Report of the International Emergency Disease Investigation Task Force on a Serious Finfish Disease in Southern Africa

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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2009

ISBN 978-92-5-106238-8

Preparation of this document

This document is the final report of the work carried out by the International Emergency Disease Investigation Task Force on a Serious Finfish Disease in Southern Africa, a joint undertaking by the Food and Agriculture Organization of the United Nations (FAO), Botswana's Department of Wildlife and National Parks (DWNP) and Department of Animal Health and Production (DAPH), the Aquatic Animal Health Research Institute (AAHRI) of Thailand's Department of Fisheries and the Network of Aquaculture Centres in Asia and the Pacific (NACA), as a result of a technical mission to Botswana undertaken from 18 to 26 May 2007 and the subsequent outcomes of laboratory analysis of field samples conducted by AAHRI.

Prior to the finalization of this report, preliminary results, through a report dated 13 June 2007, containing some of the findings of the Task Force, particularly the confirmation of the epizootic ulcerative syndrome (EUS) in Botswana and including recommended short-term actions to deal with this emergency, were conveyed to the Government of Botswana and other stakeholders through the FAO offices in Angola, Ghana, Malawi, Namibia, South Africa, Zambia and Zimbabwe, and relevant organizations such as AAHRI, NACA and the World Organisation for Animal Health (OIE).

The preparation of this report was spearheaded by Dr Melba B. Reantaso, Fishery Resources Officer (FAO) and head of the Task Force mission, with contribution from Task Force members (Dr Somkiat Kanchanakhan of AAHRI, Dr Rohana P. Subasinghe of FAO, Dr Michael J. Phillips and Dr C.V. Mohan of NACA, Dr Ben Van der Waal of Namibia, Dr Bernard M. Hang'ombe of Zambia and Mr Shaft M. Nengu of Botswana).

Abstract

In response to a request for an emergency technical assistance from the Government of Botswana in connection with a serious disease affecting freshwater fishes in the Chobe-Zambezi River system reported since October 2006, the Food and Agriculture Organization of the United Nations (FAO) formed an International Emergency Disease Investigation Task Force. The overall objectives of the Task Force were to undertake an emergency assessment of the fish disease outbreak in order to identify, as far as possible, the causative agent, to provide recommendations to prevent further spread of the disease, to recommend control measures if applicable, to develop an emergency response and contingency plan for future outbreaks to concerned governments, and to develop a possible regional project. Members of the Task Force travelled to Botswana from 18 to 26 May 2007. The mission of the Task Force, in May 2007 and subsequent work, confirmed the occurrence of the epizootic ulcerative syndrome (EUS) in the southern African region. A preliminary report containing initial findings confirming the presence of EUS in Kasane, Botswana, was submitted in June 2007, immediately after confirmation, and provided the basis for initial short-term actions to address this significant fish disease emergency.

The EUS outbreak in the Chobe-Zambezi River system had exposed serious aquatic biosecurity weaknesses in the region. The mission identified various short-, medium- and long-term actions and recommended an aquatic biosecurity programme to strengthen capacity for fish disease diagnosis and control, quarantine, responsible movement of live aquatic animals, development of appropriate policy and regulatory frameworks, and implementation of better aquatic animal health management programmes in the region. In response to the mission's recommendations, FAO approved a regional technical assistance project – TCP/RAF/3111 *Emergency assistance to combat EUS in the Chobe-Zambezi River* involving seven countries bordering the Zambezi River, namely Angola, Botswana, Malawi, Mozambique, Namibia, Zambia and Zimbabwe.

This report provides comprehensive information on the outcomes of the 2007 Task Force investigation, building on earlier reports, and including further updates on EUS occurrence in southern Africa based on an active surveillance programme that was implemented by FAO and government partners in late 2007 until 2008. It also includes other ongoing activities and developments aimed at further enhancing aquatic biosecurity in southern Africa.

FAO.
Report of the International Emergency Disease Investigation Task Force on a Serious Finfish Disease in Southern Africa, 18–26 May 2007. Rome, FAO. 2009. 70p.

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Acknowledgements

The members of the local task force from Gabarone and Kasane are gratefully acknowledged for their kind assistance throughout the field mission as well as the fish farmers and fish traders who provided some information during the mission. We also gratefully acknowledge epizootic ulcerative syndrome (EUS) experts Drs Kamonporn Tonguthai and Supranee Chinabut for their guidance in the Task Force work and confirmation of EUS. Dr Flavio Corsin (World Wildlife Fund, Viet Nam) prepared the maps in Plate 12; while Mr Jeff Jenness (FAO Consultant) and Dr José Aguilar-Manjarrez of the Aquaculture Management and Conservation Service (FIMA) of the FAO Fisheries and Aquaculture Department prepared the maps for Figures 1 and 12 – they are all gratefully acknowledged. Mr José Luis Castilla and Ms Elda Longo (desktop publishers), Ms Sylviane Borghesi of FIMA, Ms Françoise Schatto and Ms Tina Farmer of the FAO Fisheries and Aquaculture Information and Statistics Service are acknowledged for their kind assistance in the final publication. Last but not least, we thank Drs Ezzedine Boutrif (Nutrition and Consumer Protection Division), Mike Robson and Peter Kenmore (Plant Production and Protection Division) of the FAO Agriculture and Consumer Protection Department and Mr Jia Jiansan of FIMA for encouragement and for facilitating funding support to the mission and subsequent work under FAO's Programme Cooperation Agreement with Norway.

Acronyms and abbreviations

AAHRI	Inland Aquatic Animal Health Research Institute
BF	blue fin (cell line)
CPE	cytopathic effect
DWNP	Department of Wildlife and National Parks
EGA	epizootic granulomatous aphanomycosis
EPC	epithelioma papulosum cyprinae (cell line)
EUS	epizootic ulcerative syndrome
FAO	Food and Agriculture Organization of the United Nations
FIMA	Aquaculture Management and Conservation Service
GP	glucose peptone
HBSS	Hanks' balanced salt solution
H&E	haematoxylin and eosin
MG	mycotic granulomatosis
NACA	Network of Aquaculture Centres in Asia and the Pacific
NGO	non-governmental organization
NVL	National Veterinary Laboratory
OIE	World Organisation for Animal Health
PCA	Programme Cooperation Agreement
PCR	polymerase chain reaction
RAF	Regional Office for Africa (FAO)
RSD	red spot disease
SADC	Southern African Development Community
TCP	Technical Cooperation Programme
TSA	tryptone soy agar
UM	ulcerative mycosis
USD	United States dollar
WAHIS	World Animal Health Information System

1. Background

In December 2006, the Government of Botswana was informed by the Namibian Government of fish disease outbreaks in the Chobe-Zambezi River. Infected fish were first observed at Impalila Island, Namibia, by the Namibians in October 2006. Similar cases of fish infection were further seen on the Zambian side of the Zambezi River. Subsequent to this, a fish sampling team was assembled by Botswana's Department of Wildlife and National Parks (DWNP) in mid-December 2006 to undertake surveys in the area to determine if the infection had spread to the Botswana side of the Chobe River. The findings indeed confirmed that the fish infection had spread to the Chobe River. Clinical signs of infected fish included body lesions, skin ulceration and blood patches on the body. However, no dead fish were observed in the Botswana side although reports by Zambia indicated that dead fish were seen by fishers floating in the water, especially in the Kasaya River, a tributary of the Zambezi River.

After the confirmation of the occurrence of the infection in the Chobe River, water, tissue, blood and soil samples were collected for further analysis at the National Veterinary Laboratory (NVL) in Sebele, Gaborone, Botswana. A ban on fishing and fish imports from the affected areas was also instituted with immediate effect until further notice. The Government of Namibia had also imposed a fishing moratorium starting from 21 December 2006 to 28 February 2007.

The results from the NVL, which concur with those of Namibia, indicated that there was a lot of faecal bacteria in the Chobe River water rendering it unsuitable for drinking. Other fish disease causing pathogens (e.g. *Aeromonas hydropila*, *Salmonella* spp.) were isolated. However, their concentration levels were not determined. Analytic results further demonstrated that the Chobe River was the most highly polluted. The laboratory examinations carried out by both Botswana and Namibia emphasized on bacteriological analysis only; other possible aetiological agents such as fungi and viruses were not included in the tests (unpublished report dated 25 January 2007, Kasane Fish Disease Laboratory Investigation Report by Dr J.F.C., NVL, Sebele; unpublished report dated 27 March 2007, Report of outbreak of fish infection in Chobe-Zambezi River System by DWC-Kasane; unpublished report dated 30 March 2007, Report on the fish disease outbreaks in the Chobe-Zambesi River System by Mr Shaft M. Nengu (DWNP-Gaborone).

As a continuation of the investigations, another team of two experts from Rhodes University, Republic of South Africa was engaged by DWNP-Gabarone to carry out fish sampling surveys in the Chobe River on 18 and 19 April 2007. The team of two experts was joined by the DWNP, the Department of Animal Health and Production and fisheries officers from Namibia, in their sampling activity.

The preliminary findings indicate that the infections were a severe granulomatous mycosis. The lesions closely resembled those caused by the fungus *Aphanomyces invadans,* the aetiological agent associated with the epizootic ulcerative syndrome [EUS] (Enviro-Fish Africa, 2007, Pathology report by Dr K.D.A. Huchzermeyer and Dr P.A. Colly).

In response to a request to FAO by the Government of Botswana for technical assistance in investigating this serious disease outbreak, Senior Fishery Resources Officer (Aquaculture) Dr R.P. Subasinghe of the Aquaculture Management and Conservation Service (FIMA) secured funds and initiated and coordinated the organization of an International Emergency Disease Investigation Task Force on a Serious Finfish Disease in Southern Africa simply referred to in this report as the "Task Force".

2. International Emergency Disease Investigation Task Force

The Task Force, formed by FAO in April 2007, was composed of experts from FAO, the Aquatic Animal Health Research Institute of Thailand's Department of Fisheries (AAHRI, based in Bangkok) and Reference Laboratory for Epizootic Ulcerative Syndrome (EUS) of the World Organisation for Animal Health (OIE), the Network of Aquaculture Centres in Asia and the Pacific (NACA, based in Bangkok) and the Botswana Department of Wildlife and National Parks (DWNP, Gaborone, Botswana and the Department of Animal Health and Production [DAHP, Gaborone, Botswana]). The composition of the Task Force can be found in Annex 1 and Plate 1.

The overall objectives of the Task Force were to undertake an emergency assessment of the fish disease outbreak through: (a) field observations (e.g. visit to affected river system, interviews with local/district officials and local fishermen, collection of epidemiological data); (b) laboratory examination (i.e. parasitology, bacteriology, histopathology, mycology, virology) of available normal and diseased fish samples; and (c) examination of available reports and other laboratory findings, in order to identify as far as possible the causative agent of the outbreak, to provide recommendations to prevent further spread of the disease, to recommend control measures if applicable, to develop an emergency response and contingency plan for future outbreaks to concerned governments, and to develop a possible regional project.

With funding support from the Programme Cooperation Agreement with Norway (PCA Norway 2006-2007) under the D.1 Biosecurity Objective, three of the Task Force members (Dr M.B. Reantaso of FAO, Dr S. Kanchanakhan of AAHRI and Dr C.V. Mohan of NACA) travelled to Botswana from 18 to 26 May 2007.

PLATE 1

Task Force members and EUS experts at work

(All photos courtesy of M.B. Reantaso)

Last day sampling
From left, seated: S. Kanchankhan, M. Reantaso, M. Bakani. From left, standing: Coastguard1, DWNP Staff, K. Kesego, Shaft Nengu, Coastguard2. G.D. Rammusi, C.V. Mohan

First day sampling
From left, first row: Coastguard1, M. Reantaso, K. Kesego, Coastguard2, From left, second row: B. van der Waal, Shaft Nengu, B. Bernard, C.V. Mohan, K.V. Motshereganyi, S. Kanchanakhan, Driver1

Task Force members visit farmers and conduct discussion regarding fish disease

Thai EUS experts, from left, S. Chinabut. S. Tandavanitj (AAHRI Director) and K. Tonguthai, meeting with Task Force Members (12 June 2007)

Task Force members, from left, K. Tonguthai, R. Subasinghe, C.V. Mohan, S. Kanchanakhan, M. Reantaso, at AAHRI in Bangkok, 12 June 2007

3. Methodology: field observations and laboratory examinations

3.1 GENERAL PLANNING OF THE TASK FORCE WORK WITH LOCAL COUNTERPARTS

The first day was spent discussing local logistics, the type of fish sampling method to be used, the exact location of sampling in Kasane and roles and responsibilities.

The Task Force also explained in detail the procedures for investigating a disease outbreak including establishment of a case definition that will be used for the investigation. Annex 2 outlines the steps for establishing a case definition. There are 9 basic steps[1] for investigating an outbreak of a disease, however, not all steps are necessarily included in every investigation nor do they follow the same sequence, and several steps may be taken simultaneously. A case definition is a set of standard criteria for deciding whether an individual study unit of interest has a particular disease or other outcome of interest. The study unit may be an individual animal or group of animals such as a pond of shrimp, a cage of fish, an entire farm or a village, or an entire river system (Baldock *et al*., 2005).

3.2 FISH SAMPLING

The method of fish sampling used (i.e. gillnet or scoopnet) was determined on a daily basis depending on the outcome of the fish samples collected.

Two sets of experimental gillnets, each consisting of 11 panels of 10 m each with graded mesh size from 12 to 150 mm and a large scoopnet with 10 mm mesh were used to collect fish samples from several spots in the Chobe River in Kasane, Botswana. Gillnets were set up in the evening and fish were collected early the next morning. The scoopnet was used for collecting fish in shallow areas and backwashes of the river, applying the same procedures found effective by Namibian officers in collecting infected fish samples in the Zambezi River, Caprivi Region in Namibia (Plate 2).

Fish were kept in transparent plastic bags and transported live to a make-shift laboratory (adjacent to the hotel) for further examination and collection of fish tissues samples (Plate 3).

All fish collected were numbered, identified up to species level (as far as possible), length-weight measurements were taken and disease observations (see next section) collected. Photographs were taken as much as possible.

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[1] from Lilley, J.H., Callinan, R.B., Chinabut, S., Kanchanakhan, S., MacRae, I.H. and Phillips, M.J. 1998. EUS Technical Handbook. AAHRI, Bangkok. 88 p.

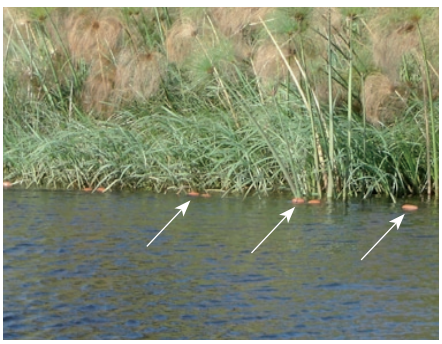
PLATE 2

Experimental gillnets and scoopnet used to collect fish during the Task Force mission in Zambezi River, Kasane, Botswana, May 2007

(All photos courtesy of M.B. Reantaso)

Gillnet

Scoopnet



Gillnet in the water

Scoopnet

PLATE 3

Make-shift laboratory in the premises of the hotel

(All photos courtesy of M.B. Reantaso)

S. Kanchanakhan preparing materials for collecting samples for laboratory examination

Local Task Force members (G.D. Rammusi, S. Nengu, M. Bakani) and C.V. Mohan taking length and weight measurements of fish samples

Local Task Force member G.D. Rammusi holding plastic bags with fish collected from sampling site

Transparent plastic bags used to keep fish samples prior to tissue sample collection

Fish samples deemed appropriate for further examination (see section 3.3 on collection of samples for laboratory analysis) were shipped using appropriate media to Bangkok, Thailand, for laboratory examination at AAHRI.

3.3 COLLECTION OF SAMPLES FOR LABORATORY ANALYSIS

3.3.1 Gross clinical signs

Fish samples were observed for gross clinical signs. Samples showing normal appearance (lack of any obvious abnormalities) were fixed in 10 percent formalin, while those showing some disease signs were subjected to further pathogen examination and/or isolation.

3.3.2 Parasitology

The skin, fins and gills of fish samples were examined by the naked eye for presence of parasites. Small fish were examined under a dissecting microscope. Similarly, fins and gills from bigger fish samples were examined under a dissecting microscope. Fresh smears (mucous) from the skin and gills were also collected and examined under a compound microscope for parasites. Large parasites found from the scrapes were fixed in 10 percent formalin.

3.3.3 Bacteriology

Fish samples showing gross clinical disease signs were subjected to bacteriological examination using standard bacteriological procedures (AAHRI, 1999; see Annex 3). The bacterial isolates were sub-cultured before transferring to transport media containing tryptone soya agar (TSA).

3.3.4 Mycology

Fish samples were examined by the naked eye for external fungal infection (i.e. presence of "tufts", nodules, obvious fungal mycelia or cotton wool-like growth and other epithelial lesions indicative of the presence of fungi). Only fish samples showing these signs were subjected to fungal isolation using standard mycological procedures (AAHRI, 1999; see Annex 3). Petri dishes containing culture media (agar plates, e.g. glucose-peptone agar or GP) were kept at 22 °C to 25 °C. Oomycete hyphae which grew in the culture plates were transferred to GP tubes prior to transport to AAHRI.

3.3.5 Virology

Only one diseased specimen was subjected to virus isolation using standardized virological procedures (AAHRI, 1999; see Annex 3). Virus extraction was carried out within 10 hrs after fish sampling. Extracts were kept in a cool box and transported to AAHRI laboratory. Cell culture and extract inoculations were carried out using two fish cell lines, epithelioma papulosum cyprinae (EPC) and blue fin (BF2).

3.3.6 Histopathology

Only live or moribund samples with clinical lesions were sampled for histopathology using standardized procedures (AAHRI, 1999; see Annex 3). Haematoxylin and eosin (H&E) and the general fungal stain (Grocott's stain) were used to observe fungal granulomas.

4. Results

The Zambezi River, with an area of 1 390 000 km^2 (or 537 000 miles) and length of about 2 574 km (or 1 600 miles), is the fourth longest river in Africa and the largest river flowing into the Indian Ocean from Africa. The main source is Kaleni Hills, Mwinilunga District in Zambia and the river flows through Angola, Zambia and then along the borders of Namibia, Botswana, Zambia and Zimbabwe to Mozambique (Figure 1).

There are an estimated 32 million people inhabiting the Zambezi river valley of which 80 percent are dependent on agriculture and the upper river's flood plains provide good agricultural land. The river is important for local livelihoods and nutrition, being fished extensively by surrounding communities; people travel long distances to fish for food. Recreational angling is also a significant activity in some parts of the river. In Zambia and Namibia, for example, there are several safari lodges which cater for tourists targeting tigerfish and other predatory fish species.

FIGURE 1
Map of Zambezi River
(prepared by Jeff Jenness and José Aguilar-Manjarrez, FAO-FIMA; source: African Water Resource Database)

4.1 GENERAL PLANNING OF THE TASK FORCE WORK WITH LOCAL COUNTERPARTS

In this particular investigation, the case definition[2] used was "a fish with granulomatous dermatitis and/or myositis and/or mycotic granulomas in tissues and organs infected with *Aphanomyces invadans* (=*A. piscicida*) found within the lesion".

The Task Force was divided into two groups: one group setting up the gillnet and collecting fish samples; the other group being responsible for processing of fish samples (identification of fish species, taking length and weight measurements, taking clinical observations and collection samples for further laboratory tests). A temporary make-shift laboratory was set-up for this purpose.

4.2 FISH SAMPLING

The first two days were devoted to gillnet sampling and since this procedure did not result in finding disease samples, the scoopnet was used during Days 3 and 4 in the shallow areas of the Chobe River west of Kasane. The scoopnet method was, based on experience by Namibia, quite effective in capturing small fish samples in the shallow part of the river.

A total of 189 fish belonging to more than 14 species (Table 1) collected by gillnets and 371 fish belonging to 27 species (Table 2) collected by scoopnet were

TABLE 1
Details of fish species collected by gillnets

Scientific name	Common name	Number of fish examined			Mean length +/-S.D (cm)	Mean weight +/- S.D (g)
		21/05/07	23/05/07	24/05/07		
Barbus eutaenia	orangefin barb	-	1	-	7	3
Bracinus lateralis	striped robber	3	10	1	10.98 (+/-1.92)	12.85 (+/-5.28)
Clarias gariepinus	sharptooth catfish	1	-	-	34.3	272
Pollimyrus castelnaui	dwarf stonebasher	-	4	-	11.15 (+/-0.75)	13.75 (+/-5.31)
Cyphomyrus discorhynchus	Zambezi parrotfish	-	2	-	19.5 (+/-1.41)	-
Hydrocynus vittatus	tigerfish	19	33	3	21.65 (+/-6.08)	90.19 (+/-97.91)
Marcusenius macrolepidotus	bulldog	2	9	1	13.44 (+/-2.74)	22.50 (+/-23.27)
Mormyrus lacerda	western bottlenose	-	8	-	13.78 (+/-2.51)	-
Petrocephalus catostoma	churchill	2	7	-	11.66 (+/-1.56)	16.8 (+/-6.65)
Schilbe intermedius	silver catfish	23	27	15	20.35 (+/-4.39)	76.63 (+/-44.76)
Serranochromis thumbergi	brownspot largemouth	1	-	-	12	22
Synodontis sp	squeaker	2	10	-	16.99 (+/-4.05)	45 (+/-48.56)
Tilapia sparmani	banded tilapia	4	-	-	9.53 (+/-1.18)	13 (+/-5.35)
*U*nidentified species		1	-	-	5.5	1

[2] Baldock *et al.* (2005) defined a case definition as a set of standard criteria for deciding whether an individual study unit of interest has a particular disease or other outcome of interest; the study unit may be an individual animal or a group of animals such as a pond of shrimp, a cage of fish, an entire farm or a village. It was indicated that a case definition is neither right nor wrong in terms of diagnosing a disease, it is simply an agreed set of rules which permits investigators to uniformly decide that a particular individual has or does not have a particular disease as defined.

TABLE 2
Details of fish species collected by scoopnet

Scientific name	Common name	Number of fish examined		Mean length (+/-S.D) (mm)
		22/05/07	23/05/07	
Aplocheilichthys johnstoni	Johnston's topminnow	7		33.2 (+/-2.05)
Aplocheilichthys katangae	striped topminnow	3	-	32 (+/-6.08)
Barbus haasianus	Sickle-fin barb	1	-	24
Barbus barotseensis	Barotse barb	4	-	46.25 (+/-6.40)
Barbus bifrenatus	hyphen barb	2	-	-
Barbus eutaenia	orangefin barb	11	2	39.4 (+/-9.48)
Barbus fasciolatus	red barb	1	-	38
Barbus multilineatus	copperstripe barb	10	-	28.67 (+/-0.58)
Barbus poechii	dashtail barb	3	-	60.5 (+/-9.19)
Barbus radiatus	Beira barb	10	38	47 (+/-5.10)
Barbus kerstenii	redspot barb	9	-	28.33 (+/-14.01)
Barbus thamalakanensis	thamalakane barb	8	-	32.42 (+/-1.13)
Barbus unitaeniatus	slender barb	28	1	44.32 (+/-6.49)
Momyrus lacerda	western bottlenose	1	-	163
Marcusenius macrolepidotus	bulldog	1	-	106
Micralestes acutidens	Silver robber	14	-	-
Pharynchochromis acuticeps	Zambezi happy	5	-	38.8 (+/-15.55)
Petrocephalus catostoma	churchill	-	-	-
Pollimyrus castelnaui	dwarf stonebasher	-	-	-
Cyphomyrus discorhynchus	Zambezi parrotfish	-	3	
Pseudocrenilabrus philander	southern mouthbrooder	57	7	33.79 (+/-6.67)
Serranochromis macrocephalus	purpleface largemouth	1	-	44
Serranochromis robustus	nembwe	1	-	-
Synodontis nigromaculatus	spotted squeaker	2	-	-
Synodontis spp.	squeaker	7	-	50.6 (+/-7.89)
Tilapia rendalli	redbreast tilapia	7	-	51
Tilapia ruweti	Okavango tilapia	4	-	38.5 (+/-12.02)
Tilapia sparrmanii	banded tilapia	43	77	49.36 (+/-9.40)

collected during a 4-day intensive sampling (21-24 May 2008). Out of these, tissue samples from 23 fish belonging to 16 species, and showing normal and abnormal clinical signs, were used for further laboratory analysis (Table 3).

4.3 FISH EXAMINATION

4.3.1 Gross clinical signs

All fish samples subjected to detailed examination were divided into three categories: (1) fish with disease clinical signs, (2) fish with skin damages from gillnet or scoop net, and (3) fish without disease clinical signs. Details are provided below.

(1) Fish with disease clinical signs. Two fish samples fall under this category, fish specimen No. 1 (*Barbus thamalakanensis*) and No. 9 (*B. poechii*) both exhibited abnormal clinical signs. *Barbus thamalakanensis* had haemorrhage at the anterior terminal of the body and showed fungal-like mycelium visible on the surface of the lesion. *Barbus poechii* showed remarkably large haemorrhagic dermatitis just

TABLE 3
Details of fish species subjected to further laboratory tests

Fish #	Scientific name	Common name	Gross clinical signs	Laboratory procedures	Findings	
					Findings on parasitology, bacteriology, mycology, virology	Findings based on histopathology
1	*Barbus thamalakanensis*	Thamalakane barb	superficial fungus on head and mouth	mycology histology	Fast-growing fungus isolated but contaminated with bacteria (discarded)	mycotic granulomas found in muscle tissues – **EUS positive**
2	*Pseudocrenilabrus philander*	southern mouthbrooder	normal	histology	-	mycotic granulomas not found in muscle – EUS negative
3	*Micralestes acutidens*	sharptooth tetra	normal	histology	-	mycotic granulomas not found in muscle – EUS negative
4	*Schilbe intermedius*	silver catfish	normal	parasitology histology	unidentified monogeneans observed	mycotic granulomas not found in muscle – EUS negative
5	*Barbus unitaeniatus*	slender barb	normal	histology	-	mycotic granulomas not found in muscle – EUS negative
6	*Aplocheilichthys katangae*	striped minnow	normal	histology	-	mycotic granulomas not found in muscle – EUS negative
7	*Pseudocrenilabrus philander* (2 fish)	southern mouthbrooder	white patch on the body	bacteriology histology	bacteria negative	mycotic granulomas not found in muscle – EUS negative
8	*Schilbe intermedius*	silver catfish	normal	histology	-	mycotic granulomas not found in muscle – EUS negative
9	*Barbus poechii*	dashtail barb	EUS-like lesion dermatitis with fungus on surface	mycology histology virology	slow growing fungus isolated virus negative using BF2 and EPC	mycotic granulomas found in muscle tissues – **EUS positive**
10	*Barbus bifrenatus*	hyphen barb	normal but with pale coloration	histology	-	mycotic granulomas not found in muscle – EUS negative
11	*Marcusenius macrolepidotus*	bulldog	minor haemorrhage at the tail and anal fin (damaged from gillnet)	parasitology bacteriology mycology histology	unidentified monogeneans observed bacteria negative fungus negative	mycotic granulomas not found in muscle – EUS negative
12	*Marcusenius macrolepidotus*	bulldog	haemorrhage at the caudal peduncle (gillnet damage)	parasitology histology	unidentified monogeneans, digeneans and sporozoans observed	mycotic granulomas not found in muscle – EUS negative

TABLE 3 (Continued)

13	*Petrocephalus catostoma*	churchill	multiple red spots (damaged by gillnet)	mycology histology	fungus negative	mycotic granulomas not found in muscle – EUS negative
14	*Petrocephalus catostoma*	churchill	single red spot (gillnet damage)	histology	-	mycotic granulomas not found in muscle – EUS negative
15	*Schilbe intermedius*	silver catfish	normal	parasitology histology	unidentified monogeneans, digeneans and sporozoans observed	mycotic granulomas not found in muscle – EUS negative
16	*Synodontis* sp.	squeaker	small white patch at tail (gillnet damage)	histology	-	mycotic granulomas not found in muscle – EUS negative
17	*Mormyrus lacerda*		normal	parasitology histology	unidentified monogeneans observed	mycotic granulomas not found in muscle – EUS negative
18	*Hydrocynus vittatus*	tigerfish	normal but with redness coloration of muscle	parasitology histology	unidentified monogeneans observed	mycotic granulomas not found in muscle – EUS negative
19	*Brycinus lateralis*	striped robber	normal	parasitology histology	unidentified monogeneans observed	mycotic granulomas not found in muscle – EUS negative
20	*Synodontis thamalakanensis*	squeaker	normal	histology	-	mycotic granulomas not found in muscle – EUS negative
21	*Synodontis* sp.	plain squeaker	normal	parasitology, histology	unidentified digeneans and sporozoans observed	mycotic granulomas not found in muscle – EUS negative
22	*Mormyrus lacerda*	western bottlenose	small skin damage (scoop net damage)	parasitology histology	unidentified monogeneans and sporozoans observed	mycotic granulomas not found in muscle – EUS negative
23	*Marcusenius macrolepidotus*	bulldog	multiple red spots at the caudal peduncle (mechanical damage)	bacteriology mycology histology	bacteria negative fungus negative	mycotic granulomas not found in muscle – EUS negative

after the anus opening to the caudal peduncle; the lesion was covered with fungal-like mycelium.

(2) Fish showing skin damage from gillnets or scoopnets. Eight fish specimens (fish specimen Nos. 7, 11, 12, 13, 14, 16, 22 and 23) fall under this category. They exhibited discoloration of body, lost scales, red spots ranging from single or multiple spots on the body surface and fins – gross signs related to mechanical damage caused by netting.

(3) Fish without abnormal clinical signs. Thirteen specimens (fish specimen Nos. 3, 4, 5, 6, 8, 10, 17, 18, 19, 20, 21) showed normal external appearance.

4.3.2 Parasitology

Monogenetic parasites were found in seven fish samples (specimen Nos. 4, 11, 15, 17, 18, 19 and 22). Digeneans and sporozoans were also observed in few fish samples (specimen Nos. 12, 15 and 21) as cysts forming in the gills or internal organs but in very low frequency. Fish observed to harbour monogenetic, digenetic and sporozoan parasites did not exhibit any gross clinical signs. No attempt was made to identify the parasites collected (see Plate 4).

4.3.3 Bacteriology

No fish pathogenic bacterium could be isolated on TSA or cytophaga media from fish specimen Nos. 7, 11 and 23. Fish with clinical lesions such as white patches or red spots/wounds were not related to bacterial infection.

4.3.4 Mycology

Fungal oomycete was successfully isolated from the muscle tissue next to the dermatitis lesion of diseased specimen No. 9. The oomycete grew slowly out of the muscle tissue and penetrated into GP agar plate at 2-3 mm in 2 days at 15-22 °C incubation temperatures. This slow growing oomycete isolate was sub-cultured and maintained in GP agar at 22 °C. The oomycete sporulated after placing the oomycete mycelium in autoclaved pond water for 4-6 hrs at 22 °C. It was confirmed as belonging to the genus *Aphanomyces* (Plate 5). The sporangia were narrow, with diameters similar to that of the hyphae. A single row of primary zoospores formed within a zoosporangium and then released through the sporangium to encyst at the apical tip to form achlyoid clusters. The main free-swimming stage of *Aphanomyces* spp. is the secondary zoospore which is discharged from the encysted primary zoospores.

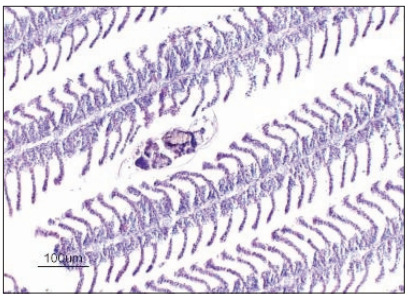
4.3.5 Virology

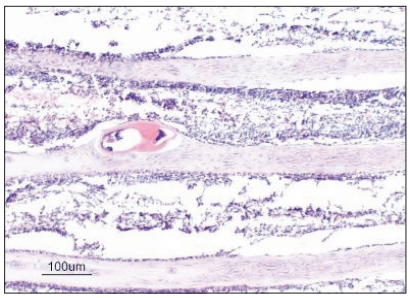
Virus isolation was attempted only for diseased specimen No. 9. No cytopathic effect (CPE) was observed in the first inoculation and subsequent blind passages. No virus could be isolated from diseased fish using EPC and BF2 cell lines.

PLATE 4

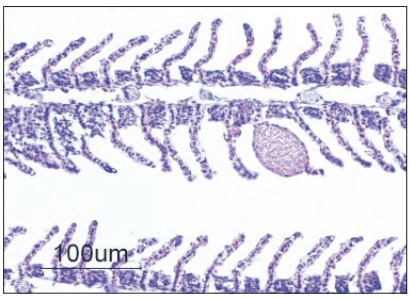
Parasites observed from fish samples

(All photos courtesy of AAHRI)

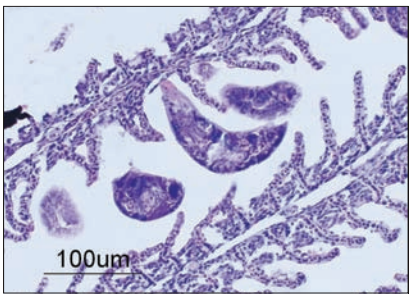




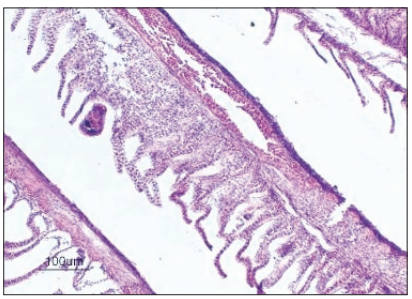
Fish sample No. 11 *Marcusenius macrolepidotus*, bulldog, was observed to harbour unidentified monogenetic parasites in the gills and kidney



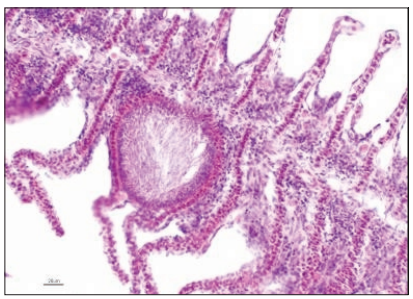
Fish sample No. 12 *Marcusenius macrolepidotus* (bulldog) was observed to harbour unidentified parasite cysts in the gill

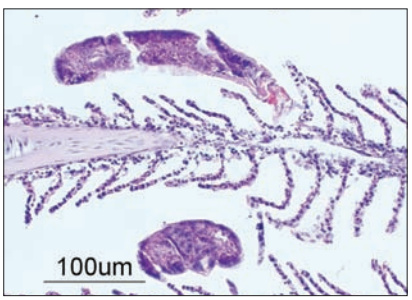


Fish sample No. 17 *Momyrus lacerda*, western bottlenose mormyrid, was observed to harbour unidentified monogenean parasite in the gills

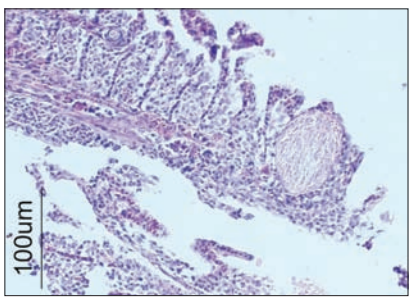


Fish sample No. 15 *Schilbe intermedius*, silver catfish, was observed to harbour unidentified monogenean and unidentified parasite cyst in the gills.





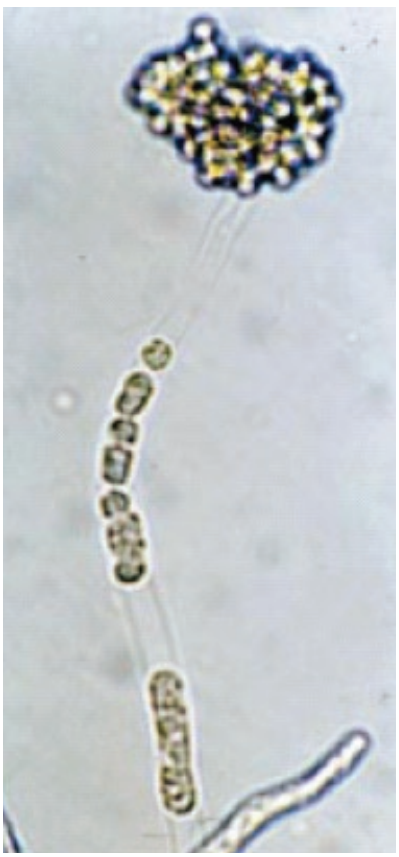
Fish sample No. 18 *Hydrocynus vittatus*, tigerfish, was observed to harbour unidentified monogenean in the gills



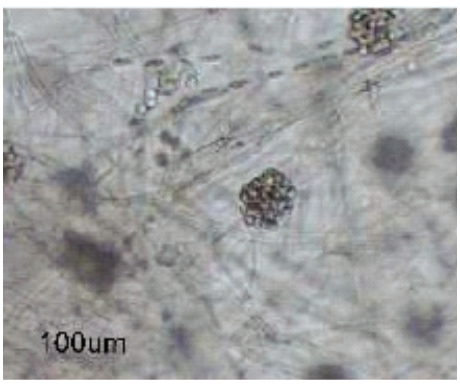
Fish sample No. 23 *M. macrolepidotus*, bulldog, was observed to harbour unidentified myxosporean and metacercarial cysts in the gills

PLATE 5

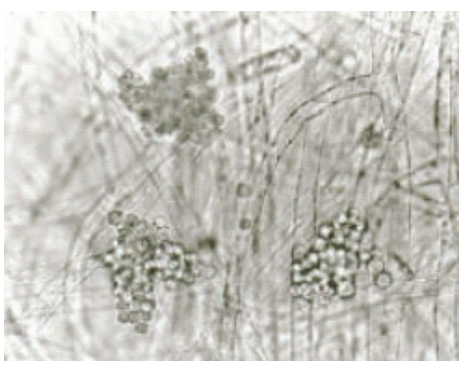
Aphanomyces sporangia (Japanese, Botswana and Philippine isolates)



Typical characteristic of *Aphanomyces* sporangium (Japanese isolate)
Source: K. Hatai and FAO Fisheries Technical Paper 402/2



Sporulation of the Botswana oomycete isolate identified as *Aphanomyces* successfully done by AAHRI (June 2007).
Source: S. Kanchanakhan (June 2007)



Aphanomyces sporangia, Philippine isolates
Source: M.B. Reantaso (1999)

4.3.6 Histopathology

(1) Fish with disease clinical signs. Fish specimen No. 1 showed swelling of the secondary gill lamellae, minor oedema and hyperplasia and blood sinusoid enlargement. Mycotic granulomas were found in the muscle tissues confirming EUS infection (Plate 6). Fish specimen No. 9 showed fungal hyphae invading the epidermis and dermis through to the musculature with necrotizing dermatitis and degeneration of muscle cells (Plate 7). Gills and internal organs were not processed for histopathology as they were used for virus isolation.

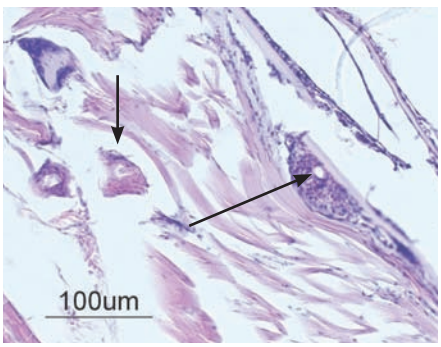
(2) Fish showing skin damage from gillnets or scoopnet. Histopathological changes in the skin lesions were related to loss of scales and epidermis or even parts of the dermis. Histopathology of gills and internal organs of fish in this group were minor and were probably not vital to fish health. These include the following observations: (i) gills of some fish showed minor hyperplasia, oedema, necrosis or inflammation. Monogeneans, metacercarial cysts of digeneans and sporozoan cysts were observed on the gills and caused necrosis or inflammation (Plate 4); (ii) kidney, liver, spleen and pancreas of most fish in this group showed normal histology. Minor histopathological changes such as pycnotic cells in some cells, melanomacrophage aggregation in internal organs of some fish, partial necrosis in kidney tubules, vacuolation in the liver of one fish and presence of unidentified digenean parasite cyst in few fish specimens.

(3) Fish without abnormal clinical signs. Some fish examined under this group showed minor histopathological changes. These changes are similar to those found in fish under the second group.

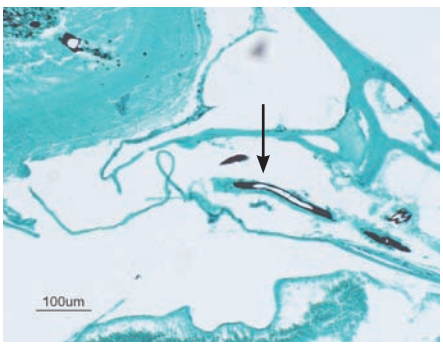
PLATE 6

Histopathology of EUS-infected Thamalakane barb, *Barbus thamalakanensis*, collected by scoopnet on 22 May 2007 in the shallow waters of Chobe-Zambezi River in Kasane, Botswana

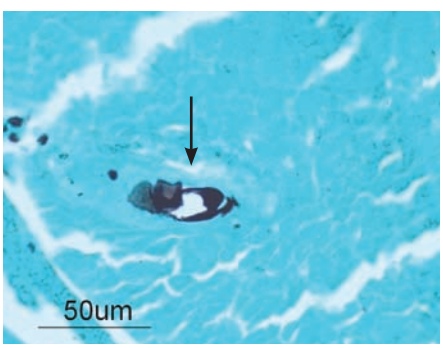
(All photos courtesy of AAHRI)



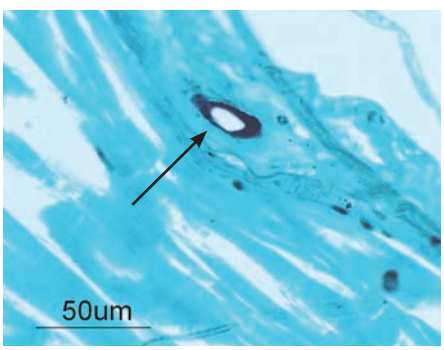
A



B



C



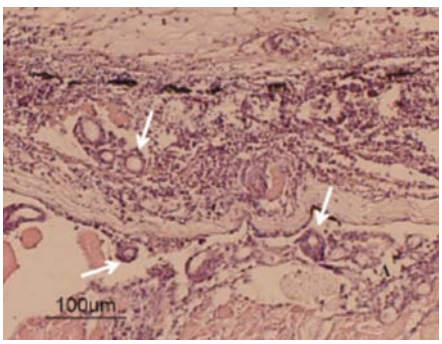
D

Typical mycotic granulomas (indicated by black arrow) found in the muscle tissue of fish sample No. 1 *Barbus thamalakanensis* (Thamalakane barb). (A) muscle tissues with mycotic granulomas (H&E); (B) oomycete hyphae penetrated into the brain of the fish; (B), (C) and (D) are stained with Grocott's stain

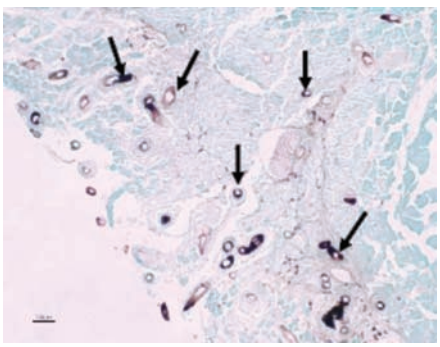
PLATE 7

Histopathology of EUS-infected dashtail barb, *Barbus poechii* (Steindachner, 1911), collected by scoopnet on 22 May 2007 in the shallow waters of Chobe-Zambezi River in Kasane, Botswana

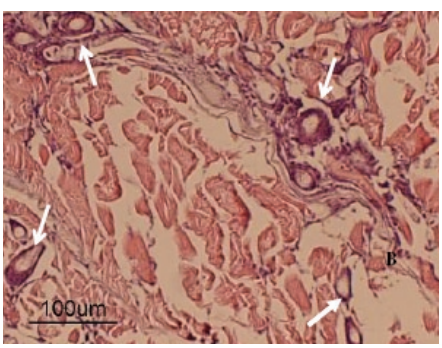
(All photos courtesy of AAHRI)



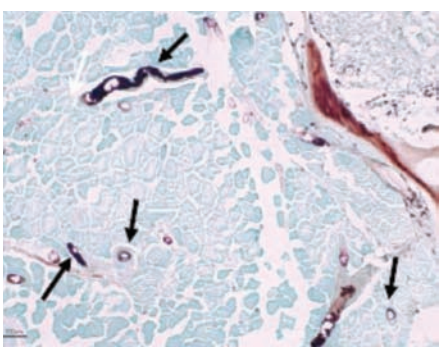
Histopathology of EUS-infected dashtail barb showing typical mycotic granulomas surrounding the invasive fungal hyphae (white arrows) in the skin layer (H&E)



Histopathology of EUS-infected dashtail barb showing typical mycotic granulomas surrounding the invasive fungal hyphae (stained black, black arrows) in the skin layer (Grocott's silver stain)



Histopathology of EUS-infected dashtail barb showing typical mycotic granulomas surrounding invasive fungal hyphae (white arrows) penetrating into the muscle layer (H&E)



Histopathology of EUS-infected dashtail barb showing typical mycotic granulomas surrounding invasive fungal hyphae (stained black, black arrows) penetrating into the muscle layer (Grocott's silver stain)

Dashtail barb, *Barbus poechii* (Steindachner, 1911), exhibiting haemorrhagic dermatitis posterior to anus and towards the caudal peduncle

5. Diagnosis

5.1 BACKGROUNDER TO EUS

EUS is a serious fish disease which has swept across Japan, Australia, many countries in Asia and the United States of America since the first outbreaks were reported in the early 1970s, causing significant loss of income to fishers and fish farmers and negative biodiversity and social impacts. Estimates of losses to EUS include the following: (i) USD 100 million in Thailand during 1983–1991; (ii) USD 4.8 million in Bangladesh during 1988-1989; (iii) USD 235 000 in Indonesia during 1980-1987; (iv) USD 300 000 in Pakistan in 1996; and (v) USD 700 000 annually in Eastern Australia. EUS is an OIE listed finfish disease, thus, OIE member countries are obliged to make an official notification to OIE in the event of an occurrence or an outbreak. EUS has caused major losses in fresh and estuarine fish species in many countries for over three decades during which time it was given several names such as: (i) in Japan, first described in 1971 as an *Aphanomyces* (fungal) infection (Egusa and Masuda, 1971) and later named as mycotic granulomatosis or MG; (ii) since 1972, an epizootic cutaneous ulcerative syndrome in estuarine fishes in Australia named as red spot disease or RSD (McKenzie and Hall, 1976); (iii) in 1986, the present name of epizootic ulcerative syndrome or EUS was given by an FAO Expert Consultation on Ulcerative Fish Disease (FAO, 1986) concerning similar conditions with dermal ulcerations and mortalities which have occurred throughout southeast and south Asia; (iv) in the United States of America, similar ulcerative lesions, named as ulcerative mycosis or UM (Noga and Dykstra, 1986) affecting estuarine fishes since 1978; and (v) since 2000, during an Expert Consultation on EUS as a special session of the Fifth Symposium on Diseases in Asian Aquaculture held in Gold Coast, Australia where 36 EUS experts from Australia, India, Japan, Philippines, Sri Lanka, Thailand, and the United States of America (Baldock *et al.,* 2002) re-examined the causal factors, case definition and nomenclature of EUS and proposed two new common names: epizootic granulomatous aphanomycosis (EGA) and ulcerative aphanomycosis. Annex 4 provides a comprehensive list of references demonstrating the range of research topics and other information about EUS spanning a period of over three decades.

More detailed information about EUS can also be found at Lilley *et al.* (1998), Bondad-Reantaso (2002), OIE (2006); reports/notification on EUS can be found at WAHIS Web site at www.oie.int/wahid-prod/public.php?page=home and the NACA Web site at www.enaca.org. Plate 8 shows some photographs of EUS-affected fish from the Philippines, Japan and Australia.

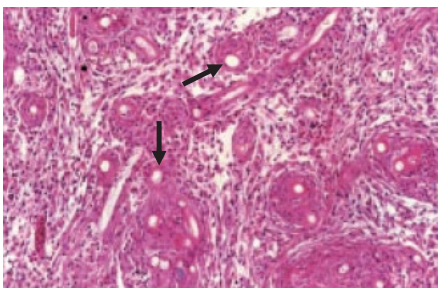
Various studies have listed a number of risk factors (see Table 4). These include temperature, rainfall and related water quality, flooding, soil and sediment characteristics. It is likely that there are a diverse group of biotic and abiotic agents/

PLATE 8

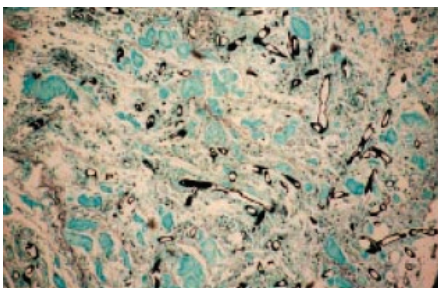
EUS-infected fish from the Philippines, Japan and Australia

(Source: FAO Fisheries Technical Paper 402/2 (2001). Asia diagnostic guide to aquatic animal diseases)

Snakehead (*Channa striata*) in the Philippines (1985) showing typical EUS lesions (dermal ulcers).



Typical severe mycotic granulomas (black arrows) from muscle section of EUS infected snakehead in the Philippines (1985) (H&E stain)

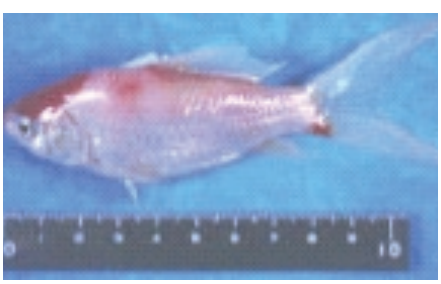


Mycotic granulomas showing fungal hyphae (stained black) using Grocott's silver stain

Ayu, *Plecoglatus altivelis*, from Japan, infected with mycotic granulomatosis

Wild mullet (M*ugil* sp.) in the Phillipines (1989) infected with EUS

EUS infected farmed silver perch *Bidyanus bidyanus* from Eastern Australia



Experimentally infected goldfish (*Carassius auratus*) (experiment conducted in Japan by M.B. Reantaso, 1999)

TABLE 4
Examples of EUS risk factors (i.e. predisposing factors, environmental conditions, biological factors)

Country	Risk factors	References
Asian outbreaks	Shipping movements, ballast water, fish migrations, ocean currents – potential pathways for pathogen movement	Morgan, 2001
	Cross border movements of fish for aquaculture and ornamental fish trade	Blazer *et al*. 2005
Australia and United States of America	Outbreaks of *Aphanomyces invadans* associated with rainfall season	Australia: Virgona, 1992 United States of America: Blazer *et al*., 2002
Bangladesh	General: use of pesticides, presence of wild fish in ponds, flooding, ponds connected to natural waters, high levels of organic wastes	Ahmed and Rab 1995
	Cross-sectional studies: wild fish observed in ponds, EUS during previous season, ponds connected to other water body; ponds flooded during rainy season, ponds not dried during pre-stocking, bottom mud not removed during pre-stocking, no liming, no fertilization, black color of water, parasites observed on fish	Khan *et al*., 2002
	Fish-level case study: 95 percent of EUS fish also with bacterial *Aeromonas* sp. infection; 49 percent of EUS fish infected with parasites, most commonly protozoan *Apiosoma* sp.	Lilley *et al*., 2001
	Pond-level case control study: low water depth, high ammonia levels, pond connection to other water body, presence of wild fish, no pre-stock liming	Lilley *et al*., 2001
India	Outbreaks in estuarine and brackishwater ponds following heavy rainfall when salinity drops below 1 ppt	Vishwanath *et al*., 1997
Philippines	Low water temperature, low alkalinity, low hardness and chloride, fluctuating pH and heavy rainfall	Bondad-Reantaso *et al*., 1992
Philippines and Australia	EUS outbreaks in wild estuarine populations associated with acidified run-off water from acid sulphate soil areas	Callinan *et al*., 1995, 1997

factors that may initiate skin lesions in freshwater and estuarine fish species and these non-specific lesions are subsequently colonized by *Aphanomyces invadans*. It is unlikely that any specific determinant is associated with EUS outbreaks and more likely that environmental determinants will vary from outbreak to outbreak depending on the agent initiating the non-specific lesions, the aquatic environment at the site and the population at risk. For EUS to occur, a combination of causal factors must ultimately lead to exposure of the dermis, attachment to it by *A. invadans*, and subsequent invasion by the fungus.

Control of EUS in natural waters is impossible, but in small closed water bodies and fish ponds several measures have been shown to reduce risks of EUS outbreaks or control mortalities. In outbreaks occurring in small, closed water bodies, liming of water and improvement of water quality, together with removal of infected fish, have sometimes been effective in reducing mortality. EUS outbreaks usually occur in the wild during cooler months of the year (below 20 °C–25 °C) where they may spread into fish aquaculture ponds. During dry and cold seasons, it is important that fish farmers closely observe wild fish. If EUS–diseased fish are present in the wild, farmers should stop water exchange. This simple measure can minimize or prevent the spread of EUS. In addition, farmers should also prevent all possible carriers or vectors such as birds or terrestrial animals as well as contaminated

fishing gears/nets from getting into the fish ponds. Table 4 lists some examples of EUS risk factors which may assist in determining appropriate risk management measures.

No official OIE notification of EUS occurrence was made by any country since 2005; in 2007, Botswana made an official notification to OIE[3] and in May 2008, Zambia. In March 2008, "ProMed mail list" circulated a message warning about the occurrence of EUS in New South Wales.[4]

5.2 CONFIRMATION BASED ON INTERNATIONALLY ACCEPTED METHODS FOR EUS DIAGNOSIS

There are three recommended confirmatory diagnosis for EUS (OIE, 2006). These are: (i) demonstration of mycotic granulomas in histological sections of affected tissues and organs using special stain such as Grocott's silver stain for fungal hyphae, (ii) isolation of *Aphanomyces invadans* and confirmatory identification, and (iii) PCR of pure isolate of *A. invadans.*

Two (i and ii) of the above three recommended confirmatory diagnostic methods were used for identifying the causative agent of the disease outbreak in southern Africa based on fish samples collected from the Chobe River, near Kasane, Botswana, situated near the Zambezi/Chobe confluence.

Of the 23 fish samples that were subjected to detailed laboratory analysis, two fish samples (fish specimen Nos. 1 and 9, *Barbus thamalakanensis* and *B. poechii*, respectively) satisfied the established case definition for this disease investigation. *Barbus thamalakanensis* and *B. poechii* both exhibited haemorrhagic dermatitis similar to EUS-lesions. The lesion found in *B. poechii* was covered with fungal-like mycelia. Plates 6 and 7 show the histopathological changes observed in both fish species. Mycotic granulomas were clearly evident in skin and muscle sections of infected fish. Oomycete was successfuly isolated from the same fish species; sporulation was undertaken and it was confirmed as belonging to the genus *Aphanomyces*.

Water temperature at the time of sampling was between 17 °C to 20 °C and air temperature was between 11 °C to 15 °C. This water temperature range was within the permissive temperature for EUS occurrence.

[3] www.oie.int/wahid-prod/public.php?page=disease_immediate_summary&selected_year=2007

[4] www.abc.net.au/rural/news/content/200803/s2195267.htm

6. Conclusions and recommendations

Most of the fish examined during the Task Force mission appeared normal. Ten of the 23 fish specimens subjected to detailed analysis showed lesions, red spots and haemorrhages many of which can be attributed to handling.

Oomycete infection was confirmed in two clinically diseased fish. Fungal-like hyphae invaded the epidermis, dermis and through to the musculature causing necrotizing dermatitis with degeneration of the muscle. Some hyphae appeared in the muscle next to the vertebral column. The oomycete fungi elicited a strong inflammatory response and mycotic granulomas formed around the penetrating hyphae, a typical characteristic of EUS.

The preliminary outcomes of laboratory analysis in early June 2007 revealed that the two diseased fish samples were infected with EUS based on histopathology. Further work enabled the successful isolation and sporulation of the putative fungal pathogen *Aphanomyces invadans.*

Because of the urgency of this fish disease situation, a preliminary report (dated 13 June 2007 or referred to as June 2007 report in this document) was submitted to the Government of Botswana and other relevant governments and organizations to inform the preliminary findings of the Task Force investigation.

The June 2007 report indicated that with the EUS pathogen now found in the upper reaches of the Chobe-Zambezi River system, downstream spread seems inevitable, particularly during the rainy season. Flow reversal along tributaries during the rainy season will also likely lead to the spread of the pathogen through floodplain watersheds. Salinity and water temperatures strongly influence spore production of the fungus. EUS outbreaks may not occur wherever salinity is ≥ 2 ppt and water temperatures remain above 30 °C. Within the Chobe-Zambezi River system, this disease condition could become pandemic, damaging both aquaculture and capture fisheries and aquatic biodiversity.

The report also emphasized that a short-term (urgent) control, prevention and preparedness programme is essential and could help to reduce social and economic impacts of the fish disease on people involved in fisheries in the Chobe-Zambezi River system, as well as aquaculture farmers.

Among the key short-term measures recommended by the mission in the June 2007 report include:

- urgent notification to the World Organisation for Animal Health (*Office international des épizooties* or OIE), of the presence of EUS in the Chobe River in Botswana by the veterinary authority of Botswana. Similarly, the Namibia

veterinary authority was also encouraged to make the same notification of the occurrence of the disease in the Caprivi region in Namibia;

- initiation of a public awareness and extension programme to raise understanding of the disease and impact reduction measures;
- conducting short-term training and awareness raising on EUS for key government officers and other key stakeholders (e.g. NGOs working on fisheries or with fishing communities) to raise awareness and implement an extension and monitoring programme;
- establishment of surveillance and monitoring programmes along the Chobe-Zambezi River system to monitor spread pattern of the disease outbreak;
- more detailed epidemiological investigation of the present EUS distribution, analysis of risks to the fisheries (people and biodiversity) in all major tributaries and lakes in the Chobe-Zambezi River system, and development of appropriate risk management responses.
- Initial dialogue among the countries sharing the Chobe-Zambezi River system, to establish a subregional disease surveillance, monitoring, preparedness and response programme and a practical action plan as early as possible.

FAO was also encouraged to ensure that the information on EUS is shared among countries in the Chobe-Zambezi River system and neighbouring watersheds to create awareness on EUS and to provide early warning of wider spread of the disease.

The EUS outbreak had exposed serious biosecurity weaknesses in the southern African region. Thus, the mission also recommended actions through a medium- to long-term programme to strengthen capacity for fish disease diagnosis and control, quarantine, responsible movement of live aquatic animals, development of appropriate policy and regulatory frameworks, and implementation of better aquatic animal health management programmes in the region.

As there is insufficient capacity to control EUS within existing facilities and human resources in Botswana and neighboring countries, the mission recommended that an immediate programme of technical assistance be established:

- to assist government authorities take immediate preventative and control measures, particularly through training of key staff and establishment of an effective surveillance, monitoring and public awareness campaign, before the possible start of the next EUS season in 2007/2008;
- to consider a longer-term programme to identify the source of EUS and take measures to reduce the spread of the disease to other parts of the region.

The June 2007 report emphasized that the disease (fish mortalities) experienced in the Chobe-Zambezi River system over the past few months was not entirely due to environmental and water quality problems but a disease outbreak caused by EUS. The causative agent of EUS does not pose any human health implications. Except for the fish exhibiting deep ulcerations and tissue decay, which could harbour secondary pathogens which may have human health implications, the fish infected with EUS do not pose human health hazards for consumers. This fact needs to be conveyed to affected communities urgently.

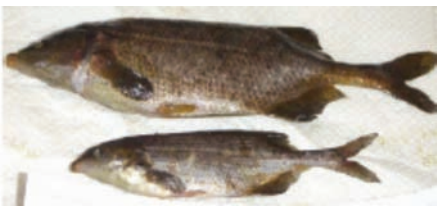
The potential negative impact to biodiversity cannot be ignored. The Zambezi River is home to more than two hundred fish species, some of which are endemic to the river. Important species include cichlids which are fished heavily for food, as well as catfish, tigerfish, yellowfish and other large species (Plate 9). An estimate of about 32 million people inhabit the Zambezi River valley; 80 percent of the population are dependent on agriculture. River communities fish extensively for food; recreational fishing is also a significant activity in some parts of the river for tourists.

There had been a number of developments since the submission of the preliminary report of the Task Force findings in June 2007. These are briefly described in the following sections.

PLATE 9

Examples of diversity of fish species in the Chobe-Zambezi River, Kasane, Botswana

(All photographs courtesy of M.B. Reantaso)



Bulldog
Marcusenius macrolepidotus

Squeaker
Synodontis spp.

Tigerfish
Hydrocynus vittatus

African catfish
Clarias gariepinus

Silver catfish
Schilbe intermedius

Churchill
Petrocephalus catostoma

Banded tilapia
Tilapia sparrmanii

Brownspot largemouth
Serranochromis thumbergi

7. Further updates on EUS status in southern Africa

Since the Task Force mission in May 2007, confirming EUS in fishes in Botswana, further confirmation of EUS occurrence in freshwater fishes in Namibia and Zambia followed. Fish samples suspected to be infected with EUS were sent to AAHRI for processing and/or confirmation.

In Zambia, based on preliminary outcome of 2007 surveillance work carried out by a team from the University of Zambia led by Dr Bernard M. Hang'ombe, prevalence can go as high as 50 percent in new areas of Zambezi and Chavuma districts where the disease is spreading. These districts are located on the upstream Zambezi River bordering Angola. The Barotse plains in the Western Province of Zambia was seriously hit by EUS in May, June, July and August 2007. However, there have been no reported cases in 2008, even though a prevalence rate of 5 percent per catch has been observed in Sesheke District where the disease was first noticed in Zambia.

In Namibia, based on surveillance work carried out by Dr Ben C.W. Van Der Waal of the Integrated Management of the Zambezi/Chobe River System Fishery Resource and a staff of the Ministry of Fisheries and Marine Resources of Namibia Project, small outbreaks of EUS have been found in 2008 in the Chobe River area of the Zambezi floodplain. A few isolated cases of infected fish were also collected in the Zambezi itself. Inspection of large fish during the 2007 and 2008 annual international angling competition demonstrated the occurrence of EUS on a considerable number of larger cichlids, especially Nembwe (*Serannochromis robustus*) in 2007 and pink bream/happy (*Sargochromis giardi*) in 2008. The occurrence seems to be sporadic and changing amongst species. In early November 2008, some tilapia [3 percent in one farm] from two fish farms in the Kavango Region of Namibia were found with lesions that could be related to EUS. Samples have been collected and confirmed by the School of Veterinary Medicine of the University of Zambia in Lusaka. The veterinary authorities are on alert and preventive measures will be taken.

The above updates bring to three the countries in southern Africa that are positively infected with EUS. The confirmation of EUS in southern Africa adds more than 20 species to the growing list of species at risk to EUS. These include the following species: dashtail barb (*Barbus poechii*), straightfin barb (*B. paludinosus*), Thamalakane barb (*B. thamalakanensis*), Longbeard barb (*B. unitaeniatus*), striped robber (*Brycinus lateralis*), silver robber (*Micralestes acutidens*), tigerfish (*Hydrocynus vittatus*), African pike (*Hepsetus odoe*), sharptooth catfish (*Clarias gariepinus*), Blunttooth catfish (*C. ngamensis*), silver catfish (*Schilbe intermedius*), Upper

Zambezi labeo (*Labeo lunatus*), Redeye labeo (*L. cylindricus*), bulldog (*Marcusenius macrolepidotus*), churchill (*Petrocephalus catostoma*), threespot tilapia (*Oreochromis andersonii*), greenhead tilapia (*O. macrochir*), banded tilapia (*Tilapia sparrmanii*), Green bream/happy (*Sargochromis codringtonii*), rainbow bream/happy (*S. carlottae*), pink bream/happy (*S. giardi*), thinface largemouth (*Serranochromis angusticeps*) and Nembwe (*S. robustus*). Plate 10 shows some photographs of EUS-positive fish from Namibia and Zambia; Plate 11 shows examples of fish from Namibia with lesions that have not been confirmed by laboratory analysis as related to EUS. Figure 2 shows the current global distribution of EUS.

The list of freshwater fish species at risk from EUS can be found in Table 5 (African fish species) and Table 6 (OIE, 2006).

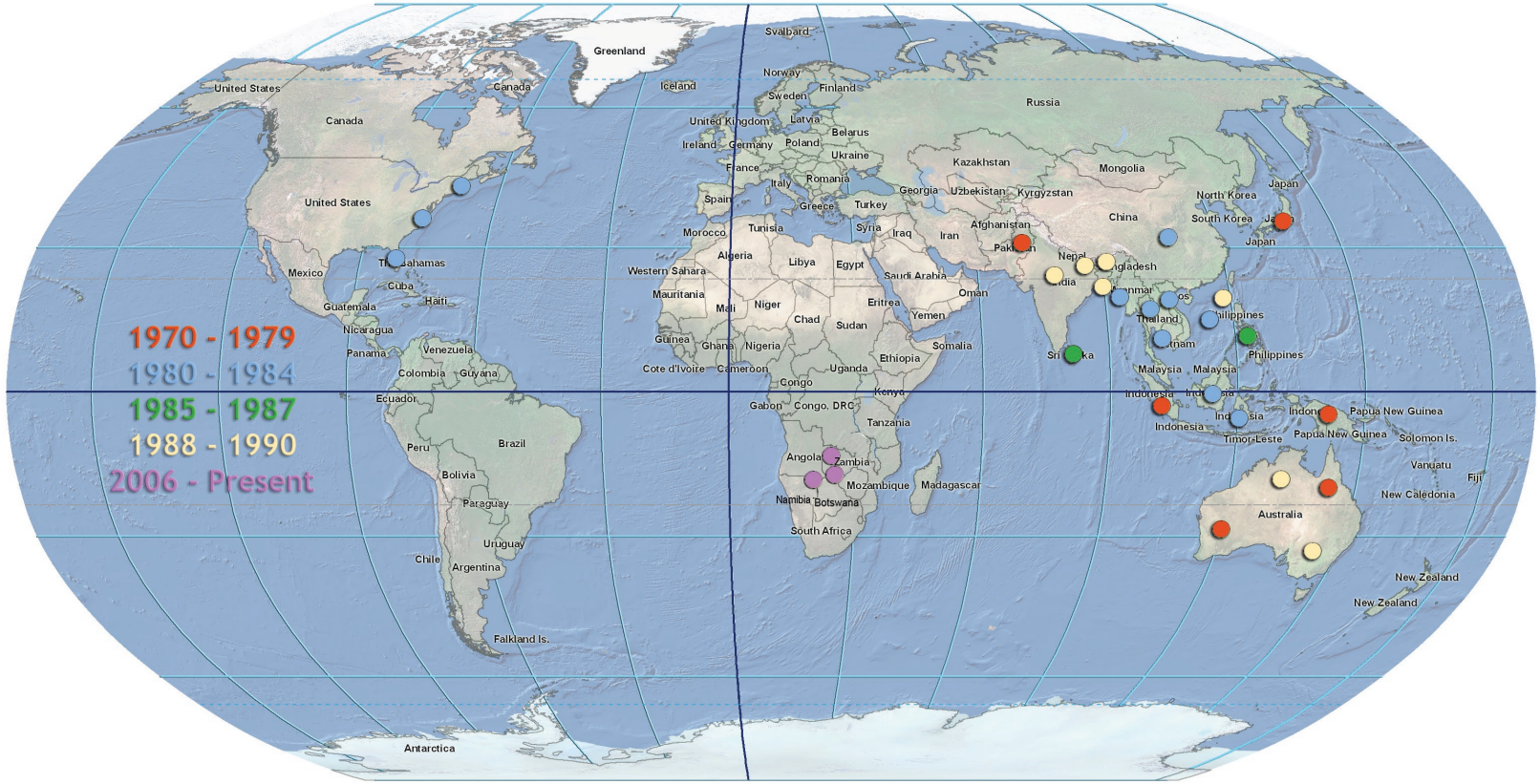
TABLE 5
Fish species susceptible to EUS in southern Africa

Scientific name	Local name	Country with confirmed diagnosis (year)
Barbus poechii	dashtail barb	Botswana (2007) Namibia (2007) Zambia (2007)
Barbus paludinosus	straightfin barb	Namibia (2007)
Barbus thamalakanensis	Thamalakane barb	Botswana (2007)
Barbus unitaeniatus	longbeard barb	Namibia (2008)
Brycinus lateralis	striped robber	Namibia (2007)
Clarias gariepinus	sharptooth African catfish	Namibia (2007) Zambia (2008)
Clarias ngamensis	blunt-toothed African catfish	Namibia (2007) Zambia (2007)
Clarias sp.	catfish	Zambia (2007)
Hepsetus odoe	African/Kafue pike	Zambia (2007) Namibia (2007)
Hydrocynus vittatus	tigerfish	Namibia (2007)
Labeo lunatus	upper Zambezi labeo	Botswana (2007) Namibia (2007)
Labeo cylindricus	red-eye labeo	Namibia (2008)
Marcusenius macrolepidotus	bulldog	Namibia (2007)
Micralestes acutidens	silver robber	Namibia (2007)
Oreochromis andersonii	three-spotted tilapia	Namibia (2007) Zambia (2007)
Oreochromis macrochir	greenhead tilapia	Namibia (2007)
Petrocephalus catostoma	churchill	Botswana (2008)
Pharynchochromis acuticeps	Zambezi River bream	Namibia (2008, suspected)
Sargochromis codringtonii	green beam/happy	Namibia (2008) Zambia (2007)
Sargochromis carlottae	rainbow bream/happy	Namibia (2008)
Sargochromis giardi	pink bream/happy	Namibia (2008)
Schilbe intermedius	silver catfish	Namibia (2007) Zambia (2007)
Serranochromis robustus	Nembwe	Namibia (2007, suspected) Zambia (2007)
Serranochromis angusticeps	thinface largemouth	Namibia (2008, suspected) Zambia (2007)
Serranochromis macrocephalus	purpleface largemouth	Namibia (2008, suspected)
Tilapia rendalli	redbreast tilapia	Namibia (2008)
Tilapia sparrmanii	banded tilapia	Namibia (2008)

FIGURE 2

Map showing the current global distribution of epizootic ulcerative syndrome

(prepared by Jeff Jenness and José Aguilar-Manjarrez, FAO-FIMA; source: African Water Resource Database)



Chronology of global occurrence of EUS; dates with question mark indicate outbreaks of ulcerative fish disease and/or unconfirmed EUS outbreaks; dates without question mark indicate year of EUS confirmation (Lilley *et al.*,1998; Baldock *et al.,* 2005)

Japan (1971); **Australia** (Queensland – 1972, New South Wales – 1989, Northern Territory – 1990 and Western Australia – 1994); **Papua New Guinea** (1975–1977?; 1982–1983?; 1986); **Indonesia** (1980?; 1993–1994); **Singapore** (1977?); **Malaysia** (1979?; 1980); **Thailand** (1981); **Myanmar, Lao PDR and Cambodia** (1983 or 1984); **Viet Nam** (1983?); **China** (1982?; 1987–1988?; 1989?); **China, Hong Kong SAR** (1988?); **Philippines** (1985); **Sri Lanka** (1987); **Bangladesh** (1988); India (1988); **Bhutan and Nepal** (1989); **Pakistan** (1996); **United States of America** (North Carolina, Florida and Connecticut – 1984); **Bostwana** (2006?; 2007); **Namibia** (2006?; 2007); **Zambia** (2007; 2008)

TABLE 6
List of fish species susceptible to EUS (OIE, 2006)

Species/Family	Local name
Acanthopagrus australis	yellowfin seabream
Anabas testudineus	climbing perch
Bidyanus bidyanus	silver perch
Brevoortia tyrannus	Atlantic menhaden
Catla catla	catla
Channa striatus	striped snakehead
Cirrhinus mrigala	mrigal
Clarias batrachus	walking catfish
Colisa lalia	dwarf gourami
Esomus sp.	flying barb
Fluta alba	swamp eel
Family Bagridae	catfishes, bagrid
Glossogobius giurus	bar-eyed goby
Glossogobius sp.	goby
Mugil cephalus	grey mullet
Family Mugilidae (*Mugil* spp.; *Liza* spp.)	mullets
Labeo rohita	rohu
Lates calcarifer	barramundi or seabass
Osphronemus goramy	giant gourami
Oxyeleotris marmoratus	marble goby
Platycephalus fuscus	dusky flathead
Plecoglossus altivelis	ayu
Puntius gonionotus	silver barb
Puntius sophore	barb, pool
Psettodes sp.	spiny turbot
Rohtee sp.	keti-Bangladeshi
Scatophagus argus	spotted scat
Sillago ciliata	sand whiting
Family Siluridae	catfishes, wells
Trichogaster pectoralis	snakeskin gourami
Trichogaster trichopterus	three-spot gourami
Toxotes charateus	common archer fish

An ongoing active targeted surveillance for EUS (see section 8.1) involving seven countries in southern Africa will further reveal the extent of spread and distribution of EUS in the African region. Preliminary results, however, indicate that EUS is spreading. As previously mentioned, three countries (Botswana, Namibia and Zambia) are now positive for EUS while two countries (Angola and Zimbabwe) have collected fish samples with suspected EUS-like lesions but these require laboratory confirmation (see Plate 12).

PLATE 10

Additional photographs of EUS positive fish species from Namibia and Zambia, southern Africa

(All photos courtesy of B.W.C. Van der Waal)

Barbus paludinosus – Straightfin barb
Lake Liambezi, Chobe River
Caprivi Region, Namibia, 2007

Brycinus lateralis – Striped robber
Zambezi River
Caprivi Region, Namibia, 2007

Barbus poechii – Dashtail barb
Lake Liambezi, Chobe River, Namibia, 2007

Hepsetus odoe – African pike
Zambezi River, Caprivi region, Namibia, 2007

Clarias gariepinus – Sharptooth catfish
Zambezi River, Namibia, 2007

Clarias gariepinus – Sharptooth catfish
Zambezi tributary, Zambia, 2008

Sargochromis codringtonii – Green bream
Chobe River, Caprivi, Namibia, 2007

Serranochromis robustus – Nembwe Zambezi River, Caprivi Region, Namibia, 2007
Note: This specimen from Namibia has not yet been confirmed; however, in Zambia Nembwe has been positively confirmed as susceptible to EUS

PLATE 11

Examples of fish from Namibia with sores that have not yet been confirmed by laboratory analysis as related to EUS

(All photos courtesy of B. Van der Waal)

Tilapia sparrmanii – Banded tilapia
Isolated small pool near Zambezi River, Caprivi, Namibia, Tested negative, 2007

Oreochromis andersonii – Threespot tilapia
Litapi Fish Farm, Caprivi Region, Namibia
Tested negative, 2007

Hepsetus odoe – African pike
Zambezi River, Namibia
Tested negative, 2007

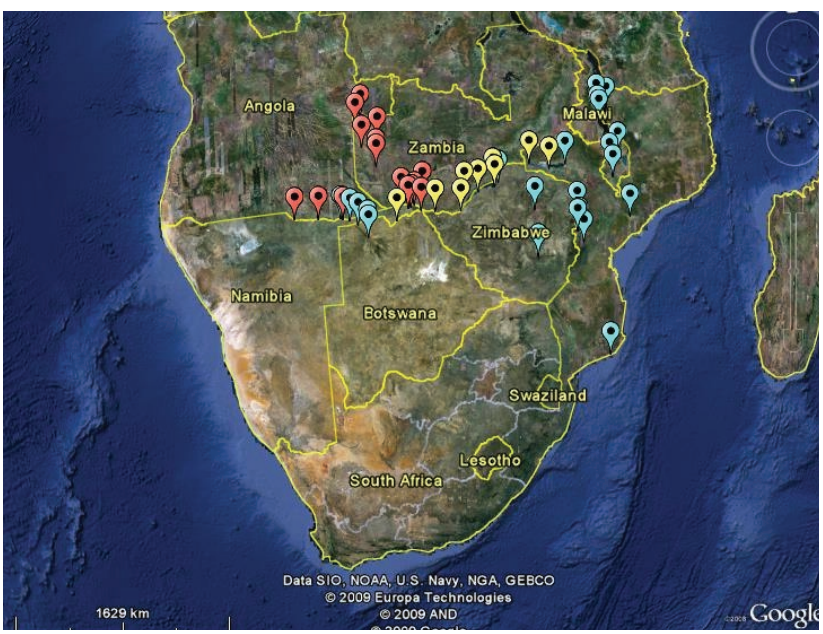
Labeo lunatus – Upper Zambezi labeo
Zambezi River, Namibia
Tested negative, 2007

PLATE 12

Maps showing EUS occurrence in southern Africa

(Courtesy of F. Corsin)

Map 1: Map of southern Africa showing the site (lower tip of the balloon) that was sampled and confirmed as EUS positive during the May 2007 Task Force outbreak investigation



Map 2: Map of southern Africa showing the EUS confirmed (red balloons), EUS suspect (yellow balloons) and EUS negative (blue balloons) sites as reported during the follow-up surveillance activities conducted in 2007 and 2008 as part of TCP/RAF/3111

8. The way forward

The incursion of EUS in southern Africa revealed serious biosecurity lapses which if not urgently addressed could lead to more risks to the communities surrounding the Zambezi River who are dependant on its resources for food and livelihood, as well as risks to animal health, fish welfare, biodiversity and environmental sustainability of the fisheries and aquaculture sectors. Since preliminary results of surveillance work indicate that EUS is spreading, further urgent intervention is necessary.

The current challenge now is to determine how EUS came about to Africa, to determine the risk factors for the African EUS outbreak, to improve capacity on aquatic animal health management in the region particularly in dealing with future outbreaks.

The work and accomplishments of the Task Force provided an impetus for further support for improving aquatic biosecurity awareness in Africa.

8.1 FAO REGIONAL TECHNICAL COOPERATION PROGRAMME (TCP/RAF/3111)

Immediately following the Task Force mission and based on the above recommendations, an FAO Regional Technical Cooperation Programme (TCP/RAF/3111 [E]) *Emergency assistance to combat EUS in the Chobe-Zambezi River* was prepared and approved for implementation beginning October 2007. Seven southern African countries (Angola, Botswana, Malawi, Mozambique, Namibia, Zambia and Zimbabwe) are participating in this regional project. The specific objectives of the project are to: (i) strengthen the capacity of competent authorities of the seven participating countries in minimizing the impacts of the disease by enhancing surveillance and diagnostic capacities, (ii) increase their ability to educate and raise awareness of communities of both affected and unaffected locations/zones of risk factors and promoting responsible trading of live aquatics and (iii) facilitate the formulation of a regional emergency response strategy and implementation.

The first major activity was a week-long training course (see Plate 13), held from 7 to 11 November 2007 at the School of Veterinary Medicine, University of Zambia in Lusaka, for 22 key staff from eight countries (including Mauritius). The training course covered lectures, laboratory and field work on basic aquatic animal health management, EUS diagnosis and preparation of a targeted surveillance design for EUS.

PLATE 13

Activities undertaken during the first workshop of the FAO TCP/RAF/3111 [E] *Emergency assistance to combat EUS in the Chobe-Zambezi River*, Lusaka, Zambia, 7-11 November 2007

(All photos courtesy of M.B. Reantaso)

R.P. Subasinghe (FAO) giving a lecture on global aquaculture development and importance of aquatic animal health management

S. Kanchanakhan (AAHRI) demonstrating fish necropsy and procedures for collecting fish tissue samples for laboratory analysis

F. Corsin (FAO consultant) discussing with G Kanyerere (Malawi) a surveillance design for EUS

Participants experience field work

Twenty two key staff from eight countries (including Mauritius) participated in the workshop held at the University of Zambia from 7–11 November 2007 which trained participants on basic aquatic animal health management, EUS diagnosis and preparation of a targeted surveillance design for EUS

8.2 FAO REGIONAL WORKSHOP ON DEVELOPMENT OF AN AQUATIC BIOSECURITY FRAMEWORK FOR SOUTHERN AFRICA

As part of FAO's continuing assistance, a capacity assessment questionnaire survey was undertaken from January to March 2008 to evaluate national capacities for managing aquatic biosecurity (i.e. capacity to manage risks associated with exotic or emerging pathogens of aquatic animals and invasive aquatic species). Nine countries (Angola, Botswana, Kenya, Malawi, Mozambique, Uganda, Tanzania, Zambia and Zimbabwe) participated in the survey which covered a number of areas with direct relevance to assessing aquatic biosecurity performance and include the following aspects: (1) international affiliations, (2) trade activity, (3) border control, (4) surveillance/monitoring, (4) incident/emergency response, (5) diagnostic capacity, (6) research/training, (7) expertise (knowledge base), and (8) challenges. The outcomes of the survey was presented during the FAO Regional Workshop on Development of an Aquatic Biosecurity Framework for Southern Africa held in Lilongwe, Malawi, from 22 to 24 April 2008, attended by 18 representatives from 9 countries participating in the survey, the OIE and FAO (see Plate 14). The workshop identified a number of key regional capacity building activities to address aquatic biosecurity gaps or lapses in the southern African region, foremost of which is a request to FAO to develop a follow-up project to assist countries in reviewing institutional and legal frameworks to better address current biosecurity issues, especially addressing aquatic animal health management, trans-boundary movement of live aquatics and maintaining aquatic biodiversity. Additional recommendations include the following: (i) countries in the region to work closely in collaboration with FAO and OIE and regional partners to collectively address matters pertaining to aquatic animal health and biosecurity; (ii) recognizing the University of Zambia's School of Veterinary Medicine as a potential regional diagnostic centre and Uganda as a regional coordinating centre; (iii) development of a regional model/template on import risk assessment for introductions and transfers of live aquatic animals; and (iv) holding of a ministerial level meeting for southern African countries to raise the issue of aquatic animal biosecurity. This workshop also recommended that the FAO focal points on aquatic animal health participate in the OIE Regional Seminar (see 8.2) in Maputo, Mozambique. The full recommendations from the FAO Lilongwe Workshop can be found in Annex 5.

8.3 OIE REGIONAL WORKSHOP ON OIE STANDARDS, A LEVER FOR GROWTH IN THE FISHERIES AND AQUACULTURE SECTOR IN SOUTHERN AFRICA

The OIE Regional Workshop on "OIE standards, a lever for growth in the fisheries and aquaculture sector in Southern Africa" organized by the OIE Sub-Regional Representative, was held in Maputo, Mozambique, from 10 to 12 June 2008. Major recommendations resulting from this regional workshop relevant to fisheries and aquaculture include: (i) to promote dialogue between veterinary authorities and other competent authorities, as well as the private sector, to identify their respective roles and responsibilities with respect to aquatic animal health matters; (ii) to review the national legislative framework for allowing the development of

PLATE 14

Photographs during the FAO Workshop on Development of Aquatic Biosecurity Framework for Southern Africa, Lilongwe, Malawi, 22-24 April 2008

(All photos courtesy of M.B. Reantaso)

A,B,C: Opening ceremony guest speakers FAO Representative Mr Mazlan Jusoh, Malawi Department of Fisheries Director Mr Alex Bulinari and Dr Rohana Subasinghe of FAO.
D: Prof Eli Katunguka-Rwakishaya (OIE) making a presentation; **E**: Dr Patrick Bastiensen (OIE) with regional participants; **F**: Eighteen participants representing nine countries (Angola, Botswana, Kenya, Malawi, Mozambique, Tanzania, Uganda, Zambia, Zimbabwe) participated in this regional workshop

the fisheries and aquaculture sector; (iii) to prioritize aquatic animal diseases of concern and fast tracking the implementation of surveillance programmes; (iv) to enhance cross-border cooperation between competent authorities to control aquatic animal diseases; and (v) to coordinate and support the establishment of a regional aquatic animal health network for fisheries and aquaculture in southern Africa in close collaboration with relevant bodies at national, regional and international level. The full recommendations of the OIE Maputo Workshop can be found in Annex 6.

8.4 FAO SPECIAL PROGRAMME FOR AQUACULTURE DEVELOPMENT IN AFRICA (SPADA)

The Special Programme for Aquaculture Development in Africa (SPADA) is a new and innovative programme recently launched by FAO as recommended by the twenty-seventh session of FAO Committee on Fisheries (COFI) in recognition of the growing importance of aquaculture in the Africa region as well as the region's underutilized aquaculture resources. Strengthening aquatic biosecurity is included in one of the seven programme arenas of SPADA, under the theme *Strengthening institutions and enabling frameworks*.

8.5 FAO TRAINING/WORKSHOP ON BASIC AQUATIC ANIMAL HEALTH MANAGEMENT AND INTRODUCTION TO RISK ASSESSMENT IN AQUACULTURE

Participants from Botswana, Ghana, Kenya, Malawi, Mozambique, Namibia, South Africa, Uganda, Zambia and Zimbabwe attended this training/workshop which was held from 9 to 15 February 2009 at the School of Veterinary Medicine, University of Zambia in Lusaka, Zambia. This is a follow-up activity of two previous FAO training/workshops held in Lusaka (November 2007, see section 8.1) and Lilongwe (April 2008, see section 8.2) under the ongoing TCP/RAF/3111 "Emergency assistance to combat epizootic ulcerative syndrome (EUS) in the Chobe-Zambezi River System" and the Aquatic Biosecurity Project funded under FAO's Programme Cooperation Agreement with Norway, respectively. The training/workshop was preceded by a one-day session on updating the implementation of TCP/RAF/3111 particularly the targeted surveillance work for EUS and the EUS educational materials. Plate 15 shows some photographs taken during the workshop. The main objective is to provide continuous training opportunities that will support capacity building on aquatic biosecurity for sustainable fisheries and aquaculture development focusing in the areas of aquatic animal health management and risk analysis.

The workshop identified a number of follow-up activities under the broad heading of establishing a coordination team for aquatic animal health activities in the African region; assessing the current aquatic animal health status in the African region; drafting a regional surveillance system for Africa; facilitating the proposed Regional TCP on Aquatic Biosecurity in Africa; and following-up on the other recommendations identified during the Lilongwe Workshop in April 2008.

PLATE 15

FAO Training/Workshop on Basic Aquatic Animal Health Management and Introduction to Risk Assessment in Aquaculture , University of Zambia Lusaka, Zambia, 9-15 February 2009

Participants (Botswana, Ghana, Kenya, Malawi, Mozambique, Namibia, South Africa, Uganda, Zambia and Zimbabwe) attended the FAO Training/Workshop on Basic Aquatic Animal Health Management and Introduction to Risk Assessment in Aquaculture held at the University of Zambia in Lusaka from 9-15 February 2009. The workshop was implemented by FAO officers, Drs Rohana Subasinghe and Melba Reantaso, supported by international consultants, Dr Flavio Corsin, Prof. Mohammed Shariff and Dr Richard Arthur.

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Annex 1

COMPOSITION OF THE INTERNATIONAL EMERGENCY DISEASE INVESTIGATION TASK FORCE ON A SERIOUS FISH DISEASE OUTBREAK IN THE CHOBE/ZAMBEZI RIVER SYSTEM

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Annex 2

PROCEDURES FOR INVESTIGATING A DISEASE OUTBREAK

There are 9 basic steps[5] for investigating an outbreak of a disease, however, not all steps are necessarily included in every investigation nor do they follow the same sequence, and several steps may be taken simultaneously:

9 Basic steps		Information/Action required		
1	Establish a diagnosis	Provisional diagnosis based on: species of fish affected clinical signs gross pathology seasonality (if applicable) Verification of provisional diagnosis based on laboratory results		
2	Define a "case"	A 'case definition' is simply an agreed set of rules which permits investigators to uniformly decide that a particular individual has or does nor have a particular disease as defined; it is important to develop a set of rules that will define both suspect and confirmed cases. By definition, a case definition is a set of standard criteria for deciding whether an individual study unit of interest has a particular disease or other outcome of interest. The study unit may be an individual animal or group of animals such as a pond of shrimp, a cage of fish, an entire farm or a village, an entire river system.		
3	Confirm that an outbreak is actually occurring	It is important to know the normal percentage of a mortality event versus an outbreak caused by a disease, for example. Confirmation that an outbreak is actually occurring is particularly required in cases where a disease is endemic or prevalent.		
4	Characterise the outbreak in terms of time, afected/ unaffected fish, and place	**Time:** • What is the exact period of the outbreak? • Given the diagnosis, what is the probably period of exposure? • Is the outbreak most likely a common source (e.g. intoxication, contaminated water or equipment), propagated (e.g. animal to animal transmission as in infectious agents) or both?	**Fish:** • Any characteristic about the fish for which specific attack rates vary? • Which groups have the highest and which have the lowest attack rates? Example of computation for Attack Rate (AR): AR_1 = Number with Disease/Total # of fish in a sample	**Place:** • Significant features of the geographical distribution of cases? • Relevant attack rates?
		Time: Duration of an outbreak is influenced by: the # of susceptible animals exposed to a source of infection which become infected; the period of time over which susceptible animals are exposed to the infection source; the minimum and maximum incubation period of the disease. Fish: species, age, sex and geographical origin Place: for example in farmed fish, looking at patterns in different ponds, making diagram are useful; type of fishery ital information about the Chobe-Zambezi River system, e.g. fish species, fish stocking activities, water quality and other environmental data, etc.		

[5] From Lilley, J.H., Callinan, R.B., Chinabut, S., Kanchanakhan, S., MacRae, I.H. and Phillips, M.J. 1998. EUS Technical Handbook. AAHRI, Bangkok. 88 p.

9 Basic steps (Continued)		Information/Action required
5	Analysing the data	Include specific factors such as species, age, sex, etc. Analysis of time, place and fish data
6	Working hypothesis	Based on outcomes of 5: Whether an outbreak is common source or propagating? If common source, whether it is point or multiple exposure? Mode of transmission – contact, vehicle or vector? Any hypothesis should be compatible with facts.
7	Intensive follow-up	Clinical, pathological and microbiological examinations; water quality data analysis; relevance of recent meteorological data Epidemiological follow-up – search for additional cases Flow charts of management and movements of fish, water and equipment Transmission trials
8	Control and prevention	Recommendations and advice to terminate the outbreak (if possible) and reduce the risk of similar or future outbreaks
9	Reporting	Written report to serve a permanent record as reference for future outbreaks. Background, methods, results, case definition, hypothesis, financial impacts, recommendations, appendices containing laboratory reports, etc. All other relevant information, for e.g.: Any human health implications Analogy to other disease outbreaks Marketing of fish Local fish disease diagnostic capacities (fisheries, veterinary and/or other relevant departments/universities)

Annex 3

STANDARDIZED PROCEDURES FOR PARASITOLOGY, BACTERIOLOGY, VIROLOGY AND HISTOPATHOLOGY

Bacteriology examination

Only clinically diseased specimens were subjected to bacterial isolation using tryptone soya agar (TSA) or cytophaga agar (CA). Fish with white patches on the body were subjected to flexibacterial isolation using CA while fish with haemorrhagic lesion on the body or showing abdominal swelling were used for bacterial isolation in TSA.

Fish were sacrificed by a pit in the brain or a cut in the notochord. For external surface, the wound surfaces were cleaned with a tissue paper or cotton. External contamination were disinfected using hot spatula or wiped with 75 percent alcohol. Using a sterile scalpel blade, a cut was made through the wound surface and a sterile bacterial loop was used to isolate bacteria in the muscle tissue beneath the wound. For internal organs, fish abdomen was aseptically opened with a sterile pair of scissors or a scalpel blade. A small cut in the liver, kidney or spleen was made using a sterile scalpel blade and bacteria was isolated using a sterile bacterial loop and placed in TSA medium. Agar plates are incubated at room temperature under moisture container. Isolated bacteria may need to be sub-cultured before transferring into transporting medium containing TSA.

Mycology examination

Only clinically diseased specimens with visible oomycete infection were subjected to oomycete isolation using glucose-peptone agar (GP).

For large fish, those showing moderate, pale, raised, dermal lesions are most suitable for oomycete isolation attempts. Scales around the periphery of the lesion were removed and the underlying skin was seared with a red-hot spatula to sterilise the surface. Using a sterile scalpel blade and a sterile fine-pointed forceps, a cut was made through the stratum compactum underlying the seared area and the underlying muscle was exposed by cutting horizontally and reflecting superficial tissues. In order to prevent contamination, instruments should not make contact with external surface. Using aseptic techniques, affected muscle, approximately 2 mm^3, were carefully excised and placed in a petri dish containing GP agar with penicillin G (100 units/ml) and oxolinic acid (100 µg/ml).

Specimens smaller than <20 cm in length can be sampled by cutting the fish into two using a sterile scalpel and slicing a cross-section through the fish at the edge of the lesion. Flame the scalpel until red-hot and use this to sterilise the exposed surface of the muscle. Use a small sterile scalpel blade to cut out a circular block of

muscle (2-4 mm^3) from beneath the lesion and place it in GP agar plate. Seal plates, incubate at room temperature or at 22 °C – 25 °C and examine daily. Emerging hyphal tips should be repeatedly transferred on to fresh plates of GP agar until cultures are free of contamination.

During the field visit, the GP plates were incubated or kept on top of small refrigerator in the hotel room to keep the plate warm at 22 °C–25 °C. As the hotel's room temperatures were around 15 °C–20 °C, the oomycete hyphae were transferred from GP plates to GP tubes before transport to AAHRI.

Virology examination

Only clinically diseased specimens were subjected to virus isolation. One gram of pooled organs was placed in a vial containing transporting medium, Hanks' balanced salt solution (HBSS) containing penicillin (800 IU/ml), streptomycin (800 µg/ml) and 2 percent serum (one volume of organs in nine volumes of transportation fluid). The specimens were kept in HBSS vials and stored in a cool box until virus extraction.

Virus extraction had been carried out within 10 hrs after fish sampling using the following procedures. Decant transporting medium from organ sample, homogenize organ pools using a mortar and pestle until a paste is obtained followed by dilution in fresh transport medium at a dilution rate of 1/10. Sterile fine sand was added to facilitate grinding. Tissue debris and sand were separated using a hand centrifuge to obtain clear tissue extract. Extracts were diluted using HBSS (1:50 final dilution) and filtered through 0.45 micron syringe-attached disposable filter units. Extracts were kept in cool box prior to transport to AAHRI in Bangkok.

Simultaneous cell culture and extract inoculations were carried out using 2 different fish cell lines, EPC and BF2. Viral isolation was conducted in 24-well plates. The following steps are general procedures practised at AAHRI:

1. The 24-well plate is first seeded with a single cell suspension of the fish cell line in maintenance medium (L-15 medium containing 2 percent fetal calf serum, 100 IU/ml penicillin and 100 µg/ml streptomycin).
2. Each well receives 1.3-1.4 ml of cell suspension. Cells with complete monolayer in 25 cm^2 tissue culture flask is sufficient to produce 80 to 90 percent confluent monolayer in 1 day after seeding in one 24 well tissue culture plate.
3. One tissue extract (1:50 dilution) is immediately inoculated into 2 wells. First well receives 200 µl inoculum; while the second well receives 50 µl inoculum. The same numbers of replicate wells are used as negative controls for each plate.
4. The tissue extract-inoculated cells are incubated at 22 °C and observed daily for cytopathic effect or CPE for at least 14 days.
5. A first blind passage of culture fluids is performed on days 7 to 10. Viral passage or subculture is done by transferring 200 µl of supernatant from each well to fresh culture 24-wells plate. CPE observation is still continuing

in the old plates for a further 5 to 7 days. Second blind passage was also carried out.

6. Samples showing CPE in which the cell monolayer changed, disintegrated, sloughed off the surface of the tissue-culture wells or ended with cell lysis, will be passaged to provide larger quantities of suspect virus. If viruses are isolated, the supernatants will be collected, aliquoted in tubes with 1 ml quantities and stored, some tubes at 4 °C and some tubes at –20 °C or –80 °C, for further characterisation.

Histology examination

Procedures for collecting samples for histology follow the steps below:

1. Sample only live or moribund specimens of fish with clinical lesions.
2. Take samples of skin/muscle (<1 cm^3), including the leading edge of the lesion and the surrounding tissue. Parts of internal organs may also be collected. For small fish, the fish operculum and abdomen were cut and opened.
3. Fix the tissues or fish specimens immediately in 10 percent formalin. The amount of formalin shall be 10 times the volume of the tissue to be fixed. The tissues were properly fixed for at least 24 hour.
4. Transfer the fixed tissue into small bags with formalin-moistened tissue paper them wrap properly to prevent leakage or smell.
5. Transport the bags of fish tissue in a cool box to AAHRI for analysis.

Histological procedures include processing of the fixed tissue involves dehydration through ascending alcohol grades, clearing in a wax-miscible agent and impregnation with wax in an automate tissue processor. The blocks of fish tissue are cut at about 5 µm and mounted on a glass slide. Before staining, the section must be completely de-waxed and stained in haematoxylin and eosin (H&E). H&E and general fungus stains (e.g. Grocott's stain) will demonstrate typical granulomas and invasive hyphae.

Annex 4

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Annex 5

RECOMMENDATIONS OF THE FAO LILONGWE WORKSHOP

Recommendations, outputs and agreed follow-ups of the Lilongwe Workshop

In order to improve aquatic biosecurity in Southern Africa, the participants at the FAO Workshop on Development of an Aquatic Biosecurity Framework for Southern Africa held at the Sunbird Hotel in Lilongwe, Malawi from 22-24 April 2008 made the following recommendations:

1. Participants strongly recommended that the countries in the region should work closely in collaboration with the Food and Agriculture Organization and the World Organisation for Animal Health in addressing matters pertaining to aquatic animal health and biosecurity.
2. FAO should write to participating governments participating in the Regional TCP Project to highlight the importance of establishing formal focal points (akin to those established under an OIE initiative), asking for nominations. It was suggested that workshop attendees be the FAO focal point for aquatic biosecurity issues. It would be necessary to develop terms of reference for the focal points, including responsibilities and accountability, including raising awareness.
3. FAO should develop a follow-up project aimed at aquatic biosecurity capacity building in Southern Africa. There is also an urgent need for a regional project for evaluating legal frameworks for aquatic biosecurity (with the need to link biodiversity, production and trade). Several countries advised of their intention to write a letter of support/request to the FAO for a regional project addressing both legal and capacity building issues.
4. The University of Zambia's School of Veterinary Medicine, through Dr Hang'ombe Bernard Mudenda, was identified as potential regional diagnostic centre and Uganda as a regional coordination centre.
5. FAO should develop a Southern Africa regional model on import risk assessment for introductions and transfers of live aquatic animals.
6. Ministerial level meeting for Southern African countries should be held to discuss aquatic animal biosecurity needs.
7. There is a need for a Web site on aquatic biosecurity to assist the Southern African region on aquatic biosecurity issues. Participants recommended the establishment of a regional aquatic biosecurity information network including a dedicated website. As the first step, Mr Wilson Waiswa Mwanja

of Uganda would coordinate establishment of an email group for networking on aquatic biosecurity issues.

8. The participants identified the need for a joint FAO/OIE/Workshop statement as an outcome of the workshop.

Follow-up activities

The follow-up activities listed below are being initiated/completed.

- The Aquatic Biosecurity Framework which will contain the broad development needs and recommendations for projects and activities with associated timelines aimed at enhancing southern African region's (as well as individual participating countries) capacity to effectively manage aquatic biosecurity risks and the Workshop Report are being finalized.
- Correspondence concerning establishing a communication platform on aquatic biosecurity among fisheries and focal points (FAO and OIE) in southern Africa had been initiated. The representative from Uganda volunteered to take a lead on this.
- At the recommendation of this regional workshop, a number of FAO focal points participated in the OIE seminar on "OIE international standards, a lever for growth in the fisheries and aquaculture sector in Southern Africa" organized by the OIE Sub-Regional Representative, held in Maputo, Mozambique, from June 10-12, 2008.
- Discussion are being made to include the southern Africa aquatic biosecurity framework in the broad FAO programme of work on SPADA (Special Programme for Aquaculture Development in Africa) and the aquatic biosecurity workshop participants to be included in the newly established Aquaculture Network for Africa (ANAF).

Annex 6

RECOMMENDATIONS OF THE OIE MAPUTO WORKSHOP

"OIE international standards, a lever for growth in the fisheries and aquaculture sector in southern Africa" **Maputo, Mozambique, 10–12 June 2008**

Recommendations

Considering

- OIE's mandate and responsibilities to promote aquatic animal health; and
- the international resolve and numerous instruments on fisheries and aquaculture in relation to food security, trade, environmental concerns, income generation and achievement of the *Millennium Development Goals;* and
- the potential benefits from sustainable fisheries and aquaculture and the opportunities to meet increasing demand for food from fish and other aquatic animals, as well the enhancement of natural resources; and
- the need to improve skills, knowledge and information exchange on aquatic animal diseases in the OIE Members in the SADC region; and
- the crucial role played by veterinary and other aquatic animal health professionals in the development and sustainability of the fisheries and aquaculture sector in the OIE Members in the SADC region; and
- the need for harmonised development of the fisheries and aquaculture sector across the SADC region, both at private and public levels; and
- the international obligations of the countries in the region as Members of both the OIE and the *World Trade Organisation* (WTO); and
- the recent *epizootic ulcerative syndrome* (EUS) outbreak in the Chobe-Zambezi river catchment and the questions it raises with regard to preparedness and disease intelligence at national and regional levels;

the OIE seminar on International Standards : a level for growth in the fisheries and aquaculture sector in Southern Africa, recommends:

To the OIE Members in southern Africa :

1. To ensure that OIE Delegates appoint the aquatic animal health focal points and that these appointees be officially communicated and regularly updated to the OIE Central Bureau.
2. To provide national focal points with adequate resources in order to fulfill their terms of reference.

3. To ensure that the OIE Delegates provide the nominated national OIE focal points with the reports from the *Aquatic Animal Health Standards Commission* and that the focal points coordinate the in-country consultation to provide a consolidated national response for submission to the OIE through the OIE Delegate and hence take an active part in the OIE standard setting process.
4. To ensure that national OIE focal points assist the OIE Delegate so as to comply with reporting requirements to the OIE through the WAHIS reporting system.
5. To encourage twinning between national diagnostic laboratories and with OIE Reference Laboratories. To encourage similar agreements with OIE Collaborating Centers.
6. To encourage the inclusion of aquatic animal health issues into the veterinary, fisheries and aquaculture curricula and provide opportunities for continuous education.
7. To promote dialogue between veterinary authorities or other relevant competent authorities, as well as the private sector, to identify their respective roles and responsibilities in aquatic animal health matters.
8. To review the national legislative framework for allowing the development of the fisheries and aquaculture sector.
9. To prioritise aquatic animal diseases of concern and fast track implementation of surveillance programmes in line with art. 13.9 of the *SADC Protocol on Fisheries* (2001) and OIE guidelines. To enhance cross-border cooperation between competent authorities to control aquatic animal diseases.

To the OIE Central Bureau and the Sub-Regional Representation for Southern Africa:

10. To facilitate OIE Members in the surveillance and notification of aquatic animal diseases by supporting training on the use of WAHIS.
11. To coordinate and support the establishment of a regional aquatic animal health network for fisheries and aquaculture in southern Africa in close collaboration with relevant bodies at national, regional and international level.
12. To promote the inclusion of aquatic animal health training into the ongoing process of harmonisation of the veterinary curriculum.

Endorsed by all participants on 12 June 2008 in Maputo, Mozambique.

Source:

www.rr-africa.oie.int/docspdf/en/Mozambique%202008%20Recommendations.pdf

This document is the final report of the work carried out by the International Emergency Disease Investigation Task Force on a Serious Finfish Disease in Southern Africa, a joint undertaking by the Food and Agriculture Organization of the United Nations (FAO), Botswana's Department of Wildlife and National Parks (DWNP) and Department of Animal Health and Production (DAPH), the Aquatic Animal Health Research Institute (AAHRI) of Thailand's Department of Fisheries and the Network of Aquaculture Centres in Asia and the Pacific (NACA), as a result of a technical mission to Botswana undertaken from 18 to 26 May 2007 and the subsequent outcomes of laboratory analysis of field samples conducted by AAHRI. This report provides comprehensive information on the outcomes of the 2007 Task Force investigation, building on earlier reports, and including further updates on epizootic ulcerative syndrome (EUS) occurrence in southern Africa based on an active surveillance programme that was implemented by FAO and partners in late 2007 until 2008. It also includes other ongoing activities and developments aimed at further enhancing aquatic biosecurity in southern Africa.

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