be required for each research project.

The Research Ethics Panel will review the **protocols of all studies conducted by WorldFish that collect primary data** from human participants or animal subjects. The Research Ethics Panel will give feedback to the PIs/project leads to ensure that the protocols meet the WorldFish standards for both quality and research ethics. The protocols will be evaluated for:

- Requirement for external ethics submissions to an IRB (human ethics) or IACUC (animal ethics)
- Completeness of the protocol
- Adherence to WorldFish policy for animal and human ethics
- Rationale and evaluation of the impacts on animal and human ethics
- Research quality as outlined in the quality rubric

The Research Ethics Panel will complete an evaluation form, and the Research Support Unit will serve as the point of contact for feedback to the submitting principal investigator.

Submissions

The Research Support Unit will be the point of contact for all project leads and principle investigators to submit their protocols for evaluation. The PI or project lead will submit the document through the "Research Panel Submissions" MS Teams channel by answering the affiliated questions and attaching the protocol. Questions on the process can be directed to wf-ethics@cgiar.org

The Research Ethics Panel will give feedback on research quality, along with recommendations for human/animal ethics. This feedback will be communicated and documented by the Research Support Unit. It is expected that the investigators incorporate the recommendations made by the Research Ethics Panel. Any rebuttal or disputes will be handed as per 4.3.

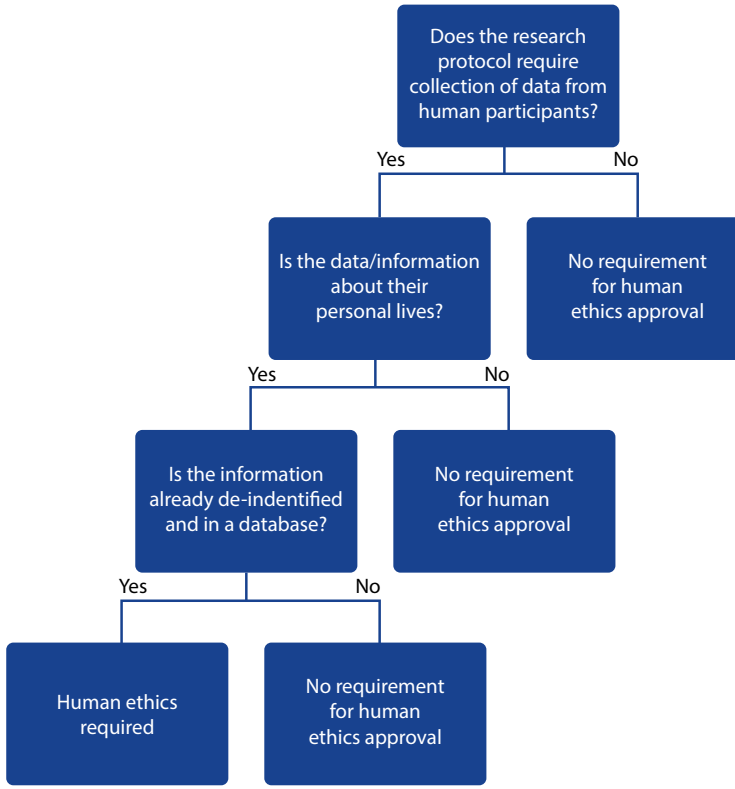
Dispute resolution

If the researchers disagree with the Research Ethics Panel decision and believe that ethical approval is not required for their project, then a submission to the panel must be made out of "normal" session, to revisit the requirement. The scientist must present the reasons why their experiment or research project does not require ethical approval. If there are disagreements that require escalation to the executive, a complaints form must be filled in to keep record of the formal complaint. The final decision in the case of a dispute will be made by the WorldFish Director of Aquaculture and Fisheries Sciences (or as delegated by the Director General).

Human Ethics Approval

Do I need to seek human ethical approval?

At WorldFish, human ethical approval will be required for some research projects, commonly sought through an external IRB. Once your research protocol is submitted for evaluation by the Research Ethics Panel, a decision will be made on whether human ethical approval is required using the decision tree below.



Note: If you are interviewing farmers about their private or family life, then this is considered personal information. This extends to any information about the individual from which they are readily identifiable, so sometimes will include work- or business-related information.

Prerequisites

WorldFish staff members are required to be inducted into the WorldFish human ethics policy and are also required to take an external course through an IRB on human ethics prior to commencement of any research that involves human subjects. The link to the UNICEF course on human ethics can be found here. You can make an account and take the course called *Introduction to Ethics in Evidence Generation*. The certification of each PI and co-PI should be submitted with the protocol. WorldFish staff members who are not familiar with issues around Human Ethics may refer to some collated resources found on the Teams Channel.

Process for obtaining human ethics approval

Steps	Approximate time needed	Person responsible
The protocol is developed and adequately reviewed for quality. It should also be approved by the Research Lead at this point	months	Principal investigator (PI) or project lead
The protocol or is submitted to the Research Ethics Panel via MS Teams	1 day	Principal investigator (PI) or project lead
The Research Ethics Panel will give feedback on whether a submission is required for Human Ethics. If Human Ethics is required, then the PI or Project lead will be required to submit to an external IRB.	2 weeks	Research Ethics Panel and Research Support Unit
The protocol is refined based on the feedback from the Research Ethics Panel and resubmitted if requested by the panel	1 month or less	Principal investigator (PI) or project lead in collaboration with their respective teams
The protocol/application is submitted to an IRB (Institutional Review Board) which oversees human ethics	2-3 months	Principal investigator (PI) or project lead with support from the Research Ethics Panel, if needed
Administration fee is paid (if applicable)	5 days	Research Support Unit and WF Finance team
Interview is scheduled between IRB and PI (or host country representative)	1 month	Principal investigator (PI) or project lead

Steps	Approximate time needed	Person responsible
Approval letter is sent to the Research Support Unit for recording keeping	1 day	Principal investigator (PI) or project lead
Research Support Unit will keep all records of correspondence with IRBs on MS Teams	In perpetuity	Research Support Unit

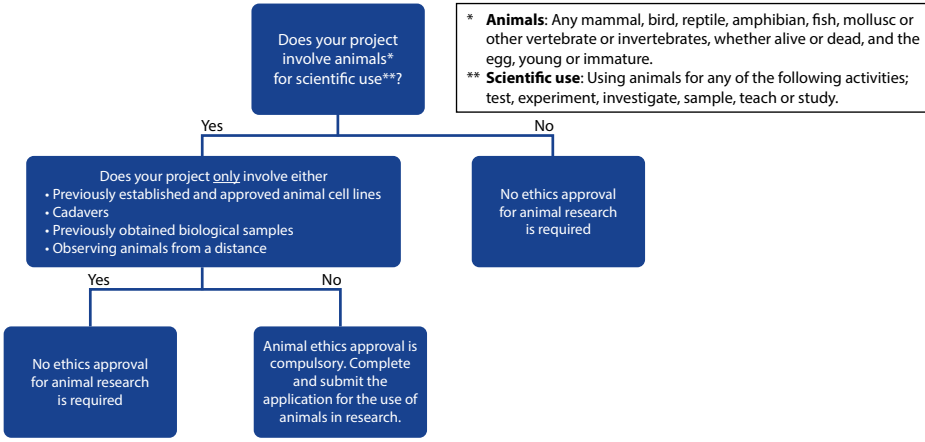
Additionally:

- The PI lead may seek approval of the protocol from the funders, as needed. Often, M and E plans will likely already be reviewed or approved by the funder.
- The PI/project lead must send the revised protocol to the Research Support Unit after ethical approval is granted, if the protocol or application was changed during the ethical review process where it will be stored as the final version

Animal Ethics

Do I need to seek ethical approval for animal research?

At WorldFish, animals are used for various scientific purposes in both research and teaching. All staff and students using any animals (i.e. any mammal, bird, reptile, amphibian, fish, mollusc or other vertebrate or invertebrate) require approval from the Institutional Animal Care and Use Committee (IACUC) prior to commencing any animal experiments. Once your research protocol is submitted to the Research Ethics Panel, there will be a decision made by the panel on the requirement of animal ethics using the decision tree below:



Pre-requisites

All researchers who intend on submitting experimental or research protocols to be reviewed by the Research Ethics Panel must undertake the WorldFish animal ethics training at a minimum. In some instances, further training is required from the partner organization if their IACUC is used for ethics approval. Be sure to check with your partner researcher.

All staff members that are supervising the handling of animals for scientific purposes must also undertake the WorldFish animal ethics training and must also perform procedures according to WorldFish standard operating procedures when available.

Process for obtaining animal ethics approval

WorldFish uses external IACUCs for the purpose of animal ethics submissions and assessments. Animal ethics approval may be sought through a partner organization, but it will be up to the project lead or principal investigator to determine if this is feasible. If this avenue is chosen, then the Research Support Unit will be sent the animal ethics approval letter prior to commencing any experiments. The steps are outlined in the below table:

Step	Approximate time needed	Person responsible
The protocol is developed and adequately reviewed. It should also be approved by the Research Lead at this point	months	Principal investigator (PI) or project lead
The protocol is submitted to the Research Ethics Panel via MS Teams or to: wf-ethics@cgiar.org	1-2 days	Principal investigator (PI) or project lead
The Research Ethics Panel will give feedback on whether a submission is required for Animal Ethics. If animal ethics is required, and the PI or Project lead would like to use a partner organization for approval follow steps 4, 5 and then 13. If animal ethics is required, and the PI or Project lead would like to use an approved Worldfish IACUC then follow steps 6 to 13.	2 weeks	Research Ethics Panel and Research Support Unit

Step	Approximate time needed	Person responsible
The protocol/M and E plan is refined based on the feedback from the Research Ethics Panel.	2 weeks	Principal investigator (PI) or project lead in collaboration with their respective teams
A researcher from the partner organization will submit the animal ethics submission form to the partner organisation's IACUC. The partner organization and associated researchers will be fully responsible for animal ethics approval.	1-2 months	Researcher from the partner organization
Principle investigator will fill out the Worldfish Animal Ethics Submission Form using the refined research protocol	In own time – but must submit according to the schedule published by the Research Support Unit	Principal investigator (PI) or project lead
The Worldfish Animal Ethics Submission Form is sent into the Research Support Unit		Principal investigator (PI) or project lead
The protocol/application is submitted to an IACUC (previously approved by Worldfish) by the Research Support Unit	1-2 months	Principal investigator (PI) or project lead with support from the WorldFish Research Panel, if needed

Step	Approximate time needed	Person responsible
Administration fee is paid (if applicable)	5 days	Research Support Unit, Project Lead and WF Finance team
Interview is scheduled between the IACUC and the Principle Investigator	2-3 weeks from IACUC sitting/ interview	Research Support Unit will schedule
Approval letter, letter of decline or resubmission letter is sent to the Research Support Unit	4 weeks from IACUC sitting/ interview	Research Support Unit to communicate
The Research Support Unit will communicate with the Principle Investigator the result in order to: • Resubmit • Answer questions from the IACUC • Appeal the decision if there are grounds for appeal	4 weeks from IACUC sitting/ interview	Research Support Unit and Project Lead or Principal Investigator
Research Support Unit will keep all records of correspondence with IACUCs on sharepoint and will update the Research Ethics Panel sitting dates and participants	In perpetuity	Research Support Unit

Roles and responsibilities

Research Support Unit: Coordinate the research panel, and feedback to all submissions from the Research Ethics Panel. The RSU will also apply for all animal ethics applications through external IACUCs and act as an administrative service to ensure that there is appropriate record keeping of all submissions, approvals, rejections and amendments.

Animal and Human Ethics Experts: Provide expertise in human or animal ethics, to inform the Research Ethics Panel.

Scientists and Post-doctoral scholars: Scientists and post-docs are responsible for developing protocols that are of high quality using a rigorous review process. They are also responsible for submitting the applications to the ethical review boards as needed, and documenting approvals and renewals.

Principal Investigators/Project Leads: Project leads who are overseeing projects that collect primary data may be responsible for developing a high-quality protocol, and in some cases seeking external ethical approval. Project leads and principle investigators are expected to induct staff members that report to them, and to confirm that scientists that are involved in the project have adequate training to perform the tasks/procedures in an ethical and competent manner that complies with Worldfish standards.

Research Leads: Research leads are responsible for approving the protocols presented to them by the scientists, post-docs and/or project leads who report to them.

Country Directors: The country directors ensure that all project that collect primary data (whether from animals or humans) have been vetted by the Research Ethics Panel and have obtained official ethical approval as needed.

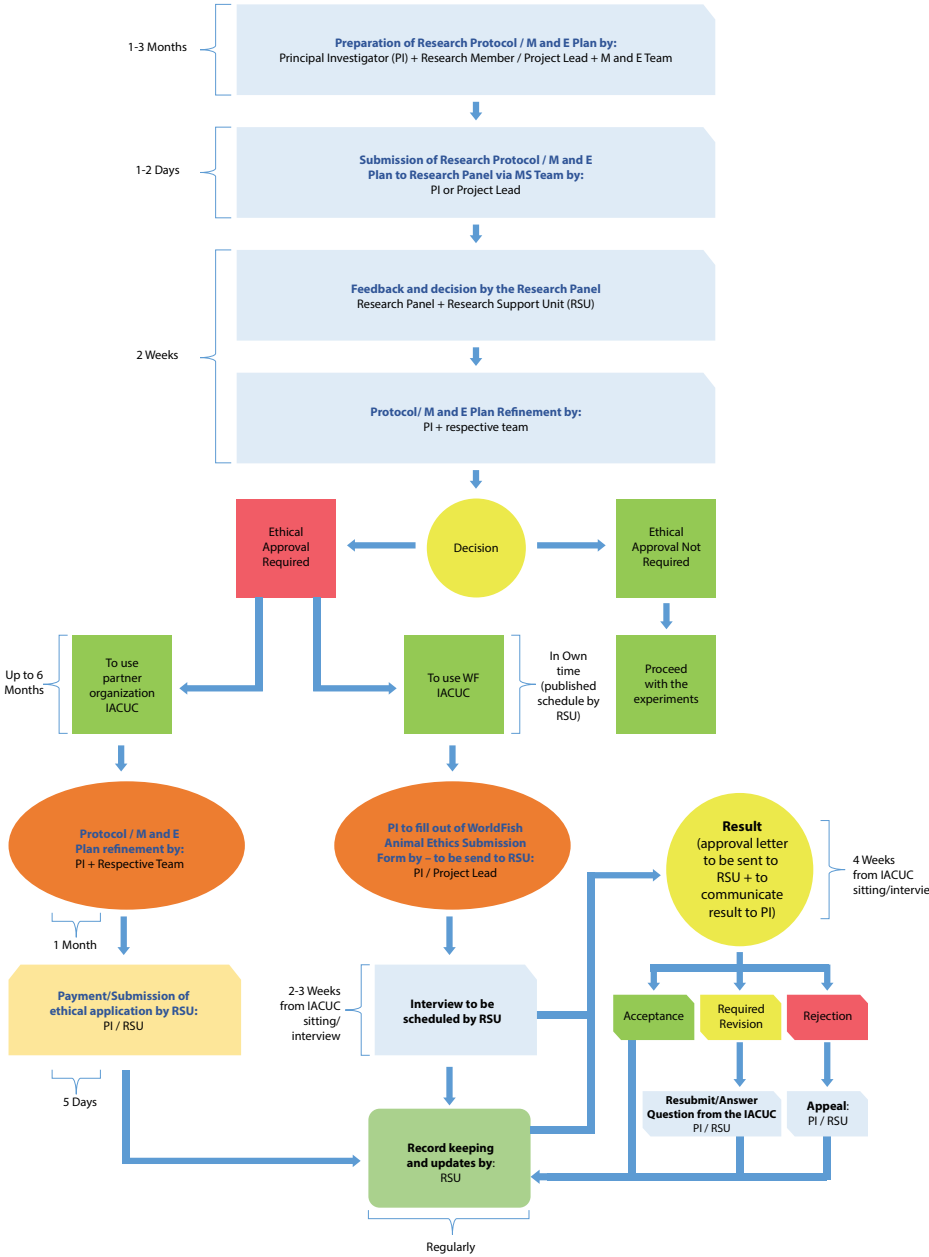
Research Director: The Director of Aquaculture and Fisheries Sciences/Research Director will appoint scientists to the roster for serving on the Research Ethics Panel. The Research Direction will also settle any disputes that may arise on whether or not a protocol should be submitted for ethical approval.

Resources for preparing and reviewing the protocol

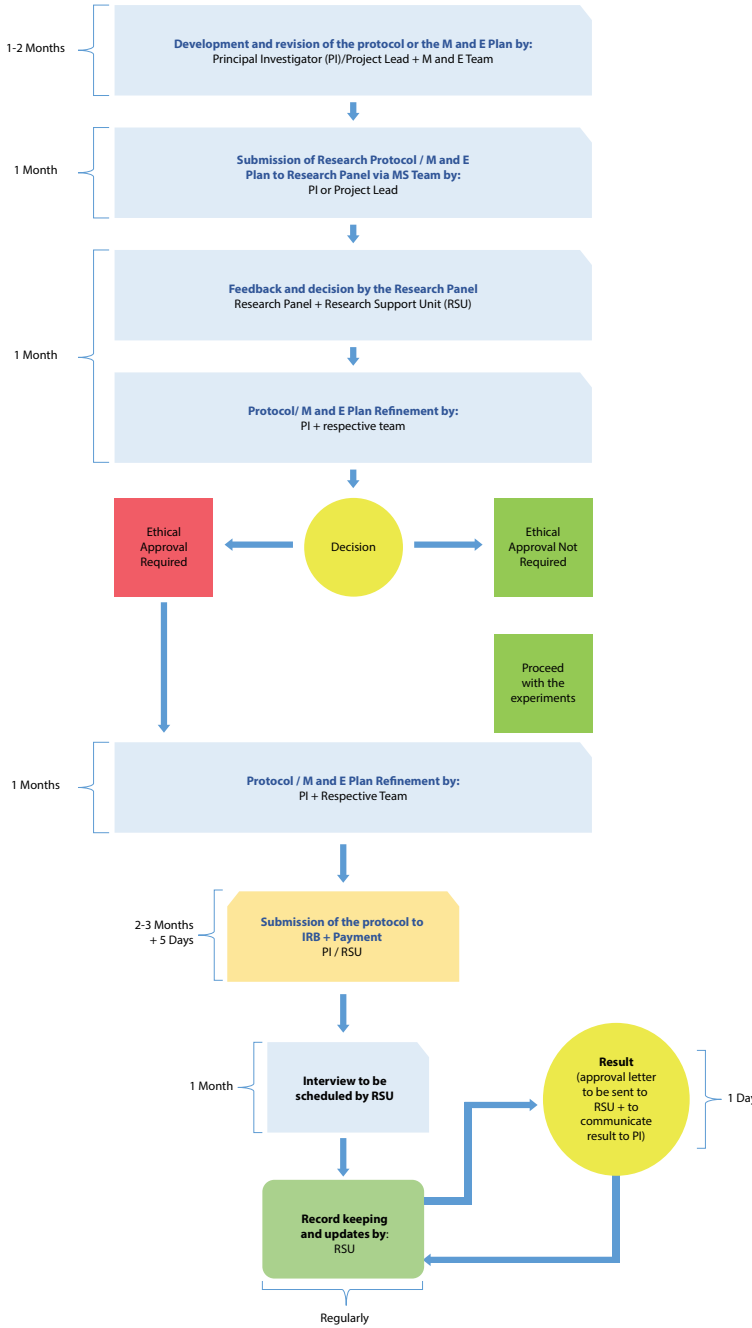
Please see below for resources on preparing the protocol.

Appendix A: Link to protocol template for research on humans
Appendix B: Link to protocol template for research on animals
Appendix C: Link to protocol review rubric – document to use when sending the protocol out for review by peers/external scientists

Ethical Approval Process – Animal Ethics



Ethical Approval Process – Human Ethics

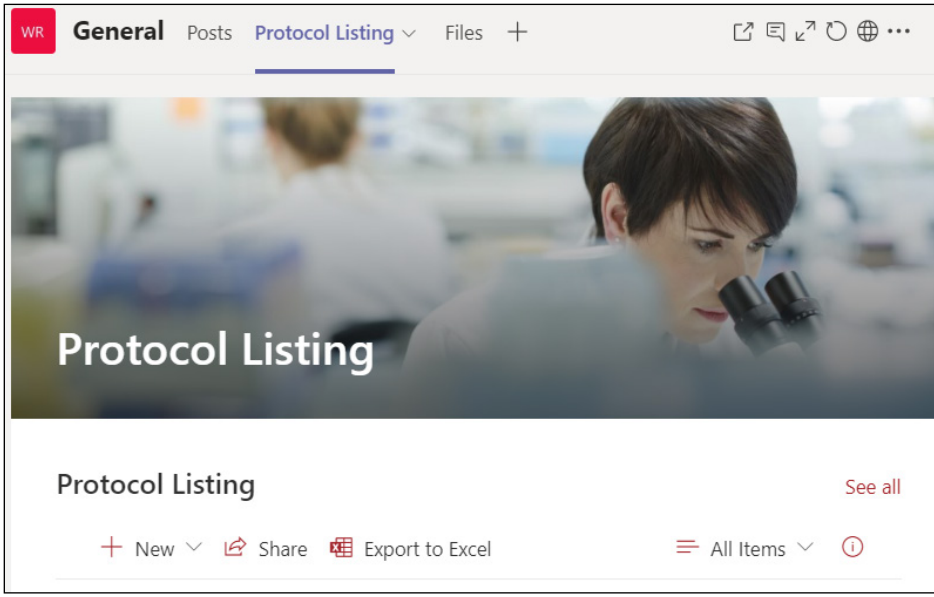


Instructions for Submission of Protocol Online Procedure

Instructions for Submission of Protocol Online Procedure

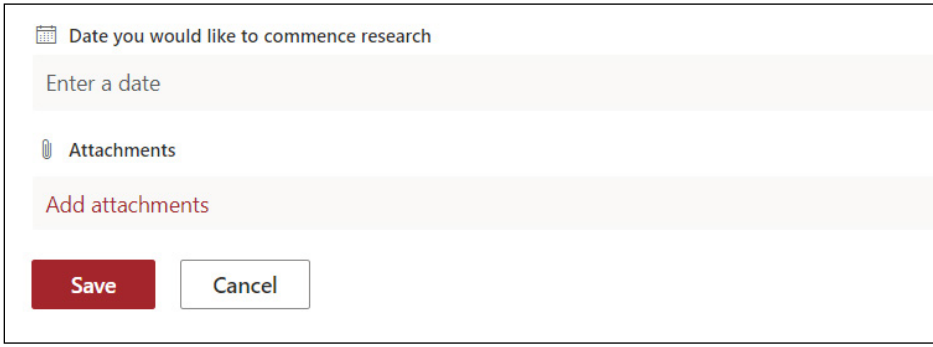
1. Login and open the **MS Team Application**
2. Scroll down and look for **"WF-Research Quality and Ethics"** channel – Click on the channel
3. Click on the **"General"** section

4. From the **"General"** tab (on the bottom-right of your screen), click on the **"Protocol Listing"** tab – select **"New".**

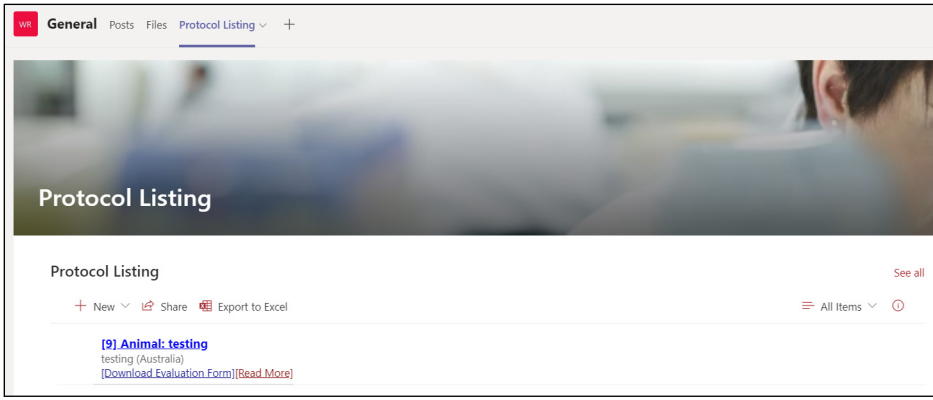


5. Click **"New"** and you may start filling in the relevant information.
**There might be amendments to the online submission form from time to time.*

6. Once all the required information have been provided, please click "Save" to avoid loss of information.



7. Once the form is saved, you may view the submitted forms by clicking on the **"Protocol Listing"** tab.



Research Protocol Template (Animal Ethics)

The purpose of this document is to guide the project/study from the initial planning stages. In discussions with the planning team (both internal and external partners) you will record key details here. This document can then be shared with additional academic/implementing partners who wish to know more about the study. It can also be used to seek approval from an ethical review board. Additionally, it can be used to develop the methods section of an academic manuscript. The audience for this document includes all researchers engaged in the project, country directors, and heads of the implementing partners. It does not necessary include field staff. This document may be modified during the life of the project. This protocol should be reviewed by academics who are not engaged in the project using the protocol review rubric form.

Investigators and institutional affiliations

Principal investigator

Principal Investigator/ Responsible Scientist	
Position and Organization	
Contact details	
Application title	
Expected duration of project	Start date:
	End date:
Qualification (PhD, MSc, DVM, BSc, etc.)	

Co-investigators

Coinvestigator 1:

Name	
Contact details (phone and email)	
Position and organisation	
Qualification	
Contact details	
Application title	

Co-investigator 2:

Name	
Contact details (phone and email)	
Position and organisation	
Qualification	
Contact details	
Application title	

Abstract/summary

Brief summary of the project in < 200 words. Include the why the project is important, the aims and expected outcomes. This should include: purpose, objectives, location, data type, study duration and outcomes.

Study protocol

Rationale/background and need for the study

This section describes the context of the study and defines the research problem that the experiment will tentatively solve. Here, the investigators demonstrate the relevance (importance, opportunity, etc.) and originality (new, innovative, etc.) of the research. They succinctly present the justification, including what knowledge gaps the study will fill and for whom. How will this project the study build on (not duplicate) previous/existing knowledge? Be sure to cite 1-2 keys papers that highlight the research gap.

Research Objectives

This section presents the goal and general objective of the experiment, as well as the specific objectives. The specific objectives are components of the general objective, which once completed, lead to the achievement of the general objective. This section ends with the setting of the research hypothesis, which is an affirmative statement. This section can be drawn from the research proposal and include:

1. Overall purpose
2. Specific objectives (research questions)
3. Who will use the findings generated by this study (partners, policy makers, programs, civil society – aim to be specific in these) and how/for what?

Materials and Methods

This section must be as detailed as possible, in order to not only allow the reproducibility of the research, but also allow the reader (and reviewer) to detect any flaws in the materials and methods and suggest and/or take corrective actions.

Experiment Design

This section describes the experimental design (eg. completely randomized design, completely randomized block design, split-plot design or latin square design) that will be applied, the treatments (eg. the different water temperatures, the different diets, the different vaccines or the different strains of fish) and the experimental units (eg. fish, ponds, tanks or aquaria). This needs to be as detailed as possible for the assessor.

Facility

This section thoroughly describes the rearing site and facility. It includes information on:

- The research site (on-state, on-farm)- include a google earth image of the farm
- The type of culture/holding facility (eg. pond, sea-cage, aquarium or tank)
- The source of water (eg. well, mains, or surface water),
- Tolerated water quality parameters (eg. temperature, pH, ammonia, nitrite, nitrate, etc.) that will be maintained throughout the experiment
- Actions planned to maintain the desired water quality (water exchange, adding of chemicals, etc.), the flow system (recirculated, flow-through or stagnant) and the water flow rate (eg/ in l/min per tank or aquarium)
- Filters (mechanical and biological) that will be applied to the water (if applicable)

Particularly for ethics approvals, this section needs to be very detailed to ensure that animals are held in a low-stress culture environment and that water quality requirements are met specific to the species.

Diets and Feeding

In this section, the detailed composition of the diets, the physical characteristics of the feeds (floating or sinking), feed size (in mm), the supplier of the feeds, and the storage condition of the feeds must be provided. In addition, information on feeding rate (percentage body weight or to apparent satiety) and frequency (number of meals per day and time of each feeding per day), feeding method (automated or hand feeding), and the duration of the feeding must be provided.

Fish

At a minimum, the following information needs to be specified:

- Species of the fish (and common name),
- Gender (number of males and females used),
- Supplier,
- Health certification from supplier (if applicable),
- Average initial size and weight and predicted end-size,
- Number of fish used,
- And health status of the experimental fish must be provided.

Information on the animal ethics committee that will review and approve this research and the timeline for submitting the application to this committee must be provided.

Sample collection and anticipated analyses

This section presents the detailed description of all the sampling methods including (not limited to):

- Weight/length collection
- Fecal collection
- Behavioural observations
- Necropsy
- Samples for diagnostic testing e.g. fresh preparations, histology, PCR, bacterial cultures etc.
- Water samples and anticipated analyses
- Swabs (external or internal)

Other items that need to be described:

- Timing or chronology of collection
- Information on sample processing
- Information on the laboratory analysis:
    - Detailed analytical techniques
    - If the analytical technique is new, developed by the lab or adapted from a conventional technique – it must be thoroughly described
    - If the technique is standard or conventional then a reference should be cited that uses the same technique
    - If there are deviations from the standard or conventional technique, then a description of the deviation should be written

Statistical analysis

In this section, the statistical procedure (or combination of procedures) that will be used to analyse each parameter must be described, eg ANOVA, ANCOVA, polynomial regression, contrast procedure, multiple comparison test, etc. In addition, the significance level that will be used should be mentioned, eg. 0.05. Finally, information on the statistical software and the version of the software that will be used must be provided.

Field teams and research management

If field teams are required, then a field team structure is required, including supervisors and reporting. This may be applicable in situations where there are fisheries assessments, or teams of researchers on commercial aquaculture sites with concurrent projects.

Mechanisms should be in place to monitor the quality control of data collection. The team needs to have identified who is responsible for reviewing data collection practices, accurate recording of data, and data submission on a real time or timely basis. Those responsible will need to be able to liaise with the project PI and others on issues and problems arising in the field. In addition, they should organize weekly supervisory meetings and monthly refresher trainings / feedback meetings with field teams, especially if the study period is long.

Together the PIs, research team coordinator and data collection teams to:

a. ensure that participants are selected properly;
b. ensure that the research is conducted in an ethical manner;
c. ensure that supplies and materials are in adequate supply for team operations; and
d. carry out conduct quality control assessments including observations and document / database review to ensure that measures are taken as per the protocol, recorded on the forms and entered into the database accurately – and improvements made rapidly as needed.

The PI, coordinator and field teams are responsible for deciding together (sign off by PI) how to overcome unexpected problems/any needed changes to the research protocol. Each problem encountered and decision made should be promptly recorded and included in the supervision report. If possible, he/she should organize weekly or bi-weekly de-briefing sessions with team members to discuss problems concerns, review progress, forms, etc. to ensure that no pieces of data have been left out.

Data management

Data will need to be managed and cleaned. A description of how the data will be managed, stored and cleaned is important for record keeping, and business continuity for WorldFish. Information on the data management plan should be included in this section and comply with WorldFish's policy on data sharing and open access.

Set of tables and flow charts

This section contains all the tables that were referred to in the text above. These tables include, but are not limited to:

- A table on the research timeline (Gantt chart)
- A table on the summary of the sample that will be collected
- A table on the summary of the data that will be collected
- A table on the composition of the experimental diets

Examples of tables

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Distillers/brewers grain						
Gluten (corn)						
Soybean meal (48 solv)						
Corn (7.5% CP)						
Wheat bran						
Canola oil						
Fish oil						
Dicalcium Phosphate						
Trace mineral premix						
Vitamin C						
Vitamin premix						
Lecithin - Soy (70%)						
DL-Methionine						
L-Lysine						
L-Glutamic acid						
L-Threonine						
Total						
DM%						
Ash%						
GE MJ/kg						
DE MJ/kg						
CP%						

Table 1. The composition of the experimental diets.

Activity	Time
Protocol and formulations	by March
Feed manufacture	by April 20...
Animal sourcing and acclimating	by April 20...
Start of experiment	by May 20...
Final sampling	by August 20...
Sample Preparation	by August 20...
Biochemical Analysis	by September 20...
Data analysis	by October 20...
Reporting	by November 20...

Table 2. Timeline- this is better to be displayed in a gantt chart for tracking purposes.

Diets	Initial Fish	Final Fish (per tank)
500 g/ diet	2*2 pools	1 fish pool x 36 tanks
6 samples	4 samples	36 samples (in duplicate?)

Table 3. Samples to be sent to a local laboratory for analysis of diet and carcass proximate composition.

Fish	Intermediate sample	Feed	Daily
Dry Matter (%)	Fish weight (g)	Dry Matter (%)	Water quality
Total Ash (%)	Number of fish	Total Ash (%)	Feed intake
Crude Fat (%)		Crude Fat (%)	Mortalities
Crude protein (%)		Total N (%)	
Crude fiber (%)		Crude fiber (%)	
Gross Energy (Kcal/100 g)		Gross Energy (Kcal/100) g)	
Amino acid profile		Amino acid profile	
Weight (g)			
Length (cm)			
Liver weight (g)			
Gonad weight (g)			
Survival (%)			

Table 4. Data collection sheet example.

Budget

You can link to the budget in the proposal or provide a short summary (table preferred). A budget for the project should be included:

- Total cost for the project
- Analysis costs
- Required FTE allocated hours

Impact of the project

The impacts of the project need to be clearly articulated. This should be stated clearly in dot points, relating to:

- Commercial outcomes
- Environmental outcomes
- Industry and community outcomes
- Science outcomes and new knowledge generated

References

This section presents the full information on the references that were cited in the text. Here is an example:

Tacon AG. 2018. Global Trends in Aquaculture and Compound Aquafeed Production. World Aquaculture, 49(2), 33-46.

Research Protocol Template (Human Ethics)

For use with quantitative, qualitative, and mixed methods studies

The purpose of this document is to guide the project/study from the initial planning stages. In discussions with the planning team (both internal and external partners) you will record key details here. This document can then be shared with additional academic/ implementing partners who wish to know more about the study. It can also be used to seek approval from an ethical review board. Additionally, it can be used to develop the methods section of an academic manuscript. The audience for this document includes all researchers engaged in the project, country directors, and heads of the implementing partners. It does not include field staff, etc. This document will not remain static, but may be modified during the life of the project. However, it does provide an important starting point. This protocol should be reviewed by academics who are not engaged in the project using the protocol review rubric.

Investigators and institutional affiliations

Principal investigator

List here the principal investigators and indicate their institutional affiliations.

Co-investigators

List here all co-investigators and indicate their institutional affiliations.

Abstract/summary

Briefly describe the study's purpose and objectives, location, the type of data and findings that will be produced, the target sample (sample size), and the study duration.

Study protocol

Rationale/background and need for the study

This section describes the study in greater detail. Important to include here is the justification, research gaps, and the contribution of the current study in filling the gaps. Information used in this section comes from the research proposal. Here you will succinctly present the justification, including what knowledge gaps the study will fill for whom

Research Objectives

The purpose and specific objectives are listed. This information comes from the research proposal.

1. Overall purpose
2. Specific objectives

Conceptual and/or analytical Framework (if applicable)

Conceptual and/analytical framework to be applied (and why this is appropriate)

Research Approach, Design, Methods and Sites

Overall design: experimental (with a control group) or nonexperimental?

Methods

Overall: What methods will be used (if mixed-methods)

Identify the type (sequential, etc) and describe who

Study site(s)

Criteria and rationale for site selection (a) overall country, districts; (b) specific sites (villages etc.)

Study population

Identify study population – include inclusion and exclusion criteria if needed

Description of the Intervention

If applicable

List of variables to be collected (might consider a table here)

Can give broad categories as well. It also might be useful to list what is your primary variable, secondary variables, etc. Can also list what confounding/effect modifier variables you will collect.

Qualitative methods description (if needed – delete if not)

1. Describe, including key dimensions, core modules.
2. Identify how data will be captured (audio recording vs notes; transcription, translation…) and how the choices minimize risk of bias, loss of accuracy

Data collection tools

1. Attach the questionnaire as an appendix to the protocol.
2. Describe how surveys will be administered including how data will be recorded (e.g. tablets, mobile phone) and uploaded to a secure database at what frequency

Sampling

The sample size is a very important aspect of the protocol. It is important to justify the number of people you will interview for the study. The sample should also support inclusiveness, and to avoid excluding certain groups (i.e. women, some ethnic groups), unless there is a strong reason for excluding them.

Qualitative sampling, if applicable

1. Who are the intended participants (link with above)
2. Present the qualitative sampling (numbers of different groups for each method) and rationale.

Quantitative sampling, if applicable

1. Size determination (who are the intended participants (link with above) How will the sample size be determined? A clear description of power analysis may be helpful. Other determinants of sample size including budgetary constraints should be highlighted as well
2. Sample selection Indicate who are the study participants and how will they be identified. If you have any ads or letters to recruit participants you can include it as an attachment.

To what extent are the qual and quant samples intended to overlap or not?

Explain and indicate why. How will this be ensured?

Field teams and implementation

Team composition and distribution

1. What is the field team structure? For example, how many enumerators? How many supervisors? In what language will the survey be conducted in?
2. How will the field team be constituted to meet the needs of the study? (gender balance, language skills etc)

Recruiting and training fieldworkers

1. How many days of training, covering what components, when?
2. How will the training allow sufficient room for role-playing and field practice to ensure that the field team fully understand the study and its implementation?

Pre-testing and finalizing the tools

After the training and pretest, the survey tools and procedures should be adapted as necessary. Final versions of tools should then be submitted to the review board if appropriate

Community permission and timing

1. Who, how and when will the team engage with community leaders for permission and to sensitize on the study objectives and procedures;
2. Gather info needed to schedule fieldwork at times and places that will allow participation and minimize attrition of women and men.
3. How does the research comply with the local laws?

Implementation and supervision

What are the mechanisms between field teams and 'supervisors' (researchers) to:
1. ensure data is checked for quality and completeness in a timely and effective way?
2. improve and correct?
3. trouble shoot and adapt effectively as field issues arise?

Tips: Mechanisms should be in place to monitor quality control of data collection. The team needs to have identified who is responsible for reviewing data collection practices, accurate recording of data, and data submission on a real time or timely basis. Those responsible will need to be able to liaise with the project PI and others on issues and problems arising in the field. In addition, they should organize weekly supervisory meetings and monthly refresher trainings / feedback meetings with field teams, especially if the study period is long.

Together the PIs, research team coordinator and data collection teams to: a) ensure that participants are selected properly; b) ensure that the research is conducted in an ethical manner; c) ensure that supplies and materials are in adequate supply for team operations; and d) carry out conduct quality control assessments including observations and document / database review to ensure that measures are taken per the protocol, recorded on the forms and entered into the database accurately – and improvements made rapidly as needed.

The PI, coordinator and field teams are responsible for deciding together (sign off by PI) how to overcome unexpected problems/any needed changes to the research protocol. Each problem encountered and decision made should be promptly recorded and included in the supervision report. If possible, he/she should organize weekly or bi-weekly de-briefing sessions with team members to discuss problems concerns, review progress, forms, etc. to ensure that no pieces of data have been left out.)

Coordination

The principal investigators should ensure the overall coordination and implementation of the study.

Laboratory analysis

Describe laboratory analysis, if any.

Data management

The CGIAR requires that deidentified data are made open access so that others can access the data and study materials. Please describe how you will keep any personal information of the study participants protected, and accessible only by those who need it.

Before making the dataset Open Access, and hosting it on Data Verse, you must take care that all personal information is removed. Describe here how you will protect participant data, and adhere to the CGIAR Open Access Data policy.

Statistical analysis

Describe what kinds of statistical analysis will need to be run to answer which questions and in relation to which data sets.

Tip: This section is not meant for a detailed description of the statistical analysis to be conducted. But it is important to explain briefly what analysis will be conducted and why

You can list here where the dataset will be hosted once it is ready to be made public.

Timeframe

Might consider a gantt chart here

Ethical considerations

Identify what the study (or if the larger project) has in place to assess UNINTENDED and potential negative consequences of the study early and rapidly enough to mitigate those. For example, will you engage participants who are particularly vulnerable? (e.g. illiterate farmers, minors, or people with diminished cognitive capacity, migrants, the elderly, or women in a traditional patriarchal society) If so, how will you will you take steps to insure that their rights are protected? This might include taking steps to make sure the interview will take place in a comfortable, neutral location where they can feel free to talk. You might also consider if the study necessitates engaging with vulnerable populations, or if there is someone else you could talk to. If you will engage with vulnerable populations, care needs to be taken to modify the consent form (appendix 14.1) with consultation with the local community. You may consider translating, and back-translating the form for clarity in the local language.

If you will survey people who fall under the legal definition of a minor (usually someone under 18) you will need an assent form attached to the consent form (see appendix 14.1).

Communities and study participants should be informed of the study and sensitized by the research team to its activities and purpose through community meetings and meetings with local leaders. An informed consent must be obtained from all study participants. As part of the informed consent process the participants should be informed on the purpose and procedures of the study, any potential risks (unintended), maintenance of the confidentiality of personal data, continued storage of data, possibility to refuse the consent without having to justify the refusal (see appendix 14.1 for an example of an informed consent form).

For more information on ethics policies at WorldFish, please see the WorldFish policy on research involving humans, and the CGIAR Research Ethics Code

Expected application of the results

Who will use the findings generated by this study (partners, policy makers, programs, civil society – aim to be specific in these) and how/for what?

References

Budget

If needed

Appendices

Consent form

Good morning/afternoon. We are coming from [*Name of local partner organization*]. In collaboration with WorldFish Center, we are conducting a study to understand fish consumption, how consumers make their choices when buying fish and fish products, and knowledge about food safety.

You have been randomly selected to participate in the study. Your name and any other personal identifiers will not appear in any data that is made publicly available. The information you provide will be used purely for research purposes. With your permission it will be stored and used by other scientists, but there is no way the information will be linked to you.

The study has two parts. The first part is a simple market exercise that will give you an opportunity to actually buy fish. This part will take about 40 minutes. In the second part, I will ask you a few questions. This part will take about 20 minutes. In total, therefore, both activities will take 1 hour. Your participation in the study is voluntary and you do not have to participate if you don't want to. There are no risks to participating in this study, beyond those encountered in daily life.

If there are questions that you would prefer not to answer then we respect your right not to answer them. You may ask questions before agreeing to participate, at any time during the study, and if after the study you have any questions, you can contact [*Name of local partner*] from [*Institution*] at [*phone number*] or [*name*] from WorldFish Center at [*phone*]. You may also contact the Institutional Review Board (IRB) Chairperson at IFPRI, [*name*] with any concerns or complaints through email: [*email address*] or phone call: [*phone*].

Consent	Do you accept to provide information?	
	1=yes, continue with interview	1=yes
	0=no, terminate the interview and record the reason for refusal.	0=no

Enumerator's full name: _____ Date: _____

Signature or thumb print: _____

Consent form approved by IFPRI IRB on [date _____]

Asset Statement

IF THE PARTICIPANT IS UNDER 18 YEARS OF AGE:

I confirm that I am the legal guardian of the child named above and therefore may grant permission for this release on behalf of the child:

_____

Name of Legal Guardian, Relationship to Child _____ Signature and Date

Data collection tools (survey instruments, FGD and SSI guides, etc)

_____

_____

_____

Animal Ethics Feedback form for PI/Project Lead from WorldFish Research Ethics Panel

Protocol title: _____

Submitted by (PI): _____

Co-PIs: _____

Submission Date: _____

Meeting date protocol review by Research Panel: _____

Date form returned to PI: _____

Date rebuttal submitted: _____

Date form archived on MS Teams: _____

Attendees of session: _____

	Question	YES/NO	Comments
1*	Are the test subjects considered "animals" by law in the country that you are conducting the research?		
2*	Do the donors of the project stipulate that they require animal ethics approval, by their definition of "animal" research?		
3*	Are the test subjects considered "animals" by the WF or CGIAR policies?		
4**	Have the PIs and all of the co-PIs taken a course on animal ethics from an IACUC?		
5	Have they considered replacement of (alternatives to) the use of animals in the study?		
6	Have they assessed the numbers of animals using quantitative methods to ensure that there will be adequate benefits to the research and use of animals?		
7	Have the researchers addressed the principle of refinement in their research protocol by detailing the areas where they will minimize stress on animals and how e.g. stocking density limitations; control of water quality		

* *If "no" to all 3 Q1-3 then no ethical approval is required and the research panel can proceed in evaluating research quality using the rubric*

** *Animal ethics courses are generally run, by universities or research institutes that conduct research with animals. This is a certificate that the PIs and co-PIs can present from external universities or research institutes and need to be conducted withing 5 years prior to commencing the research project. All staff members who conduct research with animals must be inducted into the WF animal ethics policies and internal training*

Final Question	Yes	No
Does this protocol need to be submitted to an IACUC?		

General Recommendations to the PI, including suggestions and comments on how to improve the research protocol:

Rebuttal comments from PI:

References

CGIAR animal ethics policy WF animal ethics policy links

Human Ethics Feedback form for PI/Project Lead from WorldFish Research Ethics Panel

Protocol title: _____

Submitted by (PI): _____

Co-PIs: _____

Submission Date: _____

Meeting date protocol review by Research Panel: _____

Date form returned to PI: _____

Date rebuttal submitted: _____

Date form archived on MS Teams: _____

Attendees of session: _____

Checklist based on WorldFish Policy on Ethics of Research Involving People, and the CGIAR Research Ethics Code

Item	YES/NO/NA	Comments
Has the PI presented a certificate as proof of a course on human ethics (i.e. CITI or the UNICEF course for humans)?		
Does the PI address how the research is in line with the national ethics laws regarding where the research that will take place?		
Have the researchers stated how data collecting personal information will be handled (i.e. the plan for protecting data, and anonymizing it prior to the data being open access)?		
Have the PIs included an **informed consent statement** as an appendix?		
If yes to above, has adequate thought been put into the informed consent statement (e.g. does it use simple language, what language will it be delivered in, and does it make clear what will happen with participant data?)		
Does the research involve any vulnerable participants? (e.g. illiterate farmers, minors, or people with diminished cognitive capacity, migrants, the elderly, or women in a traditional patriarchal society) and if so do they address this? See ethics section of the protocol template		

Item	YES/NO/NA	Comments
If the research involves minors (typically people under the age of 18), is there an assent form included in the consent form?		
Do the benefits of participating in the study outweigh the risks? Have the researchers thought through any potential negative or unintended effects on those participating in the research?		
Are the risks and benefits of the study evenly distributed among participant populations (i.e. one group is not being treated better than another?)		

Final questions	Y/N	Additional comments
Does this protocol adequately treat human participants with respect and dignity, and follow the universal guidelines on human ethics in research?		
Does this protocol need to be submitted to an external ethics review board?		

General Recommendations to the PI, including suggestions and comments on how to improve the research protocol:

Rebuttal comments from PI:

References

Brydensholt HH and Axelsen NH. 2004. Research ethics. *Ugeskrift for Laeger, 166*(24), 2335–2336.

Research Protocol Review Rubric

Reviewer Name: _____

Reviewer Affiliation: _____

Date Reviewed: _____

Please use this document as you review the protocol to provide specific feedback that may be achievable. Consider the following dimensions and give a ranking for each.

Dimension	Quality ranking (1-10) [One per dimension]	Briefly explain responses. Please provide suggestions for improvement, if needed.
1. Are the research questions and project objectives: 1.1. Clearly stated? 1.2. Sufficiently compelling, and has the gap in the literature been established (i.e. is there an established need/problem that this question will address)?		
2. What is the research design described here? 2.1. Is it appropriate? 2.2. Is it clearly described? Types of overall **designs/paradigm approaches**: a. Experimental b. Non-Experimental (observational) c. Qualitative d. Mixed methods		
3. Is the choice of Research Design: 3.1. Appropriate? (ie will the design be **effective in answering the Research Questions**?) For example, if the study/M&E needs to understand cause-and-effect, will the design capture that? Most M&E designs should include room for negative or unintended consequences – will the design enable that?		
4. Methods 4.1. Are the methods appropriate to answer the research questions? (E.g., if 'why' and 'how' questions are asked/needed, does the study include qualitative? If 'how much' questions -- quant?) 4.2. Are the methods clearly defined and justified? You can refer to the following appendices for more information on individual methodological approaches • Observational trials (appendix 1, link) • Randomized controlled trials (appendix 2, link) • Qualitative trials (appendix 3, link) • Lab-experiments (I.e. fish feed experiments)		
5. Study population 5.1. Appropriate? 5.2. Clearly stated?		

Dimension	Quality ranking (1-10) [One per dimension]	Briefly explain responses. Please provide suggestions for improvement, if needed.
6. Sampling 6.1. Quantitative (if applicable): Is the sampling design effective to produce the needed statistical power? Including will it enable gender disaggregation and intersectional analysis (age, wealth group, other) as needed 6.2. Qualitative sampling (if applicable): Does the protocol present a qualitative sampling strategy that is appropriate to the study questions and population? Is it clearly described – ideally presenting a sampling framework or at least a narrative regarding saturation?		
7. Rigour 7.1. Are the dimension of rigour and the strategies for achieving them appropriate to the study approach and design? 7.2. Does the protocol clearly describe what dimensions of rigour are being considered and how they will be ensured? Including potential weaknesses and how they will be addressed?		
8. Is the study (approach, design, methods, and sampling) feasible within the timeline and resources allocated?		
9. Is there a plan to share the data with the data manager and make the dataset public?		
10. Are clear roles assigned?		
11. Is the appropriate language being used (i.e. no causal language if it is an observational study)		
12. Are any contingency plans in place in case plan A does not work out? Are there systems in place for updated the protocol (and reporting to the IRB) as needed?		
13. Are ethical issues taken into consideration?		
Average score		

Please give insight as to whether this protocol needs minor or major revisions. If major revisions are needed for this project or program to be successful, please list the revisions needed here.

Overall comments on protocol:

Appendix 1. Observational studies

1. Does the protocol explain the settings? Including location, relevant dates, periods of recruitment, and methods for data collection?
2. Are the inclusion and exclusion criteria for the study participants explained?
3. Are all variables (outcome, predictors, potential confounders, and effect modifiers) clearly defined? Are diagnostic criteria described, if applicable?
4. For each variable, are sources given? Does the protocol describe how the variables will be measured?
5. Is the method for reducing bias delineated?
6. Was the sample size calculation described?
7. Are the analysis methods clearly described? Explained how all quantitative variables will be handled (and presented?). Are the analysis methods appropriate to the question being asked?

Appendix 2. Randomized controlled trials

1. Description of trial design (such as parallel, factorial) including allocation ratio
2. Are the inclusion and exclusion criteria for the study participants explained?
   - Settings and locations where the data will be collected
   - Completely defined pre-specified primary and secondary outcome measures, including how and when they will be assessed
   - How sample size was determined
   - Method used to generate the random allocation sequence
   - Type of randomisation; details of any restriction (such as blocking and block size)
   - Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned
   - Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions
   - If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how
   - If relevant, description of the similarity of interventions
   - Statistical methods used to compare groups for primary and secondary outcomes
   - Methods for additional analyses, such as subgroup analyses and adjusted analyses

Appendix 3. Qualitative studies

1. Is the qualitative methodology clearly laid out, and is it appropriate? i.e. is the methodology right for addressing the research goal?
2. Is the recruitment strategy described and is it appropriate to the aims of the research?
3. Are the data collection methods clearly described? Was there evidence for planning for trustworthiness of the data? Assessed by:
   - credibility – will the data adequately recorded and triangulated?
   - transferability – plans to record adequate aspects of the population and setting?
   - dependability – will there be evidence of an audit trail or peer review? The audit trail describes the decision points throughout the research process
4. Are there methods for reducing bias? Is there a plan to check with colleagues about the ideas and interpretation of the data?
5. Are the plans for data analysis sufficiently rigorous?

Appendix 4. Experimental

1. Is the information on the fish (gender, strain, age, size) and supplier of the fish provided?
2. Is the information of the facility (pond, tank, aquarium) and the rearing system thoroughly provided?
3. Is the information of the type (floating or sinking) and size (in mm) of the feeds as well as feeding frequency provided?
4. Are the treatment and experimental design well defined and appropriate to address the research problem?
5. Are the plans for data analysis sufficiently rigorous?

Euthanasia of Finfish

Version Control Tracking			
Issue Date	**Summary of Changes**	**Distribution**	**Version Number**
20 June 2019	Initial version	Internal	1.0.0
30 December 2021	Revised version	Internal	1.2.0

Associated Standards

- Handling of Finfish
- Sedation and Anaesthesia of Finfish

Summary

Application

In some instances (e.g. active/passive surveillance for health sampling and culling/sorting), humane euthanasia of finfish is necessary. WorldFish does not condone the inhumane killing of fish. Euthanasia should be as quick, and as painless as possible. Fish under the care of WorldFish should not be left to suffocate in the air or to experience pain in water if practical human interventions to avoid this are possilbe.

Prior to euthanasia, all fish should be handled in accordance with the WorldFish Handling of Finfish Standard Operating Procedure.

Benefits and Risks

Benefits

Humane euthanasia may be used for:

- In-field routine health sampling
- During a diease outbreak to euthanise moribund animals
- In-laboratory sampling throughout an experiment
- Culling of fish at the end of an experiment
- Culling of fish during breeding programs
- Culling of fish for production purposes

Risks and Mitigation

Refer to the **Risk** and **Mitigation** section of the Associated Standards

Risks:

- If using physical methods, staff may be injured by sharp instruments (e.g. if using Iki Jime or percussive stunning methods) or blunt instruments (e.g. if using angler's priests or sharp instruments).
- If using anaesthetic overdose, anaesthetics can be very irritating through skin and aerosol exposure
- If using anaesthetic overdose, pollution of waterways and soil with anaesthetics

Mitigation:

- If using physical methods on large fish, it is recommended that anaesthesia is considered as a first step. Sedation of larger animals results in them becoming easier to handle before performing Iki Jime or other euthanasia methods.
- If using anaesthetic overdose, the risk reduction methods detailed in the WorldFish Sedation and Anaesthesia of Finfish Standard Operating Procedure should be implemented.

Procedure/Protocol

Methods of euthanasia such as exsanguination (i.e. draining of blood through the cutting of gills and/or caudal vein) or decapitation are **unacceptable** without prior anaesthesia or percussive stunning (or explicit exemption from the animal ethics committee)..

Any equipment used must be in good working order and checked prior to performing euthanasia. All equipment should be cleaned and disinfected as needed or between uses.

Any investigator or person involved in the euthanasia of fish is to be well informed about the pharmacological and physiological impacts of their proposed method of euthanasia (see Neiffer & Stamper, 2009; Readman et al., 2017)).

Anaesthetic Overdose for euthanasia

Ideally euthanasia by anaesthetic overdose should be a two-step process with the first step being anaesthesia (at least to the point at which fish lose their equilibrium) followed by another physical or chemical method. This can be applicable in tank situations, where there is a more controlled environment. However, WorldFish recognises that this cannot always be the case when working in remote field locations.

Acceptable physical techniques of euthanasia following anaesthesia are pithing to the brain (or physical destruction of the brain) and brainstem, decapitation at the cervical vertebrae (using a sharp knife), or exsanguination via severance of the vessels to the gills or the caudal vein.

The sole use of chemical methods within the water to euthanize fish (i.e. overdose with aesthetic) is acceptable if adequate monitoring time is performed to ensure death. It is recommended that fish are immersed for at least 1 hour after being declared dead. Death can be declared in a fish that has not shown any signs of respiratory effort or opercula movements for at least 10 minutes.

Drug	Dosage (mg/L)	Duration (min)
Eugenol	Five times the normal heavy anaesthetic dosage (at least 400-500 mg/L)	5 minutes, and leave fish in anaesthetic bath for 1 hour until after opercular movements cease
MS-222	Five times normal heavy anaesthetic doses of 500 mg/L (min)	5 minutes, leave fish in anaesthetic bath for 1 hour after opercular movements cease

Iki Jime (or pithing)

- This method should be used with experienced operators only, and the use of an Iki Jime gun should be considered.
- If an Iki Jime gun is not available, insert a sharp probe into the brain and move it around to destroy the brain and brain stem.
- Push the sharp probe down the spinal column to destroy the nervous tissue.

Percussive Stunning

- The use of concussive forces (i.e. priesting), in conjunction with pithing or exsanguination is acceptable with reservation. There must be supervision of staff during this process, and the correct methods used.
- Percussive stunning is achieved by a blow of sufficient strength to the head applied above or immediately adjacent to the brain in order to damage the brain.
- The blow on the head should be placed on top of the head, right behind the eyes
- It is best that after a blow is delivered, and the fish is stunned, to ensure death by exsanguination or pithing. Sometimes, stunning is not enough, and fish can recover if the blow is not delivered with enough force.
- Using this method of euthanasia can affect health samples - if you need to collect brain or fresh gill preparations, it is not recommended as blood clots can form rapidly, reducing the ability to examine the gills. For this application, it is recommended that fish are overdosed with anaesthetic.

Additional Information

External reference materials:
Neiffer DL and Stamper MA. 2009. *Fish Sedation, Anesthesia, Analgesia, and Euthanasia: Considerations, Methods, and Types of Drugs*. *50*(4). doi: 10.1093/ilar.50.4.343

Readman GD, Owen SF, Knowles T G and Murrell JC. 2017. Species specific anaesthetics for fish anaesthesia and euthanasia. *Scientific Reports*, January, 1–7. doi: 10.1038/s41598-017-06917-2

Product safety data sheets (SDS)*:
- MS-222: https://www.sigmaaldrich.com/AU/en/sds/aldrich/e10521
- Clove oil: https://www.sigmaaldrich.com/AU/en/sds/sigma/c8392
- Benzocaine: https://www.sigmaaldrich.com/AU/en/sds/sigma/e1501

* *Note that the above SDS are for reference only. Please look up the manufacturers specific SDS for the product you are using.*

Exemptions

Where adherence to these Standard conflicts with proposed work, the WorldFish IACUC (or delegated IACUC) may grant exemptions to all or part of the Standard. To seek exemption, applications should clearly outline how the proposed work deviates from the Standard and justify the need for this. Before seeking exemption, it is recommended that you consult with the head of WorldFish Aquatic Animal Health or their designate.

Unexpected Adverse Incidents

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Fin Clipping of Finfish

Document Summary	
SOP title:	Fin Clipping of Finfish
SOP owner:	Sustainable Aquaculture Research Program
SOP sponsor:	Director of Aquaculture and Fisheries Sciences
Responsible office:	Sustainable Aquaculture Research Program
Effective date:	20 June 2019
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Version Control Tracking			
Issue Date	Summary of Changes	Distribution	Version Number
20 June 2019	Initial version	Internal	1.0.0
30 Dec. 2021	Reviewed version	Internal	1.2.0

Associated Standards

- Anaesthesia/Sedation of Finfish
- Handling of Finfish

Summary

Application

Fin clipping involves the removal of a small amount of tissue from a fin in order to obtain genetic material for genotyping. If done correctly, fins should regenerate within two weeks. The removal of one or more of the adipose and pelvic fins has been shown to have no effect on growth rate, survival and early sexual maturity (Gjerde & Refstie, 1988).

Benefits and Risks (Staff and Fish)

Benefits

Ability to perform non-lethal sampling of fish in order to obtain genetic material for genotyping.

Risks and Mitigation

Refer to the **Risk** and **Mitigation** section of the Associated Standards.

Risks:
- Injury to handlers from sharp objects and/or fish spines
- Over-handling and associated stress on fish

Mitigation:
- Users should be experienced or trained in the proper handling of fish

Procedure/Protocol

Pre-procedure Preparation

Note: Cross-contamination of fin-clip samples occurs when collectors use the same instrument for fin clipping of multiple individuals during the same sampling event without cleaning and sterilizing the instrument between samples. If just a few fish cells/mucus, or even portions of fish cells and mucus are left behind on the instrument, this is enough to cause cross-contamination.
- All surfaces and materials used for tissue collection should be sterile. Surfaces used for the procedure should be cleaned with ethanol prior to set up. The fish should not be exposed to ethanol directly, as it can be irritating and dry out the slime coat (mucous)
- The cutting tool (e.g. scissors or hole puncher???) should always be sharp, and any surgical equipment should be sharpened professionally on a regular basis
- The cutting tool should be freshly unwrapped from an autoclaved pack (if possible) or sterilized initially with ethanol
- Sample tubes should be labelled prior to fin-clipping

Procedure

- The fish should be anaesthetised prior to handling and fin-clipping – fish should be at a surgical level of sedation/anaesthesia (refer to the WorldFish Anaesthesia/Sedation of Finfish Standard Operating Procedure)
- Gentle handling techniques are required (refer to the WorldFish Handling of Finfish Standard Operating Procedure)
- In between fish, the scissors should be cleaned (no mucous and scales) and disinfected with 70% ethanol in order to prevent cross-contamination of genomic material
- Avoid sampling damaged fins
- Cut a section of fin, approximately 5 mm x 15 mm or less in surface area. Do not cut more than what is necessary
- Samples should be stored appropriately:
    - Exposure of samples to water and air must be minimisedTubes should contain at least 9 parts 95% molecular grade ethanol (undenatured) to 1 part tissue sample. Tissue samples should be fully submerged in ethanol and sample tube lids must be firmly closed. Ideally samples should be stored in a freezer.
- Sample identifiers must be recorded against tube label identifiers.
- Care should be taken to ensure that no other parts of the fish are cut/injured during this process
- All samples should be recorded in the WorldFish sample database managed by the Penang Genetics Laboratory Supervisor

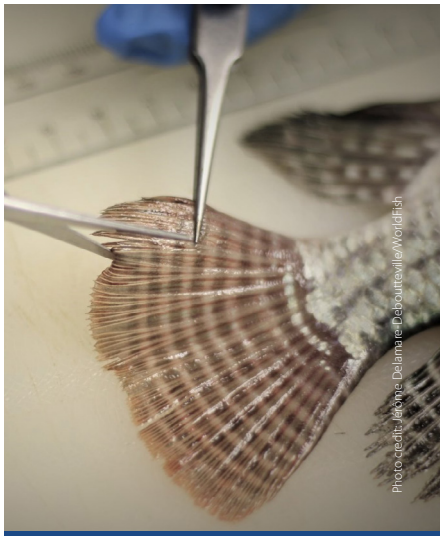

Plate 1. Fin-clipping procedure on the dorsal fin.

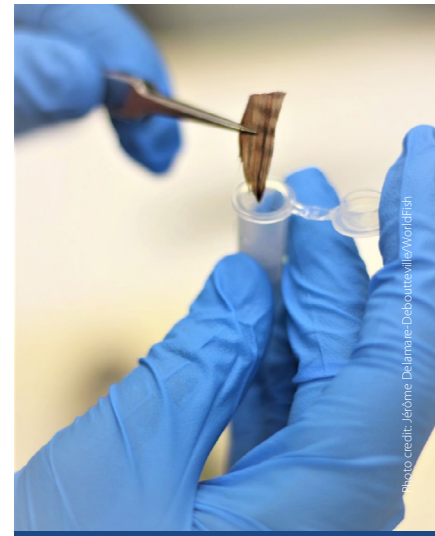

Plate 2. Fin clip placed into an 1.5 mL Eppendorf tube with 95% molecular grade ethanol for preservation.

Additional Information

None

Exemptions

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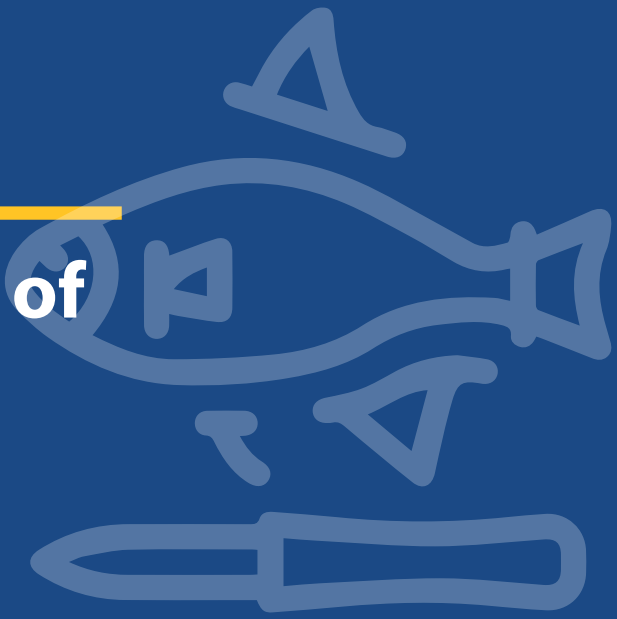
References

Gjerde B and Refstie T. 1988. The Effect of Fin-Clipping on Growth Rate , Survival and Sexual Maturity of Rainbow Trout. *Aquaculture*, *73*, 383–389.

Handling of Finfish



Document Summary	
SOP title:	Handling Procedure for Finfish
SOP owner:	Sustainable Aquaculture Research Program
SOP sponsor:	Director of Aquaculture and Fisheries Sciences
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Issue Date	Summary of Changes	Distribution	Version Number
20 June 2019	Initial version	Internal	1.0.0
30 December 2021	First revision	Internal	1.2.0

Associated Standards

None

Summary

Application

Handling procedures are used for transporting, examining, sampling, splitting groups of, grading, measuring and spawning fish. This is a necessary procedure in WorldFish breeding programs, research experiments and when actively sampling stock for health surveillance and disease investigation. Sometimes, fish will also be sampled for fisheries surveys, when other species of fish will be handled.

Benefits and Risks (Staff and Fish)

Benefits

Handling of fish enables the observation and measurement of fish parameters, required for surveillance, research and breeding activities. Handling during aquaculture practices can be stressful for finfish (Barton, 2000; Barton & Iwama, 1991). The impacts of stress should be minimised whenever possible, by limiting time out of water, using appropriate handling methods and, where possible, by utilising chemical sedation.

Risks and Mitigation

Risks:

- Damage to the scales and delicate slime coat (mucus) of fish
- Zoonotic diseases that can be transmitted from fish to humans and *vice versa*

Mitigation:

- Nitrile gloves should be worn where practical to reduce the risk of transmission of zoonotic diseases through open wounds.
- Fish should be handled on wet surface to avoid damage of scales and mucus (never touch a fish skin with hands or dry gloves)
- All equipment used should be smooth and prevent potential damage to the scales and slime coat of fish. This includes the use of sanitized non-knotted (smooth) nets, fish chutes, fish grading equipment, fish cradles or fish pumps.
- Fish handlers should be aware of the potential for and consequences of zoonotic diseases transmission (e.g.. Streptococci, vibriosis, Mycobacterium, Erysipelothrix, various fungal infections).

Procedure/Protocol

- Investigators have a responsibility to determine and use the least amount of restraint necessary to perform their procedure in a humane manner, with minimal distress or suffering caused to the animal. In some cases, this may include the use of sedative or anaesthetic agents, especially for species which are venomous or capable of inflicting serious injuries on themselves or those handling them and for large specimens.
- Fish should be fasted for an appropriate amount of time to prevent regurgitation or high amounts of waste production and provided with high water quality post restraint or handling (see....? Ashley, 2007; Davis & Gaylord, 2011).
- Fish should be handled gently on wet surface and as quickly as possible in order to minimise stress and damage to scales and slime coat.
- Avoid handling fish in brightly lit areas or in direct sunlight. Where applicable shade should be provided to elicit a calming effect in fish.

- The time spent handling fish out of water should be minimised, ideally to less than 30 seconds.
- Fish should always be placed on a wet surface or cloth: Never place fish on a dry surface to prevent damage to their skin and mucus.
- When handling fish there is the potential for personnel to be injured (i.e. bitten, stabbed). Some species of fish are venomous or poisonous and any wounds or even open skin contact may have potentially serious/fatal consequences. Investigators should be familiar with the defensive strategies of their target species and other non-target species (bycatch) that may be encountered in the field.
- Maintaining a barrier between the researcher and the animal may help to reduce the risk of injury. The use of traps that allow the animal to be visible to researcher prior to opening it is encouraged. For potentially hazardous fish, such as sharks or venomous species, chemical restraint is strongly advised and may be required. ***This is more applicable for those working with commercial or small-scale fishing techniques.***

Additional Information

None

Exemptions

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References

Ashley PJ. 2007. *Fish welfare : Current issues in aquaculture. 104*, 199–235. doi: 10.1016/j.applanim.2006.09.001

Barton BA. 2000. Salmonid Fishes Differ in Their Cortisol and Glucose Responses to Handling and Transport Stress. *North American Journal of Aquaculture, 62*(1), 12–18. doi: 10.1577/1548-8454(2000)062<0012:sfditc>2.0.co;2

Barton BA and Iwama GK. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases, 1*(C), 3–26. doi: 10.1016/0959-8030(91)90019-G

Davis KB and Gaylord TG. 2011. Effect of fasting on body composition and responses to stress in sunshine bass. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology, 158*(1), 30–36. doi: 10.1016/j.cbpa.2010.08.019

Sedation and Anaesthesia of Finfish

Document Summary	
SOP Title:	Sedation and Anaesthesia of Finfish
SOP Owner:	Sustainable Aquaculture Research Program
SOP Sponsor:	Director of Aquaculture and Fisheries Sciences
Responsible Office:	Sustainable Aquaculture Research Program
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Issue Date	Summary of Changes	Distribution	Version Number
20 June 2019	Initial version	Internal	1.0.0
4 January 2022	Revised version	Internal	1.2.0

Associated Standards

- Handling of Finfish

Summary

Application

Procedures may need to be performed with fish that require sedation or anaesthesia.

Sedation: When a drug is administered, by immersion for finfish, to induce a state where the indivdual animal has a reduced awareness of its surroundings and stimuli but is still conscious. Animals in this state can still feel pain, so sedation techniques are normally used for non-invasive procedures where the animal must only be restrained.

Anaesthesia: An artificially induced insensitivity to pain. For finfish, this application is used to perform more invasive procedures such as minor surgical procedures (e.g. PIT tagging, fin-clipping or blood-sampling).

Anaesthetic: The drug used to induce sedation or anaesthesia (e.g. eugenol, MS-222, benzocaine hydrochloride).

This SOP does not cover euthanasia with anaesthetic overdose (link to overdose SOP).

Benefits and Risks (Staff and Fish)

Benefits

- Reduced pain perception in fish for more invasive techniques by use of anaesthesia; and to reduce stress on, and risk of injury to, fish when being handled by sedation.
- Reduced risk of injury to a person performing a procedure, as fish movement is reduced.

Risks and Mitigation

Refer to the **Risk** and **Mitigation** section of the Associated Standards.

Risks:

- Fish death due to overdose
- Anaesthetics can be very irritating through skin and aerosol exposure
- Wear protective clothing, nitrile gloves and goggles when handling eugenol.
- Wear nitrile gloves to handle animals exposed to eugenol.
- When making eugenol solutions:
  - Work inside a fume hood (if in a laboratory setting) or in the open air if in field, to prepare a concentrated stock solution with ethanol.
  - Dilute the stock solution with ethanol further, as required to manage dosage
- When disposing of eugenol waste:
  - Eugenol should be collected and disposed of as chemical waste where possible.
  - Do not discard eugenol directly into sinks, drains, surface water, storm water conveyances or catch basins where possible. There is the recognition that eugenol could be used in a pond environment and will degrade over time.
- Thoroughly wash hands after handling or administering anaesthetics
- Always read and follow product labels to ensure that anaesthetics are used appropriately and that any risks associated with handling anaesthetics or fish treated with anaesthetics are understood by staff. A summary of this information can be found on the product safety data sheet (SDS), and this should be read and understood by any person who comes into contact with the product. It is the role of the Site Manager to ensure that the SDS has been reviewed, and adequate personal protective equipment (PPE) is worn while chemicals are being used.

Procedure/Protocol

When working with a new anaesthetic protocol or species it is advisable to anesthetize a few animals and follow these animals through to full recovery to ensure drug dosages, concentrations and techniques are safe and provide enough anaesthetic depth for the intended procedures.

- Where sedation is required for handling, or other drugs are to be given, investigators must ensure they are familiar with the drug, dose, concentrations, mode of delivery, expected effects and potential side-effects of its administration.
- Fast fish for 12–24 hours prior to sedation or anaesthesia. This reduces faecal contamination and risk of regurgitation.
- Maintain adequate oxygenation during the procedure: supply via an air stone or similar device. Oxygenate all water chambers during anaesthesia and recovery when fish are in tanks or in higher densities. In field, a portable battery powered air supply is adequate if individual fish are being sedated. Oxygen level should ideally be monitored through this process.
- Ensure a clean water source is used to hold fish under sedation/anaesthesia. Using the water source in which the fish are being held is preferable, if clean.
- Maintain water temperature at the species' normal optimum during both sedation, anaesthesia and recovery where possible.
- The effects of anaesthetic drugs are affected by both dosage and duration of exposure. The longer fish are left in an anaesthetic bath, the greater the anaesthetic depth.
- Ensure that anaesthetic depth is monitored throughout the experiment.

Anaesthesia

Below is a table of acceptable doses for anaesthetic at WorldFish. When adding anaesthetic solutions, it is recommended that 70% of the recommended dosage is added to the bath initially, and small doses are added to intended effect.

Anesthetic agent	Dose	Comment
MS-222 (tricaine methanesulfonate)	75-125 mg/l (induction) and 50-75 mg/l (maintenance)	Sodium bicarbonate should be added to stock solution to maintain neutral pH.
Clove oil	40-100 mg/l (depending on fish species and anesthetic depth)	Active ingredient is eugenol. Clove oil stock solution (100 mg/ml) made with 95% ethanol. Stock solution is added to induction chamber at 40-100 mg/l
Benzocaine hydrochloride	25-100 mg/l	Sodium bicarbonate may need to be added to stock solution to maintain neutral pH. Small margin of safety between effective and lethal doses.

The respiratory rate and gill colour of fish must be regularly monitored while they are exposed to, and recovering from, anaesthesia or sedation. The planned procedure must not be undertaken unless fish are at a sufficient level of sedation or anaesthesia.

Methods of evaluating respiratory rate and gill colour:
- Observe movement of the operculum (rigid flap that covers the gills) as it opens and closes to assess rate.
- Observe gill colour: should be dark pink to light red.
- If respirations become extremely slow or stop, place the fish in anaesthetic-free recovery oxygenated water until respirations resume (this is recovery)

Indicators that fish are in a sedated state:
- Fish are at a handleable state when they are slower to respond to stimuli – i.e. able to touch the fish without them reacting and swimming away rapidly
- Fish are still able to maintain equilibrium

This state is used for intramuscular (IM) and intraperitoneal (IP) injections in field, and collection of weight and length samples.

Indicators that fish are in an anaesthetized state:
- Total loss of equilibrium and muscle tone
- Decreased respiratory rate
- No response to stimuli: squeeze at the base of the tail to determine response to stimuli.

This state is used when the procedures are more invasive e.g. PIT tagging, finclip or blood sampling

Additional Information

Product safety data sheets (SDS)*:
MS-222: https://www.sigmaaldrich.com/AU/en/sds/aldrich/e10521
Clove oil: https://www.sigmaaldrich.com/AU/en/sds/sigma/c8392
Benzocaine: https://www.sigmaaldrich.com/AU/en/sds/sigma/e1501

** Note that the above SDS are for reference only. Please look up the manufacturers specific SDS for the product you are using.*

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Transportation and Acclimation

Document Summary	
SOP title:	Transport and Acclimation
SOP owner:	Sustainable Aquaculture Research Program
SOP sponsor:	Director of Aquaculture and Fisheries Sciences
Responsible office:	Sustainable Aquaculture Research Program
Effective date:	20 Jun 2019
Last updated:	04 January 2022
To be reviewed:	04 January 2023

Version Control Tracking			
Issue Date	Summary of Changes	Distribution	Version Number
20 Jun 2019	Initial version	Internal	1.0.0
04 January 2022	Revised version	Internal	1.2.0

Associated Standards

- Handling of Finfish

Summary

Application

When conducting research on finfish, it is often necessary to transport experimental animals from one place to another. Fish may be transported into a facility for research or could be transported from a hatchery system onto farm. Sometimes, fish may also be transferred internationally, depending on the research project. It is important for fish health, welfare and sound experimental design to minimise stress during any transportation activity, and appropriate acclimation to the new holding environment is essential.

Risks (Staff and Fish)

Refer to the **Risk** and **Mitigation** section of the Associated Standards.

Risks:
- Stress and possible death of fish
- Crushing or lifting injuries to staff

Mitigation:
- If vehicles are used, then a reasonable speed limit must be enforced, as fish can become stressed from vehicle movements.
- Ensure all water quality parameters remain within acceptable limits.
- Ensure all safety policies and procedures are adhered to and risks to staff are identified and controlled.

Procedure/Protocol

Transport

- The specific type of transport container will depend on the size, number and species of fish.
- Transport containers selected must be suitable for fish transport. This includes selecting containers that prevent injury to fish, and that are either disposable (e.g. plastic bags) or with surfaces that are easy to clean and disinfect.
- Staff should aim to keep the water temperature slightly cooler than the fish's usual environment and avoid placing the transport container in direct sunlight. Overheating can result in rapid reductions in dissolved oxygen which can quickly become fatal. Transporting fish in extreme hot weather should be avoided.
- Only compatible fish and fish species should be housed together.
- As water quality is paramount to fish health, all reasonable precautions and steps must be undertaken to ensure water quality parameters remain within acceptable limits. This includes the use of appropriate water quality monitoring equipment (i.e. sensor, meters) and water quality enhancement equipment (i.e. aeration, filtration). Aeration can be provided by many methods, including battery powered air pumps and stones. For oxygen sensitive species consider aerating the water with oxygen.
- Water can be sourced from the site of fish collection or externally sourced but must be of a suitable quality as outlined in the species-specific water quality parameters.
- Using one or two strong plastic bags, fill 1/3 full of water (preferably using the water the fish came from). Place the sampled fish in the water, remove the air, and then fill the bag with compressed oxygen. Ensure oxygen does not escape. Twist and double over the neck of the bags and securely tie using tape, cord, zip ties or rubber bands.

Bags should then be packed in a watertight container, sealed and clearly labelled.

- When possible, avoid the feeding of fish for at least 48 hours prior to transport as fish are liable to empty their digestive tracts into the transport container which will can lead to a significant deterioration in water quality.
- Where overnight stays at field sites or camps are required, it is the investigators responsibility to ensure suitable housing is available with an appropriate degree of climate control, aeration and water quality requirements. Water quality parameters should be checked, and fish visually inspected whenever possible. If water quality parameters or conditions deteriorate, attempts should be made to improve the water quality.
- The time from first capture (for wildfish) to placement into an approved facility should not exceed 24 hours. In exceptional circumstances (such as international or long-distance travel), this may be extended but ideally should not exceed 48 hours.
- Transport containers and vehicles can be used but must be fitted with appropriate oxygenation/aeration for the stocking density and species of fish.

Acclimation

- Transport and/or capture are highly stressful events for fish (Barton & Iwama, 1991) and great care must be taken to mitigate the negative effects that may occur. An acclimation period is necessary to allow the animals to adjust to their surroundings, which can include a change in diet, lighting, temperature water quality and housing.
- Changes in water quality should be done slowly over time. Ideally fish transported in plastic bags of water should be secured to the new enclosure and left floating to allow the temperature to slowly equilibrate. Changes in water temperature of more than 2-3 °C can induce thermal shock in fish.
- Fish should not be used for procedures or investigations until they have had adequate time to acclimate. This is to ensure they have time to recover from stress to avoid aberrant results caused by fish being in a stressed physiological state.
- The time period required for acclimation will vary across the species, and wild-caught fish will require additional time to acclimate compared to those born in captivity. A recommended starting point for captive-bred animals or those previously acclimated and maintained in captivity is 7 days to adjust to the new facility before beginning experiments. Wild-caught animals may need to acclimate over 3-4 weeks, unless experience with the species suggests a different time period is required.
- Fish should be acclimated to a system that is already established. In ponds, the appropriate pond preparation and fertilisation regime must be completed prior to introducing new fish. In a tank system which is semi-recirculating or full recirculation, there must be an established biofilter.
- Daily monitoring by visual inspection, food intake, activity, and behaviour is essential for any new animal. Body weight or biomass can be measured prior to fish being transferred into their new enclosures.
- It is recommended that handling fish be kept to a minimum for at least 2 weeks after transfer.

- Signs of acclimation in fish are demonstrated by normal behaviour, activity and feeding well. Fish will need to transition to their new diet slowly and where possible should receive the feed used by their previous facility. Wild-caught specimens may have special feed requirements which should be considered and available prior to capture.
- Fish that are not feeding, are losing weight and/or appear to be visually unwell or abnormal over the first few weeks in the facility, may not be acclimating well. This should be raised to the Site Manager as well as the Principal Investigator/Scientist.
- Where fish fails to acclimate, then humane killing may be required to ensure it does not suffer. This may be observed as sustained mortality, sustained poor feed intake, or post-transfer disease outbreaks.

Additional Information

A list of recommendations for transport conditions and acclimation of various species and further information about stress physiology as a function of transport duration can be found in references below).

Exemptions

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References

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Weight and Length Measurement

Document Summary	
SOP Title:	Weight and Length Sampling
SOP Owner:	Sustainable Aquaculture Research Program
SOP Sponsor:	Director of Aquaculture and Fisheries Sciences
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04 January 2022	Revised version	Internal	1.2.0

Associated Standards

- Handling of Finfish
- Sedation and Anaesthesia of Finfish

Summary

Application

Weight and length sampling is a crucial aspect of collecting performance data, and is used to calculate growth and condition metrics such as specific growth rates (SGR or) condition factor. This type of data collection can be applied to:

- Nutritional studies
- Genetics (e.g. assessing fish in progeny tests)
- Health and performance monitoring during disease outbreaks, and against models as a form of early detection of disease

Benefits and Risks (Staff and Fish)

Benefits

Weight and length samples are a non-invasive way of gathering information. During this process, other observations can be recorded such as deformities and clinical signs of disease.

Risks and Mitigation

Refer to the Risk and Mitigation section of the Associated Standards

Procedure/Protocol

- The frequency of measuring fish is context dependent but it should be noted that measuring fish will always cause some level of stress which may affect research outcomes.
- Measurement of weight or length will generally require anaesthesia and handling. If this is the case, fish should be deprived of food for 24-h prior to handling. Note that this will influence feed intake and growth on any given day.
- Measurement of weight or length should be undertaken efficiently and quickly to reduce the amount of time the fish are removed from the water and always handled with wet gloves to prevent injury to scales and the slime coating. This also protects the handler from fish slime which can contain infectious agents.
- Sufficient chemical restraint (sedative) and aeration/oxygenation should be employed to minimise stress (see Sedation and Anaesthsia SOP link). After chemical restraint and measurements, fish must be observed until they have recovered and resumed normal swimming and behaviour. In a pond situation, a small netted area with an air stone (i.e. recovery net such as hapa) can be used to allow fish to wake up from the sedative.
- Any individual fish that is removed from a group of fish to be measured, must recover fully before being returned to their pond or tank.
- Prior to handling fish for measurement, the investigator or carer must first observe them in their rearing system.
- Fish should be assessed for their behaviour and activity levels, physical appearance, social interactions and respiratory effort by watching the movement of the operculum and mouth. Fish should not be handled for measurement if they show signs of stress.
- The response of fish to stimuli should be observed.
- If the response is abnormal (e.g. slow or rapid), then consultation with the Manager of the Facility is required.

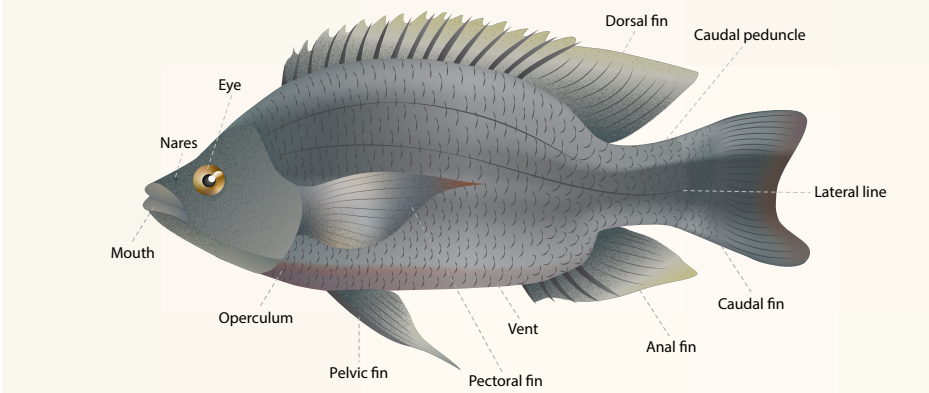
Weights

- Waterproof digital scales with 1-2 decimal places should be used to weigh fish, with small kitchen scales being suitable to most species under 100 g. Larger commercial scales can be used for larger fish.
- Individual fish can be weighed by placing fish directly on a scale, or by placing them in a container of water positioned on a scale.
- Taking the weight in water reduces error due to fish movement but may not be practicable for large fish.
- Bulk weights (i.e. measuring multiple fish at once) should be performed by doing the following:
    - place a suitably sized container of water on scales, and tare.
    - Net Fish into the container and record the weight.Fish are then counted out of the container into either another container of water, or back into their holding tank. Total biomass is determined. Average individual weight is calculated by bulk weight divided by the number of fish in the container.

Lengths

- Fish length is measured using a measuring board on which the anterior end (snout) of a fish is placed against a stop at the beginning of a measuring scale. The fish should be measured with the mouth closed, and the body positioned on its right side with the head to the measurers left. Any one of three measurements can be taken: total, fork or standard length.
- Total length is the greatest length of a fish from its anterior most extremity (usually the mouth) to the end of the tail fin. For fish with a forked tail, the two lobes should be pressed together, and the length of the longest lobe should be taken.
- Fork length is measured from the anterior end of the fish to the tip of the middle rays of the tail.
- Standard length is the length of a fish from the anterior end of the fish to the tip of the middle rays of the tail. Standard length is the length of a fish from the anterior end to where the base of the median tail fin rays joins the caudal peduncle. This spot can be located by bending the tail sharply. A crease should form where the tail fin rays end. Determination of standard length is very difficult on some species.

Illustration: Sabrina Chong/WorldFish



Reference for further reading
http://www.fao.org/3/F0752E/F0752E03.htm

Additional Information

None

Exemptions

Where adherence to these Standard conflicts with proposed work, the WorldFish IACUC (or delegated IACUC) may grant exemptions to all or part of the Standard. To seek exemption, applications should clearly outline how the proposed work deviates from the Standard and justify the need for this. Before seeking exemption, it is recommended that you consult with the head of WorldFish Aquatic Animal Health or their designate.

Unexpected Adverse Incidents

An unexpected adverse event is any event, which impacts negatively on the wellbeing of animals, and which was not anticipated, or has occurred at a frequency or severity in excess of what was anticipated in line with the WorldFish IACUC (or delegated IACUC) approval. This can be a single or cumulative event, and will normally involve unexpected mortality, morbidity or injury. Anyone identifying an unexpected adverse event must act to remove and/or minimise any immediate risk to animals. Immediately thereafter, there must be a report to the WorldFish IACUC (or delegated IACUC), and relevant head of WorldFish Aquatic Animal Health or their designate (within a 24 hour period).

Injection and Blood Sampling of Finfish

Document summary	
SOP title:	Injection and Blood Sampling of Finfish
SOP owner:	Sustainable Aquaculture Research Program
SOP sponsor:	Director of Aquaculture and Fisheries Sciences
Responsible office:	Sustainable Aquaculture Research Program
Effective date:	20 June 2019
Last updated:	04 January 2022
To be reviewed:	04 January 2023

Version Control Tracking			
Issue Date	Summary of Changes	Distribution	Version Number
20 June 2019	Initial version	Internal	1.0.0
04 January 2022	Revised version	Internal	1.2.0

Associated Standards

- Handling of Finfish

Summary

Application

Injection and blood sampling of finfish are used:

- For the injection of hormones in broodfish for breeding activities
- For the injection of vaccines
- For the injection of microorganisms in infectivity, susceptibility challenges
- Drawing of blood for sampling for diagnostic testing
- Lavage is sometimes used in challenge trials for extraction of cells in the coelomic cavity (gut cavity)

Benefits and Risks (Staff and Fish)

Benefits

- Allows quick absorption of chemicals and drugs that may be used in an experiment.
- Blood sampling can also be performed using non-lethal methods, with the use of anaesthesia.

Risks and Mitigation

Refer to the **Risk** and **Mitigation** section of the Associated Standards

Risks:
- Needle stick injury and exposure to hormone and microorganisms to staff members
- Muscle tearing and injury to internal organs of fish that are thrashing vigorously

Mitigation:
- If the animal cannot be adequately restrained or are large, sedation of fish should be considered (refer to Sedation and Anaesthesia SOP link).

Procedure/Protocol

Investigators must be familiar with the anatomy and vasculature of their target species prior to attempting injections. Injections should always be administered between the scales. Sites for injection vary depending on the procedure and species of fish. Listed below are general guidelines for injections in fish; however, due to species-specific variations, different approaches may be required. Aquatic veterinary, fish expert, or Animal Welfare Officer advice should be sought in such cases.

Intramuscular injection

- Intramuscular injection may be administered between the scales into the large dorsal epaxial and abdominal muscles taking care not to inject into the lateral line and ventral blood vessels.
- For intramuscular injection of breeders (catla, rohu, grass carp ect.), removal of one scale is necessary, otherwise the needle will have to perforate one scale while detaching another scale. This results in a more substantial injury than removal of just one scale.
- Injection of breeders is to be done under sedation if it taken out of water, and should be placed on plastic-covered sponge, to prevent damages to ovary or other injuries.
- Intra-peritoneal injection of breeders can be done at the base of pelvic or pectoral fins, where no scales are covering the body.

- Breeders preferably injected without taking out of water. Sedation may be applied, but not necessary if done by experienced hatchery operators: the eyes of fish should be covered, as shown in the inserted picture (see picture in additional information below).

Intracoelomic (Intraperitoneal) injections

- Intracoelomic injections may be made into the coelomic cavity between the scales and taking care to avoid penetration of coelomic viscera.
- Noxious substances known to cause extreme inflammation and irritation should not be injected into the coelomic cavity as they may cause severe intracoelomic adhesions.

Teleost (bony) fish blood sampling

- Most healthy fish greater than 7.5 cm in length can sustain the removal of 1 mL/1kg of blood volume from their circulatory system. Volumes greater than 1 mL/kg for non-lethal venipuncture sampling must be justified.
- Haematocrit recovery of fish is temperature dependent and highly variable between species.
- Typically, blood circulating volume of healthy fish is 5% (compared to 10% in mammals). However due to their physiology they can sustain greater levels of total blood volume collection of up to 30% (compared to 10% in mammals).
- The preferred site of non-lethal blood sampling in fish is the caudal vertebral vein or artery which is located along the ventral midline of the tail via either a ventral or lateral approach.
- Needle gauge size varies with the size of the fish. The following guideline can be used: 22-26G can be used in smaller fish, 20-25G for medium-sized fish, and 18-22G for larger fish.
- Care should be taken to apply pressure at the site of any venipuncture for 30-60 seconds after needle removal to give the blood a chance to clot at the site of collection. If bleeding occurs from the site after this time, gentle pressure should be applied until haemostasis can occur and no blood is noted.
- Fish should be sedated during these procedures and gloves should be worn to prevent damage to both scales and the slime coat.
- Ventral approach - Restrain the animal on its side on an appropriate surface (clean, smooth, and non-slippery) or in its back in a fish cradle. Introduce the needle between the scales along the ventral midline near the base of the caudal peduncle and advance towards the ventral vertebrae. Once the needle has contacted the vertebral body, slightly withdraw the needle, and withdraw the plunger slightly until blood enters the hub. Slowly draw the sample out, allowing breaks in the suction for the vessel to refill. It may be necessary to slowly and gently rotate the needle and syringe if blood flow has ceased. Once the sample is acquired, remove the needle and place gentle pressure on the site for 30-60 seconds.

- Lateral approach - Restrain the animal on its side on an appropriate surface (clean, smooth and non-slippery). Introduce the needle between the scales few millimetres below the lateral line near the base of the caudal peduncle. Direct the needle towards the midline slightly below the ventral and gently withdraw the plunger slightly until blood enters the hub. Slowly draw the sample out, allowing breaks in the suction for the vessel to refill. It may be necessary to slowly and gently rotate the needle and syringe if blood flow has ceased. Once the sample has been collected, remove the needle and place gentle pressure on the site for 30-60 seconds.
- For very small fish, a tail snip can be taken with blood collected in capillary tubes.
- Blood collected from most species of fish should be immediately stored at 4°C to prevent deterioration. Depending on the parameters to be sampled and the species of fish the choice of anticoagulant may vary. Investigators should be aware of the ideal anti-coagulant agents and storage temperature required for their analysis and respective species of fish.
- Large teleost fish may need to be sampled differently and investigators must demonstrate familiarity with venipuncture techniques, anatomy and vasculature of their target species prior to attempting blood collection.

Non-Teleost fish and other species

The preferred site of non-lethal blood sampling in non-teleost fish can vary. It is recommended to contact an aquatic veterinarian, fish expert, or the Animal Welfare Officer for further and more detailed information.

Additional Information

For a comprehensive review on best practices for non-lethal blood collection via the caudal vasculature, refer to (Lawrence et al., 2020).

For a video demonstrating blood collection, download mp4 “supporting information” from doi: 10.1111/jfb.14339

Quick fish sampling guide for disease diagnostics - Blood sampling guide
https://digitalarchive.worldfishcenter.org/handle/20.500.12348/4839

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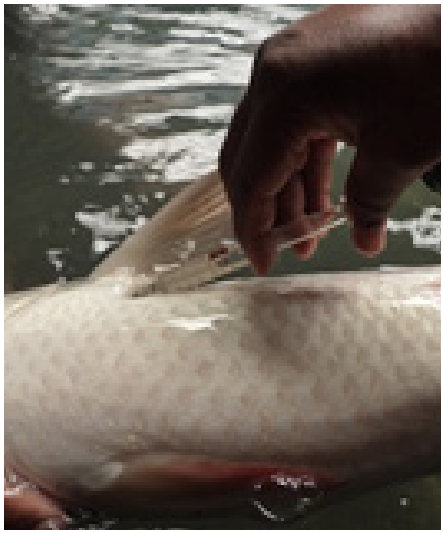
References

Lawrence MJ, Raby GD, Jeffries KM, Danylchuk AJ, Hasler CT, Clark TD, Teffer AK, Eliason EJ and Cooke SJ. 2020. *Best practices for non-lethal blood sampling of fish via the caudal vasculature. January*. doi: 10.1111/jfb.14339



Appendix

WorldFish Animal Care and Welfare Policy

WorldFish Policy on Ethics or Research Involving People

PPT – Briefing on Human and Animal Ethics: WorldFish Research Ethics Panel

PPT – Introduction to the Human and Animal: Ethics Approval Process

PPT – The care and use of animals for scientific purposes: How to obtain ethics approval for research

PPT – Ethics in Human Research at WorldFish: An Update and Refresher

CGIAR Open access and data management policy

CGIAR research ethics code

Handbook on Research Ethics

About WorldFish

WorldFish is a leading international research organization working to transform aquatic food systems to reduce hunger, malnutrition and poverty. It collaborates with international, regional and national partners to co-develop and deliver scientific innovations, evidence for policy, and knowledge to enable equitable and inclusive impact for millions who depend on fish for their livelihoods. As a member of CGIAR, WorldFish contributes to building a food- and nutrition-secure future and restoring natural resources. Headquartered in Penang, Malaysia, with country offices across Africa, Asia and the Pacific, WorldFish strives to create resilient and inclusive food systems for shared prosperity.

For more information, please visit www.worldfishcenter.org