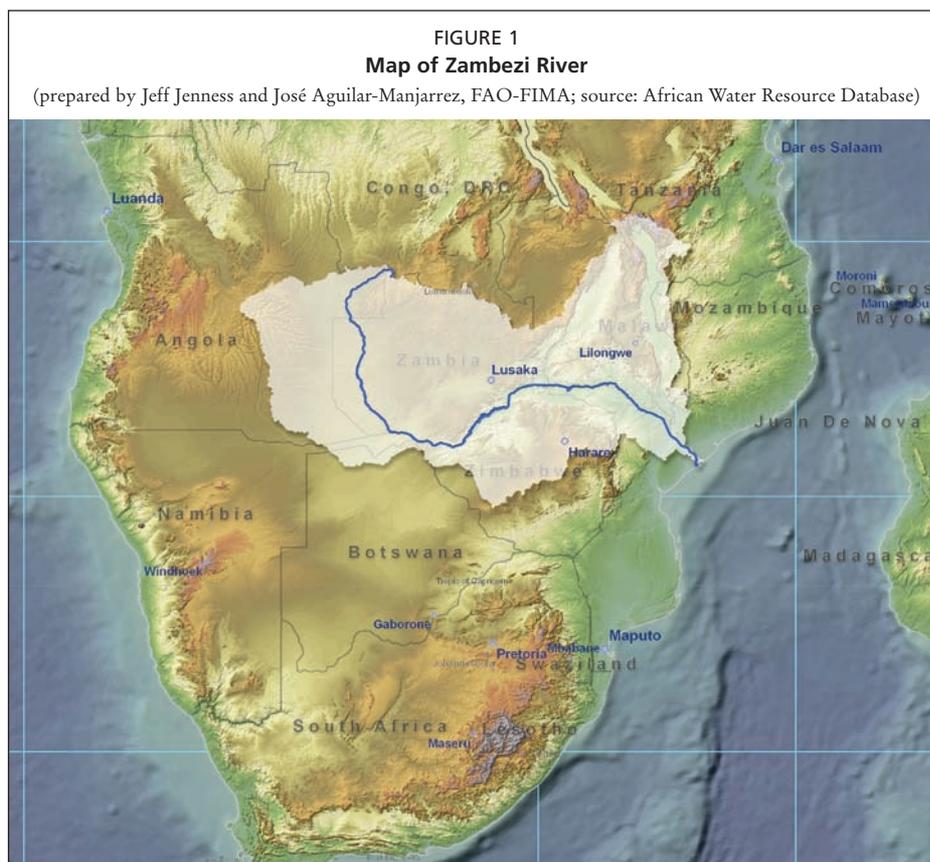


4. Results

The Zambezi River, with an area of 1 390 000 km² (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.



4.1 GENERAL PLANNING OF THE TASK FORCE WORK WITH LOCAL COUNTERPARTS

In this particular investigation, the case definition² 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		
<i>Barbus eutaenia</i>	orangefin barb	-	1	-	7	3
<i>Bracinus lateralis</i>	striped robber	3	10	1	10.98 (+/-1.92)	12.85 (+/-5.28)
<i>Clarias gariepinus</i>	sharp-tooth catfish	1	-	-	34.3	272
<i>Pollimyrus castelnaui</i>	dwarf stonebasher	-	4	-	11.15 (+/-0.75)	13.75 (+/-5.31)
<i>Cyphomyrus discorhynchus</i>	Zambezi parrotfish	-	2	-	19.5 (+/-1.41)	-
<i>Hydrocynus vittatus</i>	tigerfish	19	33	3	21.65 (+/-6.08)	90.19 (+/-97.91)
<i>Marcusenius macrolepidotus</i>	bulldog	2	9	1	13.44 (+/-2.74)	22.50 (+/-23.27)
<i>Mormyrus lacerda</i>	western bottlenose	-	8	-	13.78 (+/-2.51)	-
<i>Petrocephalus catostoma</i>	churchill	2	7	-	11.66 (+/-1.56)	16.8 (+/-6.65)
<i>Schilbe intermedius</i>	silver catfish	23	27	15	20.35 (+/-4.39)	76.63 (+/-44.76)
<i>Serranochromis thumbergi</i>	brownspot largemouth	1	-	-	12	22
<i>Synodontis</i> sp	squeaker	2	10	-	16.99 (+/-4.05)	45 (+/-48.56)
<i>Tilapia sparmani</i>	banded tilapia	4	-	-	9.53 (+/-1.18)	13 (+/-5.35)
Unidentified 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	
<i>Aplocheilichthys johnstoni</i>	Johnston's topminnow	7		33.2 (+/-2.05)
<i>Aplocheilichthys katangae</i>	striped topminnow	3	-	32 (+/-6.08)
<i>Barbus haasianus</i>	Sickle-fin barb	1	-	24
<i>Barbus barotseensis</i>	Barotse barb	4	-	46.25 (+/-6.40)
<i>Barbus bifrenatus</i>	hyphen barb	2	-	-
<i>Barbus eutaenia</i>	orangefin barb	11	2	39.4 (+/-9.48)
<i>Barbus fasciolatus</i>	red barb	1	-	38
<i>Barbus multilineatus</i>	copperstripe barb	10	-	28.67 (+/-0.58)
<i>Barbus poechii</i>	dashtail barb	3	-	60.5 (+/-9.19)
<i>Barbus radiatus</i>	Beira barb	10	38	47 (+/-5.10)
<i>Barbus kerstenii</i>	redspot barb	9	-	28.33 (+/-14.01)
<i>Barbus thamalakanensis</i>	thamalakane barb	8	-	32.42 (+/-1.13)
<i>Barbus unitaeniatus</i>	slender barb	28	1	44.32 (+/-6.49)
<i>Momyrus lacerda</i>	western bottlenose	1	-	163
<i>Marcusenius macrolepidotus</i>	bulldog	1	-	106
<i>Micralestes acutidens</i>	Silver robber	14	-	-
<i>Pharynochromis acuticeps</i>	Zambezi happy	5	-	38.8 (+/-15.55)
<i>Petrocephalus catostoma</i>	churchill	-	-	-
<i>Pollimyrus castelnaui</i>	dwarf stonebasher	-	-	-
<i>Cyphomyrus discorhynchus</i>	Zambezi parrotfish	-	3	-
<i>Pseudocrenilabrus philander</i>	southern mouthbrooder	57	7	33.79 (+/-6.67)
<i>Serranochromis macrocephalus</i>	purpleface largemouth	1	-	44
<i>Serranochromis robustus</i>	nembwe	1	-	-
<i>Synodontis nigromaculatus</i>	spotted squeaker	2	-	-
<i>Synodontis spp.</i>	squeaker	7	-	50.6 (+/-7.89)
<i>Tilapia rendalli</i>	redbreast tilapia	7	-	51
<i>Tilapia ruweti</i>	Okavango tilapia	4	-	38.5 (+/-12.02)
<i>Tilapia sparrmanii</i>	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 based on histopathology
1	<i>Barbus thamalakanensis</i>	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	<i>Pseudocrenilabrus philander</i>	southern mouthbrooder	normal	histology	-	mycotic granulomas not found in muscle – EUS negative
3	<i>Micralestes acutidens</i>	sharptooth tetra	normal	histology	-	mycotic granulomas not found in muscle – EUS negative
4	<i>Schilbe intermedius</i>	silver catfish	normal	parasitology histology	unidentified monogeneans observed	mycotic granulomas not found in muscle – EUS negative
5	<i>Barbus unitaeniaius</i>	slender barb	normal	histology	-	mycotic granulomas not found in muscle – EUS negative
6	<i>Aplocheilichthys katangae</i>	striped minnow	normal	histology	-	mycotic granulomas not found in muscle – EUS negative
7	<i>Pseudocrenilabrus philander</i> (2 fish)	southern mouthbrooder	white patch on the body	bacteriology histology	bacteria negative	mycotic granulomas not found in muscle – EUS negative
8	<i>Schilbe intermedius</i>	silver catfish	normal	histology	-	mycotic granulomas not found in muscle – EUS negative
9	<i>Barbus poechii</i>	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	<i>Barbus bifrenatus</i>	hyphen barb	normal but with pale coloration	histology	-	mycotic granulomas not found in muscle – EUS negative
11	<i>Marcusenius macrolepidotus</i>	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	<i>Marcusenius macrolepidotus</i>	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	<i>Petrocephalus catostoma</i>	churchill	multiple red spots (damaged by gillnet)	mycology histology	fungus negative	mycotic granulomas not found in muscle – EUS negative
14	<i>Petrocephalus catostoma</i>	churchill	single red spot (gillnet damage)	histology	-	mycotic granulomas not found in muscle – EUS negative
15	<i>Schilbe intermedius</i>	silver catfish	normal	parasitology histology	unidentified monogeneans, digeneans and sporozoans observed	mycotic granulomas not found in muscle – EUS negative
16	<i>Synodontis</i> sp.	squeaker	small white patch at tail (gillnet damage)	histology	-	mycotic granulomas not found in muscle – EUS negative
17	<i>Mormyrus lacerda</i>		normal	parasitology histology	unidentified monogeneans observed	mycotic granulomas not found in muscle – EUS negative
18	<i>Hydrocynus vittatus</i>	tigerfish	normal but with redness coloration of muscle	parasitology histology	unidentified monogeneans observed	mycotic granulomas not found in muscle – EUS negative
19	<i>Brycinus lateralis</i>	striped robber	normal	parasitology histology	unidentified monogeneans observed	mycotic granulomas not found in muscle – EUS negative
20	<i>Synodontis thalalakanensis</i>	squeaker	normal	histology	-	mycotic granulomas not found in muscle – EUS negative
21	<i>Synodontis</i> sp.	plain squeaker	normal	parasitology, histology	unidentified digeneans and sporozoans observed	mycotic granulomas not found in muscle – EUS negative
22	<i>Mormyrus lacerda</i>	western bottlenose	small skin damage (scoop net damage)	parasitology histology	unidentified monogeneans and sporozoans observed	mycotic granulomas not found in muscle – EUS negative
23	<i>Marcusenius macrolepidotus</i>	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

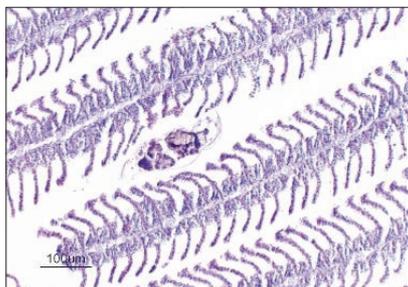
Fungal oomycete was successfully isolated from the muscle tissue next to the dermatitis lesion of diseased specimen No. 9. The oomycete grew slowly out of the muscle tissue and penetrated into GP agar plate at 2-3 mm in 2 days at 15-22 °C incubation temperatures. This slow growing oomycete isolate was 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

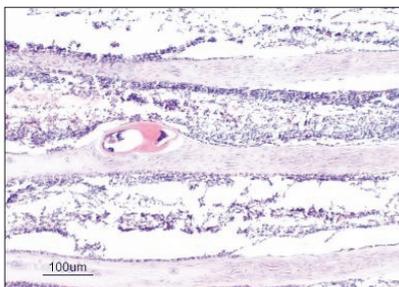
Virus isolation was attempted only for diseased specimen No. 9. No cytopathic effect (CPE) was observed in the first inoculation and subsequent blind passages. No virus could be isolated from diseased fish using EPC and BF2 cell lines.

PLATE 4
Parasites observed from fish samples

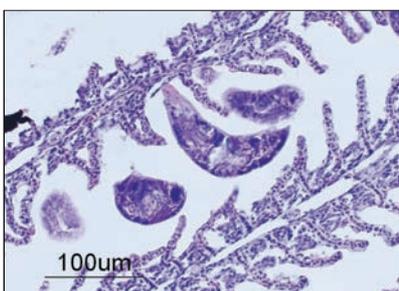
(All photos courtesy of AAHRI)



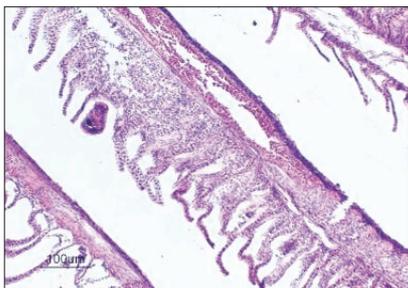
Fish sample No. 11 *Marcusenius macrolepidotus*, bulldog, was observed to harbour unidentified monogenetic parasites in the gills and kidney



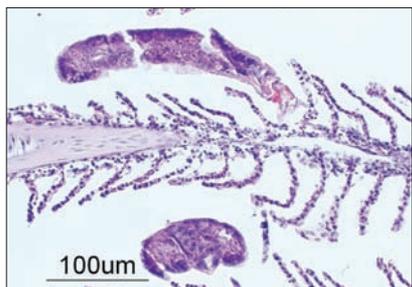
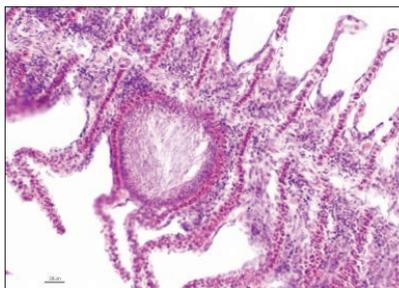
Fish sample No. 12 *Marcusenius macrolepidotus* (bulldog) was observed to harbour unidentified parasite cysts in the gill



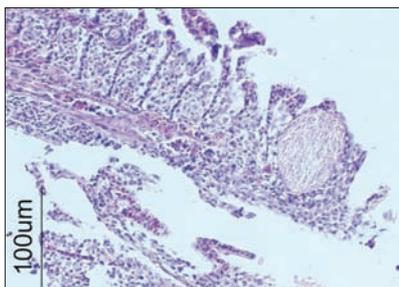
Fish sample No. 17 *Momyrus lacerda*, western bottlenose mormyrid, was observed to harbour unidentified monogenean parasite in the gills



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

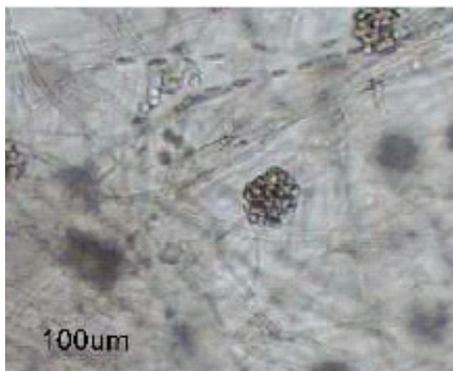


Fish sample No. 23 *M. macrolepidotus*, bulldog, was observed to harbour unidentified myxosporean and metacercarial cysts in the gills

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.

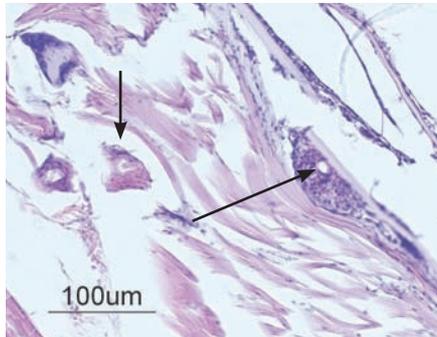
(2) Fish showing skin damage from gillnets or scoopnet. Histopathological changes in the skin lesions were related to loss of scales and epidermis or even parts of the dermis. Histopathology of gills and internal organs of fish in this group were minor and were probably not vital to fish health. These 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.

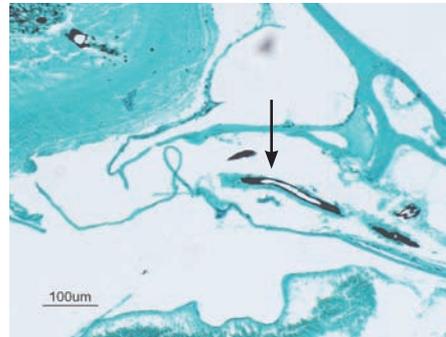
PLATE 6

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

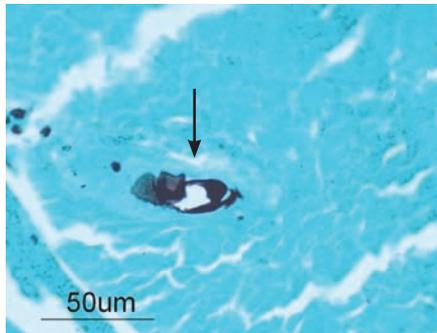
(All photos courtesy of AAHRI)



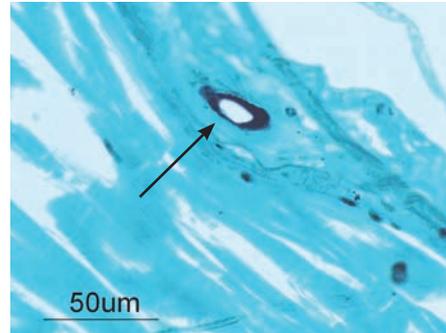
A



B



C



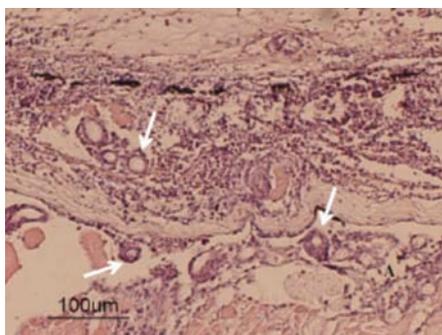
D

Typical mycotic granulomas (indicated by black arrow) found in the muscle tissue of fish sample No. 1 *Barbus thalakanensis* (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

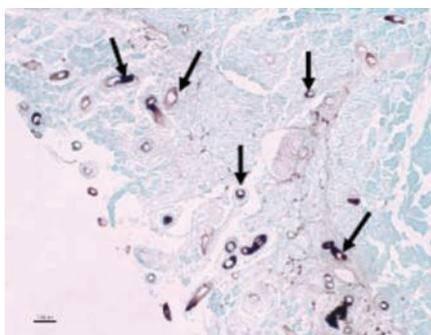
PLATE 7

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

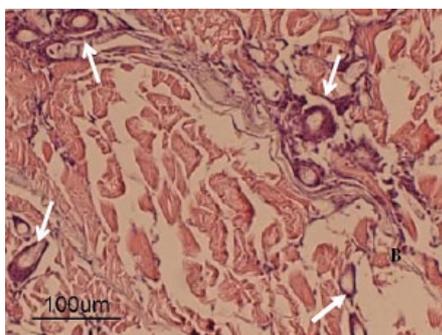
(All photos courtesy of AAHRI)



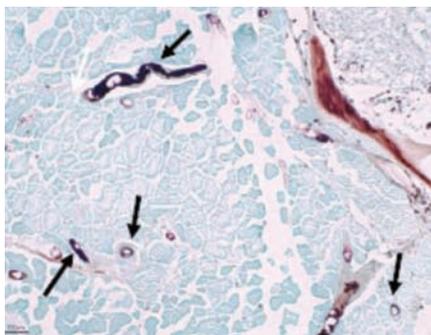
Histopathology of EUS-infected dashtail barb showing typical mycotic granulomas surrounding the invasive fungal hyphae (white arrows) in the skin layer (H&E)



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 poechnii* (Steindachner, 1911), exhibiting haemorrhagic dermatitis posterior to anus and towards the caudal peduncle