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دليل الأمن الحيوي للأسماك المستزرعة في المملكة العربية السعودية NATIONAL FISH BIOSECURITY MANUAL Kingdom of Saudi Arabia

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The General Directorate of Fisheries Ministry of Environment, Water and Agriculture Kingdom of Saudi Arabia

The General Directorate of Fisheries hereby declares that:

- This National Fish Biosecurity Manual stipulates the official regulation and procedures concerned with Biosecurity and Fish health management in Kingdom of Saudi Arabia.
- The General Directorate of Fisheries of Ministry of Environment, Water and Agriculture (GDF-MEWA) shall be the Competent Authority in Kingdom of Saudi Arabia in dealing with all matters related Biosecurity, Aquatic animal health, emergency preparedness, and to issue Aquaculture Licensing, inspection of Production, approval for imported live aquatic species and issuance of HC for Export.

The General Directorate of Fisheries hereby approves the stated contents of National Fish Biosecurity Manual to be followed in all matters related to the Biosecurity and Fish producton in the Kingdom.

Any interim decision(s) taken by concerned government agencies shall be incorporated in the manual during subsequent revisions.

Director General – General Directorate of Fisheries Ministry of Environment, Water and Agriculture

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1. Introduction

Diseases are part of any livestock production and present a business and sustainability risk. Biosecurity is a tool to reduce the economic impact of diseases and protecting the investment using preventive measures, diagnostic techniques and establishing the contingency plan to act in emergency situations.

Biosecurity must work integrated into the production process with an active and supportive roll. Wellestablished and executed biosecurity measures are key to business and should be considered an investment as their cost can be very low compared to disease outbreaks. The Biosecurity for the marine fish industry in the Kingdom of Saudi Arabia is a priority due to the presence of exotic and endemic diseases, which pose a potential risk for its growing aquaculture industry.

The objective of this Biosecurity Manual is to establish the national guidelines for sustainable fish production under best aquaculture procedures, welfare and environmental care by following the procedures and Standard Operating Procedures (SOP) furnished in this manual.

Below table reflects marine species that are allowed to import and culture in Kingdom of Saudi Arabia as of November 2019 (for more recent updates, please see the Saudi Aquaculture Society).

Sr. No.	Common name	Species name
1	Asian seabass	Lates calcarifer
2	European seabass	Dicentrachus labrax
3	Gilthead seabream	Sparus aurata
4	Silver seabream	Sparidentex hasta
5	Red seabream	Pagrus major
6	Amberjack	Seriola dumerelli
0	Amberjack	Seriola lalandi
7	Tilapia	Oreochromis sp.
8	Meagre	Argyrosomus regius
9	Sobaity	Sparindentex hasta

Table 1: Fish species allowed for importation by KSA government

2. Biosecurity Strategy

Biosecurity strategy should be a combination of pathogen exclusion coming from outside, control of pathogen spread within a zone/establishment and fish health management. Within a commercial scale, there is hardly such thing as a profitable zero risk approach. A realistic approach is to mitigate the risk with practical cost-effective measures.

Main biosecurity risks are related to fish (introduction, movements between zones and harvest), water (incoming water and water management). Live feed control and feed quality control, and



infrastructure/equipment which have been in contact with fish and culture water. The efforts (investments) should prioritize these components.

A qualified and dedicated team of aquaculture specialists / animal health experts under General Directorate of Fisheries Ministry of Environment, Water & Agriculture (GDF-MEWA) is assigned to support the fish production industry on Biosecurity procedural support and execute the National surveillance program, which covers all production stages for all endemic and some of the exotic fish diseases, which are economically important for KSA (*See Annexure #5 Surveillance program*). The team is also responsible to conduct Biosecurity audits of production facilities on a regular basis to verify the biosecurity of the industry compliances with national biosecurity standards. Most importantly, GDF-MEWA Biosecurity team also extends its support to emergency/disease outbreak episodes whenever is required. (See annexure #1 – SAS Organogram and Biosecurity functionalities).

3. National Reference Laboratory

Jeddah Fish Health and Safety Laboratory at Fisheries Research Center in Jeddah is an ISO 140025 certified National Reference laboratory for fish and crustacean diseases. The lab is managed directly by GDF-MEWA. All the samples collected as part of National surveillance program are analyzed here. The lab includes a comprehensive range of services from basic water quality parameters analysis to molecular diagnostics like Polymerase chain reaction (PCR) and also histopathological analysis of both fish and shrimp samples. The National Reference laboratory also participates and garnishes perfect score in inter-laboratory proficiency test conducted annually by OIE reference laboratories.

4. Use of Specific Pathogen Free (SPF) stocks

Use of SPF stocks is key point to successful and sustainable aquaculture production. SPF animals offer an advantage to a country introducing a species as it offers some assurance that the imported animals will not introduce the listed pathogens to native species. Only SPF animals from authorized suppliers will be permitted for import for aquaculture activities in KSA. GDF-MEWA together with SAS, listed authorized SPF suppliers for different fish species. *(See Annexure #3 Procedures for importation/introduction of live fishes to KSA for aquaculture purposes)*. This list is updated upon request of the stakeholders after audit and approval by MEWA. Samples from all the stages of fish production must be analysed to monitor and ensure the SPF status of the stocks.

Based on the species cultivated in the Kingdom, there are a series of known pathogens which are targeted within this biosecurity strategy. Some of these are considered primary pathogens and the aim is to exclude them from the system. Secondary pathogens are part of the normal micro flora and can be dealt with through best aquaculture practices and mitigation measures such as prophylactic treatment and vaccination.



5. KSA listed pathogens

		NACA	GDF-	
Disease	OIE listed	listed	MEWA	Status in
	(2018)	(2018)	listed	KSA
Epizootic haematopoietic necrosis disease	Yes	Yes	Yes	Absent
Epizootic ulcerative syndrome, EUS (Aphanomyces	Yes	Yes	Yes	
invadans)				
Infection with Gyrodactylus salaris	Yes	Yes		Absent
Koi herpesvirus disease, KHV	Yes	Yes	Yes	Absent
Red sea bream iridoviral disease	Yes	Yes	Yes	Present
Viral haemorrhagic septicaemia, VHS	Yes	Yes	Yes	
Big Belly syndrome, BBS	No	No		Present
Streptococcosis	No	No		Present
Viral nerval nerosis, VNN	No	Yes	Yes	Present
Tilapia Lake virus, TiLV	No	Yes	Yes	Absent

The major marine fish diseases listed by OIE, NACA and GDF-MEWA and their status in Kingdom of Saudi Arabia are provided in the below table:

(See annexure #13 - Major fish diseases their prevention and treatment).

A routine quarterly Biosecurity workshop is conducted with representatives from all the fish production companies in the kingdom also officials from SAS and GDF-MEWA. All the companies are required to present their production and sanitary status for the last quarter and discussions on major issues conducted to find out the need for support. SAS will also present the results of national surveillance program for the previous quarter to update the national sanitary status. Biosecurity experts are also invited to share information on relevant biosecurity issues and sustainable production, which help to create awareness.

All operations conducted during farming take into consideration the welfare of the animals which is reflected in the productivity performance. All Standard Operation Procedures in fish production units must consider proper welfare (including humane slaughter) and safety aspects.

Saudi Aquaculture Biosecurity Technical Group, SABTG is a committee formed by SAS Biosecurity team and representatives from industry and MEWA. A technical meeting is arranged quarterly where biosecurity concerns are discussed and also to review the strategies and progress being employed in the biosecurity program is reviewed and, where necessary, improvements to the system.

The objectives of the SABTG are:

• Approve changes to the biosecurity plan

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- Critical review of biosecurity actions and outcomes in accordance with the agreed biosecurity strategies
- Recommend improvements in the program and adaptation to the new situations.

6. Approval certificate for animal movement

Movement of live aquatic animals presents a high risk of pathogen introduction and spread. Therefore, every movement of live fish needs to be accompanied of an approval that includes endemic listed pathogens in KSA.

In order for GDF-MEWA to issue a live animal movement certificate inside KSA, the below mentioned necessary information must be followed.

- I. Written request from the end user at least 5 days prior to the movement.
- II. Must include the following information
 - a. Description of to/from the fish will be moved
 - b. Date of the movement
 - c. Species and stage
 - d. Origin of the stock
- III. PCR results for endemic pathogens from the National Reference laboratory.
- IV. Only stocks that are negative for endemic pathogens are allowed to be moved.

7. Risk ranking levels

In any aquaculture production system, different activities involve different levels of risk to the business. Based on this, functional areas are ranked by their level of business risk impact starting from the highest risk to the lowest risk and prioritized within their risk category.

Priority	Business Impact Risk Categories							
Level	High Risk	Moderate Risk	Low Risk					
1	Quarantine	Pre-shipment cages	Processing plant					
2	Brood stock facilities	Off shore cages	Transit areas					
3	Hatchery (Larval rearing)	Fish ponds	Accommodations					
4	Live feeds	Outdoor tanks	Laboratory					
5	Nursery							
6	Pre-grow out							
7	Harvest							



8. Geographic Zoning

Based on risk ranking, three zones are established following the same rational. Movement of staff, vehicles and equipment within the same zone or lower risk is unrestricted while movement to zones of higher risk should not be allowed.

- Zone 1: High Risk : Quarantine, brood stock facilities, hatchery (larval rearing), Live feeds, Nursery, Pre-grow out, and harvest process
- ✓ Zone 2: Moderate Risk : Pre-shipment cages, off shore cages, fish ponds
- ✓ Zone 3: Low Risk : Processing plant, site accommodation, laboratories, transit roads and township
 ✓ (See annexure # 2 Satellite map of fish farms in KSA with zone info).

9. Biosecurity Requirements

The following table describes the key biosecurity requirements for each risk level and relevant areas. **M**: mandatory; **R**: Recommended; **N/A**: not applicable; *: unless massive mortalities

		High Risk Moderate Risk						Moderate Risk		Low Risk		
	Quarantine	Brood stock facilities	Hatchery (Larval rearing)	Live feed	Nursery	Pre-grow out	Harvest	Pre-shipment cages	Offshore cages	Processing plant	Transit areas	Township
Primary pathogen free status	м	м	м	м	м	м	N/A	N/A	N/A	N/A	N/A	N/A
Water treatment (UV/Ozonation)	м	м	м	м	м	R	N/A	N/A	N/A	N/A	N/A	N/A
RAS	R	R	R	R	R	R	N/A	N/A	N/A	N/A	N/A	N/A
Indoors	м	м	м	м	м	R	N/A	N/A	N/A	N/A	N/A	N/A
Covered / enclosed	N/A	N/A	N/A	N/A	N/A	N/A	N/A	R	R	N/A	N/A	N/A
Effluent treatment	м	м	м	м	м	R	N/A	N/A	N/A	м	N/A	N/A
Solid wastes treatment	м	N/A *	N/A *	N/A *	N/A *	N/A *	м	М	м	м	м	м
Restricted access	м	м	м	м	м	м	м	М	R	R	N/A	N/A
Eggs disinfection	N/A	м	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
vaccination	N/A	N/A	N/A	N/A	N/A	R	N/A	N/A	N/A *	N/A	N/A	N/A
Periodic dry out	м	м	м	м	м	м	N/A	N/A	N/A	N/A	N/A	N/A

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Fallow period (one month)	N/A	R	N/A	N/A	N/A	
Periodic nets cleaning	N/A	М	м	N/A	N/A	N/A

Water treatment described above does not apply to borehole fresh water which is expected to be free of pathogens.

The following highlights the specific biosecurity requirements for each risk level and relevant aquaculture activities.

9.1 High Risk Activities

All activities within the high risk category must be indoors and operated under strict biosecurity measures.

9.1.1 Quarantine

Introduction of any aquatic animals into KSA are allowed only from GDF-MEWA certified hatcheries (see Annexure #3 Procedures for importation of live fishes to KSA). The scope of this facility is to validate that the animals introduced into Kingdom are pathogen free before transferring them to production. This is achieved by performing suitable analysis for OIE listed pathogens and other known pathogens. Quarantine is performed indoors (water treatment), all effluents must be treated and solid wastes incinerated/buried (see Annexure #4 Quarantine protocol)

Disease surveillance is done per batch (see annexure # 5 – Surveillance program and Procedure) and using the most sensitive methods (Annexure #7 - Animal Health Monitoring Procedure).

The next risk mitigation frame must be followed for the movement of aquatic animals into and within the country, and complying with national legislation requirements:



The prequalification of supplier is achieved by a successful biosecurity audit conducted by representatives from SAS, GDE-MEWA with the help of a disease expert (*see Annexure #3* Procedure for importation of live fishes to KSA). All broodstock must be vaccinated against relevant pathogens as part of the targeted eradication program.

9.1.2 Brood stock and egg production

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Considering that broodstock has gone through quarantine, incoming water must be treated; also, feeds are the major biosecurity risk at this stage. Use of thrash fish as broodstock feed is not allowed as they can carry diseases and pass it to broodstock. Hence, the use of dry/semi moist feed is necessary to avoid introduction of diseases.

Dead brood stock must be analyzed, then chopped and kept in formic or acetic acid for 24 hours before final disposal in a well-framed pit far from production units. All mortality disposals must be done in properly avoiding any possible contamination. All discarded eggs must be neutralized with formalin or chlorine before elimination (*Annexure #10 Handling mortalities, organic residues and silage*).

Eggs must be disinfected before being transferred (formalin, iodine, etc.; Annexure #11 - Recommended eradication and disinfection procedures & treatments)

9.1.3 Larval Rearing, nursery and pre-grow out and grow out

Production is performed indoors; all incoming water must be treated to avoid pathogen introduction. Fresh feeds used are to be tested regularly prior to feeding.

In case of primary pathogen detection or high mortality, the contingency plan should be followed (*see annexure #09 - Contingency Procedure*).

Handling of animals (grading, vaccination or transfer) must be carried out under best aquaculture practice procedures and considering the welfare of the animals. The vaccination against relevant diseases is done routinely. A period of 10 days for recovering after vaccination should be respected prior to the transportation to off shore operations *(See annexure #12 Fish Health Management – Vaccination, Antibiotic and Formalin treatments)*.

Dead larvae and juveniles must be treated with disinfectant (formalin or chlorine 50 ppm) prior to their release on the effluents. Biological solid wastes from both nursery and pre-grow out must be treated with formic or acetic acid before final disposal in a well framed pit far from production units.

Records of tank traceability after grading in nursery and pre grow out should be kept.

9.1.4 Live feeds production

Live feed production is performed indoors and all incoming water must be treated to avoid pathogen introduction. All biological material should be treated with disinfectant (formalin, chlorine, etc.) before final disposition.

The microbial quality of live feeds (rotifers and Artemia cysts, Nauplii and biomass) is the key for hatchery performance. Hence, all the measure should be taken to avoid bacterial contamination (e.g.



Vibrio). Routine microbiological analysis should be performed to verify the quality of the live/fresh feed prior to feeding.

9.1.5 Biological wastes treatment from processing plant and laboratory

Biological effluents must be properly treated, and all waste materials must be disposed in a bio-secured manner. Solid wastes from processing plant that can be used for rendering; chitin production, shrimp/fish meal or silage needs to be handed cautiously to prevent any spread of pathogens within the facilities or to the environment.

Solid wastes from laboratory services are categorized as high risk and should be handled cautiously; it must be incinerated or buried with chlorine

9.1.6 Harvest

Harvest is an activity that presents high risk of spreading pathogens. Bins for transporting fish must be cleaned and disinfected with Chlorine (50 ppm) in the processing plant before sending them to off shore. Any equipment that gets in contact with fish during the harvest process must be cleaned and properly disinfected with Chlorine at 50 ppm.

Harvest process on field must be performed taking into consideration the welfare of the animals, best aquaculture practices and safety procedures. In case of bleeding fish is performed, blood must be properly contained. Before final disposal all water waste recovered must be properly disinfected.

9.1.7 Laboratory Services

Movement of laboratory staff, tools and equipment towards production areas are only allowed under clear disinfection and biosecurity procedures. As much as possible, fixed samples should be submitted to the lab to prevent contamination.

9.2 Moderate Risk

9.2.1 Offshore cages

Offshore cages are operated outdoor and therefore pathogen exclusion is not achievable.

Mortality needs to be removed on a daily basis, separated by primary and visual causes, and recorded. Samples to be collected based on surveillance program and whenever there is clinical signs/suspect of infectious diseases. Divers involved in mortality collection must disinfect the tools and the diving suit between cages to avoid possible contamination, always start with the cage with lower mortality (normal) according to the last register. In case the mortality is uniform, divers should start with the cage with the youngest animal.



In case any symptomatic animals are found, it must be collected for diagnostic purposes as early as possible (*See Annexure #11 - Animal Health Monitoring Procedure*).

In case of high level of mortality, the contingency protocol must be applied within the shortest possible time frame to minimize the risk of disease spreading and economic losses (annexure #09 - Contingency Procedure). This procedure could consider the use of drugs, emergency harvest (depending on the commercial value of the stock and only if the antibiotic withdrawal period is already finished) or slaughtering (annexure #10 Handling of mortalities, organic residues and silage).

All mortality at off shore farms must be treated with formic acid under silage process and must be delivered for final disposal in sealed containers with no leaking.

All materials used during fish handling as counting, sampling, grading, net changing, mortality removal, must be carefully cleaned and disinfected. All materials and equipment movement must be disinfected in the origin (*Annexure #11 - Recommended eradication and disinfection procedures & treatments*).

9.2.2 Stocking

All fish transferred from Pre-grow out to off shore farms must be moved considering their safety and welfare. All tanks, landing craft and trucks must be properly disinfected before and after fish movements. All materials used in the stocking process must be cleaned and disinfected as well *(Annexure #11 - Recommended eradication and disinfection procedures & treatments).*

9.3 Low Risk

9.3.1 Processing Plant

Vehicles delivering seafood raw material must be disinfected after delivery and before loading ice in the case of harvest vehicles. The same applies to harvest equipment before going back to the farms and after each harvest.

Wastes from processing and harvesting are categorized as high risk should be contained and disinfected before release.

9.3.2 Site accommodations

Movement of staff, catering goods and other housing related items are permitted within site accommodations, however depending on the biosecurity status of the unit, the site access can become restricted.

9.3.3 Township



Township is an unrestricted access area except for vehicles transporting seafood and live aquatic animals which must have a pass and must be inspected before entering to the premises

9.3.4 Transit areas

These buffer zones are used by the different operators, visitors and external services.

Biosecurity gates are placed at strategic locations and act as check points to ensure restricted vehicle and staff access. Biosecurity gates can be operated for vehicle disinfection.

10. Movement of vehicles and staff between biosecurity zones



Drivers are responsible to ensure that people, equipment or goods transported in their vehicles have proper biosecurity clearance.

Requirements regarding movement of staff outside their zone of operation:

Movement of sta	ff between zones	Access		
From	То			
Zone 1	Zone2	Forbidden		
Zone 2	Zone 1	Only for fish delivery and restricted to outside the hatchery		
Zone 3	Zone 1 or 2	Only for service purposes		
Zone 1 or 2	Zone 3	Unrestricted except to processing plant, laboratory and harvest		
		areas		

Requirements regarding movements of people within a production unit:



Always proceed from the cleanest area (lowest biosecurity risk) to the dirty area (highest biosecurity risk).

• All production units



11. Biosecurity Status of production units:

As soon as a production unit starts operating, a flag is recommended to be placed at the entrance in order to communicate the biosecurity status of the unit.

The unit production manager is responsible for communicating to the biosecurity department as soon as an abnormality is detected and for raising the biosecurity status of his unit to match the corresponding alarm level.

The biosecurity department will immediately dispatch a team onsite to support the unit production manager regarding all relevant biosecurity measures.

Biosecurity Status Notification



Normal situation (Green color flag).



Any abnormal situation such as abnormal behavior, clinical signs, or mortalities above standard rates but without confirmation of a pathogen (Yellow color flag).



Detection of pathogens with mortalities or clinical signs (applicable to all zones: Red color flag).



In the case of red flag, all vehicles must undergo disinfection before leaving the affected areas a risk mitigation measure. Annexure #07 - Contingency Procedure must be followed.

In case if any antibiotic treatment is defined following Contingency plan instructions, all treatment applied must be supported by a laboratory result and the treatment must be signed by a veterinarian.

12. Compulsory reporting of disease outbreaks

Diseases are inevitable part of livestock production. Regular monitoring and on time reporting of disease events or unexplained mortalities are key to successful and sustainable farming. Therefore, it is mandatory to report immediately to GDF-MEWA in case of any disease outbreaks are encountered during production. A technical team will visit from GDF-MEWA to conduct sampling and to provide technical support to the effected production units. It includes initiation of contingency plan for containment of the outbreak in order to avoid/minimize the risk of spread of disease to other farms

13. Emergency response and Contingency plan

Early detection and responsive actions are critical for the containment of disease outbreak to minimize the economic impacts of diseases. A well-established and practiced clear emergency/contingency protocols should be developed and understood by all technical staff to avoid mistakes or delays during disease outbreak. There should be clearly defined triggers for identifying emergency situation for the activation of contingency protocols. Simulation of contingency protocols is recommended annually.





What	Who	How	
1- Investigation	GDF-MEWA		
	Biosecurity Supervisor • In-house Biosecurity team	Investigate the incident (conduct re-sampling, traceability, field diagnostics, disease history and mortality trend)	
	Production team	Treatment : In case the decision is treatment follow veterinerian advise Harvest / Elimination: initiate contingency protocol	
2- Information Outbreak	GDF-MEWA Biosecurity Supervisor	E mail to all internal and external involved entities	

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Mortality Management of Stock fish Elimination

What	Who	How	When	Reference
1. Materials	Production	 Eliminated biomass must be carried in strong and safe containers 	Case-wise	Procedure for
required	team	(IBC/Bins), with no crevices or leaking.		disposal of dead
inspection	 Biosecurity 	 All containers must be properly disinfected before get into 		animals
	team	installations, visual inspection and check enough number according the		
		Biomass to be eliminated		
		• Before start filling the containers with fish, 10 % of Biomass container		
		capacity of Formic acid/Chlorine/ Hydrated lime must be added (*)		
2. Drain the	Production	Once defined the tanks to be eliminated, water level must be drained	Case-wise	Procedure for
tank water	team	according the effluent disinfection capacity of the system		disposal of dead
	• Biosecurity			animals
	team			

3. Animals	Production	• Add anesthetic to reduce stress of the animals, taken in to consideration	Case-wise	Procedure for
removal	unit	of animal welfare.		disposal of dead
	manager	• Using hand nets fish must be moved into buckets to carry the fish to the		animals
	 Biosecurity 	final container, adding the minor amount of water possible		
	team	• When 50 % of total capacity of container is reached, 10 % of total		
		biomass container capacity of formic acid/ Chlorine/Hydrated lime must		
		be added (*)		
		 Water must be constantly drained to increase fish density 		
		• Finish filling the container with fish following the second step		
		Add 10 % of total biomass container capacity of formic		
		acid/Chlorine/Hydrated lime and seal the container		





		• Continue with same procedure until finish to fill all containers and get all tanks empty		
4. Tanks Disinfectio n	 Production team 	 As soon tanks get empty must be perfectly cleaned and disinfected as per the disinfection procedure (*) 	Case-wise	 Procedure for disposal of dead animals Recommended eradication and disinfection procedure

5. Containers movement	 Production team Animal Health Manager 	 Once the truck is loaded, a visual inspection of any leaking must be done Dead fish must be disposed at the designated area (pit). This procedure must be directly supervised The pit must be properly covered to avoid the exposure of eliminated biomass Trucks and containers must be properly disinfected 	Case-wise	 Procedure for disposal of dead animals
6. Materials	 Production team 	 All materials used must be properly disposed 	Case-wise	 Procedure for disposal of dead animals
7. Dry out and disinfection	 Production team 	 All installation must be dried out and disinfected as per disinfection procedure 	• Case-wise	 Procedure for disposal of dead animals Recommended eradication and disinfection procedure

(*) Safe personal protection must be wear



14. Rational use of drugs

Chemicals and antibiotics are useful tools for health management in aquaculture. However, their use has a series of negative secondary effects such as toxicity for the animals and the environment, the development of resistance and also the production cost will be increased. Therefore, their use is minimized at all times and management alternatives that do not require drugs are prioritized. The rational use of chemicals in this document is controlled by the suggesting products, the dosage and way to be used (*See Annexure #11 - Recommended eradication and disinfection procedures & treatments and Annexure #12 Fish Health Management – Vaccination, Antibiotic and Formalin treatments*). In case of more than two antibiotics are used, rotation of drugs is recommended to reduce bacterial resistance.

The reduction of antibiotic usage in diseases control is achieved by efficient disease eradication program, well established and updated surveillance program (*Annexure #10 - Surveillance Program and Procedure*). Antibiotic treatment and vaccination are only used after approval by a veterinarian and following manufacturer recommendations

15. Control of importation of live fish

Importing live aquatic organisms in KSA is subjected to all mentioned regulations in these instructions, which are updated every year. In addition to other regulations that General Directorate of Fisheries in Ministry of Environment, Water & Agriculture has; which follow-up and verify the following:

- 1- Conduct a Biosecurity audit to accredited companies by a technical team from the ministry (GDF-MEWA) with a disease expert to make sure about its technical capabilities, and then choose the best according to KSA requirements. The audit should be performed at least once in two years to verify the sanitary status of the supplier.
- 2- If any company would like to import aquatic organisms from a company or hatchery, not accredited in this regulation, in this case; importer has to submit the application to GDF-MEWA to make a preliminary assessment. Following that, a Biosecurity audit with representatives from GDF-MEWA and disease expert will be done; if the audit is successful the import permit will be granted for a period of 2 years.



15.1 Pre-approved supplier of any live imported aquatic animals

GDF-MEWA publishes the list of pre-approved suppliers various species of live fishes who have successfully completed the Biosecurity audit. The List of the suppliers and the procedures are described in Annexure #3 Procedures for importation/introduction of live fishes to KSA for aquaculture purposes). This list is dynamic and will be updated based on stakeholder triggered interest.

15.2 Quarantine and testing on reception

It is mandatory that live fish introduced to KSA must be from the GDF-MEWA certified live aquatic animal suppliers list. Only these animals will be allowed to enter the quarantine facilities. A primary quarantine is the one to receive the animals from abroad and will hold them until all the required testing has shown not to pose an infectious risk. Samples will be collected by ministry officials and will be analyzed at National Reference Laboratory. A primary quarantine is used for the first introduction of a particular supplier. A secondary quarantine is a private sector quarantine that needs approval from GDF-MEWA before the reception of animals released from the primary quarantine. Based on GDF-MEWA approval, a primary quarantine may be a government or private sector facility. In case that the primary quarantine is a private sector facility, a secondary quarantine might not be required.

Disease	surveillance	for	quarantine	animals u	ipon recei	otion
Discuse	Juivennunee		quarantine	anninais a	ponicee	ption

Production facilities	Animal stage	Target sample	Target pathogen	Frequency	Diganostic method	Min targetted prevalence	No of animals per PCR reaction	# of tanks/ ponds/ cages	No. of animals / sample
	Quarantine Development	Poutine	VNN/VER, Iridovirus	Once per shipment	PCR	2%	10	per shipment	150
Querentine		Koutine	All	Once per shipment	Histology	10%	N/A	per shipment	30
Quarantine			VNN/VER, Iridovirus	Once per shipment	PCR	-	1 for lethal, 5 pooled (faeces) for BBS	per shipment	3 for lethal sampling, 100% for BBS (faeces)
Brood	Broodstock	Routine	All	Once per shipment	Histology	-	N/A	per shipment	3

16. Ban on the use of wild and cage raised broodstock for commercial purposes

Wild or cage raised broodstock are not allowed to be used for commercial aquaculture production. However, they can be used to develop breeding program by domestication after individual screening.



Introduction of wild cage raised fish for breeding

In some cases, if the production industry would like to involve in the production of species which is not available with any certified suppliers and/or would like to develop broodstock from wild population for aquaculture purposes. It is only allowed if the following specific criteria are met.

- ✓ It should go through a primary and secondary quarantine.
- ✓ ELISA analysis (using blood serum) should be done for all the major susceptible diseases of individual fish (100%).
- ✓ Lethal sampling of at least 3 fish should be done to perform PCR (e.g. VNN) and histology to know if there is any unknown pathology.
- ✓ Every batch of larvae/offspring must be tested by PCR for VNN.

17. Restriction on aquatic products based on the SPS agreement of the WTO

Sanitary and Phytosanitary (SPS) agreement of the World Trade Organization (WTO) applies to all sanitary and phytosanitary measures which may, directly or indirectly, affect international trade. Such measures shall be developed and applied in accordance with the provisions of this Agreement. Members shall ensure that any sanitary or phytosanitary measure is applied only to the extent necessary to protect human, animal or plant life or health, is based on scientific principles and is not maintained without sufficient scientific evidence. Based on the SPS agreement each country will have the rights to restrict the importation of food products (seafood also) from countries with sanitary status lesser than importing country. Nevertheless, SPS measures should not arbitrarily or unjustifiably discriminate between members where identical or similar conditions prevail, including between their own territory and that of other Members. Sanitary and phytosanitary measures shall not be applied in a manner which would constitute a disguised restriction on international trade.

Reference for more details: <u>https://www.wto.org/english/tratop_e/sps_e/spsund_e.htm</u>



Annexure # 1 Saudi Aquaculture Society Organogram

A qualified and dedicated team of aqua-culturists/animal health experts under Saudi Aquaculture Society, SAS is created to support the fish production industry on Biosecurity procedural support and execute the National surveillance program. The team is also responsible to conduct Biosecurity audit of production facilities on a regular basis to verify the biosecurity of the industry compliances with national biosecurity standards. Most importantly, SAS Biosecurity team also extends its support to emergency/disease outbreak episodes whenever is required. SAS is directly reports to GDF-MEWA.





Annexure # 2 Satellite view of fish farms in KSA









Ministry of Environment Water & Agriculture المملكة العربية السعودية Kingdom of Saudi Arabia





Annexure #3 Procedures for importation of lives fishes to Saudi Arabia

Fish species allowed being imported:

Fish species that are currently allowed to be imported to the Kingdom for aquaculture purpose is specified in the table below. Stakeholders interested to import a species not recorded in the list have to apply to GDF-MEWA while demonstrating that this species is existing in the Red Sea or the Arabian Gulf or inland waters, its rearing had been proven, and there is an economical feasibility of its farming that justifies risk of importation. The ministry will verify its source and request documents deemed necessary to ward off importation risks to the Kingdom. Stakeholders must also provide sufficient information about the party that desires to import from; on quality and productivity of this source.

The lists of fish pathogens to be tested for are the ones by OIE and MEWA, will be limited to those species which are currently allowed to be imported for aquaculture purpose.

Aquatic organisms importing Regulations

Importing aquatic organisms in KSA is subjected to all mentioned regulations in these instructions, which are subjected to a periodical updating every year, in addition to other regulations that General Directorate of Fisheries in Ministry of Environment, Water & Agriculture has; which follow-up and verify the following:

- 1. Conduct a Biosecurity audit to accredited companies by a technical team from this ministry (GDF-MEWA) with a disease expert to make sure about its technical capabilities, and then choose the best according to KSA requirements. The audit should be performed at least once in two years to verify the sanitary status of the supplier.
- 2. If any company would like to import aquatic organisms from a company or hatchery, not accredited in this regulation, in this case; importer has to submit his application to GDF-MEWA to make a preliminary assessment. Following that, a Biosecurity audit with representatives from GDF-MEWA and disease expert will be done, if the audit is successful the import permit will be given.
- 3. Representative samples must be collected from each batch prior to shipment. The Biosecurity audit team will select laboratories in aquatic organisms exporting countries or neighboring countries (*Annexure #14- Reference diagnostic laboratories (National and International) for testing fish diseases*) and attach analysis results with the consignment documents.
- 4. Two years after the audit import permit of supplier could be updated automatically if there have been at least 4 introductions/year with satisfactory health certification in quarantine.
- 5. Suppliers whose fish have proven to be infected upon reception, will be removed from the authorized import list immediately and only allowed to apply to import after 2 years satisfying the process again



Lists of aquatic organisms supplier companies as of November 2019

Aquatic organisms' supplier companies for aquaculture purpose:

Company	Country	Web Site	Telephone/ Address	Date of approval
Mainstream			61-3-9734-1912	Oct 2010
Aquaculture	Australia	www.mainaquaculture.com	Paul Harrison	Oct-2019
			65 - 9151 2221	
Marine Life	Cinganoro	http://marinelife-	Frank Tan	
Aquaculture	Singapore	aquaculture.com	franktan@marinelife-	
			aquaculture.com	
Australian			61-8-9239-8030	
Centre for			Greg Jenkins	
Applied	Australia	www.challenger.wa.edu.au	Ũ	
Aquaculture			greg.jenkins@challenger.w	
Research			a.edu.au	
Asia Tropic	Theilend	· · · · · · · · · · · · · · · · · · ·	66 - 818692411	
Zone Co. Ltd	Thailand	www.asiatropic.com	info @asiatropic.com	
Sask		www.cask.com.au	61(0) 415-960-349	
International	Australia	www.sask.com.au	Steven Kons	
Seafood			Sk@sask.com.au	

Asian Sea bass (*Lates calcarifer*)

Gilthead Sea bream (Sparus aurata)

Company	Country	Web Site	Telephone/ Address	Date of approval
Selonda	Greece	www.selonda.com	30 - 210 37 24 900	
Nireus	Greece	www.nireus.com	30 - 210 66 98 145	
Kilic				
Aquacultur	Turkey	www.kilicdeniz.com.tr	90 - 252 559 0283	
е				
Sagro		Anyiou Andreou Street		
Aquacultur	Cyprus	Postal Code 3508,	26 - 999220296	Jul-2017
e ltd.		Limassol		
Ferme				
Marine de	France	www.douhet.com	33 (0)5 46 76 58 42	
Douhet				
Andromeda	Greece	www.adromedagroup.eu	30 - 210 664 0963-7	Jul-2017



Company	Country	Web Site	Telephone/ Address	Date of approval	
Selonda	Greece	www.selonda.com	30 - 210 37 24 900		
Nireus	Greece	www.nireus.com	30 - 210 66 98 145		
Kilic	Turkey	www.kilicdeniz.com.tr	90 - 252 559 0283		
Aquaculture	Turkey	www.kiiicueiiiz.coiii.ti	90 - 232 339 0283		
Sagro		Anyiou Andreou Street			
Aquaculture	Cyprus	Postal Code 3508,	26 - 999220296	Jul-2017	
ltd.		Limassol			
Andromeda	Greece	www.adromedagroup.eu	30 - 210 664 0963-7	Jul-2017	

European seabass (*Dicentrachus labrax*)

Amberjack (Siriola Dumerili and Seriola lalandi)

Company	Country	Web Site	Telephone/ Address	Date of approval
Futuna Blue	Spain	www.futunablue.com	34 - 956 560 657	
Universidad de las Palmas Gran Canaria	Spain	www.giaqua.org	34 - 928 132 900	
Acuicola del Norte S.A Hatchery "punta sin Nombre"	Chile	www.acuinor.cl	+56 9 6308 3413 Juan Enrique Gaete L. <u>Jeg@acuinor.cl</u>	
University of Miami	USA	www.rsmas.miami.edu	786-553-5557 Daniel Benetti dbenetti@rsmas.miami.edu	

Silver sea bream (Sparidentex hasta) supplier companies

Company	Country	Web Site	Telephone/ Address	Date of approval	
Kuwait					
Foundation					
for	Kuwait		+965 2227 8100		
Advancement	Kuwali	www.kfas.org			
of Science					
(KFAS)e					
National					
Aquaculture	Bahrain		+97336088288	Mar-2019	
Center					



Company	Country	Web Site	Telephone/ Address	Date of approval	
Til-Aqua	Holland	www.til-aqua.com	+31 493470225	Oct-2018	
International	Tiolianu	www.th-aqua.com	+31 493470225	000-2018	
Asia Tropic	Thailand	www.asiatropic.com	66 -818692411		
Zone Co. Ltd	Thananu	www.asiati.opic.com	00-010092411		
			941-744-9698		
AquasafraInc	USA		Mr. Mike Picchietti		
			picchietti@aol.com		
White Brook	USA	www.tilaniacourco.comw	816-866-1172		
Tilapia Farm	USA	ww.tilapiasource.comw	info@tilapiasource.com		
Genomar As	Norway	www.genomar.com	47-22341000		

Tilapia (Oreochromis spp.) supplier companies



Biosecurity audit checklist for live aquatic animal supplier pre-qualification

Fish Hatchery Audit for compliance with GDF-MEWA requirements for fry/juvenile exports by a third country hatchery and import

Audit Result					
Compliance with ADMOEWA/SAS requirements and standards for Yes No					No
import of juveniles to the KSA					
Hatchery is app	Hatchery is approved for export of marine fish juveniles to the				
KSA					
Name	Auditor 1	Auditor 2		Auditor	3

Company name:				
Hatchery name:				
Audit Conducted by :				
Date:				
Signature				
Hatchery deta	ails			
Country				
Location				



Full Company name and contact details	
Unit/ Facility Name and contact details	
Approval code	
(Veterinary or other relevant	
National Authority)	
Details of relevant	
National Authority	
Hatchery and juveniles certified by GLOBALG.A.P. or B.A.P. standards	
Total Annual Production Capacity (licensed)	
Total Annual Production (last 3yrs AVG)	
Species produced	
(Scientific Name & Commercial	
Name)	

Г

I	Licensing	Yes	No	N/A	Notes/ Comments
1	Valid Operating License				
2	Valid Environmental License				
3	Valid Veterinary License				
П	Infrastructure	Yes	No	N/A	Notes/ Comments
1	Independent maturation facility				
2	Broodstock back up facility				



3	Independent spawning /hatching facility				
4	Independent larval /post larval facility				
5	Artemia Facility				
6	Indoor algae/strain room				
7	Algal culture facility				
8	Nuursery facility				
9	Quarantine facility to receive breeders				
III	Sea water supply and treatment VALIDATION	Yes	No	N/A	Notes/ Comments
	system				
1	Type of water source				
2	Rapid Sand filters and carbon filters				
3	Reservoir facility for disinfection activities				
4	Cartridge filtration facility				
5	Ultraviolet provision				
6	Ozone provision				
7	Records of regular water quality analyses				
	(inflow, outflow)				
8	Effluent water treatment				
IV	Feed, Raw material and Storage	Yes	No	N/A	Notes/ Comments
1	Records of feeds and Raw materials used				
2	Details of feed ingredients provided by the supplier				
3	Live feeds PCR testing for primary pathogens (VNN, Iridovirus)				
4	Bio secure Broodstock feed (no trash fish)				
5	Feed and RM supplier pre-qualification system				
	in place				
6	Specifications for feed and raw material				
	<i>If yes, check records for conformity of purchased to specifications</i>				


7	Residue monitoring program in place				
	If yes provide the details whether residue				
	analysis report available for the products				
	purchased. are feed & RM analyzed for				
	presence of antibiotic residue, pesticides, PCB				
	and dioxins				
8	If feed contains drugs, is the withdrawal				
	period mentioned, followed and documented.				
	Feed tested/declared by the supplier free of				
9	potential pathogens				
	Fresh feed used is tested for pathogens.				
10	Storage facility for formulated feeds /Artemia				
	Storage facility for Frozen feed (ie squid) in - 18'C				
	Feeds are stored in labeled packing. Label				
	contains at least name of the product, product				
	composition, lot/batch number and shelf life.				
11	Separate storage area for				
	medicines/chemicals				
	If Yes, check for records of invoices, product				
	details and mode of use.				
V	Origin of Broodstock	Yes	No	N/A	Notes/ Comments
1	Source of Broodstock				
	Does the facility have a system to check the				
	specific pathogen free status of BS?				
2	Selected Breeding Program				
	<i>If yes, give details (how many generations, years)</i>				
3	Records of antibiotic treatment of broodstock,				
	larvae and juveniles for the last 2 years				
VI	Quality Control & Traceability system	Yes	No	N/A	Notes/ Comments
1	Broodstock – offspring traceability system				
2	Feed traceability for each batch				
3	Disease & treatment traceability for each batch				
4	Batch performance records (avg. growth, disease, FCR)				



	Quality constant output for for /in cosiles							
5	Quality control system for fry/juveniles							
	produced							
VII	Biosecurity and Disease Prevention		Notes/ Comments					
1	Detailed biosecurity plan and SOP (e.g.							
	Biosecurity Manual, SOPs for quarantine,							
	contingency, health management)							
2	Staff familiar with biosecurity plan and SOP's;							
	is there any specific training program for the							
	responsible staffs?							
3	Independent staffing for every unit							
	Usage of uniforms, boots/Foot Dip							
	Facility for hand wash							
	Personal safety equipment's							
4	Surveillance Program covering all the critical							
	stages of production							
	Sanitary history (Records of detections,							
	diseases & treatments (min last 2 years))							
5	Records of antibiotics and other treatments							
	used.							
	(If yes provide the details(Name of the							
	product, dosage and frequency of application))							
VIII	Diagnostic Capacity - Laboratory Analyses &	Yes	No	N/A	Notes/ Comments			
VIII		163			Notes/ comments			
	Checks for Pathogens							
1	Diagnostic Laboratory facility							
	If yes, for what type of analyses							
	· · · · · · · · · · · · · · · · · · ·							
2	Veterinary Support							
3	System to check for pathogens of fry/juveniles		1		•			
	before selling/ exporting in accordance to	No						
	listed diseases/ pathogens in ANNEX I							
	FILL TABLE I							
IX	Customer Evaluation of Supplier	Yes	No	N/A	Notes/ Comments			
1	Supply of fry in KSA farms in the past							
2	If yes, Customer(s) satisfaction level	High	Medium	Low				



CONSULT TABLE 2		

TABLE 1: List of Pathogens tested

Species Name						
	Che	ecked	Refere	nce Lab	Freework	Notes/
Disease / Pathogen	Yes	No	Yes	No	Frequency	Comments



TABLE 2: Customer Satisfaction Form

	Country					
Supplier Name	Company N	ame				
(exporter)	Hatchery na	ame				
	Location of Hatchery					
Customer Name (importer)						
Species Imported	l/received		Quar	ntities		Date(s)
Customer Satisfact	ion Rating	High	Mediu m	Low	Notes / O	Comments
Supplier cooperation Customer for provisor relevant export documentation						
Supplier's service q (order, delivery, fol	-					
Accuracy of delivered quantities						
Growth performance of imported fry/juveniles						
Health performance imported fry/juven						
OVERAL SATISFACT RATING	ION					



List of Fish Diseases & Pathogens (indicative)

		Re	efer	enc	e Sp	ecie	s*	
Disease / Pathogen	1	2	3	4	5	6	7	8
Red Seabream Iridoviral Disease - Iridovirus	X X X X V V X							
Iridovirus Infection					Х	Χ		Χ
Epizootic ulcerative syndrome – Aphanomyces invadens	Х							
Big belly syndrome	Χ	Χ						
Viral encephalopathy and retinopathy (VER) or Viral nervous necrosis (VNN) – Nodavirus	X	X	X	X	X	X	X	
Bacterial gill disease (BGD) – Flexibacter	Х							
Microcotylid infection by Sparicotyle chrysophrii		Χ						
Streptococcosis – Streptococcus iniae	Χ	Χ	Χ	Χ	Х	Χ	Χ	Χ
Streptococcosis – Streptococcus agalactiae			Х	Χ	Х	Χ		Χ
Streptococcosis – Streptococcus, parauberis				Χ	Х	Χ		
Vibriosis – Vibrio sp	Χ	Χ	Χ	Χ			Χ	Χ
Vibrio anguillarum			Χ		Х	Χ	Χ	
Vibrio ordalli			Χ					
Vibrio harveyi			Χ					
Aeromona hydrophila						Χ		
Winter Disease Syndrome (WDS) – Pseudomona anguilliseptica		Χ	Χ				Χ	
Pancreatic – hepatic necrosis – Yellowtail Ascite Virus (YAV)					Χ	Χ		
Nocardia – <i>Nocardia kampachi</i>					Χ	Χ		
Benedenia seriolae – parasite in skin					Χ	Χ		
Ichtyosporidium sp – parasite in skin					Χ	Χ		
Axine heterocerca – parasite in gill					Χ	Χ		
Cryptocaryon irritans – parasite in gill					Χ	Χ		
Amoeba like - parasite in gills						Χ		
Amyloodinium ocellatum							Χ	
Gyrodactylus sp.							Χ	
Francisella sp								Χ

* Reference Species

- 1) Barramundi Asian Seabass (Lates calcarifer)
- 2) Mediterranean / European Seabream (Sparus aurata)
- 3) Sobaity Seabream (*Sparidentex hasta*)
- 4) Grouper species (Family Serranidae)
- 5) Greater amberjack Seriola dumerellii
- 6) Pompano fish *Trachinotus genus (T.africanus* Southern pompano, *T. baillonii* Smallspotted dart, *T. blochii* Snubnose pompano)
- 7) Meagre Argyrsomus regius
- 8) Tilapia Oreochromis niloticus

The information gathered during the audit will be assessed by the biosecurity experts who may require further information and will advise MEWA on the final recommendation for MEWA decision.



Annexure #4 - Quarantine procedures

Introduction of fish from abroad

It is mandatory that live fishes introduced to KSA must be from the GDF-MEWA certified live aquatic animal suppliers list. Only these animals will be allowed to enter the quarantine facilities. A primary quarantine is the one to receive the animals from abroad and will hold them until all the required testing has shown not to pose an infectious risk. A primary quarantine is used for the first introduction of a particular supplier. A secondary quarantine is a private sector quarantine that needs approval from GDF-MEWA before the reception of animals released from the primary quarantine. Based on GDF-MEWA approval, a primary quarantine may be a government or private sector facility. In case that the primary quarantine is a private sector facility, a secondary quarantine might not be required.

Introduction of wild fish as broodstock

In some cases, if the production industry would like to involve in the production of species which is not available with any certified suppliers and/or would like to develop broodstock from wild population for aquaculture purposes. It is only allowed if the following specific criteria are met.

- > It should go through a primary and secondary quarantine.
- ELISA analysis (using blood serum) should be done for all the major susceptible diseases of the selected fish species (100%).
- Lethal sampling of at least 3 fishes should be done to perform PCR (e.g.-VNN) and also for histology to known if there is any unknown pathology.

First level quarantine facilities for imported live fish from an GDF-MEWA certified supplier

- 1. A request for quarantine must be filled up and sent to GDF-MEWA for approval.
- 2. It is recommended that the facility to be adequately isolated from all of the rearing and production areas to avoid any possible cross contamination.
- 3. It must be in an enclosed and **covered** building facility.
- 4. There must be means provided for disinfection of feet (foot dip containing hypochlorite solution at 50 ppm active ingredient/ or any other disinfectant) and hands wash facility (bottles containing 70% alcohol /iodine solution at 100 ppm) to be used upon entering and exiting the unit.
- 5. Entrance to the quarantine area must be restricted to the personnel assigned to work exclusively in this area.



- 6. Quarantine unit staff must enter through a designated room, where they put on working clothes and boots specific for the quarantine. At the end of the working shift, the sequence is reversed.
- 7. Pumped water will go through a mechanical filtration including sand filter to obtain the water filtered to at least 1 um.
- 8. Water disinfection with either 15 ppm (72 h),30 ppm chlorine (24 h) or ozone at 0.5 mg/L (10 minutes contact time; 8 minutes for 1.5 ppm) any other disinfectant to ensure proper water disinfection would be used and must be validated by bacteriology in a general media.
- 9. Water will pass through activated charcoal unit.
- 10. Water will pass through UV light at 10 ml/cm².
- 11. All the tanks must be washed with soap and water and disinfected with hypochlorite solution (100 ppm active ingredient) and rinsed with disinfected water.
- 12. All wastewater must be collected for chlorination (100 ppm for not less than 1 day) and dechlorination before released to the environment.
- Used plastic containers and hoses must be washed and disinfected with hypochlorite solution (100 ppm) or other disinfectants at an equivalent concentration, before reuse.
- 14. All the materials used in the quarantine unit must be clearly marked and should remain in the quarantine area. Facilities for disinfection of all equipment at the end of each day should be available.
- 15. On entering the quarantine area, the fish should be gradually acclimatized to the same temperature, salinity and pH of the tanks.
- 16. Once the fish are stocked in the holding tanks, the packing plastic bags, boxes, and any other disposable material related to the shrimp packing must be incinerated. Styrofoam boxes could be disinfected at 200ppm chlorine and allow to dry for 5 days.
- 17. Dead fish will be sampled for PCR for relevant pathogens. All GDF-MEWA listed pathogens must be tested. Organs/whole from each dead shrimp will be individually preserved in 95% ethanol (See Annexure #6 Sample preparation procedure for diagnostics).
- 18. Any symptomatic fish will be fixed in Davidson's fixative/10% buffered formalin. (See Annexure #6: Sample preparation procedure for diagnostics) for histopathological analysis.



- 19. Samples must be sent to National reference lab or GDF-MEWA approved laboratories. (See Annexure# 14 Reference diagnostic laboratories (National and International) for testing fish diseases)
- 20. In the case that any sample gives a positive result for any of the GDF-MEWA listed diseases (See table 3) or display histopathological changes which are not recognized and might pose concern, the whole population will be discarded.
- 21. Only fish stock free of any of the GDF-MEWA listed diseases by PCR and histology would be taken to the secondary quarantine.
- 22. Only commercial dry pellet feed is given to fishes once stocked in quarantine.

Second level quarantine

- 1. This quarantine has the same requirements in terms of infrastructure and water treatments as a primary quarantine. The second level quarantine could be a private sector quarantine approved by GDF-MEWA that could maintain fish if the following requirements are met:
 - a. Fish stock from a primary quarantine that met all the health sanitary status required by GDF-MEWA.
 - b. Fish stock from an approved secondary quarantine.
 - c. In secondary quarantine, samples for PCR and histology analysis will be taken and only if the animals are free of the GDF-MEWA listed disease, the stock would go to production area.

The check list for the quarantine is described below.

	Quarantine unit Checklist							
Т	Infrastructure	Yes	No	N/A	Notes/ Comments			
1	Independent QUARANTINE facility							
2	Availability of independent nets/buckets/tools for each section							
П	Sea water supply and treatment VALIDATION system	Yes	No	N/A	Notes/ Comments			
1	Water source							
2	RAS System water cycle							
3	Water parameter equipment(water temperature, PH, salinity)							
4	Rapid Sand filters and carbon filters (back wash record)							



				T	
5	Reservoir facility for disinfection activities record				
6	Cartridge filtration facility(change the cartridge record)				
7	Ultraviolet provision (total house operated record)				
8	Ozone provision – Microbiology test -Bromine residue analysis				
9	Records of regular water quality analyses (inflow, outflow water treatment, temperature , pH, Salinity)				
Ш	Feed, Raw material and Storage	Yes	No	N/A	Notes/ Comments
1	Records of feeds used				
2	Feed tested/declared by the supplier free of potential pathogens				
3	Storage facility for formulated feeds (check the storage condition – temperature, expirer date)				
4	Feeds are stored in labeled packing. Label contains at least name of the product, product composition, lot/batch number and shelf life.				
5	Separate storage area for chemicals(check the storage condition – temperature, expirer date)				
					_
IV	Quality Control & Traceability system	Yes	No	N/A	Notes/ Comments
IV 1	Quality Control & Traceability systemFry , fish –traceability system	Yes	No	N/A	Notes/ Comments
		Yes	No	N/A	Notes/ Comments
1	Fry , fish –traceability system	Yes	No	N/A	Notes/ Comments
1 2	Fry , fish –traceability system Disease & treatment traceability for each batch	Yes	No	N/A	Notes/ Comments
1 2 3	Fry , fish –traceability system Disease & treatment traceability for each batch Batch performance records (avg. growth, disease)	Yes			Notes/ Comments
1 2 3 4	Fry , fish –traceability system Disease & treatment traceability for each batch Batch performance records (avg. growth, disease) Daily dead fish Recorded Biosecurity and Disease Prevention Detailed biosecurity plan and SOP (eg: Biosecurity	Yes			
1 2 3 4 V	Fry , fish –traceability system Disease & treatment traceability for each batch Batch performance records (avg. growth, disease) Daily dead fish Recorded Biosecurity and Disease Prevention	Yes			
1 2 3 4 V 1	Fry , fish –traceability systemDisease & treatment traceability for each batchBatch performance records (avg. growth, disease)Daily dead fish RecordedBiosecurity and Disease PreventionDetailed biosecurity plan and SOP (eg: Biosecurity Manual, contingency, health management)Staff familiar with biosecurity plan and SOP's; is there any specific training program for the	Yes			
1 2 3 4 V 1 2	Fry , fish –traceability systemDisease & treatment traceability for each batchBatch performance records (avg. growth, disease)Daily dead fish RecordedBiosecurity and Disease PreventionDetailed biosecurity plan and SOP (eg: Biosecurity Manual, contingency, health management)Staff familiar with biosecurity plan and SOP's; is there any specific training program for the responsible staffs?Independent staffing for every unit (Method of control of movement of men and materials in the	Yes			



6	Surveillance Program				
VI	Diagnostic Capacity - Laboratory Analyses & Checks for Pathogens	Yes	No	N/A	Notes/ Comments
1	Diagnostic Laboratory facility If yes, for what type of analyses				
2	Veterinary Support				

GDF-MEWA official name and signature:

Remarks:

Official Stamp with date:



Annexure # 5 National Surveillance Program (Fish Aquaculture)

A well established surveillance program is designed and executed, covers all major endemic and exotic fish diseases and economically important emerging diseases (including fish imports and wild population). Surveillance program also describes the appropriate diagnostic methods recommended for various pathogens tested.

	FISH SURVEILLANCE PROGRAM												
Number of Scuples and Or algester													
Production facilities	Animal stage	Target sample	Target pathogen	Frequency	Diganostic method	Min targetted prevalence	No of animals per PCR reaction	# of tanks/ ponds/ cages	No. of animals / sample				
	1	-	VNN/VER, Iridovirus	Once per shipment	PCR	2%	10	per shipment	150				
0	Juveniles	Routine	All	Once per shipment	Histology	10%	N/A	per shipment	30				
Quarantine	market de	1.5.00	VNN/VER, Iridovirus	Once per shipment	PCR		1 for lethal, 5 pooled (faeces) for BBS	per shipment	3 for lethal sampling, 100% for BBS (faeces)				
	Broodstock	Routine	All	Once per shipment	Histology	1	N/A	per shipment	3				
Hatchass		Deutine	VNN/VER, Iridovirus	Monthly	PCR	2%	50	1	150				
Hatchery	Larvae	Routine	All	MONTHLY	Histology	5%	N/A	i.	60				
Nurran	Juvenile	1733	VNN/VER, Iridavirus	Marshite	PCR	5%	30	2	60				
Nursery	Juvenile	Routine	All	Monthly	Histology	10%	N/A	2	30				
			VNN/VER, Iridovirus		PCR	10%	30	a	30				
Pre growout	Sub adult	Routine	All	Monthly	Histology		N/A	3	10				
Off shore		and a local second	VNN/VER, Iridovinus		PCR	1.22	10	3	10				
cages	Adult	Routine	All	Monthly	Histology		N/A	3	5				
Fish Market/Fish ing boat samples	Adult	Routine	VNN/VER, Iridovinzs, Tilapia Lake virus (only for tilapia)	Monthly	PCR	4	5	minimum 4 sources	5				



Annexure # 6 – Sample Preparation Procedure

Sample Preparation Procedure – Wet mount

What	Who	How	When	Reference
General Examination	• Animal Health expert	• Tools needed	• Routine/ Case wise	Fish Health Sample collection procedure Wyoming Game and Fish Laboratory
Physical Examination	• Animal Health expert	 Look at the eyes for cataracts (crystalline opacity) or cloudiness and hemorrhage and gas bubbles behind the eyes Image: Image and gas bubbles behind the eyes Image and gas bubbles behind the eyes <l< td=""><td>• Routine /Case wise</td><td>Fish Health Sample collection procedure Wyoming Game and Fish Laboratory</td></l<>	• Routine /Case wise	Fish Health Sample collection procedure Wyoming Game and Fish Laboratory





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1- Prepare wet	• Animal	Gills		• Routine/	Fish Health
mount of	Health	•	Use moribund/freshly dead fish	Case	Sample collection
gills/Fin/tail	expert			wise	procedure Wyoming Game and Fish Laboratory
		•	Cut out a few gill arches and place on a clean slide		
		•	Place the gills on a slide. Use the scalpel to cut out the gill arch.		
		•	Add drop of sea water to cover the sample and coverslip it		
			Examine using bright field microscopy		





• Healthy gills will look clean and feathery.



• These gills show signs of gill disease.



• It can be hard to see the fine structure of the gills using bright field microscopy on large fish. In this case, place gill tissue in a petri dish with water





Examine under the dissecting microscope.
Fins & Tail
 Gently scrap the edge of the scalpel blade across the skin above
the fin and lateral line.
Scrap behind the pectoral fin down toward the tail
Wipe the mucus on the scalpel blade on a clean slide and add drop
of sea water and coverslip it.









Examine the slide using bright- -field microscopy ٠



• You may observe lots of cells, debris, and air bubbles, parasites on the slide





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	1			
2-Internal	 Animal 		Routine/	Fish Health
examination and	Health	• Cut from the vent up to the pectoral fin, in a half Cut from the vent	Case	Sample collection
organ imprinting	expert	up to the pectoral fin, in a half-moon shape	wise	procedure
				Wyoming Game
				and Fish
				Laboratory
		Open the fish up to expose the internal organs		
		 Make note of any hemorrhage or other abnormalities in all internal 		
		organs and check for parasites.		
		Organ Imprinting (rapid staining)		
		 Using forceps extract a samples of organ tissues (liver, spleen, 		
		kidney)		





Blot the tissue to remove excess red blood cells	
Place the tissue on a clean slide	
Take another clean slide and press down on the tissue.	
 Separate the slides.Use forceps to remove excess tissue from separated slides. 	
 Label each slide with the source, species, and tissue type 	



	 Keep it in slide box and for staining and then slide reading using microscope. 	

Sample Preparation Procedure – Microbiology

What	Who	How	When	Reference
Prepare	Animal	 Sanitize all equipments and area with ethanol and label bacteriological 	•	
microbiology	Health expert	plates with proper identification.		
samples		• Cut from just before the vent up to the pectoral fin, in a half cut from		
		the vent up to the pectoral fin, in a half-moon shape		
		 Open the fish up to expose the internal organs 		







Sample Preparation Procedure - PCR

What V	Who How	When Reference
--------	---------	----------------



1- Verify cleanliness	• Animal	Check equipment and cleanliness of the working area to avoid	Routine/Cas	Fish Health
	Health	possible contamination	e wise	Sample
	expert			collection
				procedure
				Wyoming
				Game and
				Fish
				Laboratory

2- Prepare tubes	• Animal	Add 95% ethanol in tubes	Routine/Cas	Fish Health
	Health expert	 label the tubes with the details of the source 	e wise	Sample collection procedure Wyoming Game and Fish Laboratory
3- Collect samples whole fish larvae/	• Animal	 Collect the tissue samples (brain & retina, Kidney, liver, spleen, 	• Routine/Cas	Fish Health Sample





juveniles or organs	Health	gills, internal organs) and put them in labeled tubes	e wise	collection
	expert	 Samples must be taken using sterile scissors in good ambient conditions to avoid contamination. If different batches have to be sampled, do not forget to change the 		procedure Wyoming Game and Fish
		scissors or scalpel blade or disinfect in between samples.		Laboratory
		 If you wish the samples to be analysed individually, please place one sample per tube. Tubes must be identified properly 		
		• If you wish a pooled analysis, please pool the organs or the whole fishes (larvae) in a transport tube. The tubes must be correctly identified.		

4- Clean and	• Animal	After sample preparation of one pond/tank, clean and dip the	Routine/Cas	Fish Health
disinfect the tools	Health	scissors/forceps in ethanol and flame them to remove the tissues	e wise	Sample
	expert	of previous samples, in order to avoid the chances of		collection
		contamination.		procedure
				Wyoming
				Game and
				Fish
				Laboratory



What	Who	How	When	Reference
1- Label the bottles	Animal	• Label the bottles with all the information relevant to the samples	Routine/Case	Fish Health
	Health		wise	Sample
	expert			collection
				procedure
				Wyoming
			Game and	
		10% BUFFERED FORMALIN		Fish
		A CONTRACTOR OF		Laborator
				У

Sample Preparation Procedure - Histology

2- Ensure safety	• Animal	Adequate PPE should be used (goggles, mask, gloves and lab coat)	Routine/Case	Fish Health
	Health		wise	Sample
expert	expert			collection
			procedure	
				Wyoming
				Game and
		The second se		Fish
				Laboratory



3- Prepare	Animal	Formulation of 10% Buffered formalin (1litre) – (to be prepared by a	Routine/Case
Fixative	Health	trained laboratory personnel inside a fume-hood with adequate PPEs)	wise
	expert	37-40% formalin - 100ml	
		NaH2PO4.H2O - 4g	
		Na2HPO4.H20(Dibasic) - 6g	
		Distilled water - 900 ml	
		Shake well	
		Formulation of Davidson's fixative (1 liter) (to be prepared by a	
		trained laboratory personnel inside a fume-hood with adequate PPEs)	
		95% ethanol - 330 ml	
		Formaldehyde - 220 ml	
		Glacial acetic acid - 115 ml	
		Distilled/tap water - 335 ml	
		Shake well	



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4- Fix the	Animal Health	Animals MUST be fixed alive		Routine/Case	Fish Health
animals and	expert	Gill	Liver	wise	Sample
organs		spleen	Kidney (posterior)		collection procedure Wyoming Game and Fish Laboratory
		Kidney (Anterior)	Stomach		
		Intestine	Heart and muscle		







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 Incubate for 24-72 hours depending on the size of the animals 	
(Larvae	
and frys-24 hours , Juveniles and adults -48 hours, large sized	
animals -72 hours) in room temperature.	



When

daily once for

• Minimum

nursery and

pre grow out



Reference

Health

Monitoring

program

• Production

data

Animal

of culture	Production	Feeding and feed management	Feeding and food	management	hatchery,
conditions	unit technician • Animal Health Manager	 management Water parameters as per unit specifications Larvae tanks Water quality & parameters as per unit specifications Feeding and feed management Larval quality and live feed quality 	feed managemen t • Handling and transportati on • Water quality as per the unit specification s • vaccination	 Mortality collection Diving operations Fish health check Cage net changing/cleanin g Water parameters Bird activity Farm production records 	nursery and pre grow ou • Minimum weekly once for Offshore
2-	Production	Fish Hatchery	Nursery and pre	Grow Out	Minimum
Sampling	unit	Broodstock tanks	grow-out	Ensure the nets are	daily once f
	technician	Ensure scoop nets are	Ensure the	clean and of proper size	hatcheries,
		alaan and fus a of fus ala		 Collect weak animals 	Number

Annexure #7 - Animal Health Monitoring Procedure

Fish Hatchery

Broodstock tanks

Who

• Biosecurity

Officer

What

1- General

Monitoring

	Health Manager	 Larvae tanks Water quality & parameters as per unit specifications Feeding and feed management Larval quality and live feed quality 	 Handling and transportati on Water quality as per the unit specification s vaccination 	 Fish health check Cage net changing/cleanin g Water parameters Bird activity Farm production records 	weekly once for Offshore	• Unit SOPs
2- Sampling	 Production unit technician Biosecurity Officer 	 Fish Hatchery Broodstock tanks Ensure scoop nets are clean and free of fresh feed Ensure disinfection of sampling equipment before and after use Collect symptomatic 	Nursery and pre grow-out Ensure the nets are clean Collect weak animals from the tanks	 Grow Out Ensure the nets are clean and of proper size Collect weak animals from the cage Ensure disinfection of sampling equipment before and after use and between cages 	 Minimum daily once for hatcheries, Nursery &PGO Minimum weekly once for Offshore 	 Surveillance program Animal Health Monitoring Guide Disinfection procedure- SOP

How

grow-out

Nursery and Pre

Grow out

• Feeding and feed





animals	• Ensure	Annexure
Larvae tanks	disinfection	
Ensure disinfection of	of sampling	
sampling equipment	equipment	
before and after use	before and	
Collect larvae for	after use	
checking the quality	and	
	between	
	tanks	

3. Primary	Biosecurity	Brood stock tanks			Surveillance
health	technician	Macroscopic observations			Program
check	Animal	Behavior (response to food, swimming etc.)	Normal / Abnormal		 Animal Health
	Health	Body color	Normal / blackish / Other	• Daily	Report
	Manager	Overall health status(absence of large wounds, hemorrhages, infections, parasites and necrosis)	Present / Not present		 Animal Health Monitoring Guide
		Deformities	Present / Not present		
		Internal organs(Liver, spleen, kidney, heart, brain)	Normal/Color change/enlarged/hemorrhage/other		
		Gill and eye	Normal/pale /cloudy / blind		
		Parasites- Internal and external	Present / Not present		
		Microscopic Observations			





Gills and s	skin scraping-F	Parasites	Presence/Absence of parasites/bacteria/others	
		ons, blood, skin, , either stained	Presence/Absence of parasites/bacteria/others	● In_abnormal
Gram stai	ining		Presence/Absence of bacteria	cases
Hatchery Larvae Macroscopic obs				
Criteria	If (score))	Action Plan	
Mortality(survi	Normal (10)	Go for Sur	vival check and microscopic observation	ons
val)	Abnorma (0)	l Discard		
Health status	>95% (10)	Go for N	licroscopic observations	
condition	<95% (0)	Discard		Daily
Microscopic obse	ervations			• Daily
Criteria		If (score)	Action Plan	
Deformities		<5% (10)	Go for next batch inspection	
Delomines		>5% (0)	Discard	
		<5%		
Larvae Quality		(10)	Go for next batch inspection	

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Criteria	If (score)	Action Plan	
Tank turbidity	<5% (10)	Go for next batch inspection	
	>5% (0)	Management decision	
Bacterial/fungal growth	Normal / Abnormal	If abnormal, siphon out growth from water body	
Swimming activity	Normal / Abnormal	If abnormal, sample for quality check	
Mortality	Normal / Abnormal	If abnormal, Management decision	
Tank water flow	Normal / Abnormal	If abnormal, fix the water	
		flow issues.	Daily
	ns		• Daily
Microscopic observation Criteria	ns If (score)	flow issues. Action Plan	• Daily
Criteria	ns		• Daily
	ns If (score) >60%	Action Plan	• Daily
Criteria Egg –fertilization,	If (score) >60% (10) <60%	Action Plan Go for next batch inspection	• Daily
Criteria Egg –fertilization, viability, hatching Larvae stage-Mouth	If (score) >60% (10) <60% (0) <5%	Action Plan Go for next batch inspection Management decision	• Daily





Deformities	<80% (0) <5% (10) >5% (0)	Management decision Go for next batch inspection Management decision	• Daily	
Nurseries/Pre gr Macroscopic observatio	row out/Grow out			
Behavior	Normal / Abr	normal		
Body color	Normal / Blac	ckish/ Other		
Deformities	Present/Not	present	11	
Necrosis-External body infection	Present/Not	present		
Еуе	Normal / clou	udy / blind		
Skin, fin/ tail erosion	Present/Not	present		
	Case	wise		
Gill color	Normal / pale	e / Black		Surveillance
Gill/external body	Presence/Ab	sence of parasites		Program • Animal
All internal organs	Color change	/enlarged/hemorrhage/parasites/other	Daily	• Animai health
Microscopic observation	ns			monitoring program
gills and skin scraping-Pa	=	Presence/Absence of		• Sample
putted in fresh water to	release parasites)	parasites/bacteria/others Presence/Absence of		preparation
Wet mount		parasites/bacteria/others		procedures Surveillance



		smears of possible lesions, blood, skin, gills, spleen and kidney, either stained or fresh(Rapid staining) gram staining	Presence/Absence of parasites/bacteria/others Presence/Absence of bacteria		 Program Animal health monitoring program Sample preparation procedures
				Each grading	
4- Prepare samples for further diagnostics (VNN, Strepto, others)	 Animal Health Technician Animal Health Manager 	PCR/Histology/Bacteriology samples for critical abnormalities must be prepared as per Sample Preparation Procedures.		• Case-wise	 Surveillance Program Animal health monitoring program Sample preparation procedures
5- Provide animal health recommen	 Animal Health Manager BSD HOD 	• Adequate preventive, corrective and imp observations and diagnostic results.	rovement recommendations based on	• Case-wise	Animal Health report



dations	BSD Director		



Annexure # 8 - Animal Health Monitoring Guide

What	How			
Fish				
Behavior	Normal	Moribund		
Body color	Normal	Blackish		
Deformities	Swim bladder-Normal	Swim bladder-deformity		
	Spine –normal fish	Spine deformity		


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nity-X-	ray
all.	
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Deformities	Normal-X-ray of spine	Spine deformity-X-ray
		CONTRACTOR OF THE OWNER
White patches	Normal	White patches
Necrosis-External	Rotten body	Wound on mouth
body infection		









Eye	Normal	Exophthalmic eyes
	Eye-hemorrhage, bulging and whitish	Eye with dark pigment and Melanization
	Crystalline opacity : Cataract	Cloudy eyes
Gills	Normal	Pale and chronic



Gills	Pale gills	Tricodina from the gills	
	Protozoa from the body scrapping	Tricodina from the body	
Internal organs- Liver	Normal-Liver	Liver hemorrhage	
	Pale	Dark Version of the second sec	



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Spleen	Normal spleen	Spleen enlarged	
	Normal-kidney	Kidney with white spot	
	Kidney hemorrhage	kidney hemorrhage	
others	swim bladder broken	Gastrointestinal tract with plenty of fluid	



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Others	Normal flush	Flush with red spot		
	Bulged belly	Fish with bulged end (tumour)		
Microbiology	Streaking blood agar plates	Bacterial growth on blood agar		
	Gram positive bacterial growth on blood agar	Gram negative growth on blood agar		







Annexure #9 Contingency Procedure

What	Who	How	When	Reference
1- Investigation	 GDF-MEWA Biosecurity Supervisor In-house Biosecurity team Production team 	Investigate the incident (conduct re-sampling, traceability, field diagnostics, disease history and mortality trend) Treatment : In case the decision is treatment follow veterinerian advise Harvest / Elimination: initiate contingency protocol	• Case- wise	• Diagnostic laboratory results
2- Information Outbreak	 GDF-MEWA Biosecurity Supervisor 	E mail to all internal and external involved entities	• After lab results	• Mail





3-Prepare for contingency	 GDF-MEWA Biosecurity Supervisor In-house 	Management decision to harvest, treat or terminate the batch	→	Access limited to onsite staff, biosecurity staff (and harvest staff if emergency harvest). Disnfection (for farms) of all vehicles on exit.	• Case- wise	• Animal Health Report
	 Biosecurity team Production team 			vest request to harvest team or or termination		









Mortality Management of Stock fish Elimination

What	Who	How	When	Reference
8. Materials	 Production 	• Eliminated biomass must be carried in strong and safe containers	Case-wise	Procedure for disposal
required	team	(IBC/Bins), with no crevices or leaking.		of dead animals
inspection	 Biosecurity 	 All containers must be properly disinfected before get into 		
	team	installations, visual inspection and check enough number according the		
		Biomass to be eliminated		
		• Before start filling the containers with fish, 10 % of Biomass container		
		capacity of Formic acid/Chlorine/ Hydrated lime must be added (*)		
9. Drain the	 Production 	• Once defined the tanks to be eliminated, water level must be drained	Case-wise	Procedure for disposal
tank water	team	according the effluent disinfection capacity of the system		of dead animals
	 Biosecurity 			
	team			
10.Animals	 Production 	Add anesthetic to reduce stress of the animals, taken in to	Case-wise	Procedure for disposal
removal	unit manager	consideration of animal welfare.		of dead animals



	Biosecurity	• Using hand nets fish must be moved into buckets to carry the fish to		
	team	the final container, adding the minor amount of water possible		
		• When 50 % of total capacity of container is reached, 10 % of total biomass container capacity of formic acid/ Chlorine/Hydrated lime must be added (*)		
		Water must be constantly drained to increase fish density		
		 Finish filling the container with fish following the second step 		
		 Add 10 % of total biomass container capacity of formic 		
		acid/Chlorine/Hydrated lime and seal the container		
		 Continue with same procedure until finish to fill all containers and get all tanks empty 		
11.Tanks	Production	• As soon tanks get empty must be perfectly cleaned and disinfected as	Case-wise	 Procedure for disposal
Disinfection	team	per the disinfection procedure (*)		of dead animals
				 Recommended
				eradication and
				disinfection procedure

12.Container	Production	• Once the truck is loaded, a visual inspection of any leaking must be	• Case-	Procedure for disposal
s movement	team	done	wise	of dead animals
	• Animal	• Dead fish must be disposed at the designated area (pit). This		
	Health	procedure must be directly supervised		
	Manager	• The pit must be properly covered to avoid the exposure of eliminated		
		biomass		
		 Trucks and containers must be properly disinfected 		
13.Materials	Production	All materials used must be properly disposed	• Case-	Procedure for disposal
	team		wise	of dead animals
14.Dry out	Production	• All installation must be dried out and disinfected as per disinfection	• Case-	Procedure for disposal



and	team	procedure	wise	of dead animals
disinfection				 Recommended
				eradication and
				disinfection procedure

(*) Safe personal protection must be wear



Annexure # 10 Handling mortalities, organic residues and silage





Annexure #11 - Recommended eradication and disinfection procedures & treatments

1. Disinfection Procedures

1.1 Equipment and fish farming facility (Hatchery, Nursery and PGO)

The following procedure is recommended for cleaning and disinfection of fish farm vessels and different fish production unit tanks other equipment:

- 1.1.1 Remove all gross fouling and organic matter by scraping and brushing.
- 1.1.2 Clean using a detergent solution to remove particulate matter, fats and oils.
- 1.1.3 Apply disinfectants at recommended concentration for appropriate contact time.
- 1.1.4 Rinse with clean water if required.

1.2 Well-boats and Feed Delivery Boats

The number of live fish transfers and feed deliveries made by boat is increasing and may involve serial deliveries to a number of sites. The risk of disease transmission by well-boats and feed delivery boats is highest where contact is made with fish or contaminated seawater. Well- boats and feed delivery boats may transmit disease via feed, fish, transport water, personal etc. Boats should be maintained clean after each delivery of feeds and scrape should be clean. Disinfectant all the structure with recommended chemicals

1.3 Electronic equipment

Electronic equipment (e.g. weighing scales and thermometers) may be sprayed with alcohol and allowed to air dry, paying particular attention to manufacturer's instructions particularly in the initial removal of organic fouling

1.4 Nets

1.4.1 Used nets should be transported in to designated area for cleaning. Ensure that it should be kept separate from the cleaned nets.



1.4.2 Nets should be sundried and remove fouling agents, and then it is recommended to use sodium hypochlorite solution at a concentration of 1,000 mg/l for six hours (or an alternative equally effective disinfectant at the appropriate concentration) then rinsed with fresh water. The sodium hypochlorite solution must be agitated to ensure an even concentration of hypochlorite. If nets are very heavily fouled the sodium hypochlorite concentration should be increased to at least 5 g/l.

1.5 Barges

1.5.1 Sea barges should be maintained clean after each delivery of feeds and scrape should be clean. Disinfectant the surfaces with recommended chemicals.

1.6 Staff, Site Visitors and Diving Gears

- 1.6.1 Fish diseases can be transmitted via equipment or personnel who come into contact with infected fish during daily work/visit. It is important that strict hygiene procedures are followed on a daily basis. Divers collecting mortality of an infected cage/site can act as a vector for disease transmission if there is no proper disinfection of their equipment/suit. However, it is highly recommended to collect mortality of infected cages at the end.
- 1.6.2 Dirty and disinfected suits and associated equipment should be kept separate at all times.
- 1.6.3 Footbaths and brushes should be strategically placed for the disinfection of equipment where this is not site-specific.

2. Recommended Disinfectants

For efficient disinfection, first clean the target surface to remove organic matter.

Chemical / disinfection method	Active ingredients	Dosage of active ingredient	Contact time	Scope	Application	Elimination of residues
		1000ppm	24 hours	Pathogen eradication	System disinfection (dry out)	Sun light, aeration
Chlorine	Chlorine Calcium/sodium	200ppm	1 hour	Surface disinfection	Disinfection of tanks and equipment	Dry out, sun light
		30ppm	1 min	Surface disinfection	Vehicles	Dry out, sun light
Quaternary	Quaternary	100ppm	<5 min	Pathogen eradication and	Spraying-Regular disinfection	Natural





Ammonium	Ammonium			general disinfection		breakdown
		10ml/litter	<5 min	Pathogen eradication and general disinfection	Foot bath, vehicle tyre and disinfection of equipment	Natural breakdown
Potassium Permanganate	Potassium Permanganate	350ppm	<5 min	Pathogen eradication and general disinfection	Foot bath, vehicle tyre and disinfection of equipment	Natural breakdown
		600ppm	<5 min	Pathogen eradication and general disinfection	Hand wash and egg disinfection	Natural breakdown
Povidine	lodine 30 to 50 mg/l of free iodine		10 to 30 min	Pathogen eradication and general disinfection	General disinfection	Natural breakdown
Formaldehyde (Liquid)	Formaldehyde 37% solution	100 ppm	20-60 min	External parasite/pathogen eradication	All stages of production	Natural breakdown/ water exchange
Ethanol	Ethyl Alcohol (Liquid) 99.9%	75%	Up to dry	General/Surface disinfection	Hand and work surface disinfection and tissue sampling.	Natural breakdown
Hydrogen Peroxide (Liquid)	Hydrogen Peroxide 30%		5-30 min	External parasite/pathogen eradication	All stages of production	Natural breakdown/ water exchange

Hydrochloric acid	Hydrochloric acid	PH4	24 Hrs	Pathogen eradication	System disinfection (dry out period)	Water circulation
Sodium Hydroxide	Sodium Hydroxide 100%	PH12	24 Hrs	Pathogen eradication	System disinfection (dry out period)	Water circulation
Ozone	Ozone	0.5ppm	8min for ORP values of 600-700	Pathogen eradication	Primary water treatment after mechanical filtration	12 hours by oxidation (Bromine



						must	be
						<0.05ppm)	
UV	UV light	Radiation 200-300 nm	Irradiation must reach >30,000 mws/cm2 in the incoming water flow	Pathogen eradication	End of water treatment for on land production units	N/A	

3. Facility Disinfection-Dry out

3.1 Hatchery, Nursery and Pre Grow out

- 3.1.1 Washing:
 - 3.1.1.1 Ground, tanks, walls, and surface of equipment inside the facility.
 - 3.1.1.2 Clean all with commercial food grade detergent or any other surfactant. Use broom, brush and sponges whenever applied.
 - 3.1.1.3 Spray with disinfectant solution: Quaternary ammonium (Combat) at 100ppm solution
 - 3.1.1.4 Allow the disinfectant on the surfaces for 1-2 hour, and rinse with fresh water high pressure pump.
 - 3.1.1.5 Rinse with abundant fresh water floor, tanks etc. Use a wet cloth to remove soap from the equipment that can't be sprayed.
- 3.1.2 Water Lines, Sump, bio-filters, degasser and sand filters.
 - 3.1.2.1 Water lines disinfection to be done during dry out period.
 - 3.1.2.2 Flush bio-filter to eliminate organic matter accumulated.
 - 3.1.2.3 Eliminate all salt water from the system, and rinse with fresh water.
 - 3.1.2.4 Fill up all tanks in the hatchery sump and bio-filter and initiate to circulate the water.
 - 3.1.2.5 Add approximately 50-100ppm of food grade detergent in the water, until some foam start to appear in the bio filters.
 - 3.1.2.6 Allow it to run in recirculation for around 24 hours.
 - 3.1.2.7 Drain all process water and refill same tanks, sump and bio filters with sea water and initiate to recirculate the water once again and repeat two times.
 - 3.1.2.8 Add 1000ppm Sodium Hypochlorite (12%).
 - 3.1.2.9 Allow it to run for around 24 hours, in bio filters and lines



- 3.1.2.10 Drain all process water and refill same tanks, sump and bio filters with sea water and initiate to recirculate the water once again and repeat 2 times.
- 3.1.2.11 Add 500ppm Sodium Hydroxide (49%) gradually until PH reach 11-12 and allow it to run for around 24 hours, in bio filters and lines.
- 3.1.2.12 Drain all process water and refill same tanks, sump and bio filters with sea water and initiate to recirculate the water once again and repeat two times.
- 3.1.2.13 Refill system sump and bio filters with sea water and initiate to recirculate the water.
- 3.1.2.14 Add 150-200ppm of Hydrochloric Acid (37%) gradually until PH reach between 3-4.Or Add 50-100ppm of Oxalic Acid (98%) gradually until PH reach between 3-4.
- 3.1.2.15 Let the system to circulate for with Low pH water from 24 hours.
- 3.1.2.16 Drain completely the water from the system, and rinse with fresh water.
- 3.1.2.17 Open hatchery/nursery/PGO roller doors during the day, and allow the facility to dry out (restrict entry of unauthorized). It is recommended to keep the facility dry for 8 weeks.
- 3.1.2.18 Validate disinfection by microbiological analysis
- 3.1.3 Airlines: Ensure the use of Safety equipment for this procedure.
 - 3.1.3.1 For airlines proceeds the same procedure of water lines disinfection.
 - 3.1.3.2 Evacuate the hatchery and open hatchery roller doors.
 - 3.1.3.3 Partially open all air outlets in the hatchery.
 - 3.1.3.4 Soak a cloth with disinfectant and tie it to the blower intake.
 - 3.1.3.5 Maintain the cloth wet with formalin as the blower operates sucking combat evaporation.
 - 3.1.3.6 After 15-20 minutes, remove the cloth, and continue to circulate the aeration for 12 hours.



Annexure #12 Fish Health Management – Vaccination, Antibiotic and Formalin treatments

Vaccines, antibiotics and chemicals are useful tools for health management in aquaculture. This annexure will give guidelines for vaccination and treatments of fishes at different production units.

Vaccination

- Preparation: Prepare the tanks to be vaccinated, ideally unfed. Verify fish is in good conditions for manipulation (active and healthy).
- Prepare working tabs in the grading area with inlet water, oxygen diffusers, outlet connections pipes, scoop nets.
- Prepare sedation bath adding to sea water the anesthetic (in 70 liters of sea water dissolve 5 ml of Aqui-S, or 50 ml of Benzocaine Ethanol Solution at 15% (see annexure11 for preparation). Sedation bath need to be renewed every, more or less, 2 hours, or earlier if water quality deteriorates. Fish has to be sedated for 75-90 seconds. Ensure that the bath is re-oxygenated for 30 seconds every 15 minutes of use.
- Prepare vaccine bottles (shake very well), connect injectors and air stand pipe. Fit needles on injectors, check few free shots to verify it is functioning.
- To verify that required dose would be injected (0.1 ml) collect one shot from each injector in an Eppendorf tube and compare the quantity with the reference tube provided. If a difference is noted, adjust/change the injector.
- It is the best occasion to remove unsuitable fish (deformed and stunt) from the system and record it.
- Guarantee smooth and constant supply of sedated fish on the vaccination table.
- Keep staff concentrated to minimize risk of self-injections
- Needles will accumulate fish scales during process. Clean needles routinely.
- Perform one free shot every 30 minutes, to ensure proper functionality.
- Re-suspend vaccine in the bottles every 30 minutes (shake it up and down).
- Change all needles every 2 hours.
- Clean vaccine pipes and injectors. Run hot water to clean inside. Use ethanol to disinfect outside.

Prophylactic treatment with Formalin

- After transfer/introduction of new fishes, prophylactic treatment is recommended to avoid potential infection due to handling process and also to eliminate any external parasites.
- Stop the water exchange and increase the aeration and oxygen on the tank.



- Calculate 100 ppm of formalin (or recommended chemical and dose) for the tank water volume.
- Mix the amount of formalin with fresh water in a bucket and distribute in all tank surfaces.
- During the treatment period, the oxygen saturation is maintained (90 -200%).
- After one hour of treatment, water shall be flushed with 500% water exchange for 2 hours with the low volume.
- Then water shall be raised up to normal operational level.

Antibiotic treatment by injection on Broods stocks:

- Antibiotic treatment is done as per the prescription by a veterinarian upon a confirmed diagnosed and using only MEWA approved antibiotics (see below).
- Fish shall be anesthetized in the tank previous management to reduce stress before being injected live (Figure 1)



Figure 1: Fish sampling

- The fish must be weighed and accordingly the required antibiotic dosage has to be calculated according to veterinary prescription.
- Adequate syringe has to be used to fill the antibiotic preparation and reuse of the syringe is not allowed. (Figure 2).





Figure 2: Syringe preparation

• It is advisable to inject the antibiotic below the pectoral fin. The syringe should be introduced with a small inclination into the fish body and the product has to be injected slowly (Figure 3).



Figure 3: Antibiotic injection

- Fish shall be transferred to recuperation tank.
- As soon as the fish recover from the anesthesia transfer it to the new tank.
- Fish must be checked till the total recovery into normal swimming.
- Ensure that water quality is the appropriate for the culture and daily monitoring must be carried out as a normal procedure.

Antibiotic treatment by bath for fry/juveniles:

- Antibiotic treatment is done as per the prescription by a veterinarian upon a confirmed diagnosed and using only MEWA approved antibiotics (see below).
- Stop the water exchange and increase the aeration and oxygen on the tank.
- Calculate the quantity of antibiotics required based on the veterinary prescription and considering the water volume in the tank.
- Mix the amount of antibiotics with fresh water in a bucket and distribute in all tank surfaces.
- During the treatment period, the oxygen saturation should be maintained (90 -200%).
- Monitor the feeding behavior and adjust accordingly to ensure efficient treatment.
- Follow the veterinary prescription for the antibiotic treatment course.



• After one hour of treatment (or as recommended on the veterinary prescription), water shall be flushed with 500% water exchange for 2 hours with the low volume.

Antibiotic treatment by oral for pre-adult and adult fishes:

- Antibiotic treatment is done as per the prescription by a veterinarian upon a confirmed diagnosed and using only MEWA approved antibiotics (see below).
- If the treatment is done in tanks, stop the water exchange and increase the aeration and oxygen on the tank.
- Calculate the quantity of antibiotics required based on the veterinary prescription.
- Calculate the standard feeding rate (SFR) and prepare the feed by coating the antibiotic.
- Coat the antibiotic in the feed pellets using fish oil as binder; adjust the fish oil to ensure proper fixation of antibiotics.
- Monitor the feeding behavior and adjust accordingly to ensure efficient treatment.
- Continue the antibiotic course as per the veterinary prescription.

Permitted and prohibited antibiotics at NAQUA

Permitted	Prohibited		
Oxytetracycline	Chloramphenicol		
Erythromycin	Dapsone		
Florfenicol			
Tetracycline			





English name :	Australian seabass/Barramundi
Scientific name :	Lates calcarifer

Disease/Virus	Agent	Туре	Affected/Syndrome	Stage	Measures
Nodavirus Infection	VNN	Virus	Erratic swimming, decoloured larvae, weakness, over inflation of the swimbladder, sometimes megacephalia. On larger fish, melanic fish, blindness and jaw mechanical lesions.	Larvae until 1 g, 2 to 7 week. Older fish seems seem likely to be infected but may not show clinical signs.	ls not possible
Fish Lymphocystis Disease (FLD)	Iridovirus	Virus	Grey skin tumours, like couliflowers. Possibility of toumours on internal organs.	Expression in larvae since 1 g to 120 g. Afectt juveniles. The disease continue until harvest.	Is not possible. Avoid stress factors. Eventually limit secondary infection on skin lesions with chemical treatments. Reinforce the resistance of the fish to prevent secondary infections by opportinistic germs (Vit C, selenium, inmunoestimulants)
Streptococal infection	Streptococcus iniae	Bacterial	Darkness of the fish, decoloured gills and anorexia. Internally, presence of haemorragic liquid in the visceral cavity with congestive or haemorragic internal organs.	Expression from 2 g to harvest. Main sensitive stages juveniles. Sub acute in larger fish.	Curative antibiotic treatment (Eritromicin or Oxitetracicline)
	<i>Filamentous bacteria.</i> Tenacibaculum maritimum, Flexibacter, Cytophaga Johnsonae, F Columnaris	Bacterial	Respiratory: Larvae at the surface of the tank, with open operculum. Passive swimming with hyperventilation. Presence of mucus. Can lead to focused necrosis. Often occure during the weaning stage and with poor quality and hygiene.	Expression from 1 g to 120 g	Schedule of prevention and treatment with biocide immersion treatments in open flow or static for 1 hour (Piceze- formaldehyde- peroxide and active peroxide) In the worst cases tetracycline bath, only if the biocides do not stop the problem.
bacteria.		Bacterial	Tegumentary (skin): In young juveniles, mainly with lesions near to the dorsal fin or on the body side, lesion of the causal fin and the caudal peduncle, lethargic fish. In larger fish, lesions become necrotic and ulcerative. The lower jaw can be rotten.	Expression from 1 g to 120 g	Treatment by inmertion with biocides or antbiotics.

Annexure #13 Major Marine fish diseases, their prevention and control

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Vibriosis o Photobacteriosis	Vibrio ssp, Vibrio harveyi, Photobacterium damsela damsela.	Bacterial	Enteritis & peritonitis, mainly in close system. Anorexia, melanic and lethargic larvae, showing a thin body and big head with distended addomen.	Expression from 0,2 g to 5 g	Treatment of the artemia culture (A0- A1) with Piceze to control the evolution of the bacterial flora. Improvement of live feed hygiene. Treatment of the larvae by inmersion with biocides (Peroxide, formaldehyde, Pyceze) Systemin antibiotic treatment using the live feed as support with effective antibiotic)
		Bacterial	Classic signs of septicaemia with darkness of the fish, redness of the fins, haemorrhage on internal organs, ascitis hemorrhagic liquid, ulcerative lesions with a reg edge.	Expression from 5 g to 120 g	Piceze. Antimicrobials.
Septicaemia gram (-)	Pseudomonas and other secondary pathogens.	Bacterial	Classic signs of septicaemia with darkness of the fish, redness of the fins, haemorrhage on internal organs	1 g - Harvest	Antimicrobials.
Epitheliocystis	Chlamidia or Rickettsia like microorganism. Not well established.	Bacterial	White to yellow cysts on the gills or skin.		None
	Cryptocaryon irritants (White spot disease)	Parasitic	White spots on the tegument & the fins with anorexic fish lethargic & darkness of the skin. White spts (0.4-0.8 mm) on surfaces are encysted trophonts, which feed on host epidermis. Flassh against the tank bottom or walls.		Immersion bath with formaldehyde, peroxide - copper sulphate, sodium per-carbonate, sodium chloride. Alternatively salinity treatments (lowering or increasing salinity) can be used. Filtration of water (mesh size 10 um) traps trophonts and tomonts and can ease infection intensity.
	Ichthyophthirius multifilis (White spot disease)	Parasitic			
External parasitosis by	Trichodina - Ichtyobodo necator	Parasitic	When low temperature & poor water quality, fish flashing with hyper ventilation and excess of gill mucus, lethaygy, anorexia. With Ichtyobodo, shiny skin due to scales, dark spots on the skin.		Short bath tretament formalin, copper sulphate, sodium chloride and sodium percarbonate.
protozoarios	Chilodonella	Parasitic	Lethargy and anorexia. Dense populations on the host epithelium inhibit normal physiological function, including osmoregulation, gas exchange and excretion.		Formalin, cooper sulphate, malachite green and methylene blue.
	Amyloodinium ocellatum	Parasitic	Mainly on juveniles in ponds, darkness of the skin with greenish decoloured tegument, velvet like layer in extreme infections. Hyper ventilation with opened opercula. Fish swimming at the surface. Associated with stress, poor water quality and/or poor fish health. Sometimes the fish rub their body against objects.	Juvenils	Repeated formaldehyde bath treatments or peroxide - copper sulphate treatment. Copper sulphate (less than 2 ppm), Benzalkonium chloride (up to 0.5 ppm) for up 3 days. Increase water exchange - disinfection of the pond with lime when empty.
	Piscinoodinium pillulare	Parasitic		Juvenils	
External parasitosis by	Diplectanum, Laticola and Pseudorhabdosynochus)	Parasitic	Anorexia - suffocation in worth cases with whitish gills (pale) & mucus. Darkened body, lethargy, loss of appetite.		Formaldehyde bath. Fresh water.
Trematode monogens	Neobenedenia melleni and Benedenia epinepheli.	Parasitic	Keratitis, exophthalmia, whitish spot on the skin with mucus.		Formaldehyde or peroxide bath - Praziquantel. Fresh water.



Emamectin in feed - Sealice treatment

(Diflubenzuron, Pyretrenoide)

Scale drop syndrome	Unknown agent	Probably Virus		10 to Harvest	None
(EUS) Red Spot Disease.	Oomycetes, Aphanomyces invadans	Fungus Like Eukaryotic	Motiles spores that invade the skin. Reddening in over a single scale, wich spread to the adjacents, resulting in severe ulcers. Also cloudines of the cornea accompanied or not by lesions in the skin.	Juvenils	Captive fish may respond to treatment with iodophore solution or increasing salinity.
	Hirudinea (Leeches)	Parasitic	Anemia, body discolouration, scale loss, frayed fins and restless swimming.		Formalin bath treatment and drying culture facilities in order to disiccate leech cocoons.
	Nematodes (Anisakis)	Parasitic			NA
	Cestodes (Scolex pleuronectis and Nybelinia indica)	Parasitic	Parasite in stomach, stomach wall, mesenteries, pyloric caeca, intestine		NA
Internal parasite	Trematode digeneans (Blood flukes)	Parasitic	Adult parasites release eggs into the fish's vasculr system which may be sequestered in the gills, heart, kidney, liver, spleen, pancreas or other organs, where casue inflammation and decrease the physiological and mechanical efficiency of these organs.		Praziquantel
	Trypanosoma	Parasitic	Anemia, anorexia, lethargy, scale loss, intra-ocular haemorrhage, splenomegaly and exophthalmia.	Juvenils	None
	Microsporidia Pleistosphora	Parasitic	Cysts in the muscle		None
	Myxosporidia Kudoa	Parasitic	Cysts in the gills or internal organs		None
	Isopods (Cymothoidae)	Parasitic	Blood feeders and occur on the body, mouth and branchial cavity. Infections of farmed hatchery seabas in the branchial and anterodorsal regions by Cymothoa indica resulted in skin lesions and were associated with lowered growth rates and mortality.		
External parasitosis by Crustacean	Lernanthropus latis	Parasitic	Adult females attach to the primary gill filaments, smallers males ares found on thegills or attached to females. Irreparable damage to the gills. Haemorrhages, hyperplasia and necrosis along the secondary lamellae of gill filaments.		There are no known treatments, although hydrogen peroxide bathing is currently being trialed in Australia.
	Cangus spp (Cepidemicus, Cchiastos, Corientalis, Cpagrosomi, Crotundigenitalis, Cpunctatus)	Parasitic	Skin damage and redness.	Adults in offshore	Emamectin in feed - Sealice treatment (Diflubenzuron, Pyretrenoide). Bath.

Red ulceration on the skin, weakness of the fish which lost

Parasitic

weight

Lernaea

Caligus spp (C.epidemicus,





English name :	European seabass
Scientific name :	Dicentrarchus labrax

10 Mar 1 1 1 1 1	Mar de la contra de la				
Disease/Virus	Agent	Туре	Affected/Syndrome	Stage	Measures
Viral nervous necrosis (VNN)	Nodavirus	Virus	Pale or dark colouration; erratic swimming behaviour; spiral swimming; bloating; 'fainting'; extensive vacuolation of the brain & spinal cord; generally encountered during hatchery phase. Microscopically, affected fish show characteristic severe vacuolation in the grey matter areas of the brain and in the neuronal layers of the retina.		
/iral encephalo-retinopathy	Nodavirus	Virus	Nervous symptoms	Larval and Juvenile	Good prophylaxis; good husbandry conditions
/ibriosis	Vibrio anguillarum; Vibrio ordali; Vibrio spp	Bacteria	Anorexia; darkening; skin ulcers; abdominal distension; splenomegaly; visceral petechiation; necrotic enteritis	Juvenile	Fry vaccination; antibiotic treatment
Photobacteriosis or Pseudotuberculosis	Photobacterium damsela subsp. pasteurella	Bacterium	Anorexia; darkening; splenomegaly; miliary lesions of spleen or spleen granulomatosis (chronic form)	Juvenile	Antibiotic treatment
Nyxobacteriosis	Flexibacter maritimus	Bacterium	Skin ulcers; necrosis; fin erosion	Juvenile	Antibiotic treatment
Nycobacteriosis	Mycobacterium marinum	Bacterium	Emaciation; poor growth; hypertrophic kidney and spleen with granulomas	Juvenile	Good prophylaxis
Epitheliocystis	Chlamydia-like	Bacterium	Miliary nodules on skin or gills	Juvenile	Good prophylaxis
Amyloodiniasis	Amyloodinium occelatum	Dinoflagellate	Skin darkening; skin dusty appearance (velvet disease)	Fry, Juvenile and Adults	Freshwater treatment
Cryptocaryoniasis	Cryptocaryon irritans	Ciliates	Skin lesions; white spot or multifocal white patches (marine white spot disease)	Juvenile	Freshwater treatment
Scuticociliatosis; other ciliatosis	Philasterides dicentrarchi; Uronema sp.; Te trahynema sp.	Ciliates	Skin and gill lesions; depigmentation; ulcerations; skin area haemorrhages	Juvenile	Freshwater treatment
Myxosporidiosis	Shaerospora dicentrarchi; S. testicularis; Ceratomyxa labraci	Myxosporidia	Reduced production; reduced growth rate; low mortality	Juvenile and Adult	No treatment
Microsporidiosis	Glugea sp.	Microsporidia	Reduced production; low mortality	Juvenile and Adult	No treatment
Gill fluke infections	Diplectanum aequans; D. laubieri	Monogenean trematode	Skin cloudiness; focal reddening with excess mucus production; epithelial hyperplasia; gill haemorrhages	Juvenile	Correct prophylaxis; good husbandry condition
Anisakis infection	Anisakis spp.	Nematoda	Larvae in coelomatic cavity	Larvae	Correct prophylaxis
Isopodiasis	Ceratothoa oestroides; Nerocilla orbiguyi; Anilocra physoides	Crustacea (isopods)	Growth retardation; gills and skin tissue necrosis; adults and larvae on fish	Larvae and Adults	Correct prophylaxis





English name :	Gilthead seabream
Scientific name :	Sparus aurata

Disease/Virus	Agent	Туре	Affected/Syndrome	Stage	Measures
Viral nervous necrosis (VNN)	Nodavirus	Virus	Pale or dark colouration; erratic swimming behaviour; spiral swimming; bloating; 'fainting'; extensive vacuolation of the brain & spinal cord; generally encountered during hatchery phase. Microscopically, affected fish show characteristic severe vacuolation in the grey matter areas of the brain and in the neuronal layers of the retina.		
Edwardsiella septicaemia	Edwardsiella tarda	Bacteria	Skin damage, ulcers and necrosis. Septicaemic haemorragea.		
Flavobacteriosis	Tenacibaculum maritimun	Bacteria	dara		
Septicaemia by Lactococcus garviae	Lactococcus garviae	Bacteria	Haemorragic septicaemia. Bilateral exophthalmia, petechiae over flanks or at fin bases and often haemoragic ascites.		
Mycobacterios is	Mycobacterium spp	Bacteria	Affected fish may be cachexic, darker in colour, and show swelling of the abdomen. Tubercles may be found in any organ, but specially in the liver, spleen and kidney.		
Nocardiosis	Nocardia spp	Bacteria			
Pseudo-tuberculosis	Photobacterium damsela sub. Piscicida	Bacteria	Sharp rise mortalities. Darkened sick fish gather in the corners. Whitish nodules on the spleen and posterior kidney.		

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Necrotic enteritis and peritonitis ('bloat')	Photobacterium damsela subspecies damselae	Bacteria	The consumption of excess feed, and/or larger feed pellet sand/or pellets of certain composition. It is postulated that the ingested feed may exceed the enzymatic digestive capacity of the alimentary tract and hepatopancreas, with subsequent bacterial proliferation, toxinproduction, tissue necrosis, loss of integrity of the intestinal wall	
Septicaemia by Pseudomonas	Pseudomonas sp.	Bacteria	Septicaemic haemorragea.	
Mycoba cterios is	Mycobacterium spp	Bacteria	Affected fish may be cachexic, darker in colour, and show swelling of the abdomen. Tubercles may be found in any organ, but specially in the liver, spleen and kidney.	
Nocardiosis	Nocardia spp	Bacteria		
Streptococcosis	Streptococcus iniae	Bacteria	loss of equilibrium, exophthalmia and opacity of the eye, loss of appetite, lethargy and irregular movement. Some fish displayed darkening of the skin, emaciation and proximal margins of the pectoral fins, accumulation of fluid in the peritoneal cavity hemorrhaging of the internal organs, pale livers and enlarged spleens, Darkened fish; anorexia; pale gills; reddened abdominal fluid; reddened abdominal organs & inner wall.	
Epitheliocystis	Epitheliocystis organism – Chlamydia	Bacterium	Swimming at water surface; rapid opercular movements; Microscopically, large numbers of cysts may be seen within the epithelium of the gill.	
Amyloodiniasis	Amyloodinium ocellatum	Protozoa	Found in marine conditions: In young fish opaque patches or a green discolouration of the skin; patches of skin lifting of surface & ulcersIn older fish rapid opercular movements; excess gill mucus; dark green gill colour More common in broodstock and in raceways; associated with low water temperatures or rapid drops in temperature	
Gill fluke	Diplectanum sp.;	Monogean	Rapid opercular movements; a norexia; white a reas	
Unit Huke	Dactylogyrus sp.	trematodes	on gills	
Skin fluke	Neobenedinia melleni; Gyrodactylus spp.	Monogean trematodes	Marine fish with opaque cornea; white patches on skin; skin ulcers; associated with high salinity & cool water temperatures	
Fish louse	Argulus sp.	Copepod	Disc-shaped parasite visible on skin; red foci; darkening	
Anchor worm	Lemaea sp.	Copepod	Thin body of female parasite visible on skin with small red ulcer where parasite penetrates skin	



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Cryptocaryonosis	lchthyophthirius multifiliis in freshwater, Cryptocaryon irritans in marine	Protozoa	Flashing'; rubbing skin on surfaces; anorexia; swimming at water surface; white spots on skin & fins			
Nematodes Acanthocephales	Nematodes Acanthocephales	Nematodes Acanthocephales	Low productivity			
Trypa no somos is	Trypanosoma sp.	Protozoa	lethargy,incoordination, apparent blindness and death.Exophthalmos with intra-ocular haemorrhage, together with large haemorrhagic ulcers and smaller haemorrhagicerosions of the skin			
Oodiniosis	Oodinium sp	Protozoa	Flashing'; rubbing skin on surfaces;congested gills.			
Chilodonelliasis	Chilodonella spp.; Chilodonella hexasticha	Protozoa	Swimming at water surface; rapid opercula movement; flared opercula; seen in poor environmental conditions & in weakened fish			
Trichodiniasis	Trichodina complex spp.	Protozoa	Swimming at water surface; rapid opercular movements; excess gill mucus; typically follows cold water temperatures, high organic loads & high stocking densities			
Ichthyobodosis (costiasis)	lchthyobodo necator	Protozoa	'Flashing'; rubbing skin on surfaces; opaque patches on skin; raised scales; swimming at water surface; rapid opercular movements; flared opercula			
Piscinoodiniasis	Piscinoodinium sp.	Protozoa	Found in freshwater: In young fish opaque patches or a greenish discolouration of the skin; patches of skin lifting of surface & ulcers In older fish rapid opercular movements; excess gill mucus; dark green gill colour			
Red sore disease	Epistylis sp.	Protozoa	Skin ulcers in freshwater pond fish; raised fluffy surface & secondary bacterial infections			
Red spot or Epizootic Ulcerative Syndrome (EUS)	Aphanomyces invadens	Fungi	Typically affected fish have deep red or haemorrhagic ulcers on the skin of the bodies. The ulceration may extend to and involve the eyes. Fish may become lethargic and readily fall prey to other species.			
Integumentary mycosis	Saprolegnia spp.; Achlya spp.	Fungi	Raised, fluffy growths on skin & fins; associated with low water temperatures & skin trauma			
Branchiomycosis	Brachiomyces sp.; Achlya spp.	Fungi	Swimming at water surface; rapid opercular movements; white & red patches (mottled appearance) on gills; associated with cold water temperatures & high organic loads			
Myxosporidiosis	Henneguya sp.; Kudoa sp.	Spore-forming protozoa	Disease uncommon but histologically spore cysts seen in gill filaments (Henneguya sp.) & brain (Kudoa sp.)			





English name :	Tilapia
Scientific name :	Oreochromis niloticus

Disease/Virus	Agent	Туре	Affected/Syndrome	Stage	Measures
Tilapia lake virus disease	Tilapia Like virus,TiLV	Virus	Sluggish behavior, reddened skin, and inflamed eyes and brain. And when these infected fish shared water with healthy ones, they passed on their disease, killing off more than 80 percent of their neighbours in a few days.		
Streptococcosis	Streptococcussp.	Bacteria	Bleeding inside the gills; sores on the fins; ulcers on the base of the tail, loss of equilibrium, exophthalmia and opacity of the eye, loss of appetite, lethargy and irregular movement. Some fish displayed darkening of the skin, emaciation and proximal margins of the pectoral fins, accumulation of fluid in the peritoneal cavity hemorrhaging of the internal organs, pale livers and enlarged spleens, Darkened fish; anorexia; pale gills; reddened abdominal fluid; reddened abdominal organs & inner wall	-	Oral administration of antibiotics; limiting densities in cages; good quality feed; not over-feeding; removal of infected fish
Columnaris	Flavobacterium columnare	Bacteria	Anorexia; darkening; skin ulcers; abdominal distension; splenomegaly; visceral petechiation; necrotic enteritis	Juvenile	Fry vaccination; antibiotic treatment
Aeromonas infection	Photobacterium damsela subsp. pasteurella	Bacterium	Anorexia; darkening; splenomegaly; miliary lesions of spleen or spleen granulomatosis (chronic form)	Juvenile	Antibiotic treatment
Epitheliocystis	Chlamydia-like	Bacterium	Miliary nodules on skin or gills	Juvenile	Good prophylaxis
Gill fluke infections	Monogenean trematode	Monogenean trematode	Skin cloudiness; focal reddening with excess mucus production; epithelial hyperplasia; gill haemorrhages	Juvenile	Correct prophylaxis; good husbandry condition
Isopodiasis	Ceratothoa oestroides; Nerocilla orbiguyi; Anilocra physoides	Crustacea (isopods)	Growth retardation; gills and skin tissue necrosis; adults and larvae on fish	Larvae and Adults	Correct prophylaxis





English name :	Amberjack
Scientific name :	Seriola dumerili

Disease/Virus	Agent	Туре	Affected/Syndrome	Stage	Measures
Iridovirus infection Viral Splenic Virus		Virus	Abnormally hypertrophic cells in spleen, kidney, heart, intestine and gill		Exclude potentially infected fish
Viral Nervous Necrosis		Virus	Lethargy; pale coloration and loss of appetite		Exclude potentially infected fish
Ascites viral disease (Yellow tail ascites virus, YAV)	Yellow tail ascites virus	Virus	Small fry and juvenile fish distended bellies, yellow-red fluids in heart and body cavities. High morntality rates and pale gills (anaemia) can occur.mircoscopically liver and spleen exhibits necrosis.		No vaccine or treatment available
Lymphocystis virus disease	Iridovirus	Virus	Normal, healthy looking fish with numerous balack spots within the epethelial cells such as skin, fins and gills.		No vaccine or treatment available; avoid uncooked fresh feeds for broodstock
Vibriosis	Vibrio anguillarum	Bacteria	Reddening of fins and skin; skin ulceration; muscular necrosis; haemorrhaging; lethargy		Oral administration of sulfa drugs or antibiotics; limiting densities in cages; daily surveillance; good quality feed
Pseudotuberculosis	Photobacterium damselaesubsp. <i>pi</i> <i>scicida</i>	Bacteria	White nodes on spleen and kidney		Oral administration of antibiotics; administer prophylactic doses
Nocardia	Nocardia seriola	Bacteria	Slow-swimming, thin fish with pale gills and large irregularly shaped white lumps at the base of the gill filament. Ulcerated/eroded operculum, skin lumps and ulcers and enlarged kidney and spleen with 1-2 mm yellow white spots (all other organs and fat may also contain spots), a thick brown black crusty plaque inside the swim bladder is also common.		No vaccine or treatment available



Mycobacterium	micobacteri um marinum	Bacteria	Slow-swimming, thin fish with pale gills (sometimes with white spots), enlarged kidney and spleen with 1-3 mm yellow white spots, spots in the fat, yellow red fluid in the body and heart cavities (not always), enlarged distended belly and yellow-red fluid often oozes from the vent.	No vaccine or treatment available
Streptococcosis	Streptococcus sp.	Bacteria	Bleeding inside the gills; sores on the fins; ulcers on the base of the tail	Oral administration of antibiotics; limiting densities in cages; good quality feed; not over- feeding; removal of infected fish
Epitheliocystis	Chlamydia-related organisms	Bacteria	Reduced growth; branchial; respiratory distress	Major pathological problem at early stages; minor problems in juveniles and adults; hygiene and disinfection of the culture environment is recommended
Tenacibaculosis	Tenacibaculum maritimum	Bacteria	Large patches of complete skin loss and exposed muscles on the head, tail and belly, eroded fins, if mouth are effected will be with slimy yellow film, spleen and kidney may be enlarged	avoid handling stress, treament with antibiotic and formalin is effective
Jaundice syndrome (Haemolytic anaemia)	blood borne bacteria, nutritional, biochemical oxidation problems, Low DO		Pale yellow skin and operculum (the inner surace red yellow and gelly like), yellow fins, pale gills, yellow-red fluids and gelly clots in the body and heart cavities, large spleen and yellow- red necrotic liver	No vaccine available; antiobiotic through feed will help to reduce blood borne bacteria.
Fungal infection	lchthyophonus hoferi	Fungi	Affects circulation system and other organs of the fish; clinical signs seen only when infection is well established; colour change; deformity; emaciation; loss of balance	No effective treatment, although a combination of oral and in-water medication with 2- phenoxyethanol has been recommended; prompt removal of infected fish; stop feeding raw fish or raw fish based products
Cryptocaryonosis; marine white spot	Cryptocaryon irritans	protozoan ciliate	White foci visible on skin; interconnected larger masses of whitish spots; darkened body; lethargy	Prolonged copper immersion; freshwater dips; formalin bath; salinity reduced to 20 ‰; or less; decrease system temperature to < 20 ºC
Kudoosis amami	Kudoa amamiensis	Myxosporidian parasite	Affecting the muscle; accelerate degeneration and post mortem myoliquefaction; effects on product quality	No treatment available
Beko disease	Micros poridium seriolae	Microsporidian parasite	Affecting the trunk muscles; after cyst's degeneration the neighboring muscle tissue shows necrosis; concave body surface	Vaccination; oral antibiotic treatment



Flatworm infection	Benedenia seriolae; Neobenedenia melleni (syn. <i>Girell</i> ae)	Tre ma to de	Attaches to skin; feeding on mucus and epithelial cells; secretion of viscous fluid; darkened body; erratic swimming; lethargy; loss of appetite; itching; rubbing against culture surface site; develop sores and skin peels; exposed flesh	Prevented by dipping in freshwater, periodic baths with hydrogen peroxide (500 ppm); praziquantel or formaldehyde has been recommended; handling material disinfection is recommended
Zeuxaptosis; Flatworm infection	Zeuxapta seriolae	Tre ma tode	Attaches to one or two lamellae of the gills by the haptor hooks feeding on blood which may cause a fatal anaemia; gill mucus secretion; normal skin colour and weight; slow swimming	Formaldehyde baths (300 ppm for 1 hour) every 15–30 days seem effective. Baths (alone or combined) with 300 ppm hydrogen peroxide; fresh water; copper sulphate; clove oil; praziquantel (or oral administration) has been recommended; Handling material disinfection is recommended
Sanguinicolosis	Paradeontacylixsp.	Trematode	Affects circulation system and other organs of the fish; accumulation of eggs in the gills blood vessels; multiple lesions and microhaemorrhages; anaemia	No effective treatment; regular cleaning and disinfection of nets and handling materials could reduce the risk of the parasite transmission



English name :	Meagre
Scientific name :	Argyrosomus regius

Disease/Virus	Agent	Туре	Affected/Syndrome	Stage	Measures
Vibriosis	Vibrio anguillarum	Bacterium	Fins and areas around vent and mouth become reddened; loss of appetite	Juvenile	Antibiotic in feed
Oodiniasis	Amyloodinium ocellatum		Protozoan attach to gills, producing irritation, asphyxia and hypermucosis	Juvenile	Formalin; copper sulphate
Fluke	Gyrodactylus sp.	Trematodal parasite	Parasites attached to fins and gills	Juvenile and Adult	Formalin



Annexure #14 – Reference diagnostic laboratories (National and International) for testing fish diseases

A. Red Sea bream Iridoviral Disease – Iridovirus

National Research Institute of Fisheries Science, Fisheries Research Agency, Fukuura 2-12-4, Kanagawa-ku, Yokohama-shi, Kanagawa 236-8048, JAPAN.

Tel.: (81.45) 788.76.15, Fax: (81.45) 788.50.01

E-mail: <u>RSIV-lab@fra.affrc.go.jp</u>

B. Epizootic ulcerative syndrome – Aphanomyces invadens

Aquatic Animal Health Research Institute (AAHRI), Inland Fisheries research and Development Bureau, Department of Fisheries,
PaholyothinRoad,
Jatujak,Bangkok10900,THAILAND.Tel.: (66.2) 579.41.22, Fax: (66.2) 561.39.93.E-mail: sudat@fisheries.go.th; kanchanakhan@yahoo.com

C. Viral encephalopathy and retinopathy (VER) VNN

Istituto Zooprofilattico Sperimental edelle Venezie, Dipartimento di Ittiopatologia, Via Romea 14/A, 35020 Legnaro PD, ITALY.

D. Other laboratories:

Entity	Web Site	Telephone/ Address
Australian Animal Health Laboratory , CSIRO– Australia	alex.hyatt@csiro.au	61 – 352275000
National Veterinary Institute, Technical University of Denmark (DTU) –Denmark	njol@vet.dtu.dk	45 – 72346831

2- National Laboratories for testing fish diseases

- A- Fish health and safety Laboratory, Fisheries Research Center in Jeddah. Website : www.jfrc.gov.sa
- B- Fish health and safety Laboratory, Fisheries Research Center in Dammam.