Asia-Pacific
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Guidebook on Biosecurity and Good Aquaculture Policies and Practices for small-scale farmers of tilapia (Oreochromis sp.) and rainbow trout (Oncorhynchus mykiss)

APEC Sub-Committee on Standards and Conformance

April 2023

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Produced by

Muriel Gomez Sanchez
Director of the Directorate of Health and Safety
National Health Fisheries Agency (SANIPES)
Ministry of Production (PRODUCE)
Peru

Paola Barato DVM, PhD
Aquaculture Health, Pathology and Biosecurity Expert
MolecularVet US LLC
Davie, FL, United States

For
Asia-Pacific Economic Cooperation Secretariat
35 Heng Mui Keng Terrace
Singapore 119616
Tel: (65) 68919 600
Fax: (65) 68919 690
Email: info@apec.org
Website: www.apec.org

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Preparation of this document

A project "SCSC 07 2020 - Strengthening the Management of Aquaculture Diseases to Promote Commercial Exchange and Food Production Sustainability, for Small Enterprises" was undertaken in 2022 through a desk study, online surveys, an expert workshop, and cases studies held virtually from 29 March to 29 July 2022.

The project culminated in the publication of this document, which contains technical information presented during the expert workshop, contributed by 5 specialists, and peer-reviewed by 3 experts. These include 10 steps that identified major biosecurity risks and good aquaculture practices for small-scale farmers. Also, this document contains the highlights of the Expert Workshop on Biosecurity and Good Aquaculture Policies and Practices for small-scale farmers of tilapia (*Oreochromis* sp.) and rainbow trout (*Oncorhynchus mykiss*), with 8 economies participating with 49 participants. The commissioned review papers and expert workshop were technically supervised by Dr. Paola Barato (APEC Consultant, Colombia/US), Dr. Win Surachetpong (Associate Professor of Kasetsart University in Thailand), Dr. Fernando Mardones (School of Veterinary Medicine, Universidad Católica de Chile), Dr. Carlos Iregui (Independent consultant, Colombia) and from SANIPES Ms. Muriel Gomez, Ms. Vanessa Quevedo, and Mr. Carlos Smith. The study, workshop, and publication were made possible with financial assistance from the Asian Pacific Economic Cooperation (APEC).

1. General information

Introduction

Small-scale aquaculture contributes to sustainable development, in relation to food security and nutrition, poverty reduction, and the use of natural resources (FAO, 2022). Despite their high potential, these farmers face unique and complex challenges related to biosecurity and Good Aquaculture Practices (GAP) with a lack of appropriate skills and services to access markets with healthy and safe products at a fair price (FAO, 2022). The complex frameworks of rules and regulations which govern the aquaculture value chain, specifically the wide variety of trade policies implemented by countries, can significantly influence small-scale aquaculture.

In addition, poor implementation of GAP and biosecurity in small-scale aquaculture represents a risk for domestic aquaculture health to face domestic and international trades in a growing industry such as tilapia and trout production.

Strengthened science-policy interface and empowered stakeholders (fish farmers, fish workers, legislators, and government agencies) to support decision-making in the small-scale farming sector in a participatory manner are necessary to improve the formulation and adoption of laws, regulations, policies, strategies, programs, and projects.

Aware of this situation, APEC presents these guidelines to support small-scale tilapia and trout farmers in the implementation and maintenance of GAP and biosecurity for economic and fish health security of the small farmers in supporting international opportunities for trade between APEC economies.

Basic definitions of risk analysis

Risk analysis is an analytical process to provide information regarding undesirable events (https://www.sra.org/). It is a decision-making tool, an objective, systematic, repeatable, and science-based method that contributes to answering the following questions: What can go wrong? How likely is it to go wrong? What would be the consequence of its going wrong? What can be done to reduce either the likelihood or the consequences of its going wrong? (Bondad-Reantaso, 2019)

Risk analysis does not stand alone – it supports and is supported by other components of a National Strategy on Aquatic Animal Health conducted as a joint effort (sector, domestic, and enterprise levels). The basic strength of the risk analysis process is its flexibility - it is adaptable to almost any sector/system where risk and uncertainty occur. It contributes to protect the domestic health and welfare to develop sustainable aquaculture and the success of individual aquaculture businesses and operations (Bondad-Reantaso, 2019).

The components of risk analysis are: 1) hazard identification, 2) risk assessment or characterization and analysis, 3) risk management and 4) risk communication. Aquatic Animal Health Code (2022)

2. Steps to follow to structure the Guidebook on Biosecurity and Good Aquaculture Policies and Practices

The following Guidebook is divided into 10 steps to ensure the good implementation of biosecurity and good aquaculture practices (GAP) for small-scale farmers of tilapia (*Oreochromis* sp.) and rainbow trout (*Oncorhynchus mykiss*).

Through each step, the guidebook explores the specific and important aspects of biosecurity and GAP to successfully culture these freshwater fish species.

The 10 steps include:

Step 1. Know the biology of tilapia (*Oreochromis* sp.) and rainbow trout (*Oncorhynchus mykiss*), and keep their environmental conditions to maintain welfare and GAP.
Step 2. Know what pathogens affect tilapia and rainbow trout in your economy, and/or region, and the critical points where pathogens and/or diseases caused by them can enter or leave the farm.
Step 3. Understand quarantine and the role of fish movements in the introduction and spread of infectious diseases
Step 4. Introduce only healthy fry to your system. Evaluate and monitor the disease status of the farm following levels of diagnosis I, II, and III.
Step 5. Use preventive measures to avoid pathogens entry to a farm or disease presentation and, if it is necessary, use antimicrobials responsibly.
Step 6. Use practical disinfection procedures.
Step 7. Document and register your biosecurity and good aquaculture practices.
Step 8. Environmental risk/escapes/proliferation of harmful algae.
Step 9. Surveillance area / Mobile laboratories / Participatory epidemiology.
Step 10. Know the regulation in your economy related to aquaculture biosecurity and good aquaculture practices for small-scale farmers.

This guidebook also includes the result of the risk analysis performed by the APEC economies representatives who participated in the workshop on June 22nd and 23rd, 2022. This result ranks the steps to improve biosecurity and GAP in small-scale aquaculture farms.

3. Risk analysis principles for structuring the Biosecurity plan

The scope of the risk analysis for structuring the biosecurity plan evaluated the likelihood of the implementation of each of the 10 steps by farmers and the consequences if these steps are missing in the aquaculture production system. The rating was assigned for each step by the representatives of the APEC economies participating in the workshop. Risk analysis was performed during and after the workshop to obtain the perception in the evaluation of each of the 10 steps highlighting the importance of each to improve the risk rating and risk score in the absence of such implementation. This is an example of the risk analysis for structuring the biosecurity plan on each farm.

The qualitative approach to risk assessment was ranked into 5 categories for **likelihood** and **consequences** of a pathogen and/or disease entering a farm. A number was assigned to rate each category starting from 1 to 5. For likelihood to implement each step was considered rare (1), unlikely (2), possible (3), likely (4), or almost certain (5). The consequences of the absence of each step were evaluated as very low (1), low (2), moderate (3), high (4), and extremely high (5) (Lind et al., 2014).

A risk evaluation matrix was constructed to differentiate the severity of likelihood, consequences and risk categories, and a cutoff point of an acceptable level of risk (ALOR) (Table 1).

Table 1. Example of a risk evaluation matrix, highlighting differing severity of likelihood, consequence and risk categories, and a cutoff point of an acceptable level of risk (ALOR).

		Likelihood							
Consequences	Uncertain	1	2	3	4	5	Risk analysis Rank		ALOR
	1	1	2	3	4	5	Up to 2	Low	Acceptable risk
	2	2	4	6	8	10	3 to 6	Moderate	
	3	3	6	9	12	15	8 to 16	High	Unacceptable risk
	4	4	8	12	16	20	20 to 25	Very high	
	5	5	10	15	20	25			

The combination of the likelihood and consequence scores is the risk (risk = likelihood x consequence) (Lind et al., 2014). The result of this evaluation by representatives of APEC economies pre- and post-workshop is presented in table 2. The absence of the implementation of the ten steps, and the consequences, was evaluated as a high risk for biosecurity and GAP in small-scale farms of tilapia and rainbow trout.

Table 2. Risk analysis ranking for each step proposed to be evaluated during the workshop

Step	Description	PRE-WORKSHOP				POST-WORKSHOP			
		Likehood	Consequences	Risk-analysis Ranking		Likehood	Consequences	Risk-analysis Ranking	
1	Know the local regulation of biosecurity and GAP	3	4	12	2	4	3	12	2
2	Tilapia and rainbow trout farmers know the environmental conditions for welfare and GAP	3	4	12	2	3	3	9	3
3	Main pathogens that affect tilapia and rainbow trout, and the critical points where diseases can enter or leave the farm	3	4	12	2	3	4	12	2
4	Disinfect equipment, surfaces, and materials during daily activities on the farm	3	4	12	2	4	4	16	1
5	Test the health of seeds of tilapia and rainbow trout before the entrance to the farm	3	4	12	2	3	4	12	2
6	Use preventive therapies to avoid diseases on the farm	4	4	16	1	3	3	9	3
7	Maintain good aquaculture practices and staff training	3	4	12	2	3	4	12	2
8	Document and register the biosecurity and GAP practices on the farm	2	4	8	3	3	4	12	2
9	Contingency plan in place if a disease does break out on the farm	3	4	12	2	4	4	16	1
10	Farmers are transparent and cooperative with the local plans to improve biosecurity and GAP practices	2	4	8	3	3	3	9	3

4. **Development of the model of the Biosecurity Plan**

This guidebook presents 10 steps of the Aquaculture Biosecurity Plan and Good Aquaculture Practices for small-scale farmers of tilapia and trout.

Step 1. Know the biology of tilapia and rainbow trout and keep their environmental conditions to maintain welfare and good aquaculture practices

TILAPIA (*Oreochromis* sp.)

Tilapia (*Oreochromis* sp.) is a fast-growing, resistant and easy-to-handle, characteristics for which it has positioned itself as the second most cultivated fish in the world (FAO, 2020b) with good acceptance in the national and international market; contributing to food security and economic development of many developing economies.

Figure 1. Nile Tilapia and Red Tilapia

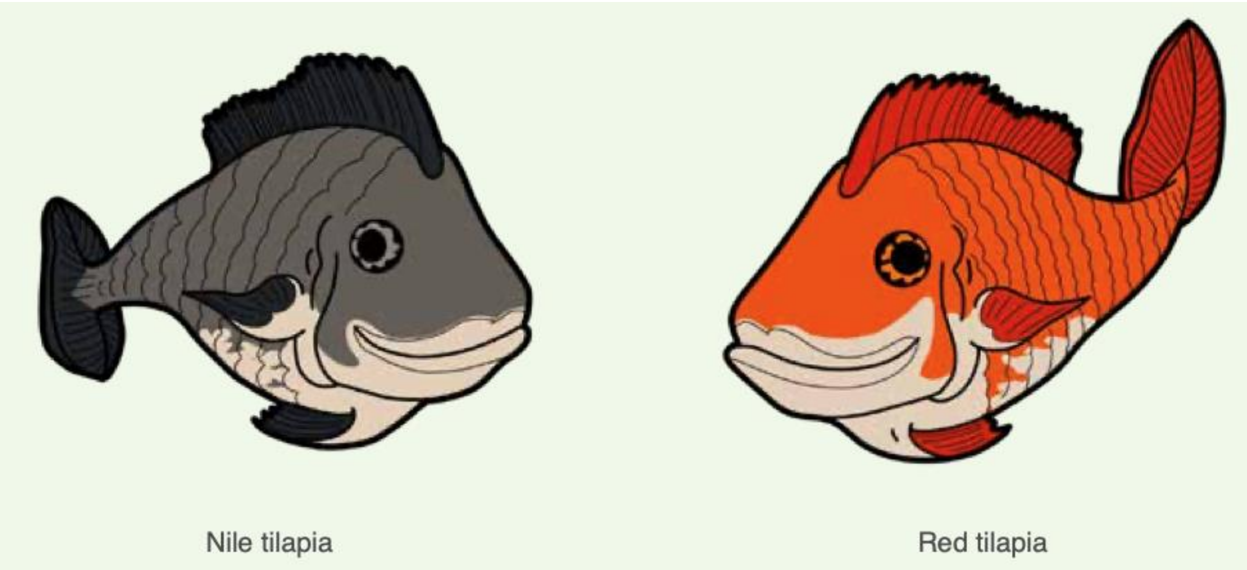


Table 3. Main tilapia species produced in the world (Tang et al., 2021)

Common (scientific) name
Nile tilapia (*O. niloticus*)
Gray tilapia (*O. niloticus* x *O. aureus*)
Red tilapia (*Oreochromis* spp.)

Red hybrid tilapia (*O. niloticus* x *O. mossambicus*)
Mozambique tilapia (*O. mossambicus*)
Mango tilapia (*Sarotherodon galilaeus*)* **Produced in Africa*

Note: If you are interested in learning about the context in Europe, the following publication is recommended: Report on Survey and Diagnosis of Fish Diseases in Europe. European Union Reference Laboratory (EURL) for Fish and Crustacean Diseases.

Table 4. Optimal temperature and water quality for Nile tilapia (Modified from Tang et al., 2021)

Growth condition	**Optimum**	**Range**	**Impact on welfare out or comfort range**
Temperature (ºC)	27–30	12–38	High temperature: Streptococcosis (>30ºC) Edwardsiellosis Low and normal temperature: TiLV (<27ºC) Francisellosis Aeromoniasis Ichthyophthirius Infectious Spleen and Kidney Necrosis Virus (ISKNV)
Salinity (ppt)	5–10* **viable 0-10*	<25	Ionic stress
Dissolved oxygen (mg/l)	>5		High: Bubble disease Low: Hypoxia Streptococcosis TiLV
pH	6–9	5–10	Out of range: TiLV Low: Streptococcosis (<5)
Ammonia (NH_3) (mg/l)	<0.1		TiLV
Nitrate (NO_3^-) (mg/l)	<27		TiLV

Rainbow trout (*Oncorhynchus mykiss*)

Rainbow trout is one of the oldest fish cultured in the world. This is also the species for which the geographical area was the most increased following the numerous introductions in several countries in the past century. In 2014, rainbow trout was the 12th most produced aquatic species globally (Teletchea, 2019). In part, the biological characteristics including that both sexes mature in captivity, spawning is easy to obtain, eggs are relatively robust, fry are sufficiently developed at hatching to directly accept pellets, relatively large tolerance to both temperature and salinity, can explain the success of its rearing throughout the world (Teletchea, 2019).

When hatching, you have to wait for the absorption of the yolk sac to start offering food in the form of fine flour. As the organism develops, pellet-based food is offered taking into account the size of the fish's mouth.

Figure 2. Rainbow trout (*Oncorhynchus mykiss*)

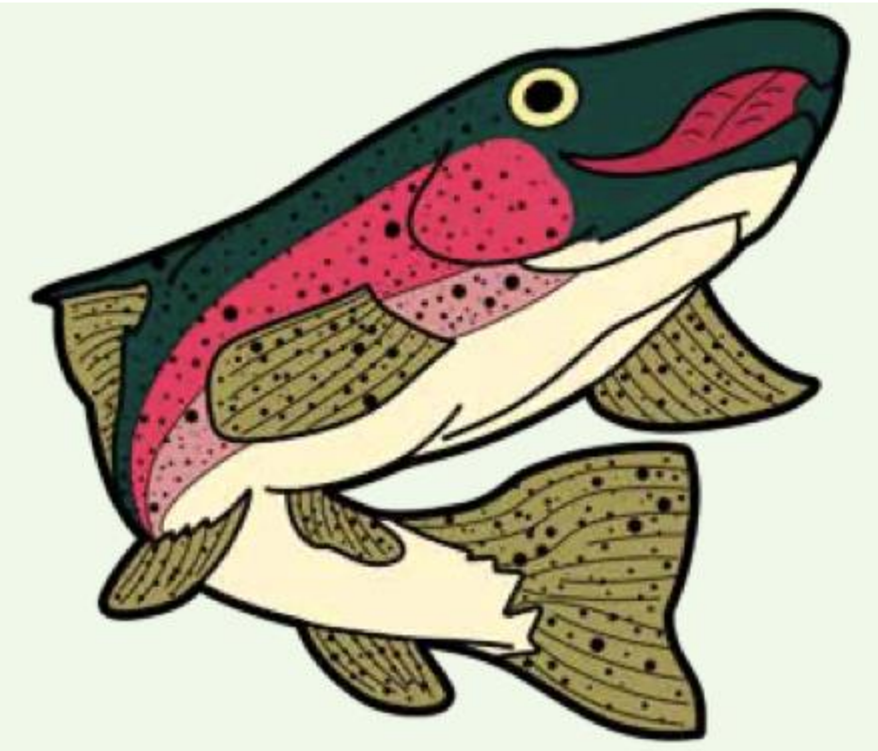


Table 5. Optimal temperature, water flow and quality for rainbow trout (Woynarovich et al., 2011)

Growth condition	**Stages**	**Optimum**	**Range**	**Impact on welfare out of comfort range**
Temperature (ºC)	Eggs - fry	6–12	4–15	Deformities Mortalities
	Growth-out	7–18	5–25	Low: Ichthyophthiriasis Coldwater disease

				SAV High: Edwardsiellosis Weissellosis
Water supply	Eggs incubation for 10000 eggs	0,25–2.5 liters/min		Mortalities
	Fry rearing for 1000 fry	3.5–4.5 liters/min		Hypoxia
	Fingerlings for 1000 fish	10–14 liters/min		Hypoxia
	Growth-out for 1000 fish	67–95 liters/min		Hypoxia Bacterial diseases
Dissolved oxygen (mg/l)	Eggs incubation and fry	5–6 mg/l close to 100% saturation		Low: Hypoxia High: Bubble disease
	Fingerlings and Growth-out	4–6 mg/ close to 100% saturation		Low: Hypoxia Bacterial diseases High: Bubble disease
pH	Eggs incubation and fry	6.5–8	5–8.5	Acute low: Acute mortality with tremors and hyperactivity, dyspnea, acute stress response Chronic low: Increased mucus production; chronic stress response High: acute and chronic stress response
	Fingerlings and Growth-out	6 - 8	4 -10	
Unionized ammonia (NH_3) (mg/l)	All phases	0.05 mg UIA/l	≤	Unionized ammonia levels ≥1 – 2 mg/l are usually lethal within 1 to 4 days. Below this level, fish might not die, but they will be stressed. If UIA is greater than 0.05 mg/l, it should be reduced as quickly as possible.

How can we measure the physicochemical water parameters?

Dissolved Oxygen (DO)
Most aquatic organisms require dissolved oxygen, often abbreviated as DO, to survive, but the source of this oxygen is not the water molecule (H_2O).

DO is gaseous, molecular oxygen in the form of O_2 originating from the atmosphere or as a byproduct of photosynthesis. Once dissolved in water, it is available for use by living organisms and can play a significant role in many chemical processes in the aquatic environment. Besides being dissolved in water, this oxygen is no different from the oxygen that humans or terrestrial animals breathe.

Optical and electrochemical sensors have some similarities. For starters, these sensors measure the pressure of oxygen dissolved in the sample. 'Raw' readings are expressed as DO%, and the only variable that affects DO% is barometric pressure, dependent on the altitude above the sea. The higher the barometric pressure (low altitudes), the more oxygen will be disolved into the water. It is important to note that DO mg/L is *calculated* from DO%, temperature, and salinity.

Temperature
The temperature of water is one of its most basic properties, and many other parameters depend on temperature for accuracy. With temperature data, we can monitor thermal loading or discharge and determine changes in the thermocline, which affect the health of aquatic organisms. Depending on their environment aquatic organisms are sensitive to high or low temperatures. The solubility of oxygen is lower in warmer water, thus limiting the oxygen supply.

pH
pH measurement is an important parameter in nearly every water quality application. In wastewater treatment, pH is regulated as part of discharge permitting and many treatment processes are pH dependent. In environmental sampling and monitoring, high or low pH values

can be indicative of pollution. In aquaculture, high pH increases ammonia toxicity and low makes heavy metals more easily soluble and bioavailable within the fish.

Ammonia, Ammonium
Ammonium (NH_4^+) — or its uncharged form, unionized ammonia (NH_3) — is a form of nitrogen that aquatic plants can absorb and incorporate into proteins, amino acids, and other molecules. High concentrations of ammonium can enhance the growth of algae and aquatic plants. Bacteria can also convert high ammonium to nitrate (NO_3^-) in the process of nitrification, however this process lowers dissolved oxygen.

Ammonia in water is either un-ionized ammonia or ammonium ion. Typically, the reported value is the sum of both forms of ammonia as total ammonia or simply - ammonia. The relative proportion of the two forms present in water is highly affected by pH.

In consequence, it is important to recognize that the risk of the aquaculture establishment being exposed to water containing pathogenic agents may be influenced by the category of aquaculture production system, the likelihood being higher for semi-open than for semi-closed and closed systems. Any water that is flowing from aquatic animals with lower or unknown health status presents a potential risk of transmitting pathogenic agents to aquatic animals of a higher health status. Aquatic Animal Health Code (2022)

Step 2. Know what pathogens affect tilapia or rainbow trout in your economy, and/or region, and know the critical points where diseases can enter or leave the farm.

Table 6. Main infectious diseases of tilapia in the Americas and Asia

Disease	**Americas**	**Asia**	**A critical point to enter in the farm**	**References**
Viral diseases				
TiLV	X	X	Healthy carriers Broodstock infected	Barato et al., 2022; Tang et al., 2021; Surachetpong et al., 2020
ISKNV	X	X	Healthy carriers Broodstock infected	Figueiredo et al., 2021; Machimbirike et al., 2019
Tilapia Parvovirus (TiPV)		X	During TiLV/ TiPV co-infection, an initial infection with TiLV may increase the ju-	Piewbang et al., 2022 Yamkasem et al., 2021

			venile host susceptibility to TiPV, or vice versa	
Bacterial diseases				
Streptococcosis			Sick fish Cannibalism Healthy carriers	Leal et al., 2019; Ortega et al., 2018; Barony et al., 2017; Ortega Asencios et al., 2016; Barato et al., 2015
S. agalactiae Ia, Ib, III	X	X		
S. agalactiae IX		X		
S. iniae	X	X		
Lactococcus garviae	X	X		
Edwardsiellosis			Sick fish Cannibalism Healthy carriers	Miniero Davies et al., 2019; Barato, 2018; Reichley et al., 2015; Griffin et al., 2013
Edwardsiella tarda, E. anguillarum, E. ictaluri	X	X		
Francisellosis			Sick fish Cannibalism Healthy carriers	Soto et al., 2019; Leal et al., 2014
Francisella noatunensis subsp *orientalis* (fno)	X	X		
Flavobacteriosis (formerly *F. columnare*)			Sick fish Cannibalism	LaFrentz et al., 2022
Flavobacterium Oreochromis	X	X		
F. davisii	X			
Aeromoniasis			Sick fish Low water temperature High manipulation	Casas et al., 1997
A. hydrophila, A. sobria, A. caviae, A. veronii	X	X		
Parasitic diseases				
Internal parasitism (digeneans, coccidia)	X	X	Intermediate host (snails, mollusks, polychaete)	Paperna, 1991

External parasitism (monogeneans, protozoa, microsporidia)	X	X	Sick fish, Presence of guppies in the system with tilapia culture	García-Vásquez et al., 2021; Maciel et al., 2018; Paperna, 1991
External parasitism (mollusks, crustacea, insects)	X	X	Sick fish, Presence of larvae and adults of macroinvertebrates in water	Paperna, 1991
Fungal diseases				
Saprolegnia and Branchiomyces	X	X	Sick fish, Low water temperature, High manipulation	François et al., 2010

Also understanding site location and neigboring risks, disease transmission pathways into and out of an aquaculture premises are important for prevention and management of disease risk. Proximity to natural bodies of water and/or other cultured populations of aquatic animals, and unintentional animal introductions or releases are transmission pathways for disease. Shared employees and equipment, employee flow on site and between any neighboring or connected premises can also pose transmission risks. The operator should have knowledge of the site and all potential hazards and risk transmission pathways on and off.

Table 7. Main infectious diseases of rainbow trout in the Americas and Asia

Disease	**Americas**	**Asia**	**A critical point to enter in the farm**	**References**
Viral diseases				
Infectious Pancreatic Necrosis Virus (IPNV)	X	X	Infected eggs; number of farms within 10 Km radius	Escobar-Dodero et al., 2019
Salmonid Alpha Virus (SAV)	X	X	Infected eggs, live animals, and neighboring farms; passive drift	Viljugrein et al., 2009
Viral Hemorrhagic Septicemia (VHS)	X	X	Infected eggs; waterborne	Escobar et al., 2018

			transmission	
Piscine reovirus Heart and Skeletal Muscle Inflammation (HSMI)	X		Infected eggs; live fish	Olsen et al., 2015
Infectious haematopoietic necrosis (IHN)	X	X	Infected eggs, water, and food	Dixon et al., 2016
Bacterial diseases				
Coldwater disease			Sick fish Cannibalism	
F. psychrophilum	X	X		Barnes and Brown, 2011
Bacterial gill disease				
F. branchiophilum	X	X	Biofilm in gills of sick fish	Speare et al, 1995
Edwardsiellosis			Sick fish; high stocking density	Rehulka et al., 2012
Edwardsiella tarda, E. piscicida	X	X		
Weissellosis			Water contamination with heavy metals, and *W. tructae*, and sick fish	
Weissella tructae	X	X		Pereira et al., 2022
Red mouth disease				
Yersinia ruckeri	X	X	Sick fish Cannibalism	Woo and Cipriano, 2017
BKD				
Renibacterium salmoninarum	X	X	Sick fish Cannibalism	Woo and Cipriano, 2017
Parasitic diseases				
External parasitism (Ich, monogeneans, protozoa, mollusks, crustacea, insects)	X	X	Sick fish	Buchmann and Bresciani, 1997
Internal parasitism (digeneans, coccidia)	X	X	Intermediate host (snails, molluscs, polychaete)	Buchmann and Bresciani, 1997

Mycotic diseases				
Saprolegnia	X	X	Sick fish, High manipulation, Viral, bacterial, or parasitic co-infection	Shin et al., 2017

Step 3. Understand quarantine and the role of fish movements in the introduction and spread of infectious diseases

Quarantine and movement restrictions should be implemented immediately upon suspicion of a viral or bacterial outbreak. Fish movement are an integral part of the many aquaculture production systems, and shipping has been recognized as a major risk for the introduction and spread of highly infectious disease in fish (Murray et al., 2002, Mardones et al, 2014). During an outbreak the farmer and/or the competent authority should establish appropriate zone and compartment designations such as spatial buffers as an strategy that may limit the spread of disease and facilitate international trade in fish and fish products. Zoning is usually under the responsibility of the competent authority; while compartments are within the production facilities, and are managed through farm-level biosecurity programs to maintain their health status (Tang et al., 2021).

Movement controls for TiLV from the affected premises (or area) should include:

- bans on the movement of live, fresh (chilled on ice) fish from the affected premises into TiLV-free areas;
- bans or restrictions on releasing live fish and pondwater from the affected premises into aquatic environments;
- restrictions on discharging of processing plant effluent within the affected premises;
- restrictions on harvesting and then transporting TiLV-infected fish in the affected premises to off-site processing plants;
- restrictions on the use and movement of equipment and vehicles between farms within the affected premises;
- control of bird access to live and moribund/dead fish within the affected premises; and
- control of the disposal of diseased fish and dead fish due to infection

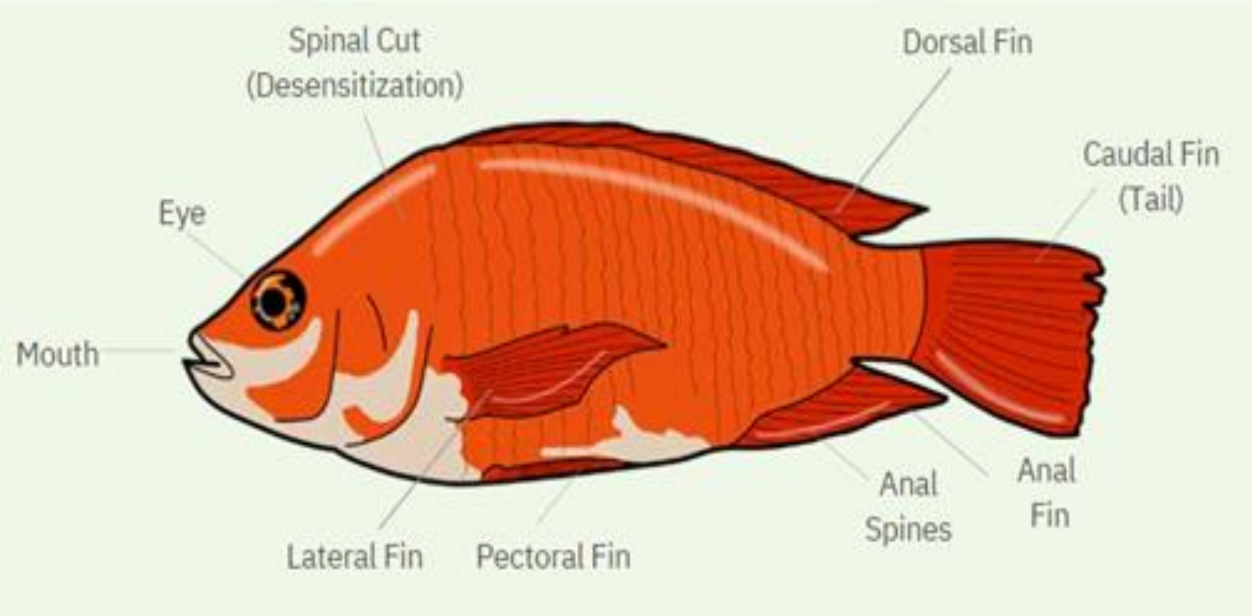
The implementation of these bans or restrictions will depend on the severity of the disease, the types of operation (such as farm location, farm size, culture system), and the response options chosen (Tang et al., 2021).

As for many diseases, distance between farms is a critical risk factor. For IPN is known that 10km is the critical distance to spread the infection between fish farms (Escobar-Dodero et al., 2019). For ISA virus it can range between 7.5 and 15 km (Mardones et al, 2009) as for *Piscirickettsia salmonis* can range from 7.5 to 10 km (Rees et al., 2014).

The risks associated with the introduction into, spread within, and release of pathogenic agents from the aquaculture establishment need to be considered for each of the following transmission pathways: Aquatic animals, Aquatic animal products and aquatic animal waste, Water, Feed, Fomites, Vectors, Personnel and visitors. Aquatic Animal Health Code (2022)

Step 4. Only introduce healthy fry to your system. Evaluate and monitor the disease status of the farm following levels of diagnosis I, II, and III

Figure 3. Tilapia (gross of external and internal appearance).



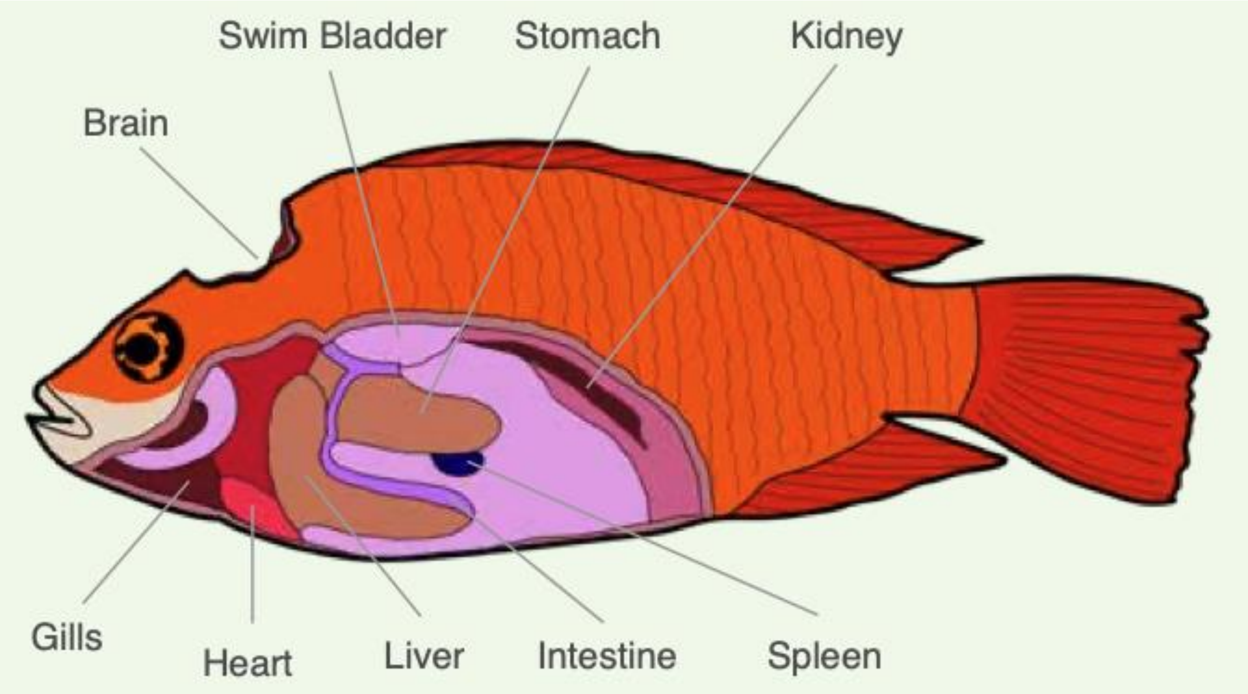
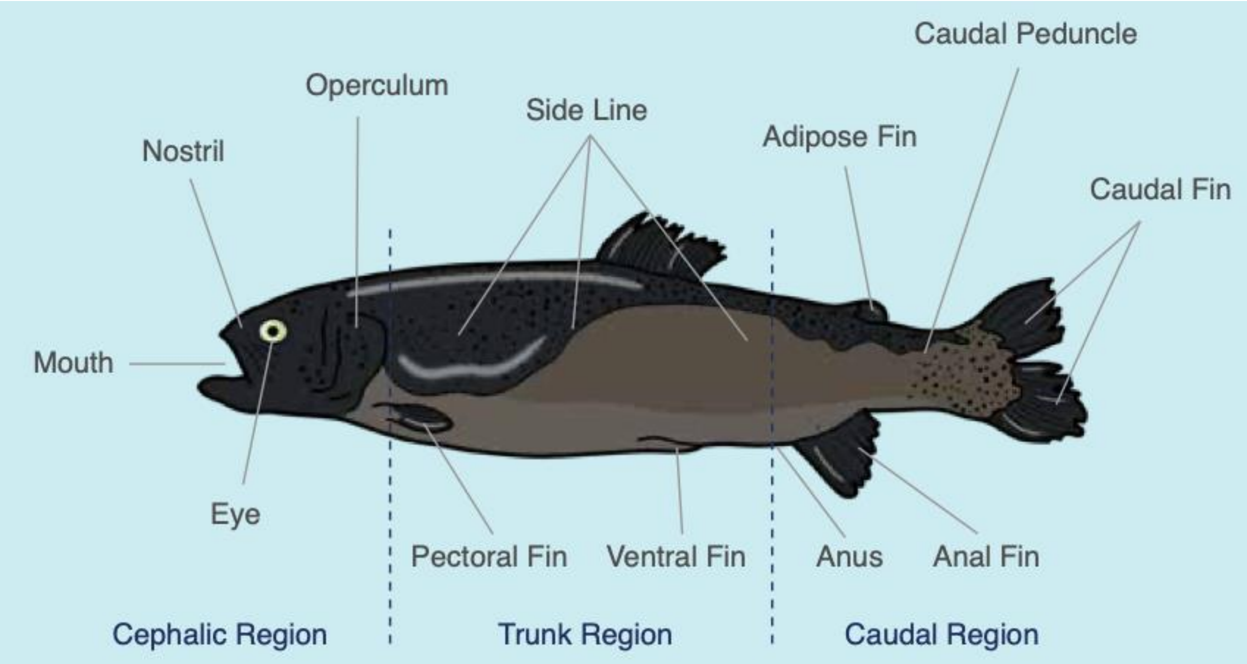
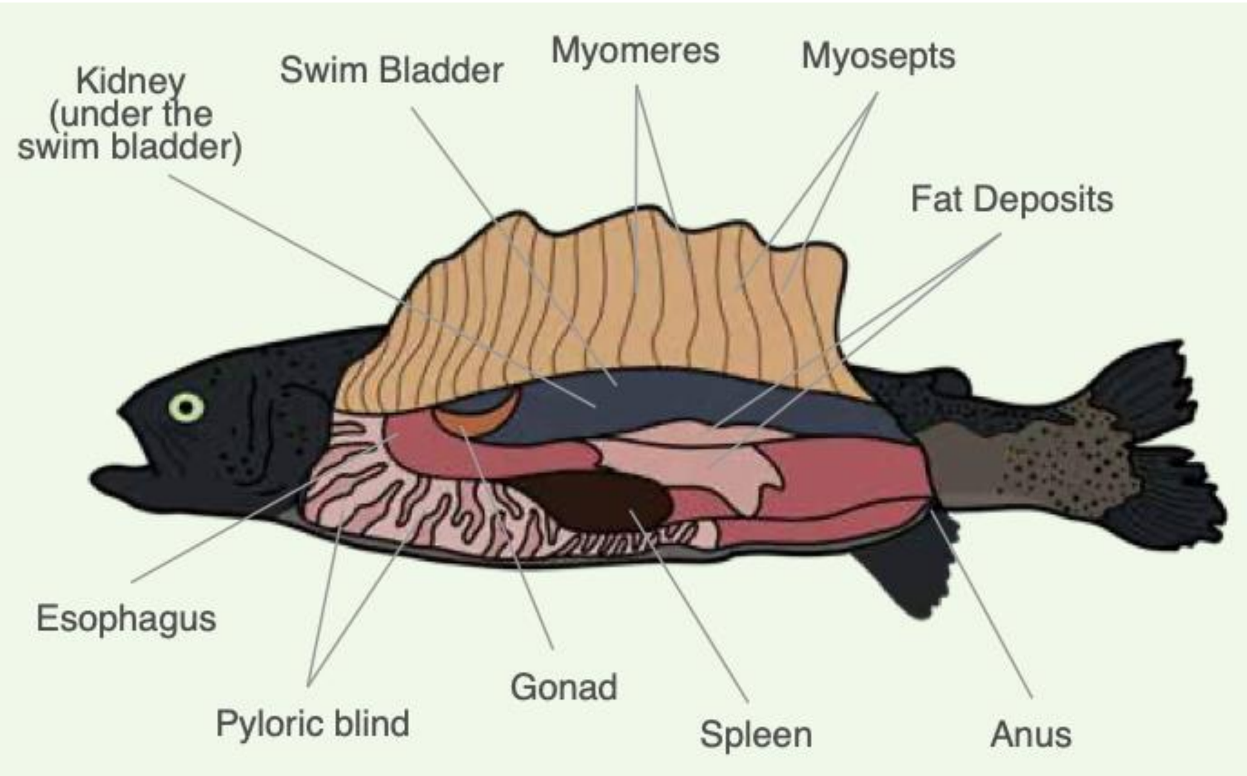


Figure 4. Rainbow trout (gross external and internal appearance)





LEVELS OF DIAGNOSIS

Level I

Includes farm/production site observations, record-keeping, and gross clinical signs – such information forms the basis for accurate results from Levels II and III diagnostic analyses. See Card 1 - Level I Gross lesions of tilapia

Level II

Includes the equipment and experience to undertake analyses that can detect and/or identify a range of pathogens. Level II laboratories may include parasitology, histopathology, bacteriology, and mycology examinations, and are, generally speaking, experienced with endemic and opportunistic disease agents in their area, region, or economy.

Level III

Diagnostics encompass techniques that target a specialized pathogen or group of pathogens or require highly specialized equipment. Virology, immunology, and molecular techniques are included in Level III, although field kits are now available for farm or pond-side use as well as in microbiology or histology laboratories for some pathogens

Step 5. Use preventive measures to avoid disease presentation, and if it is necessary use antimicrobials responsibly

Natural feed additives or phytobiotics, which combine different mechanisms of action against pathogenic bacterial species (bactericidal/ bacteriostatic activities, quorum sensing inhibition), are potential candidates for the development of prevention strategies in aquaculture. Appropriate use of preventive therapies to avoid disease presentation as phytobiotics, phytoextracts, probiotics, prebiotics, organics acids, essential oils, and other organics sources of substances with antimicrobial and immunomodulatory effects in fish is desirable to reduce the risk to develop infectious diseases.

Antimicrobials should be use only based on diagnostic test results during outbreaks, they should not be use as preventive measures or growth promoters and is a prescription of a veterinarian or other aquatic animal health professional authorized to prescribe veterinary medicines. For bacterial infections, detection should follow antibiogram to identify susceptibility to available antibiotics. It is important that any therapy, drug, or antimicrobial treatments should be done in accordance with laws/regulations of the country.

Step 6. Use practical disinfection procedures

Disinfection should be used as a routine practice in biosecurity programs designed to (1) mitigate the risk for incursion of specific diseases (prevention), (2) reduce within-farm disease incidence (control), or (3) to eliminate disease from the population (eradication). The general principles pertaining to the use of disinfection in aquaculture farms involve the application of chemical treatment in sufficient concentration, and for sufficient periods of time, to neutralize pathogens that would otherwise gain access to surrounding water systems and susceptible population (Muniesa et al. 2019).

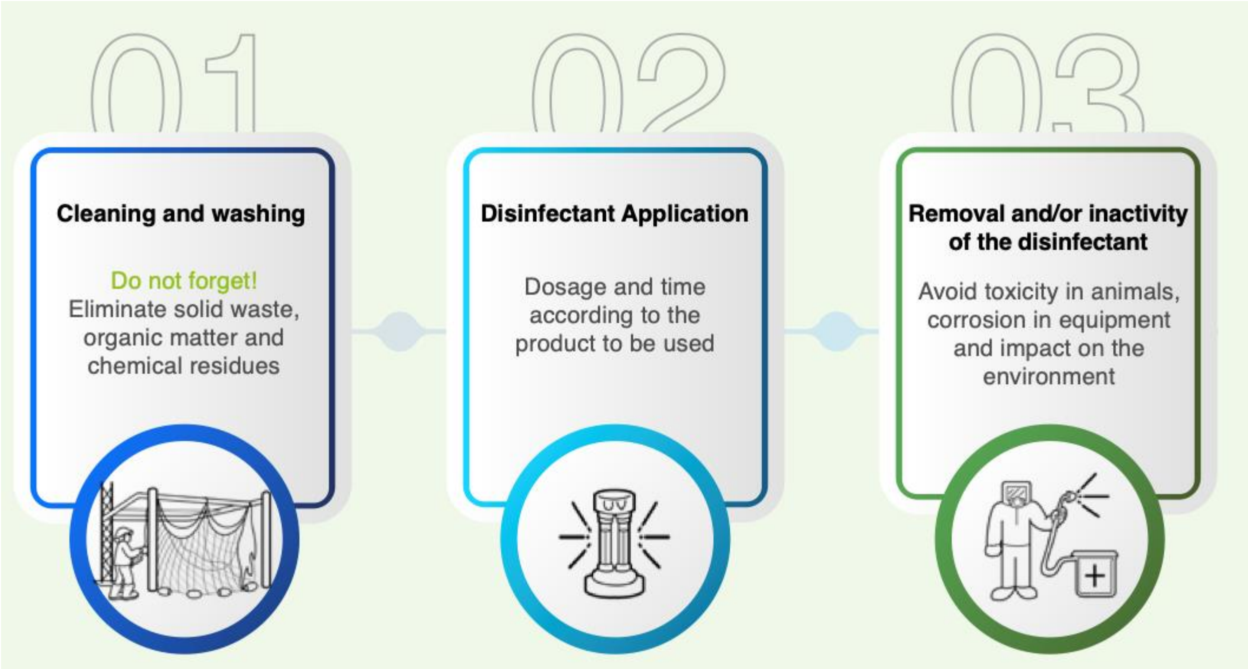


Table 8. Surface, equipment, personnel and water disinfectants with dose and time to exposition of each substance

Disinfectant	**Dose**	**Time**	**Targeted pathogen**
Desiccation	37ºC	4 days	Nodavirus
	60ºC	20 min	ISKNV
	65ºC	2 min	ISA
	70ºC	2 hours	IPN
Quaternary ammonium	125 ppm	5 min	General
	650 ppm	10 min	ISKNV
Sodium hypochlorite	100 ppm	10 min	TiLV
	1000 ppm	30 min	ISKNV

Virkon®	0.5%	10 min	TiLV
	0.5%	30 min	ISA
	1%	10 min	IPN / ISKNV
Iodine	100 ppm	10 min	General
	200 ppm	30 min	TiLV
Peracetic acid	600 ppm	30 min	TiLV
Irradiation of water	122 $mJ/cm^2/sec$	continuous	IPN
	290 $mJ/cm^2/sec$	continuous	Nodavirus
Resting transport water without fish	Resting water before stocking or for transportation of fish	3 to 5 days	TiLV

Table 9. Disinfectants types including dose and time for application on eggs and live animals

Disinfectant	**Dose**	**Time**	**Effect in which pathogen**
Hydrogen peroxide*	50–100 ppm	Aspersion over fish during feeding	General viral and bacterial diseases
Iodine for eggs*	100 ppm	10 min	General viral and bacterial diseases

** These are considered drug indications/uses in the United States*

Step 7. Document and register your biosecurity and good aquaculture practices

The minimal documentation and record of parameters to make decisions based on GAP and biosecurity must include water quality monitoring, feeding, and feed conversion ratio (FCR), aquatic animal health and behavior, daily mortalities, disease outbreaks, and use of veterinary drugs, therapeutic chemicals or disinfectants.

a. Water quality variables shall be measured, recorded, and available for inspection with minimum daily oxygen, temperature, and pH, and regular measurement of ammonia levels. See annexes F-1.

b. Feeding: The farm shall use feed for the size of fish for which the manufacturer has formulated and provided data on the inclusion rate (%) in feeds of total protein and record the quantity and inclusion rate according to age phase during the production cycle. Protein levels of all used feed, the total amounts of each feed used each year, and the total annual aquatic animal production must be registered. Sampling every 15 days to recalculate the percentage of food to be provided. See annexes F-2.

c. Feed conversion ratio (FCR): The farm shall calculate and record an average feed conversion ratio (FCR) for completed crops in a calendar year.

   Feed conversion ratio = Annual feed use ÷ Net biomass (live weight) of fish produced. The amount of feed used and the net biomass of aquatic animals produced can be reported in metric tons or kilograms, but the same units shall be used for both in the calculation. The net biomass of fish or shrimp produced is calculated by subtracting the total weight of stocked juveniles from the total live weight of the harvested individual.

d. Fish health and behavior: Regular monitoring of health status with level I of diagnosis gives information about early gross lesions or clinical signs of fish. See annexes F-3, and card 1.

e. Daily mortalities: Mortalities from acute die-offs or euthanized diseased animals must be promptly removed from culture units and disposed of responsibly by rendering, incineration, sterilization, composting, biogas production, or ensiling. Daily record of mortality is important to detect early events of unusual outbreaks to take an early decision to manage the emergency. See annexes F-4.

f. Disease outbreaks: record and report the disease outbreaks with information concerning possible causes following levels I, II, and III of diagnosis. See annexes F-5.

g. Use of veterinary drugs and/or therapeutic chemicals: Record the product name, dose, and time of use of each veterinary drugs, and/or therapeutic chemical, withdrawal period. See annexes F-6.

h. Disinfection record: the farm shall maintain or have access to regularly updated records of water quality monitoring, feeding, aquatic animal health and behavior, water quality monitoring, daily mortalities, disease outbreaks, and use of veterinary drugs, therapeutic chemicals, or disinfectants. See annexes F-7.

Step 8. Environmental risk/escapes/proliferation of harmful algae

Respect the environment, and follow the local regulation to reduce the environmental impact before and during the farming operation.

Feeding and the digestion of food by aquatic animals add substantial organic waste load, both soluble and insoluble to the water. This can create conditions that may drive photosynthetic activity in the water that can result in a proliferation of harmful algae or phytoplankton and pathogens that thrive under such conditions.

Small fish farmers must implement proper handling, maintenance, and management practices to prevent farmed tilapia and rainbow trout from escaping into the wild. This involves maintaining secure and good-condition culture ponds and nets and utilizing appropriate transportation methods. The use of nets/filters during water pumping and reservoir pond set-up can also be effective in capturing any escaped fish.

Step 9. Surveillance area / Mobile laboratories / Participatory epidemiology

Participation in the domestic program of surveillance is an important component of GAP. The information obtained from surveillance allows knowing the health status of the area where you have your farm, act together with your neighbors to reduce the risk of entry of absent pathogens or diseases, or make decisions together to mitigate the impact of those present. The following definitions as important for any monitoring and surveillance plan, including participatory epidemiology as a component for disease searching.

Surveillance: Systematic ongoing collection, collation, and analysis of information related to fish health and the timely dissemination of information to those who need to know so that action can be taken.

Monitoring: Systematic collection, analysis, and dissemination of information about the level (e.g., occurrence, incidence, prevalence) of infections or diseases that are known to occur in a specified population.

Participatory epidemiology (PE): It was originally based on combining practitioner communication skills with participatory methods to facilitate the involvement of animal caretakers and owners (embracing their knowledge, experience, and motivations) in the identification and assessment of animal disease problems, including in the design, implementation, monitoring and evaluation of disease control programs, policies, and strategies.

Small farmers are often able to describe clinical presentations, epidemiological patterns and principal pathological lesions using a vocabulary of specific disease terms in local languages that correspond to Western clinical case definitions. This body of knowledge has been termed 'existing veterinary knowledge' (EVK) (Mariner and Paskin, 2000). Participatory epidemiology learns from local knowledge, leading to disease control programs that are both acceptable to their stakeholders and effective. As experience with EVK and participatory methods increased, veterinary field epidemiologists realized that there was tremendous potential to develop participatory approaches to epidemiology as surveillance, outbreak investigation, and research tools, in a variety of rural and urban settings.

As PE evolved, an innovative participatory methodology for surveillance programs was developed in response to the needs of the Global Rinderpest Eradication Program (Mariner and Roeder, 2003). This approach is called 'participatory disease searching' and is a form of active surveillance that taps into traditional information networks to track down and diagnose outbreaks of infectious disease.

Step 10. Know the regulation in your economy related to aquaculture biosecurity and good aquaculture practices for small-scale farmers.

Be transparent and cooperative with the local, regional and domestic plans to improve the aquaculture biosecurity

Table 10. The regulatory frame of good aquaculture practice and biosecurity in aquaculture in APEC economies

Economy		**Regulation**	**Link**
AUS	Australia	Aquaculture Farm Biosecurity Plan. Generic guideline and template	https://www.awe.gov.au/agriculture-land/fisheries/aquaculture/farm-biosecurity-plan
BD	Brunei Darussalam	Guidelines on ASEAN Good Aquaculture Practices for Food Fish (17 July 2014)	https://asean.org/wp-content/uploads/2021/09/ASEAN-GAqP-for-Food-Fish-2014.pdf
CDA	Canada	Code of practice for the care and handling of farmed Salmonids Aquatic animal biosecurity	https://www.nfacc.ca/pdfs/codes/farmed_salmonid_code_of_practice.pdf https://inspection.canada.ca/animal-health/aquatic-animals/aquatic-animal-biosecurity/eng/1320594187303/1320594268146#a1
CHL	Chile	Manual de Buenas Prácticas en el uso de antimicrobianos en salmonicultura chilena Instructivo de bioseguridad para fiscalizadores SERNAPESCA Reglamento de medidas de protección, control y erradicación de enfermedades de alto riesgo para las especies hidrobiológicas.	http://www.sernapesca.cl/manuales-publicaciones/manual-de-buenas-practicas http://www.sernapesca.cl/sites/default/files/instructivo_de_bioseguridad_para_fiscalizadores.pdf https://www.subpesca.cl/portal/615/articles-83903_documento.pdf
PRC	People's Republic of China	FISHERIES LAW OF THE PEOPLE'S REPUBLIC OF CHINA (2004 REVISION)	http://extwprlegs1.fao.org/docs/pdf/chn23913E.pdf
HKC	Hong Kong, China	Hong Kong Sustainable Seafood Coalition ("HKSSC") Voluntary Codes of Conduct	https://hksustainableseafoodcoalition.org/wp-content/uploads/2018/11/HKSSC-VCOC-19Nov2018.pdf
JPN	Japan	JAS 005 Aquaculture products by artificial seedling production techniques Guideline for Disease Control of Aquatic Animals	http://www.famic.go.jp/english/jas/jas0005.pdf https://www.maff.go.jp/e/policies/ap_health/animal/attach/pdf/index-33.pdf https://www.maff.go.jp/e/policies/ap_health/animal/index.html#q_3

ROK	Republic of Korea	AQUACULTURE INDUSTRY DEVELOPMENT ACT [Enforcement Date 15. Jun, 2021.] [Act No.18289, 15. Jun, 2021., Partial Amendment] AQUATIC ORGANISM DISEASE CONTROL ACT [Enforcement Date 19. Feb, 2021.] [Act No.17036, 18. Feb, 2020., Partial Amendment]	https://www.law.go.kr/LSW/eng/engLsSc.do?menuId=2&query=AQUACULTURE%20INDUSTRY%20DEVELOPMENT%20ACT#liBgcolor0 https://www.law.go.kr/LSW/eng/engLsSc.do?menuId=2&query=AQUACULTURE%20INDUSTRY%20DEVELOPMENT%20ACT#AJAX
MAS	Malaysia	FISHERIES ACT 1985	http://extwprlegs1.fao.org/docs/pdf/mal1869.pdf
MEX	Mexico	Acciones y Programas en sanidad acuícola y pesquera Manual de Buenas Prácticas de Producción Acuícola de para la Inocuidad Alimentaria Manual de Buenas Prácticas de Producción Acuícola de para la Inocuidad Alimentaria Pliego de condiciones para el uso de la marca oficial México Calidad Suprema Tilapia y Trucha Arcoiris	https://www.gob.mx/senasica/acciones-y-programas/sanidad-acuicola-y-pesquera https://www.gob.mx/cms/uploads/attachment/file/167794/7_Manual_Tilapia.pdf https://www.gob.mx/cms/uploads/attachment/file/167793/8_Manual_Trucha.pdf https://www.gob.mx/cms/uploads/attachment/file/48460/OCP.pdf
NZ	New Zealand	Aquaculture Biosecurity Handbook Assisting New Zealand's commercial and non-commercial aquaculture to minimize on-farm biosecurity risk Guide to setting up and operating a land-based aquaculture farm	https://www.mpi.govt.nz/dmsdocument/13293-Aquaculture-Biosecurity-Handbook-Assisting-New-Zealands-commercial-and-non-commercial-aquaculture-to-minimise-on-farm-biosecurity-risk https://www.mpi.govt.nz/dmsdocument/15901-Guide-to-setting-up-and-operating-a-land-based-aquaculture-farm
PNG	Papua New Guinea	Monitoring, Control, and Surveillance	https://www.fisheries.gov.pg/monitoring-control-and-survailence

			https://www.fisheries.gov.pg/aquaculture
PE	Peru	Programa Oficial de Vigilancia y Control de Enfermedades en Animales Acuáticos. Resolución Directoral N° 009-2016-SANIPES/DSNPA Procedimiento Técnico Sanitario para la elaboración de planes de emergencia sanitaria ante enfermedades que afectan a los recursos hidrobiológicos" Programa de vigilancia de enfermedades en animales acuáticos	https://www.sanipes.gob.pe/documentos_sanipes/procedimiento/2020/e43cdadb38998281bc51a4b159292f8b.pdf https://www.sanipes.gob.pe/documentos_sanipes/procedimiento/2021/fa5d1477797b8cd0bfa323f25b85ff59.pdf http://www.sanipes.gob.pe/procedimientos/8_ProgramaOficialdeVigilanciayControldeEnfermedadesenAnimalesAcuaticos.pdf
PHL	The Republic of the Philippines	THE PHILIPPINE FISHERIES CODE OF 1998	https://www.bfar.da.gov.ph/wp-content/uploads/2021/02/Philippine-Fisheries-Code-of-1998.pdf https://www.bfar.da.gov.ph/fish-health-laboratory/
SGP	Singapore	GOOD AQUACULTURE PRACTICE FOR FISH FARMING (GAP-FF)	https://www.sfa.gov.sg/docs/default-source/section/farms/gap-ff/gap-ff-guidelines.pdf
CT	Chinese Taipei	Enforcement rules of the fisheries act Statute for Prevention and Control of Infectious Animal Diseases Agricultural Production and Certification Act	https://en.fa.gov.tw/view.php?theme=Regulations&subtheme=&id=7 https://law.coa.gov.tw/glrsnewsout/EngLawContent.aspx?lan=E&id=370 https://law.moj.gov.tw/ENG/LawClass/LawAll.aspx?pcode=M0060072
THA	Thailand	DoF regulation on registration of aquaculture establishment for	https://drive.google.com/file/d/1e27ozVdHIl-P-qZ1eMvodX0pwBu5xQen/view

		exportation of aquatic animal B.E. 2563 (2020)	
US	United States	Comprehensive Aquaculture Health Program Standards (in construction) National Aquaculture Health Plan & Standards, 2021-2023	https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/animal-disease-information/aquaculture/cahps https://www.aphis.usda.gov/animal_health/animal_dis_spec/aquaculture/downloads/national-aquacult-health-plan-standards-2021-2023.pdf

5. Implementation of Biosecurity Plan

The final objective of this guidebook is to reduce the burden of diseases on fish farms, improve the health status at farm and domestic levels, minimize the global spread of diseases, optimize socio-economic benefits from aquaculture, attract investment opportunities into aquaculture and achieve the One Health goals for small-scale farmers of tilapia and rainbow trout in APEC (FAO, 2020a).

6. Annexes (formats to be used in the Biosecurity plan)

FORMATS
F-1 WATER QUALITY VARIABLES
F-2 FEEDING
F-3 AQUATIC ANIMAL HEALTH AND. BEHAVIOUR (LEVEL I)
F-4 DAILY MORTALITIES
F-5 DISEASE OUTBREAKS (LEVELS I, II, AND III)
F-6 VETERINARY DRUGS AND CHEMICALS
F-7 DISINFECTION RECORD

CARD 1 - LEVEL I
Gross lesions of tilapia

Photos by Dr. Paola Barato and Dr. Win Surachetpong

EYES


Absence


Atrophy


Exophthalmia


Corneal opacity


Normal

SKIN


Darkening


Discoloration

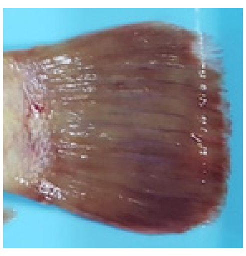

Erosions and hemorrhages

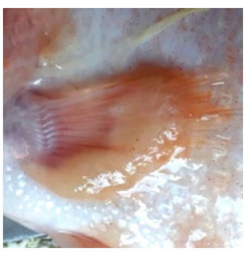

Pale skin and erosion


Normal

FINS


Erosions


Hemorrhages


Necrosis


External parasites


Normal

GILLS


Organic material

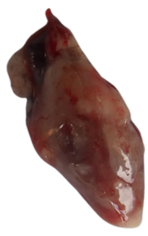

Necrosis and hemorrhage


External parasites


Necrosis


Normal

HEART


Material in surface


Normal

SKELETAL MUSCLE


Abscesses and necrosis


Normal

DIGESTIVE TRACT


Ascites

Hepatomegaly and friability

Hemorrhages in liver

Granuloma in liver

Edema gastric wall


Distended by liquid in intestine

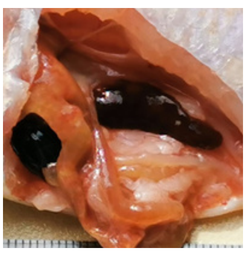
Hemorrhages intestine

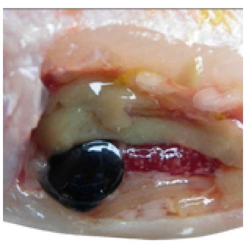
Intussusception

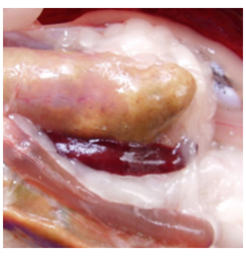
Peritonitis

Normal

SPLEEN

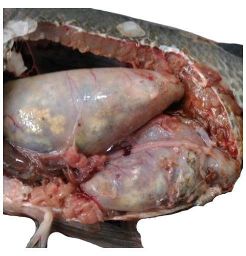

Splenomegaly


Granulomas


Normal

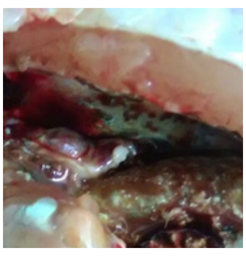
GONADS


Hardening

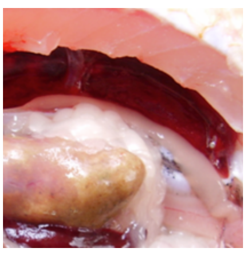

Enlarged


Normal

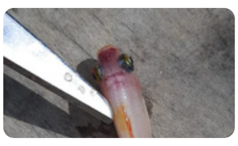
KIDNEY


Granulomas


Necrosis


Normal

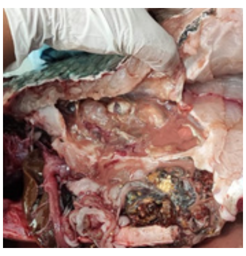
BRAIN

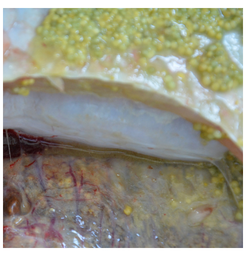

Hemorrhage surrounding the brain


Hemorrhages, friable, necrotic


Normal

SWIM BLADDER


Cysts, purulent


Normal

F-1 WATER QUALITY VARIABLES

Farm ______________________ Lot/pond ______________________

Municipality/Region ______________________

Species

Tilapia ________
Trout ________
Carp ________
Catfish ________
Other ________

Stage of culture

Eggs ________
Larvae ________
Alevins ________
Juvenils ________
Growth-out ________
Broodstock ________

Date	Disolved oxygen (mg/l)	Oxygen saturation (%)	Temperature	pH	Ammonia	Nitrite

F-2 FEEDING

Farm ______________________ Lot/pond ______________________ Municipality/Region ______________________

Species
Tilapia ________
Trout ________
Carp ________
Catfish ________
Other ________

Stage of culture
Eggs ________
Larvae ________
Alevins ________
Juvenils ________
Growth-out ________
Broodstock ________

Date sampling	Weight sample 1 (100 fish) Kg	Weight sample 2 (100 fish) Kg	Weight sample 3 (100 fish) Kg	Mean individual weight (g)	# fish per pond	Biomass (Kg/m3)	% Food according manufacturer	Kg food per day	Acummulative food (Kg)

F-3 AQUATIC ANIMAL HEALTH AND BEHAVIOUR (LEVEL I)

Date ______________ Farm ______________

Lot/pond ______________ Municipality/Region ______________

Owner ______________ Technician who perform necropsy ______________

Telephone ______________ E-mail ______________

Species			Stage of culture		
	Tilapia	______		Eggs	______
	Trout	______		Larvae	______
	Carp	______		Alevins	______
	Catfish	______		Juvenils	______
	Other	______		Growth-out	______
				Broodstock	______

Method of anesthesia used ______________

Clinical signs (behaviour)	Mark if it is present
Separation of schoolfish	
Changing position in water column (surface, bottom, shoreline, water outlet)	
Staying near the surface of water	
Lethargy	
Loss of swim axis	
Erratic swimming	
Swim in circles	
Scraping the body against the walls of the pond/raceway/etc	
Increased ventilation	
Loss of appetite	
Mortality	

Organ	Lesion	Mark if it is present
Eyes	Absence bilateral	
	Absence unilateral	
	Atrophy	
	Exophthalmia	
	Corneal opacity	
	Normal	
Skin	Darkening	
	Paleness	
	Erosions	
	Hemorrhages	
	Skin fungus	
	External parasites	
	Normal	
Fins	Erosions	
	Hemorrhages	
	Skin lumps	
	External parasites	
	Normal	
Gills	Organic material in the surface	
	Necrosis	
	Hemorrhages	
	Paleness	
	Normal	
Heart	Deposition white/yellow material in the surface	
	Normal	

Organ	Lesion	Mark if it is present
Digestive tract	Liquid in celomic cavity (ascites)	
	Hepatomegaly (enlarged liver)	
	Liver friability	
	Liver paleness	
	Liver darkening	
	Hemorrhages in liver	
	Granulomas (nodules or lumps) in liver	
	Edema in gastric wall	
	Distension with liquid in stomach/intestine	
	Parasites in the lumen	
	Hemorrhages in intestine	
	Intussusception	
	Peritonitis	
	Normal	
Spleen	Splenomegaly = enlarged	
	Granulomas in spleen	
	Paleness	
	Normal	
Gonads	Hardening	
	Hemorrhages	
	Normal	
Swim bladder	Hemorrhages	
	Cysts	
	Liquid inside	
	Purulent (pus) material	
	Normal	
Kidney	Paleness	
	Nodules or lumps in kidney	
	Hemorrhages	
	Normal	
Brain	Hemorrhages	
	Friable or lysis	
	Purulent (pus) material	
	Normal	
Skeletal muscle	Abscesses	
	Hemorrhages	
	Necrosis	
	Normal	

Probable cause			
Viral disease		Poor physico-chemical water quality	
Bacterial disease		Poor toxicological water quality	
Parasitic disease		Poor aquaculture practices	
Mycotic disease		Poor reproductive management	
Nutritional/alimentary disease		Other	

F-4 DAILY MORTALITIES

Farm ______________________ Lot/pond ______________________

Municipality/Region ______________________

Species: Tilapia ________ Trout ________ Carp ________ Catfish ________ Other ________

Stage of culture: Eggs ________ Larvae ________ Alevins ________ Juvenils ________ Growth-out ________ Broodstock ________

Date	# dead fish	Cummulative mortality	Date	# dead fish	Cummulative mortality

F-5 DISEASE OUTBREAKS (LEVEL I, II, AND III)

Date ____________________ Farm ____________________

Lot/pond ____________________ Municipality/Region ____________________

Owner ____________________ Technician who perform necropsy ____________________

Telephone ____________________ E-mail ____________________

Species			Stage of culture	
Tilapia	____________		Eggs	____________
Trout	____________		Larvae	____________
Carp	____________		Alevins	____________
Catfish	____________		Juvenils	____________
Other	____________		Growth-out	____________
			Broodstock	____________

LEVEL I (fill format F-3)

Probable cause			
Viral disease		Poor physico-chemical water quality	
Bacterial disease		Poor toxicological water quality	
Parasitic disease		Poor aquaculture practices	
Mycotic disease		Poor reproductive management	
Nutritional disease		Other	

Samples taken:			
LEVEL II		**LEVEL III**	
Wet mount		Molecular analysis	
Histopathology		Water toxicology	
Bacteriology		Water microbiology	
Other			

LEVEL II

WET MOUNT

Finding	Fins	Skin scraping	Gills	Stomach	Intestine
Trichodine					
Piscinoodinium					
Icthyobodo					
Chilodonella					
Apiosoma					
Epistylis					
Ichthyophthirius					
Coccidia					
Monogenea					
Glochidium					
Ergasilus					
Orbitids					

Lernaeids					
Argulids					
Other					

Finding	Fins	Gills	Spleen	Liver	Peritoneum
Aneurismas					
Hemorrhages					
Congestion					
Digenean					
Nematodes					
Granulomas					
Other					

HISTOPATHOLOGY RESULT

BACTERIOLOGY RESULT

LEVEL III

MOLECULAR ANALYSIS RESULT

TOXICOLOGICAL RESULT

FINAL DIAGNOSIS

F-6 VETERINARY DRUGS AND CHEMICALS

Farm ____________________ **Municipality/Region** ________________________________

Species
Tilapia __________ Catfish ______
Trout __________ Other ______
Carp __________

Stage of culture
Eggs __________ Juvenils __________
Larvae __________ Growth-out __________
Alevins __________ Broodstock __________

Date	Lot/pond	Diagnosis	MV authorized	Product name	Dose	Time to use	Biomass to be treated (Kg/m3)	Quantity of product to be applied by day	Withdrawal period

F-7 DISINFECTION RECORD

Farm ______________ **Municipality/Region** ______________

Species
Tilapia ______ Catfish ______
Trout ______ Other ______
Carp ______

Stage of culture
Eggs ______
Larvae ______
Alevins ______
Juvenils ______
Growth-out ______
Broodstock ______

Date	Area to be desinfected	Product name	Dose	Time of use	Quantity of product to be applied by day	Responsible

7. Bibliographic references

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