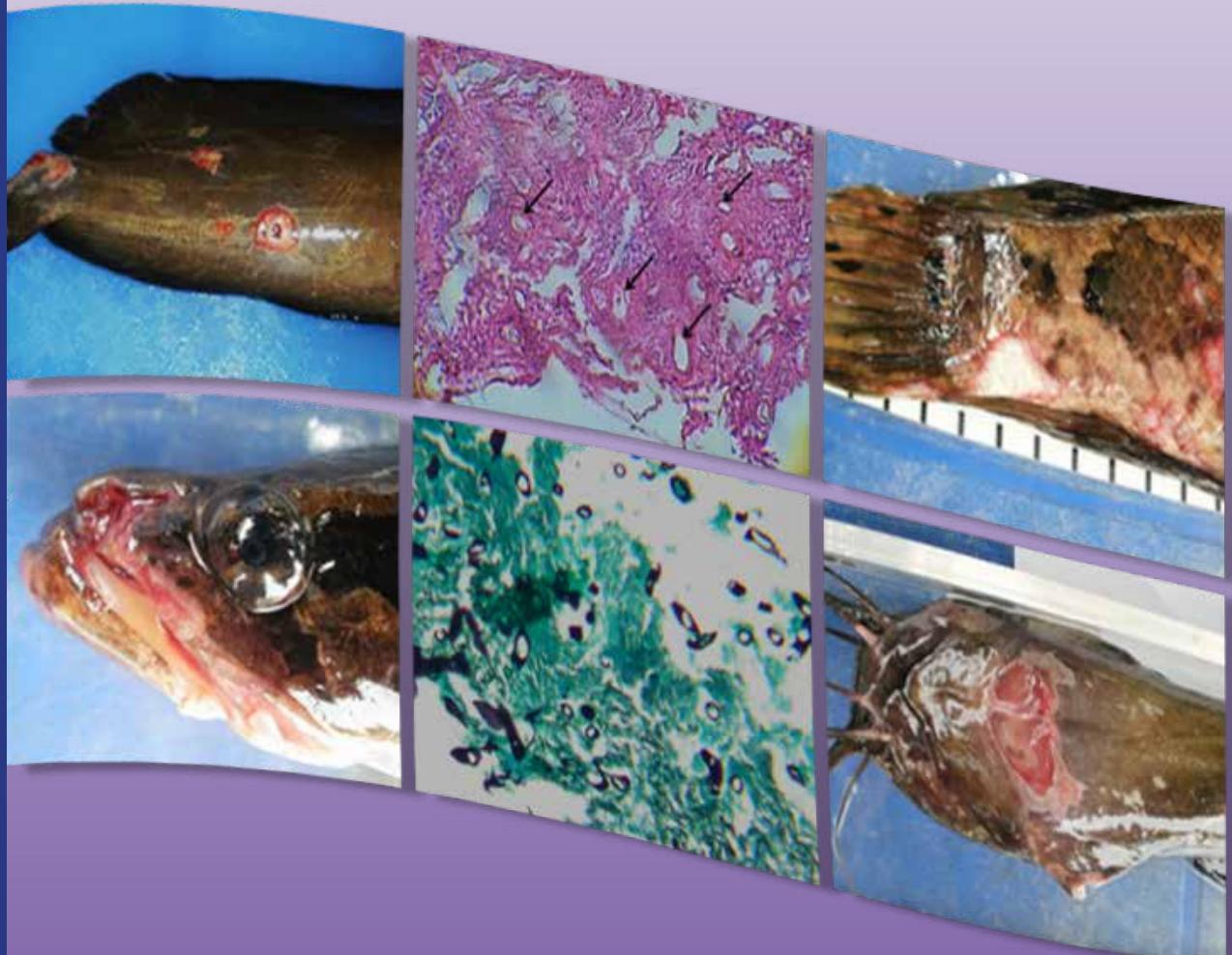




Food and Agriculture Organization
of the United Nations

Report of the International Emergency Fish Disease Investigation Mission on a Suspected Outbreak of Epizootic Ulcerative Syndrome (EUS) in the Democratic Republic of the Congo

13–19 March 2015



All photographs were contributed by Dr David Huchzermeyer (field photos) and Dr Bernard Mudenda Hangómbe (histopathology and PCR photos).

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Preparation of this document

This is the final report of the work carried out by the International Emergency Fish Disease Investigation Mission on a Suspected Outbreak of Epizootic Ulcerative Syndrome (EUS) in the Democratic Republic of the Congo, organized and funded by the Food and Agriculture Organization of the United Nations (FAO) through the Special Fund for Emergency and Rehabilitation Activities and funds from the FAO Representation in the Democratic Republic of the Congo and jointly implemented with the Government of the Democratic Republic of the Congo.

This document is based on the results of the work carried out by the Field Investigation Team to the Democratic Republic of the Congo conducted from 13 to 19 March 2015 and composed of two international consultants, Dr Bernard Mudenda Hang'ombe from Zambia and Dr David Huchzermeyer from the Republic of South Africa, with assistance from a local task force led by Dr Leopold K. Mulumba-Mfumu, Director of the Central Veterinary Laboratory (CVL) in Kinshasa, the Democratic Republic of the Congo and staff and officials of FAO Representation in the Democratic Republic of the Congo and the subsequent outcomes of laboratory analysis of field samples conducted by the team. All photographs were contributed by Dr David Huchzermeyer (field photos) and Dr Bernard Mudenda Hangómbe (histopathology and PCR photos).

The finalization of this report was under the technical oversight of Dr Melba B. Reantaso, Aquaculture Officer, Aquaculture Branch (FIAA), Fisheries and Aquaculture Department of FAO. Technical and English grammar editing was provided by Dr James Richard Arthur.

Abstract

In response to a request for an emergency technical assistance from the Government of the Democratic Republic of the Congo in connection with a serious disease affecting fish in Lokame River in Loko and in Mbanza Oton, 60 km from Gbadolite, The Food and Agriculture Organization of the United Nations (FAO) formed an International Emergency Disease Investigation Task Force. The overall objective of the Task Force was to: (1) confirm that an outbreak was happening; establish a case definition and make a presumptive diagnosis of the causative agent; (2) collect and process fish samples for relevant laboratory tests; (3) identify risk factors, confirm diagnosis and define further investigation or follow-up work; (4) recommend border/cross-border control measures to prevent further spread of the disease; (5) identify specific short-term and medium-term biosecurity action plans that the government may undertake; and (6) provide further recommendations to FAO on how to prevent the further spread of the disease.

Some members of the Task Force travelled to the Democratic Republic of the Congo from 13 to 19 March 2015, where they conducted field investigations. Subsequent laboratory tests have confirmed the presence of epizootic ulcerative syndrome (EUS) using two confirmatory tests recommended by the World Organisation for Animal Health (OIE), i.e.: (1) histopathology, which demonstrated granulomas in *Parachanna obscura* (snakehead), *Protopterus annectens* (lungfish) and *Clarias theodorae* (snake catfish) and (2) polymerase chain reaction (PCR), which confirmed the presence of genomic DNA of *Aphanomyces invadans* in collected specimens.

The Task Force concluded that permissive factors that favoured the propagation, infectivity and disease occurrence of EUS occur in the rivers and streams investigated in the Equateur Province of the Democratic Republic of the Congo. The findings also showed that environmental, climatic, water quality and human demographic conditions in the Congo River basin support the possibility of pandemic spread of the disease.

The Task Force suggested several actions which need to be undertaken to curb the spread of the outbreak. These include: active surveillance and monitoring of fish markets and other food channels used in the movement of live fish; capacity building for involved government personnel to strengthen knowledge and expertise in the identification and control of the disease through biosecurity measures; continued dialogue among the Democratic Republic of the Congo, neighbouring countries and FAO about EUS status, including subregional disease surveillance, monitoring, and response programmes; and the formulation of a national aquatic biosecurity strategy for the Democratic Republic of the Congo.

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Acronyms and abbreviations

AAHRI	Aquatic Animal Health Research Laboratory
CVL	Central Veterinary Laboratory
DAFF	Department of Agriculture, Forestry and Fisheries
EUS	Epizootic ulcerative syndrome
FAO	Food and Agriculture Organization of the United Nations
FAODC	FAO Representation in the Democratic Republic of the Congo
FAO-IAEA	International Atomic Energy Agency of the United Nations
FIAA	Aquaculture Branch (FAO)
GPA	Glucose-peptone agar
GPS	Global positioning system
H&E	Haematoxylin and eosin
OIE	World Organisation for Animal Health
PARSSA	Programmes for the Support of Water Sector Reform
PCR	Polymerase chain reaction
SARNISSA	Sustainable Aquaculture Research Networks in sub-Saharan Africa
SFERA	Special Fund for Emergency and Rehabilitation Activities
TC	Technical Cooperation Department (FAO)
TCP	Technical Cooperation Programme (FAO)
TSA	Tryptone soya agar
UNZA	University of Zambia

Executive summary

In response to a request for an emergency technical assistance from the Government of the Democratic Republic of the Congo in connection with a serious disease affecting fish in the Lokame River in Loko and in Mbanza Oton, 60 km from Gbadolite, FAO formed an International Emergency Fish Disease Investigation Task Force. The overall objective of the Task Force was to: (1) confirm that an outbreak was happening, establish a case definition and make a presumptive diagnosis of the causative agent; (2) collect and process fish samples for relevant laboratory tests; (3) identify risk factors, confirm diagnosis and define further investigation or follow-up work; (4) recommend border/cross-border control measures to prevent further spread of the disease; (5) identify specific short-term and medium-term biosecurity action plans that the government may undertake; and (6) provide further recommendations to FAO on how to prevent the spread of the disease.

Some members of the Task Force, i.e. the Field Investigation Team, travelled to the Democratic Republic of the Congo from 13 to 19 March 2015 and conducted field investigations. Laboratory tests of field samples followed, including validation of results by the World Organisation for Animal Health's (OIE) Reference Laboratory for epizootic ulcerative syndrome (EUS).

The disease. EUS is a serious finfish disease that is listed by the OIE. It is caused by an infection with *Aphanomyces invadans*, 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. In Africa, EUS was first diagnosed in the Republic of Botswana (2007) and has been confirmed in the Republic of Namibia (2007), the Republic of Zambia (2007), the Republic of South Africa (2010) and suspected in the Republic of Zimbabwe (undated). More than 50 finfish species are susceptible to EUS. In Africa alone, there are more than 20 finfish species infected (farmed and wild fish populations). EUS outbreaks threaten food security for subsistence fishers and fish farmers and subsequently people's physical health, as fish are an important source of animal protein for people in the affected countries.

Diagnosis. Fish showing clinical signs similar to EUS (small to large red spots and open dermal ulcerative lesions) were reported in the Equateur Province of the Democratic Republic of the Congo in December 2014, with a record of heavy fish mortalities. During the March 2015 investigation¹, 12 families of freshwater fish were inspected for evidence of EUS, of which members of the families Clariidae, Channidae, Protopteridae and Mormyridae were most severely affected. Confirmatory diagnosis of infection with *A. invadans* in clinically affected fish was done through OIE standards, i.e. (1) histopathology, which demonstrated granulomas in *Parachanna obscura* (snakehead), *Protopterus*

¹The Field Investigation Team to the Democratic Republic of the Congo conducted from 13–19 March 2015 was composed of two international consultants, Dr Bernard Mudenda Hang'ombe from the Republic of Zambia and Dr David Huchzermeyer from the Republic of the Republic of South Africa with assistance from a local Task Force led by Dr Leopold K. Mulumba-Mfumu, Director of the Central Veterinary Laboratory (CVL) in Kinshasa, the Democratic Republic of the Congo and staff and officials of the FAO Representation in the Democratic Republic of the Congo (FAODC). A preliminary report based on the field findings was presented to FAODC for the Government of the Democratic Republic of the Congo on 19 March 2015. Laboratory tests were conducted between April and September 2015. Analysis, validation and report finalization was completed in March 2016.

annectens (lungfish) and *Clarias theodorae* (snake catfish) and (2) polymerase chain reaction (PCR), which confirmed the presence of genomic DNA of *A. invadans* in collected specimens.

It was concluded that permissive factors favoured the propagation, infectivity and disease occurrence of EUS in the rivers (Libala, Loko and Mongala rivers and their tributaries) investigated in the Equateur Province of the Democratic Republic of the Congo. The habitat preference for well-vegetated backwaters of forest streams and floodplains that represent ideal conditions for infection with *A. invadans* explains the high prevalence of disease among fish in these four families.

The findings showed that environmental, climatic, water quality and human demographic conditions in the Congo River basin support the possibility of pandemic spread of the disease. The findings were validated by the EUS Expert at the Aquatic Animal Health Research Institute (AAHRI) and the OIE Reference Laboratory for EUS.

Control and prevention of EUS. There is no known protective vaccine nor effective chemotherapeutic for EUS. Treatment of EUS in natural waterbodies is not possible. Practical control is limited to identifying and reducing pathways of spread.

Public health significance. The agent causing EUS does not pose any direct human health threats. Except for the fish exhibiting deep ulcerations and tissue decay, which could harbour secondary pathogens which may have human health consequences, the fish infected with *A. invadans* do not pose health hazards for consumers. However, it is recommended not to eat EUS-infected fish unless properly and thoroughly cooked.

Risk of further spread. There is a high risk of spread to other African waterbodies from one lake or river system to another, endangering susceptible fish species. Factors include heavy rainfall and flooding, poor biosecurity, including movement of infected fish, as well as natural spread by fish and birds. Members of the Clariidae, Channidae and Protopteridae are of greatest concern regarding the spread of EUS, as fish in these families represent an important food commodity in the Democratic Republic of the Congo. Additionally, all three families represent air-breathing fish, and marketable fish are transported to and from markets, live thus providing an effective pathway for pathogen transfer.

Specific recommendations:

- Immediate notification by the veterinary authority in the Democratic Republic of the Congo (Animal Health Services and Chief Veterinary Officer of the Democratic Republic of the Congo) to the OIE of an outbreak of EUS (infection with *A. invadans*) in the Libala, Loko and Mongala rivers and in their tributaries in Equateur Province of the Democratic Republic of the Congo.
- Active surveillance of fish markets should be initiated immediately, with tracing of the actual source (origin) of all infected fish. Affected streams, rivers and villages should be mapped through initiation of local surveillance programmes and the spread of the disease outbreaks monitored.
- The movement of live fish between markets and between river systems in areas affected by EUS should be restricted (e.g. by quarantine and biosecurity measures); as well, a ban of fishing activities during EUS-outbreak seasons should be considered and alternative livelihood options for affected fishers should be determined.

- An initial national workshop for all personnel involved with fish and aquaculture activities should be urgently organized to initiate public awareness and extension programmes to raise understanding of the disease and the measures required to reduce its impacts (technical assistance).
- Funding should be urgently sourced to mobilize regional EUS expertise for a more in-depth epidemiological investigation of the extent of EUS in the Democratic Republic of the Congo and to provide training on EUS recognition, biology, diagnostics, pathology and surveillance procedures, emergency preparedness and contingency planning for both veterinary and fisheries officials and especially extension officers in the Democratic Republic of the Congo.
- The epidemiological investigation of the disease in the Democratic Republic of the Congo should be aimed at establishing the EUS status in the country (i.e. zones, farms etc.) and the critical areas near or along the border of affected areas in the Democratic Republic of the Congo or affected countries (e.g. the Republic of Zambia), the prevalence of EUS, the extent
- of natural waterbodies affected, the seasonality, the full range of species affected or susceptible (cultured and wild populations), potential carriers, risk factors and other pathways (e.g. fish markets, fish vendors etc.) and the risk to inland fisheries and human food security. The outcome of such an investigation should promote the development of appropriate risk management responses for the Democratic Republic of the Congo.
- The laboratory facilities at the Central Veterinary Laboratory (CVL) in Kinshasa should be urgently strengthened to enable the laboratory to initiate and perform effective fish disease investigations. This will require implementation of the fish pathology laboratory that has been initiated by the European Union and the International Atomic Energy Agency of the United Nations (joint division FAO-IAEA).
- Capacity building for local staff, particularly veterinary, fisheries and aquaculture officials, is urgently needed to enable them to educate the populace (consuming public, capture fishers, fish farmers, fish vendors, sports fishers/anglers) on the significance of this disease, the importance of implementing biosecurity measures, and the need for vigilance in collecting relevant field information; so that they can provide appropriate advice and technical assistance (e.g. who to contact and what they need to do when some mortalities are observed).
 - for consuming public: that the disease has no public health significance;
 - for capture fishing communities: season/time of year that the disease is likely to occur (heavy rainfall, flood events, low temperature season, e.g. when water temperature ranges between 18–25 °C), and reporting of any observed mortalities;
 - for farming communities: not to culture susceptible species or to avoid farming susceptible species during the EUS season; implement farm level biosecurity; reporting of observed mortalities.

General recommendations

- *Aphanomyces invadans*, the causative agent of EUS is not known to be infectious to humans and warm-blooded animals, and poses no direct health risk to human populations. Fish suffering from EUS may have extensive changes to the muscles underlying visible lesions on the skin. The quality of such flesh is poor. This may be compounded by secondary

bacterial invasion of the tissues rendering such fish unsuitable for human consumption. It is therefore recommended not to eat EUS-infected fish unless properly and thoroughly cooked. Such information needs to be conveyed urgently to human communities in affected areas.

- A clear risk communication strategy should be formulated so that accurate information can be provided and to avoid creating panic among relevant stakeholders.
- Dialogue on information-sharing systems about EUS status should be initiated. Neighbouring countries and an FAO Regional Technical Cooperation (TCP) Programme for EUS covering Central African countries should be established to initiate a practical action plan for the region to include subregional disease surveillance, monitoring, preparedness and response programmes. Dissemination of the findings to countries of the region should be undertaken as part of an early warning of potential wider spread of the disease.
- Authorities managing natural waterbodies in the region should be informed and considered as important stakeholders to address this EUS problem.
- Countries in the region (including the Democratic Republic of the Congo) should be encouraged to formulate national aquatic biosecurity strategies as part of long-term plans.
- A study should be undertaken to assess: (1) the socio-economic impacts of EUS in affected countries; (2) the efficacy of mitigation measures and other actions implemented by countries in Africa and elsewhere; and (3) other lessons learned from other aquatic disease investigations.

The way forward. The current challenge is to formulate concrete, coordinated and effective responses and actions by the Government of the Democratic Republic of the Congo, with the support of FAO, OIE and other relevant organizations and stakeholders to curtail the spread of the disease.

Strong and timely collaboration among different internal and external stakeholders is needed. Collaboration may begin with the fish farmers and Democratic Republic of the Congo government officials having close contact with each other. A strong and open communication may lead to timely identification and control of the disease at its beginning stages. Ensuring that government officials' capacity to identify, monitor and manage aquatic animal diseases, particularly in dealing with the current and future outbreaks, will enhance the success of relevant interventions. It is also essential that dialogue among the Democratic Republic of the Congo, its neighbouring countries, FAO and OIE on the status of the current EUS outbreak continues with timely and relevant information shared. Lastly, it may involve a consultative process of establishing clear short- and medium-term guidelines and policies aimed at responding to current and future EUS outbreaks.

The work and accomplishments of the International Emergency Fish Disease Investigation Task Force provide an impetus for further support to improve aquatic biosecurity awareness in the country.

1. Background

The Democratic Republic of the Congo is endowed with a vast Congo Basin drainage system of rivers, which are shared with the Republic of Angola, the Republic of Burundi, the Central African Republic, the Republic of the Congo, the Republic of Rwanda, the Republic of the Sudan, the United Republic of Tanzania, the Republic of Uganda and the Republic of Zambia. Of these neighbours, the Republic of Zambia has reported cases of epizootic ulcerative syndrome (EUS) in indigenous species of fish since 2007 (Andrew et al., 2008; FAO, 2009; Hang'ombe, Huchzermeyer and Mulumba-Mfumu, 2015), whereas the Democratic Republic of the Congo, sharing its waters with the Republic of Zambia, has until recently had no reports of this disease.

EUS is a serious disease of fish that is notifiable (infection with *Aphanomyces invadans*) to the World Organisation for Animal Health (OIE) (OIE, 2014b). The disease is caused by an infection with *A. invadans* or (also formerly known as *A. piscicida*), an oomycete or water mould (OIE, 2014b). OIE member countries are obliged to notify the OIE on the occurrence of new outbreaks of EUS. Many countries have specific-pathogen-free import certification requirements that include disease-status guarantees relevant to *A. invadans*.

In 2014, information and news received from sources including the Sustainable Aquaculture Research Networks in sub-Saharan Africa (SARNISSA), the private sector and OIE delegates from the Democratic Republic of the Congo indicated fish mortalities in the country, particularly in Lokame River in Loko and Mbanza Oton, 60 km from Gbadolite. Based on reported clinical signs and affected species, the disease was suggestive of EUS. This disease has swept across Japan, Australia, many countries in Asia, parts of the United States of America and recently Africa. In Africa, the disease has been recorded in the Republic of Botswana, the Republic of Namibia, the Republic of South Africa, the Republic of Zambia and the Republic of Zimbabwe since 2006, but never before from the Democratic Republic of the Congo. From the southern parts of the Republic of Zambia, the disease has now covered all major river systems in the country, with a serious threat of the disease spreading to other parts of Africa.

Since the first outbreaks were reported in the early 1970s, and as the disease affects both farmed and wild fish species, fishers and fish farmers have suffered significant loss of income. Farmed and wild fish worldwide are affected, with natural infection having been reported for some 76 finfish species (e.g. barbs, breams, catfish, gourami, eel, mullet, pike, tigerfish, tilapias and snakehead) (FAO, 2009).

First reports of the disease were from Japan in 1971 (Egusa and Masuda, 1971; Hatai and Egusa, 1978, 1979; Hatai et al., 1977), which identified *A. piscicida* as the cause of the commonly known disease ulcerative mycosis. Subsequent reports from Australia in 1972 (Callinan et al., 1995) implicated the same organism as the cause of red spot disease. In the United States of America, the disease was first described as ulcerative menhaden disease in 1978 (Blazer et al., 2002), and in South and Southeast Asia in 1986 as EUS (Das and Das, 1993; Lilley et al., 1997). First reports of EUS from southern Africa followed an outbreak in 2006 (Andrew et al., 2008; FAO, 2009; Mudenda, 2010; Huchzermeyer and Van der Waal, 2012; Nsonga et al., 2012; Songe et al., 2012). EUS now affects 24 countries on four continents (Africa, Asia, Australia and North America) (FAO, 2009). Most countries, particularly those in Asia (e.g. Bangladesh, Cambodia, India, Nepal, Philippines, Thailand and Viet Nam), but also Australia and Japan, have reported EUS among wild fish populations (OIE, 2014a).

In Africa, the disease has been reported in the Republic of Botswana, the Republic of Namibia, the Republic of South Africa, the Republic of Zambia (FAO, 2009; OIE, 2014a). the Republic of Zambia is by far the most severely affected country in the southern African region. For example, the western province of the Republic of Zambia, with a population of over 850 000, is solely dependent on subsistence fishing, and represents one of the poorest regions of the Republic of Zambia, with 18 percent HIV/AIDS prevalence, and where more than 85 percent of the population is living in villages along the Zambezi River. Over 2 000 villages are affected because of EUS (Musumali *et al.*, 2009). In most of the countries currently experiencing outbreaks of EUS, the decision by respective governments to ban fishing during the EUS season has further negatively impacted the livelihood and food-fish source of the communities dependent on subsistence fishing (Bondad-Reantaso, Subasinghe and Hang'ombe, unpublished paper).

The African region is home to a wide variety of indigenous and enzootic species, at least 3 200 freshwater species having been reported (FishBase, 2004). Many of these have been evaluated as suitable aquaculture candidates, the most important being tilapia and catfish (Brummett, 2007). With the exception of the Nile tilapia, *Oreochromis niloticus* (OIE, 2014a), other varieties of tilapia and catfish are susceptible to EUS. In Africa alone, there are more than 20 finfish species infected (farmed and wild fish populations). Thus, there is a high risk of EUS being spread through the movement of live fish for aquaculture purposes. Movement of live fish from one river or lake system to another with the same or closely related fish fauna within the African continent may occur through several pathways, such as movement of fish species for aquaculture, marketing, angling and ornamental trade, as well as by the natural upstream and downstream movement of fish.

The importance of fisheries in Africa for food security has been documented, and significant effects of EUS on fisheries and local communities in the Republic of Zambia, the Republic of Botswana and the Republic of Namibia have been reported (WorldFish Center, 2005). The outbreak of fish mortality in the Democratic Republic of the Congo is alarming and needs to be attended seriously. The disease will potentially affect the livelihood and food security of communities dependent on affected river systems. These communities are already under pressure because, apart from fishing and hunting, rural populations in the Equateur Province in the Democratic Republic of the Congo do not have other major subsistence activities. As hunting is a major risk factor to a broad range of zoonotic diseases like ebola, monkey pox, yellow fever, Crimean Congo fever, etc. this is justifying the “Projet d’Appui à la Réhabilitation et la Relance du Secteur Agricole/Agriculture Rehabilitation and Recovery Support Project” (PARSSA) funded by the World Bank in the South and North Ubangi districts (World Bank, 2009).

There is high risk of EUS spreading further into other African waterbodies due to heavy rainfall and flooding that may interlink drainage systems, the activities of humans not conforming to appropriate biosecurity measures, and other poorly studied risk factors such as bird migrations.

Fish is a major source of protein and income in the Democratic Republic of the Congo; as in other countries affected by EUS, there is a threat to food security for subsistence fishers and fish farmers, and subsequently people's physical health. As only a few people are engaged in livestock farming, fish plays an important role in food security and rural economies. The Congo Basin is home to a wide variety of indigenous and enzootic species, a number of which are good aquaculture candidates. The catchment forms a large, well-watered area covering most of the country, with a distinct annual flood regime in response to seasonal rainfall cycles. The rivers include young as well as mature stretches with extensive floodplain and swamp reaches. This makes the basin an important fish habitat and highlights the need to know the causative agent of fish mortalities in the Democratic Republic of the Congo.

In response to a request for technical assistance to investigate this serious disease outbreak, an International Emergency Fish Disease Investigation Mission on a Suspected Outbreak of Epizootic Ulcerative Syndrome (EUS) in the Democratic Republic of the Congo was organized by the FAO, led by the Aquaculture Branch (FIAA) in close coordination with the Technical Cooperation Department (TC) and FAO Representation in the Democratic Republic of the Congo (FAODC).

2. International Emergency Fish Disease Investigation Task Force

The International Emergency Fish Disease Investigation Task Force on EUS (subsequently referred to as EUS Investigation Task Force) was composed of EUS experts and officers from FAO (headquarters in Rome, Italy and representation office in Kinshasa, the Democratic Republic of the Congo), two international experts on EUS and a local task force consisting of officials and staff of the Central Veterinary Laboratory (CVL). The composition of the EUS Investigation Task Force can be found in Appendix 1.

The responsibilities of the EUS Investigation Team are indicated below:

- FAO headquarters staff to:
 - mobilize resources to support the field investigation mission;
 - organize an international team of experts;
 - provide technical oversight in the general planning, operational aspects and overall implementation of the mission, including organizing of Skype and teleconference calls;
 - consult with the OIE Reference Laboratory for EUS and other relevant laboratories;
 - finalize the report of the investigation on the suspected EUS outbreak in the Democratic Republic of the Congo; and
 - provide feedback to SFERA and other relevant authorities.
- International experts and local Democratic Republic of the Congo Task Force (Field Investigation Team) to:
 - undertake a field mission from 13 to 19 March 2015;
 - confirm that an outbreak was happening; establish a case definition and presumptive diagnosis of the causative agent;
 - collect and process fish samples for histopathology, polymerase chain reaction (PCR), microbiology and mycology and water samples for relevant laboratory tests;
 - identify risk factors, confirm diagnosis and define further investigation or follow-up work (EUS active surveillance);
 - recommend border/cross-border control measures to prevent further spread of the disease;
 - identify specific short-term and medium-term biosecurity action plans that the government would undertake;
 - provide additional recommendations to FAO on how to prevent the further spread of the disease; and
 - participate in video/teleconference or Skype calls to discuss technical and operations issues.

- Staff to:

- Mobilize resources to support the field investigation mission;
- coordinate with the Government of the Democratic Republic of the Congo and other relevant local agencies and institutions to assist in organizing a local task force;
- provide logistics and operational support to the Field Investigation Team;
- organize a briefing and debriefing session for the Field Investigation Team; and
- participate in video/teleconference or Skype calls to discuss technical and operations issues.

Funds were secured through the Special Fund for Emergency and Rehabilitation Activities (SFERA) and from the FAODC to conduct an investigation of the aforementioned fish mortalities.

The Field Investigation Team was composed of two EUS experts and a local task force. Appendices 1 and 2, respectively, show the members of the EUS Investigation Task Force and the itinerary of the Field Investigation Team, who undertook the mission from 13 to 19 March 2015. The Field Investigation Team visited several areas in the Democratic Republic of the Congo where the outbreak was reported (Figure 1).



FIGURE 1 Map of the Democratic Republic of the Congo showing the area where the disease investigation was carried out (red circle)

3. Methodology: field observations and laboratory analysis

3.1 General planning for the disease outbreak investigation (with local task force)

On 13 March 2015, general planning of the field mission was discussed with the FAO Representation in Kinshasa. The Field Investigation Team would be taken to the sampling sites as indicated in Appendix 2. Methods to collect fish depended on the conditions at each site. If fish were needed to be collected using gill nets, those already made up and set by local fishers would be used in exchange for new nets, if necessary. Alternatively, scoop nets would be used to catch fish in the shallows as far as these might be accessible. Where possible, fish markets would be inspected to determine prevalence of the disease among fish on offer. The planning session was followed by a security briefing. Field sampling materials sourced from Kinshasa (including portable car fridge, folding table and chairs) and sampling materials supplied by the CVL (i.e. microscope, formalin, ethyl alcohol, disinfectant gel, sampling containers and liquid nitrogen) were briefly inspected for completeness, and the field mission was equipped with a global positioning system (GPS) and satellite phone for use during the field work. A GPS reading was to be taken at each sampling site and water temperature and pH were to be recorded from all waterbodies visited. In addition, a 500 ml water sample would be collected for later analysis in Kinshasa.

Immediately on arrival at each sampling point, discussions on local logistics were held with resident staff to understand what was required and how the sampling would proceed. The realistic time situation required for fish sampling and specimen collection in the area was discussed. Local staff highlighted the difficulties of accessing fishing sites, as these were located deep in the forests. It was agreed to target live fish markets, fishers encountered coming from the river and if possible, active sampling in river sections that could be accessed along the road.

The outbreak investigation was carried out through physical inspection of fish at live fish markets. Suspected fish exhibiting red spots and evidence of ulceration were purchased from fish traders. At river sites, local people were engaged in collecting samples using a scoop net, while the Field Investigation Team was involved in interviewing fishers and other concerned local people. After the collection of fish samples, the specimens were processed through identification of fish species, taking length and weight measurements, clinical observations and collection of samples for further laboratory examination. For this purpose, a make-shift laboratory was set-up at various points.

3.2 Fish sampling

As decided during the planning session, the Field Investigation Team made provision for collection of fish samples by gill nets and by scoop nets. Gill nets were purchased in Kinshasa and two scoop nets were purchased in the Republic of South Africa. Decision on which collection method to use was left until the respective sites were reached. Few sites represented active fishing locations, and inspection of local catches on the river banks was not possible. The Gemena market selling live and large numbers of three air-breathing species of fish provided material for a preliminary prevalence determination and collection of suitable specimens for further laboratory evaluation. During the March 2015 investigation, 12 families of freshwater fish were inspected for evidence of EUS.

3.3 Description of field procedures for gross examination of fish and collection of samples for laboratory analysis

Field laboratories were set up at the hotel in Gemenia, at Karawa town, at the convent in Businga and at the hotel in Gbadolite. Depending on species, live fish were euthanized by decapitation, stunning or by an exposure to an overdose of eugenol. The species, sex and length of each fish were recorded. For larger fish, the weights were also recorded. The type and distribution of lesions was assessed and recorded, and, where systemic pathology was evident, this was also recorded. All fish specimens were allocated an identification code and this was photographed together with each sampled fish. Where the identification of a species was unclear, a small tissue sample was fixed in 70 percent ethanol and, in the case of small fishes, the entire fish was fixed in 10 percent formalin for later taxonomic identification. The same code was used to identify samples collected from the fish for laboratory analysis. Dissection of all the euthanized fish was performed, internal organs were examined and samples were collected from diseased tissues. In the case of skin lesions, one section of muscle was dissected from the edge of the lesion and placed onto culture medium for microbiology. Triplicate sections of muscle were removed extending from the center of a skin lesion through the perimeter into healthy tissue. These samples were fixed in 70 percent ethyl alcohol for molecular diagnosis. A second, but larger, set of triplicate samples was collected in the same manner and fixed in 10 percent formalin for histological examination. A further sample taken in the same fashion from a lesion was placed into liquid nitrogen for storage. Tissues preserved in liquid nitrogen during the trip were transferred for storage to a freezer at minus 90 °C at the CVL immediately after arrival in Kinshasa. From one lungfish, tissue from the edge of a skin ulcer and from the intestine was fixed in 2.5 percent glutaraldehyde for storage in the eventuality that electron microscopic examination might be necessary. From several lungfish, a full set of organ samples was collected in 10 percent formalin for histological examination. Wet-preparation tissue squashes were prepared from affected muscle tissue from one snake catfish and two lungfish, and from affected intestine from one lungfish. These were examined by light microscopy for presence of oomycete hyphae.

3.4 Water quality, meteorological and other environmental data

At each sampling point, a GPS reading was recorded, including coordinates and elevation above sea level. A number of sites were inspected for water quality, through visual inspection by checking the colour, turbidity and pH level. In all cases, the rivers and streams had sand bottom substrate with evidence of decaying vegetation, which seemed to influence the colour of the water.

Water temperature and pH were recorded and 500 ml of river water was collected. River sites were photographed. Water samples were transferred to the CVL in Kinshasa for future testing of the water for salinity, hardness and alkalinity.

3.5 Consultation with women fish marketers, fishers, fish farmers

Women selling fish at the market in Gemenia and Businga were interviewed about the origin of the fish and presence of sick fish. Fishers in Karawa, Businga, Loko, Mobayi Mbonge and Bobotu were shown photos of southern African fish suffering from EUS and were interviewed about the incidence and prevalence of sick fish in their respective areas.

3.6 Laboratory investigation procedures

3.6.1 Gross clinical signs (on site)

The collected fish were examined for gross clinical signs that included red spots on the body surface, head, opercula or caudal peduncle. Large red ulcers with brown necrosis at the periphery were also noted, as were large superficial lesions on the dorsum. Since the main sampling method was through live fish purchases, purchased fish were selected for presence of one or more of the above lesions. All collected fish with gross clinical signs were subjected to further pathogen examination and analysis.

3.6.2 Parasitology (on site)

The skin, fins and gills were examined for any evidence of parasites by the naked eye, with particular attention being paid to ulcerated areas of the skin.

3.6.3 Bacteriology (on site)

Fish samples showing gross clinical disease signs were subjected to bacteriological examination using standard bacteriological procedures (AAHRI, 1999; Table 8). The bacterial isolates were subcultured before transferring to transport media containing tryptone soya agar (TSA). Some samples from fish showing gross clinical disease lesions were collected in Carry-Blair transport medium and brain heart infusion broth for further bacteriological analysis.

3.6.4 Mycology (on site and at the laboratory facility at UNZA)

Fish with ulcerative lesions were observed for external fungal growth or infection. Using aseptic technique, sections of the ulcerative lesions were carefully excised and placed on Petri dishes containing glucose-peptone agar (GPA) for oomycete hyphal growth. The Petri dishes were sealed using Parafilm and were transported to the University of Zambia (UNZA) laboratory for further culture and processing.

3.6.5 Virology (on site and at the laboratory facility of CVL)

Tissue specimens from ulcerative lesions were collected and stored at minus 196°C in liquid nitrogen for possible viral analysis. On return to Kinshasa, the specimens were immediately transferred for storage to a freezer at minus 90°C at the CVL.

3.6.6 Histopathology (on site and at the laboratory facility of UNZA and University of Pretoria, the Republic of South Africa)

Specimens from fish samples with clinical lesions were collected for histopathology using standard procedures. Sections from selected lesions, kidneys, spleen, liver and heart were collected and immediately placed in 10 percent formalin. In the case of the lungfish, intestines were also collected. After the tissues had been fixed in 10 percent formalin for at least 24 hours, the fixed tissues were then placed into small ziplock bags with formalin-soaked tissue paper for transportation to the laboratory. In the laboratory, tissue samples were processed by standard histological technique and 5 µm thick sections were stained with haematoxylin and eosin (H&E). The sections were examined

using a standard light microscope for presence of mycotic granulomas and other pathology in the tissue. Some sections showing mycotic granulomas were stained with Grocott's stain to demonstrate oomycete (mycotic) structures.

3.6.7 Polymerase chain reaction (PCR) (on site and at the laboratory of UNZA and DAFF)

Samples that included infected tissue, spleen and kidney were collected for the direct detection of DNA belonging to *A. invadans*. A small section of affected tissue was excised and placed in 70 percent ethanol. In the laboratory, genomic DNA was extracted using the DNA extraction commercial kit (ZR genomic DNATM-Tissue Mini Prep Catalog No. D3051) following the manufacturer's instructions (Zymo Research Irvine, California, United States of America). The extracted DNA was subjected to PCR using species-specific primer sites located in the ITS1 and ITS2 regions. The forward primer ITS11 (5'-GCC-GAA-GTT-TCG-CAA-GAA-AC-3') and the reverse ITS23 (5'-CGT-ATA-GAC-ACA-AGC-ACA-CCA-3') were used. The PCR product was analysed by agarose gel electrophoresis to observe the targeted product of 550 bp (Phadee *et al.*, 2004).

3.7 Analysis of available reports and other correspondences/information based on initial information received (i.e. clinical signs and affected species)

Reports received from several sources raised suspicion that a serious fish disease outbreak was affecting wild fish at several locations in the Democratic Republic of the Congo. From descriptions of sick fish received, it appeared that the disease of concern may be what is known as EUS. Information from the following sources was taken into account: SARNISSA network, private-sector observations and information from delegates of the Democratic Republic of the Congo participating in the OIE Aquatic Animal Health Conference in Viet Nam on 20 January 2015. A report viewed at the CVL in Kinshasa indicated that *Clarias* spp. with ulcerative lesions had already been observed at Kasangulu, near Kinshasa in 2013, and it appeared to be common knowledge that fish were imported from the Republic of Zambia for sale in the markets of Kinshasa.

4. Results

4.1 General planning for the disease outbreak investigation (with local task force)

4.1.1 Case definition

For the purposes of this disease investigation, the case definition used was "fish with cutaneous lesions including red spots, erosions, ulcers and wounds". This definition conforms to that observed in previous outbreaks in other countries, and as demonstrated by the relevant photographs that were brought for comparison during this field investigation. Fish with scale loss resulting from netting injuries were precluded.

4.2 Sampling location and field activities

The Field Investigation Team (Drs Hang'ombe, Huchzermeyer and Mulumba) travelled to Gemenza, met with the FAO field officer, Mr Prudent Landon and other officials (Appendix 3) and further

discussed the field activities and received local security briefing. A live fish market in Gemena was visited where a substantial number of fish were on offer for sale, including many fish showing lesions suggestive of EUS. Figure 1 shows the location where the disease investigation was carried out. Fish samples were examined and specimens for laboratory analysis were collected. Several fish culture ponds on the outskirts of the city were also visited to ascertain the type of aquaculture practices in use (Figure 2).



FIGURE 2 Inspecting private fish culture ponds near Gemena with no observed sick fish

In another location, Karawa, where the Libala River (Site 1) is located, the Field Investigation Team first met with the local chief and veterinary field officers (Appendix 3) and was informed that sick fish had been collected and had been placed in a freezer at a nearby house, but the owner was unavailable. The Field Investigation Team showed two cardboards with photographic illustrations of species of southern African fish suffering from EUS. These proved very useful, as the lesions were immediately recognized as similar to those noticed by fishers in local fish since December 2014. The fishers reported very high morbidity and high mortality among fish at the outset of the outbreak, and described having seen large numbers of dead fish floating in the streams. At the Libala River (Figure 3), two fishers were asked to use the scoop nets along the vegetated river banks, which yielded three fish, two of which had lesions typical of EUS. There was no sign of gill net use in the river and there was no landing site where fishers brought in catches.



FIGURE 3 Field Investigation Team at the Libala River in Karawa

A further fish market was visited in Businga, where local veterinary officers sourced a number of live fish with lesions from the Mongala River (Site 2) (Figure 4). The fish were examined and dissected for sample collection. After a courtesy visit to the local chief (Appendix 3), it was established, as in Karawa, that fishers first became aware of sick fish with lesions suggestive of EUS during December 2014. As in the case of Karawa, large fish mortalities were observed in December 2014.

The Field Investigation Team then proceeded to the Mongala River, some distance from Businga. Water samples were collected and water temperature and pH were recorded. A number of small fish species were observed in the sandy shallow portion near the river bank but these appeared to be healthy.

Local veterinary officers were sent ahead to Loko River (Site 3) near Loko Village to collect fish with scoop nets. The Field Investigation Team followed and along the way inspected several forest streams. Large numbers of people were washing in almost all of the streams crossed by the road. Few fish were observed in these streams, and there was no evidence of fishing at these sites. On arrival at Loko Village, the Field Investigation Team found that the veterinary officers had successfully collected a larger number of small species of fish from the Loko River. These were kept alive in a large bucket. Slow progress on bad roads forced the Field Investigation Team to delay examination of these fish until they reached Gbadolite, several hours drive from Loko. At Loko River, the Field Investigation Team came across a fisher woman carrying a large bowl with recently caught fish. These fish were visually inspected for prevalence of suspected EUS lesions and the species were recorded. In Gbadolite, the Field Investigation Team set up the next dissection station. Examination and dissection of the fish collected at Loko River continued.



FIGURE 4 Dissecting a munguso fish with lesions at Businga, Mongala River

On 17 March 2015, the Field Investigation Team was driven to the Ubangi River at Mobayi Mbonge where they first met with the Chief of Fisheries Cell for North Ubangi District (Appendix 3). The veterinary officers with the scoop nets had travelled ahead but were able to catch only one fish at this site, as the large river, at this point, travels with a strong current through a narrow gorge below the hydroelectric turbines. The Field Investigation Team did have an opportunity to inspect a number of tiger fish and one large chessa caught by fishers from pirogues in the rapids using cast nets. There was some uncertainty about the identity of the Boy River (Site 5). It appeared that the term “Boy” referred to the Ubangi River at Mobayi Mbonge. The road between Gemena and Gbadolite apparently does not cross the Pambwa River (Site 4). On the return trip from Gbadolite to Gemena, the Field Investigation Team stopped at Bobutu Village (Bodangabo Sector) where local officials (Appendix 3) informed them that the Pambwa River can only be reached by foot after a 12 km hike from the road, which was not possible due to time constraints. Veterinary officials at Bobutu Village had, however, collected sick fish from the Mbo and Gbo rivers near Site 4. These fish, all *Clarias theodorae*, had been stored in a cooler box in the clinic at Bobutu Village and were inspected by the Field Investigation Team. The fish all showed lesions suggestive of EUS but were no longer fresh. The Field Investigation Team fixed the fish in formalin for further examination in Kinshasa. The remainder of the day was spent travelling by road back to Gemena, with several stops to inspect forest streams.

As lungfish examined earlier in Gemena had shown severe systemic lesions, a message was sent ahead to the FAO field officer in Gemena to purchase more lungfish with lesions from the fish market in Gemena. These fish were examined and dissected after the Field Investigation Team returned from Gbadolite.

4.3 Field investigation results

4.3.1 Fish species, gross pathology and prevalence

Fish collected and observed during the field investigation were identified to species level as far as possible with the assistance of Skelton's *A Complete Guide to Freshwater Fishes of Southern Africa* (Skelton, 2001) and FishBase (Centre for Ecology and Hydrology, 2015). A total of 117 fish were collected and examined during the three-day period. Of these fish, 43 were caught using scoop nets. Sick fish exhibited ulcerated dermal lesions and haemorrhagic swellings with a characteristic distribution depending on fish species. Several air-breathing fish species that form an important live trade commodity appeared to be severely affected. The results of field investigations are presented in Tables 1–8. Tables 1–7 show the species, lingala and common names, gender and observations of the Field Investigation Team, while Table 8 provides the results of laboratory analysis for EUS based on sampled fish species.

TABLE 1
Preliminary findings by the Field Investigation Team in Gemena live fish market: affected species, sample identification and gross pathology

Species	Lingala name	Common name	Sample ID	Sex	Length (cm)	Lesions
<i>Clarias theodora</i>	Ngolo	Snake catfish	GCLG1	M	<30	Ulcer on maxilla
<i>C. theodora</i>	Ngolo	Snake catfish	GCLG2	F	<30	Ulcer below operculum
<i>C. theodora</i>	Ngolo	Snake catfish	GCLG3	F	<30	Ulcer on maxilla
<i>C. theodora</i>	Ngolo	Snake catfish	GCLG4	M	<30	Ulcer on dorsum of head
<i>C. theodora</i>	Ngolo	Snake catfish	GCLG5	F	<30	Ulcer on tail
<i>C. theodora</i>	Ngolo	Snake catfish	GLCG6	M	<30	Deep ulcer on side of head behind operculum
<i>Parachanna obscura</i>	Mungusu	Snakehead	GPACH1	M	30	Ulcer on tail, red erosions on body
<i>Parachanna obscura</i>	Nzombo	Lungfish	GLF1	F	45	Ulcer on rostrum and ventrally on mandible, swollen red vent, severe, extensive, necrotic, pseudomembranous enteritis
<i>P. annectens</i>	Nzombo	Lungfish	GLF2	Imm	25	Ulcer on mandible and caudal to operculum an on head, swollen red vent, severe, extensive, necrotic, pseudo-membranous enteritis

TABLE 2

Preliminary findings by the Field Investigation Team in Karawa, Libala River: affected species, sample identification and gross pathology

Family/ Species	Lingala name	Common name	Sample ID	Sex	Length (cm)	Lesions
Mormyridae <i>Cyphomyrus discorhynchus</i>	Mbongo	Zambezi parrotfish	LM1	F	13	Ulcer at pectoral fin base, extensive erosions and scale loss on both sides of body. Liver irregular with red-brown discolouration, attached to ovary and intestine, peritonitis, ovary with follicular atresia
<i>Schilbe yangambianus</i>	Lale	Yangambi butter barbel	LC2	F	8	None
Clariidae	Dongbo	Long eel-like catfish	LC1	F	23	Multiple white blotches on skin, follicular atresia. Found sick by fishers. Moderate autolysis.

TABLE 3a

Preliminary findings by the Field Investigation Team in Businga live fish market: affected species and prevalence of gross lesions suggestive of EUS

Species	Lingala name	Common name	Lesions
<i>Clarias theodorae</i>	Ngolo	Snake catfish	Present in 3 out of 13 fish inspected
<i>Parachanna obscura</i>	Mungusu	Snakehead	Present in 8 out of 8 fish inspected

TABLE 3b

Preliminary findings by the Field Investigation Team in Businga, Mongala River: affected species, sample identification and gross pathology

Species	Lingala name	Common name	Sample ID	Sex	Weight (g)	Length (cm)	Lesions
<i>Parachanna obscura</i>	Mungusu	Snake head	MPACH1	Imm	1 210	50	Extensive erosion and ulceration on abdomen and white ulcer at base of tail
<i>P. obscura</i>	Mungusu	Snake head	MPACH2	Imm	290	34	2 small ulcers on each side of body, one ulcer at base of tail, extensive reddening of ventral skin
<i>P. obscura</i>	Mungusu	Snake head	MPACH3	Imm	350	37	Ulceration deep into bones of maxilla and rostrum, small ulcer at base of tail
<i>Clarias theodorae</i>	Ngolo	Snake catfish	MCL1	M	510	47	Ulcer caudal to pelvic fin base and on ventral body
<i>C. theodorae</i>	Ngolo	Snake catfish	MCL2	M	110	27	Paired ulcer on ventral aspect of pectoral girdle, confluent through ventral midline
<i>C. theodorae</i>	Ngolo	Snake catfish	MCL3	M	120	27	Paired ulcer on ventral aspect of pectoral girdle, confluent through ventral midline

TABLE 4a
Preliminary findings by the Field Investigation Team in Loko Village: affected species and prevalence of gross lesions suggestive of EUS

Species	Lingala name	Common name	Lesions
<i>Gnathonemus petersii</i>	Mbongo	Elephant fish	In 15 out of 27 inspected
<i>Parachanna obscura</i>		Snakehead	None in one fish inspected
<i>Tilapia rendalli</i>	Libundu	Red breast tilapia	None in 3 fish inspected
<i>Schilbe mysticus</i>			In 2 out of 5 fish inspected
Characidae			None in 2 fish inspected
<i>Hepsetus odoe</i>	Muenge	Pike	None in one fish inspected
<i>Synodontis</i> sp.	Mpoka		None in one fish inspected

TABLE 4b
Preliminary findings by the Field Investigation Team in Loko River: affected species, sample identification and gross pathology and prevalence of lesions suggestive of EUS

Family/Species	Lingala name	Common name	Sample ID	Length (cm)	Lesions
<i>Mormyridae/</i> <i>Petrocephalus</i> spp.	Mbongo	Possibly Northern Churchill	LOKOM1		8 out of 19* fish had ulcers and red swelling at base of pectoral fin, unilateral
Family Mormyridae		Unknown but similar to LOKOM1	LOKOM4		No lesions (included in counted*)
<i>Hippopotamyrus ansorgii</i>	Mbongo	Slender stone basher	LOKOM1b		4 out of 13 fish had ulcers and red swelling at base of pectoral fin, unilateral
<i>P. catostoma</i>		Northern Churchill	LOKOM3		Ulcers on body
<i>Mormyridae/</i> <i>Gnathonemus petersii</i>		Elephant fish	LOKOEM1		No lesions in one fish sampled
Family Mochokidae/ <i>Synodontis</i> sp. 1	Mpoka	Small pale squeaker	LOKOSY1		Extensive ulcers on body in one fish sampled
Family Mochokidae/ <i>Synodontis</i> sp. 2	Mpoka	Small brown squeaker	LOKOSY2		No lesions in 3 fish sampled
Family Osteoglossidae/ <i>Pantodon buchholzi</i>		Butterfly fish	LOKOBF1		No lesions in one fish sampled
<i>Clarias theodorae</i>	Ngolo		LOKOC1	10–15	No lesions in 4 fish sampled
Family Cichlidae		Dwarf cichlid	LOKOT1	8	No lesions in one fish sampled
Family Polypteridae/ <i>Polypterus bichir</i>		Lungfish	LOKOLF1	14	No lesions in 2 fish sampled
Crab			LOKOCRAB		

TABLE 5

Preliminary findings by the Field Investigation Team in Mobayi Mbonge, Ubangi River: species examined and prevalence of lesions suggestive of EUS

Family/Species	Lingala name	Common name	Sample ID	Sex	Weight (kg)	Length (cm)	Lesions
Family Distichodontidae / <i>Distichodus schenga</i>	Mpoto	Chessa	No sample	N/S	3–4	50–60	No lesions
<i>Hydrocynus vittatus</i>	Mbenga	Tigerfish	No samples		0.50–0.80	35–40	No lesions in 11 fish inspected
Family Distichodontidae/ <i>Nannocharax</i> spp. (presumptive)							No lesions in 1 fish

TABLE 6

Preliminary findings by the Field Investigation Team in Bobuto Village: affected species, gross pathology and prevalence of lesions suggestive of EUS

Species	Lingala name	Common name	Length (cm)	Lesions
<i>Clarias theodorae</i>	Ngolo	Snake catfish	10–20	Ulcers on tail and body in 12 out of 12 fish inspected. Fish already autolyzed.

TABLE 7

Preliminary findings by the Field Investigation Team in Gemenia fresh fish market: affected species, sample identification and gross pathology

Species	Lingala name	Common name	Sample ID	Sex	Weight (g)	Length (cm)	Lesions
<i>Clarias theodorae</i>	Ngolo	Snake catfish	GCLT1	M	100	25	Ulcer on dorsum and belly
<i>Protopterus annectens</i>	Nzombo	Lungfish	GLF3	F	130	35	Ulcer on top of rostrum and on ventral mandibular region. Numerous white patches on body. Extensive necrotic and pseudomembranous enteritis
<i>P. annectens</i>	Nzombo	Lungfish	GLF4	F	250	40	Ulcer on top of rostrum and on ventral mandibular region, erosion of all pectoral and pelvic fins, vent reddened but intestine normal
<i>P. annectens</i>	Nzombo	Lungfish	GLF5	F	260	50	Ulcer on top of rostrum and on ventral mandibular region, intestines and vent normal

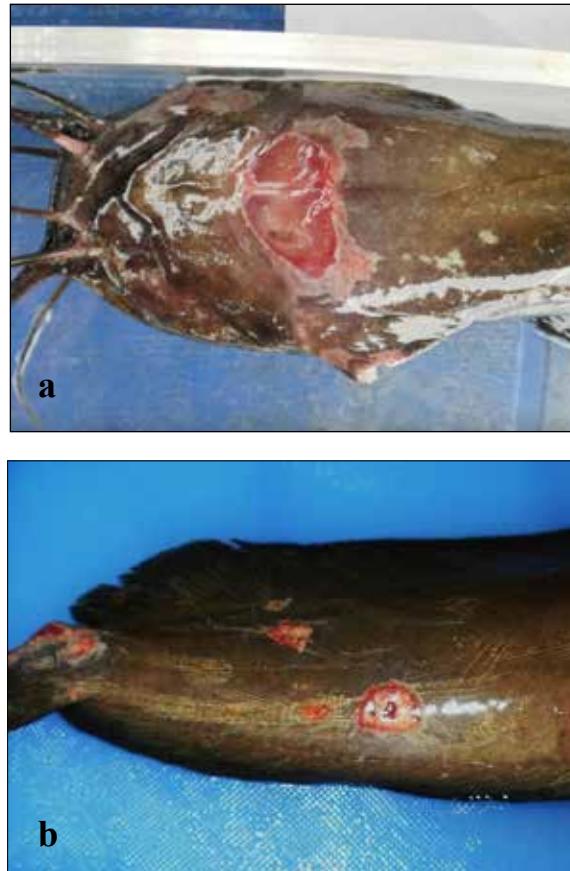


FIGURE 5 (a) Typical position of lesions over the ventral pectoral region in *Clarias theodorae* (ngolo or snake catfish)
(b) Typical EUS lesions in the tail region of *C. theodorae* purchased from Gemenia live-fish market

Sick fish were found at all the live fish markets. The three air-breathing fish species, *C. theodorae* (snake catfish/ngolo, family Clariidae), *P. annectens* (lungfish/nzombo, family Lepidosiveridae) and *P. obscura* (snakehead/mungusu, family Channidae) represented an important live fish commodity found in the markets at Gemenia and Businga. In the Gemenia market, a quick estimate of prevalence indicated that approximately five percent of *C. theodorae* presented for sale showed either hyperaemic or ulcerative dermal lesions (Figure 5). The prevalence among lungfish and snakehead appeared higher. Almost 100 percent of snakehead offered for sale in the Businga market had lesions suggestive of EUS, whereas the prevalence in lungfish was estimated to be between 10 and 20 percent and that of snake catfish at 23 percent (3 out of 13 randomly selected fish) (Table 3a). Members of the family Mormyridae had the highest prevalence of lesions. In the case of 27 specimens of *Gnathonemus petersii* (elephant fish) inspected in a fisher woman's bowl at Loko River, a prevalence of 60 percent was determined (Table 4a). At the same time, a prevalence of 40 percent was determined in five specimens of *Schilbe mysticus* being carried by the same fisher woman.

Snakeheads appeared to show the most severe skin lesions, with erosions and ulcers distributed over the entire body, but with the deepest lesions present on the head and at the base of the tail. In many instances ulceration of the tissues of the upper jaw extended into the bones of the head (Figure 6).

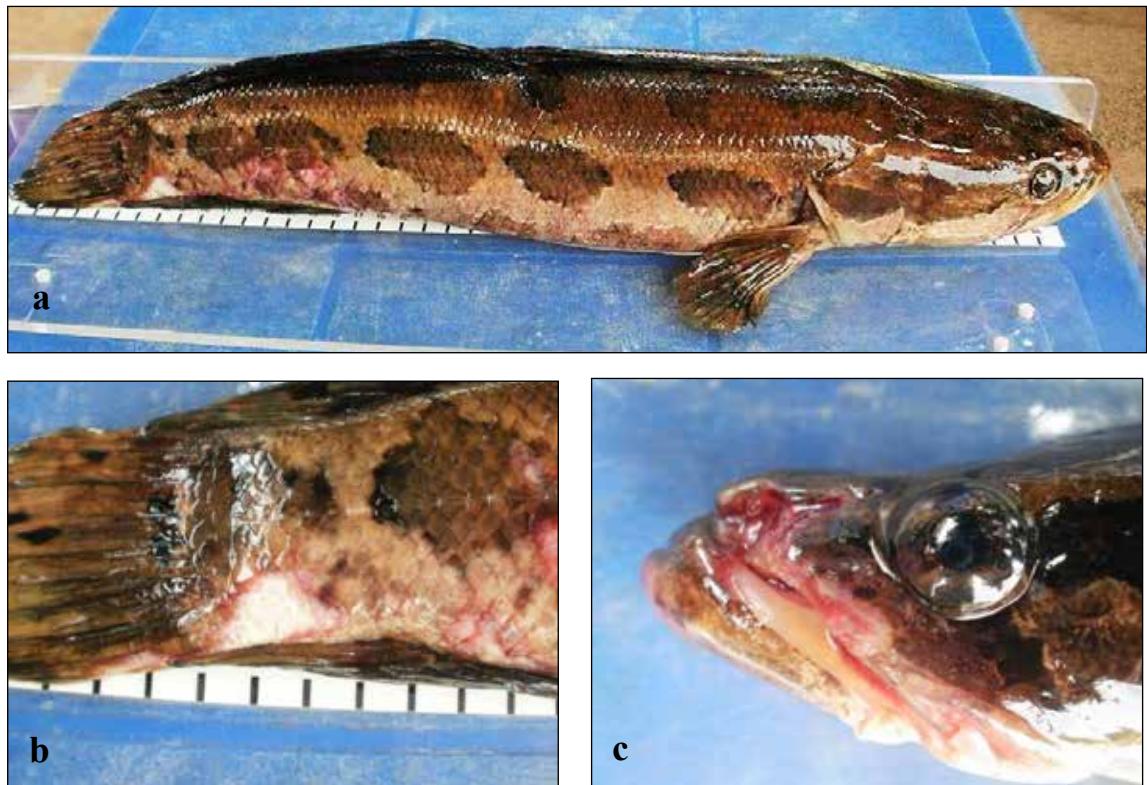


FIGURE 6 (a) *Parachanna obscura* (mungusu or snake head) with EUS lesions. (b) Typical position of lesions on tail of *P. obscura*. (c) Typical position of EUS lesions on head of *P. obscura*. Note deep ulceration into the bony structure of the maxillary portion of the head

In the snake catfish, the predilection for lesions was on the ventral aspect of the body in the region of the pectoral girdle extending bilaterally from the midline just caudal to the opercula. In lungfish, ulcers were evident on the dorsum of the rostral region of the head and ventrally on the maxillary region of the lower jaw (Figure 7). In addition, lungfish showed focally disseminated white lesions from 1–10 mm in diameter over large parts of the body. In the majority of lungfish examined, the vent (cloaca) appeared hyperaemic and swollen. Dissection of the abdominal cavity revealed a severely inflamed intestine adhering to the abdominal wall with evidence of extensive, necrotic and pseudomembranous enteritis resulting in obliteration of the intestinal lumen (Figure 8). A number of these fish showed yellow fibrin accumulations in and around the intestine.



FIGURE 7 (a) Typical position of lesions on the head of a *Proptopterus annectens* (nzombo or lungfish) (b) *P. annectens* for sale at the live-fish market in Gemenia



FIGURE 8 Ulcer ventrally in mandibular area and the severe lesions affecting the intestines (intestines dissected from body cavity) in a *Proptopterus annectens* (nzombo or lungfish). Fish purchased from Gemenia live-fish market

All sampling sites except Site 5 (Ubangi River) yielded sick fish. Fish belonging to the family Momormyridae (*Marcusenius macrolepidotis*, *Petrocephalus* spp., *Hippopotamyrus ansorgii* and *P. catastoma*) sampled from the Libala and Loko rivers were particularly severely affected by lesions (Figures 9 and 10), as was *Channallabes apus* (eel catfish) sampled from the Libala River. Typically, raw ulcers in mormyrids were present unilaterally at the base of the pectoral fins, but some fish showed extensive ulceration and hyperaemia along the sides of the body. One *Synodontis* species presented with extensive hyperaemic lesions on the sides of the body, whereas a different *Synodontis* species showed no lesions. One unidentified dwarf cichlid (family Cichlidae) from the Loko River showed no lesions. All fish examined from the family Channidae (snakeheads) had lesions typical of EUS, whereas the few fish observed belonging to the family Osteoglossidae (*Heterotis niloticus* and *Pantodon buchholzi*) showed no lesions. In contrast to the high susceptibility of *P. annectens* (lungfish) to EUS lesions, two juvenile *Polypterus bichir* (family Polypteridae/lungfish) appeared to be healthy. Smoked specimens of *P. bichir* were present in large numbers in the fish markets among numerous other larger species of fish, but no live specimens of *P. bichir* were observed in the markets (Table 8).

TABLE 8
Results of laboratory analysis for EUS according to sampled fish species

Fish #	Family/ Species	Common Name	Sample ID	Laboratory Procedures	Findings
1	<i>Clarias theodorae</i>	Snake catfish	GCLG1	PCR Histology Mycology	Positive Mycotic granulomas found in muscle tissue – EUS positive No growth
2	<i>C. theodorae</i>	Snake catfish	GCLG2	PCR	Positive
3	<i>Parachanna obscura</i>	Snakehead	GPACH1	PCR Histology Mycology	Negative Mycotic granulomas not observed No growth
4	<i>P. annectens</i>	Lungfish	GLF1	PCR Histology Mycology	Positive Mycotic granulomas not observed No growth
5	<i>P. annectens</i>	Lungfish	GLF2	PCR	Positive
6	Mormyridae/ <i>Cyphomyrus discorhynchus</i>	Zambezi parrot fish	LM1	PCR Histology Mycology	Negative Mycotic granulomas not observed No growth
7	Clariidae/ <i>Channallabes apus</i>	Eel catfish	LC1	PCR Histology Mycology	Negative Mycotic granulomas not observed No growth
8	<i>P. obscura</i>	Snakehead	MPACH1	PCR Histology Mycology	Positive Mycotic granulomas not observed No growth
9	<i>C. theodorae</i>	Snake catfish	MCL1	PCR	Negative
10	<i>P. obscura</i>	Snake head	MPACH2	PCR Histology	Positive Mycotic granulomas found in muscle tissue – EUS positive
11	<i>C. theodorae</i>	Snake catfish	MCL3	PCR	Negative
12	<i>P. obscura</i>	Snake head	MPACH3	PCR	Negative
13	Mochokidae/ <i>Synodontis</i> sp.1	Small pale squeaker	LOKOSY1	PCR	Positive
14	Mormyridae/ <i>Petrocephalus</i> spp.	Northern Chruchill	LOKOM1	PCR	Positive
15	<i>Hippopotamyrus ansorgii</i>	Slender stone basher	LOKOM1b	PCR	Positive
16	<i>Petrocephalus catostoma</i>		LOKOM3	PCR	Positive
17	<i>H. ansorgii</i>	Slender stone basher	LOKOM1b2	PCR	Negative
18	Crab		LOKO CRAB	PCR	Negative
19	<i>C. theodorae</i>	Snake catfish	GCLT1	PCR Histology Mycology	Negative Mycotic granulomas not observed No growth

TABLE 8 (continued)

Fish #	Family/ Species	Common Name	Sample ID	Laboratory Procedures	Findings
20	<i>P. annectens</i>	Lungfish	GLF3	PCR Histology Mycology	Positive Mycotic granulomas found in muscle tissue – EUS positive No growth
21	<i>P. annectens</i>	Lungfish	GLF4	PCR Histology Mycology	Positive Mycotic granulomas found in muscle tissue. – EUS positive No growth
22	<i>P. annectens</i>	Lungfish	GLF5	PCR	Positive



FIGURE 9 Typical position of EUS lesion in a mormyrid fish (*Marcusenius macrolepidotus*) sampled from the Libala River at Karawa



FIGURE 10 Examples of fish species collected from various sampling sites. (a) *Channallabes apus*, (b) *Schilbe mysticus*, (c) *Hemichromis fasciatus* and (d) a member of the family Mormyridae

4.3.2 Water quality, meteorological and other environmental data

Water quality, elevation and GPS coordinates are given for the respective sampling sites in Table 9. The pH of water tested from all rivers and streams was 4.5 with the exception of water from the Loko River, where the pH was 5. These low-pH streams and rivers have a striking reddish-brown colour typical of the so-called black waters found in tropical rainforests. Water temperature ranged from 28 to 30 °C. Elevation of sampling sites ranged from 354 m to 505 m above sea level. All sampling points lay between Gemené (N03° 14'23.0"E019°46'47.9") in the south and Mobayi Mbonge (N04°18'20.7"E021°11'06.0") in the north (Table 9).

TABLE 9
Sampling location, GPS coordinates, elevation and water temperature of sampling sites

Sampling location	Date	GPS coordinates	Elevation (m)	pH	Water temp (°C)
Gemené live fish market	14/03/2015	N03°14'23.0"E019°46'47.9"	433		
Karawa, Karawa Sector, Libala River	15/03/2015	N03°20'22.7"E020°18'12.9"	390	4.5	28
Businga live fish market	16/03/2015	N03°19'49.9"E020°52'22.9"	374		
Businga, Mongala River (at the bridge)	16/03/2015	N03°20'13.1"E020°53'37.1"	354	4.5	29
Loko Village, Loko River, fisherladies bowl	16/03/2015	N03°37'11.6"E020°46'27.6"	371	5	28
Loko River	16/03/2015	N03°37'11.6"E020°46'27.6"	371	5	28
Mobayi Mbonge Uba	17/03/2015	N04°18'20.7"E021°11'06.0"	362	4.5	
Bobuto Village (Bodangabo Sector), Mbo and Gbo rivers	17/03/2015	N03°34'59.0"E020°30'54.8"	505		
Gemené fresh fish market	19/03/2015	N03°14'23.0"E019°46'47.9"	433		

Table 10 shows the water quality parameters, sites and dates visited, GPS coordinates, elevation and temperature of sampling locations without fish samples taken.

TABLE 10
Water quality parameters of small black-water forest streams (no fish samples)

Sampling location	Date	GPS-coordinates	Elevation (m)	pH	Water temp (°C)
Just past Businga on road returning to Gemené	17/03/2015	N03°25'54.8"E020°48'12.9"	379	4.5	30
Shallow stream on road from Businga to Karawa at broken bridge	17/03/2015	N03°31'11.9"E020°26'20.1"	406	4.5	30
Forest stream after rain at Gbogbagbama	17/03/2015	N03°11'52.6"E020°05'09.0"	392	4.5	29.5

Rainfall patterns in the northern part of the Democratic Republic of the Congo are indicated in Figure 11, and differ distinctly from those of the central and southern parts of the country (Centre for Ecology and Hydrology, 2015). Rainfall patterns are relatively stable with a strong seasonal cycle. Within the basin, major forested areas receive around 2 000 mm/year rainfall. Air moisture generated by the forest has a feedback on subsequent rainfall, and in general this region is humid with almost daily rainfall, even during the dry season. The highland areas of the rift valley in the southeastern corner of the basin consistently receive the least rainfall. The central and southern parts of the Democratic Republic of the Congo experience high rainfall year round, whereas the northern regions have a short relatively dry season starting in November up to the end of February, with December and January being the driest months (Centre for Ecology and Hydrology, 2015). Interviews with local officials confirmed that the disease outbreak coincided with the onset of the dry season. Officials in Karawa were concerned that weather patterns had been disrupted in recent times. This was thought to be a consequence of global warming.

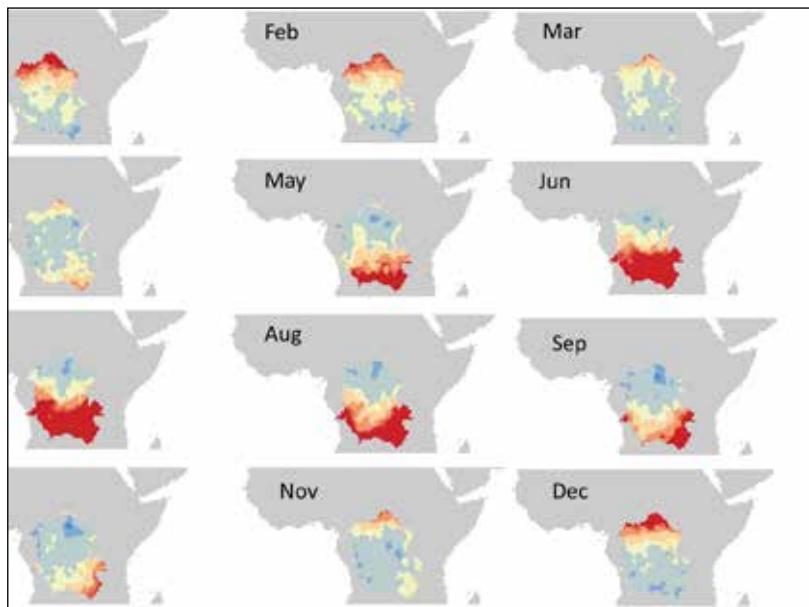


FIGURE 11 Monthly rainfall patterns in an average year over the Congo River basin (scale represents average total rainfall in mm/year)¹

4.3.3 Consultation with women fish marketers, fishers, fish farmers

The active groups of people in this area were the women fish marketers and a few fishers. The women fish marketers indicated the existence of fish with ulcerations from their supply source. Fish with marked ulcerations were discarded and not brought to the market. Fish with moderate ulcers were brought to the market, as observed from the purchased fish with ulcers.

Field interviews with ladies selling fish in the market in Businga indicated that they were aware that the fish they were selling had disease and that, although personally they would not eat fish with wounds, they were prepared to sell these fish. These ladies could provide no information on where the fish they were selling were caught.

¹ Adapted from Centre for Ecology and Hydrology, 2015). Note the relatively low precipitation from December to March in the northwest of the Democratic Republic of the Congo.

The Field Investigation Team observed snake catfish and snakeheads being transported in a bucket by air between Gemena and Kinshasa. On arrival in Kinshasa at the airport, the bucket tipped over on the luggage conveyor, spilling out about 20 fish with some showing typical EUS lesions. Although these fish may be transported live over long distances, as was observed by the Field Investigation Team on the flight from Gemena to Kinshasa, it is most likely that they are sourced close to the market.

Field interviews conducted with fishers and veterinary officers at Karawa, Businga, Loko and Bobutu (Sector Bodangabo) (Appendix 3) indicated that high fish mortalities had been observed in the surrounding rivers since December 2014. The few fishers consulted in Karawa indicated heavy fish mortalities starting in December 2014, while fishers in Businga reported that sporadic fish mortalities first occurred in January 2015. The initially large numbers of dead fish observed in the water were no longer present, but lesions typical of EUS were still being observed in catches of fish. Fishers had observed that mortalities among nina (electric fish) were very high and that many fish with lesions were observed. Nets, hook and bait, and spoon lures were used by these fishers. Spoons onto which small fish were attached were used to catch mungusu (snakeheads). Fishers said that when mungusu were sick, they could not be lured with spoon lures and that sick fish could be touched by hand, but would then swim away. The fishers interviewed at Mobayi Mbonge on the Ubangi River had observed no mortalities in this river and were unaware of fish with lesions suggestive of EUS.

4.3.4 Aquaculture

Only one fish farm was visited. This belonged to the owner of the hotel in Gemena and was situated some distance away beyond the outskirts of the city. The farm, consisting of approximately a dozen shallow, stagnant, earthen ponds with a slow inflow of top-up water, was briefly visited by the Field Investigation Team in the company of the owner's son. A breeding population of *Tilapia rendalli* could be observed in some of the ponds. The Field Investigation Team was informed that a second local species of fish was stocked in some of the ponds and that these fish grew quite large. The fish were not visible and the son of the owner could not name these, referring to them as "lamer". Both types of fish were fed on rice waste. No sick fish were observed on the farm. Enquiries revealed no evidence of Nile tilapia being introduced into the region. In the market at Karawa, ladies selling smoked lungfish of the genus *Polypterus* referred to these as "lamer". As these are predatory fish, it is unlikely that they would thrive on rice waste under farming conditions, and it is likely that the fish referred to as "lamer" on the farm was in fact a completely different species.

4.4 Laboratory analysis results

A total of ten fish sample species were collected for histopathology and culture analysis, while 22 samples were collected for the direct detection of DNA of *Aphanomyces invadans* through PCR (Table 8). All the fish samples collected for further laboratory analysis had lesions suggestive of EUS in one way or another.

After preliminary establishment of the case definition, it was clear that the disease was suggestive of EUS and hence, the diagnosis needed to be narrowed according to the terms of reference to confirm the presence of infection with *A. invadans*. The results on direct PCR examination (Figure 12) indicated that most samples collected had evidence of *A. invadans* DNA. Every marketable species examined was affected. These included species of the following genera: *Clarias*, *Protopterus*, *Parachanna*, *Synodontis*, Momormyridae/Petrocephalus and *Hippopotamyrus*.

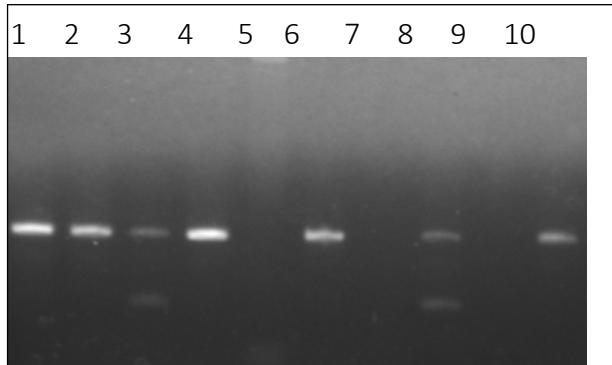


FIGURE 12 PCR results of the direct detection of genomic DNA of *Aphanomyces invadans* from infected tissues collected during the International Emergency Fish Disease Investigation Mission on a suspected Outbreak of Epizootic Ulcerative Syndrome (EUS) to the Democratic Republic of the Congo. The figure represents electrophoresis gel bands representative of the EUS-positive results (lanes 1, 2, 4 and 6). Lanes 9 and 10 are negative and positive controls respectively, while lanes 3 and 8 have non-specific amplicon.

On histopathology, mycotic granulomas were found in the muscle tissues, confirming EUS infection in *Protopterus annectens* and *Parachanna obscura* (Figures 13a, 13b, 13c, 13d, 14a and 14b) and in *Clarias theodorae* (snake catfish). In the sections from *P. obscura*, there was an active host inflammatory response, with a small granuloma having a clear center within which the oomycete hypha was located. In sections from *P. annectens*, a typical enveloping inflammatory response was evident surrounding the granuloma with myofibrillar degeneration characteristic of EUS.

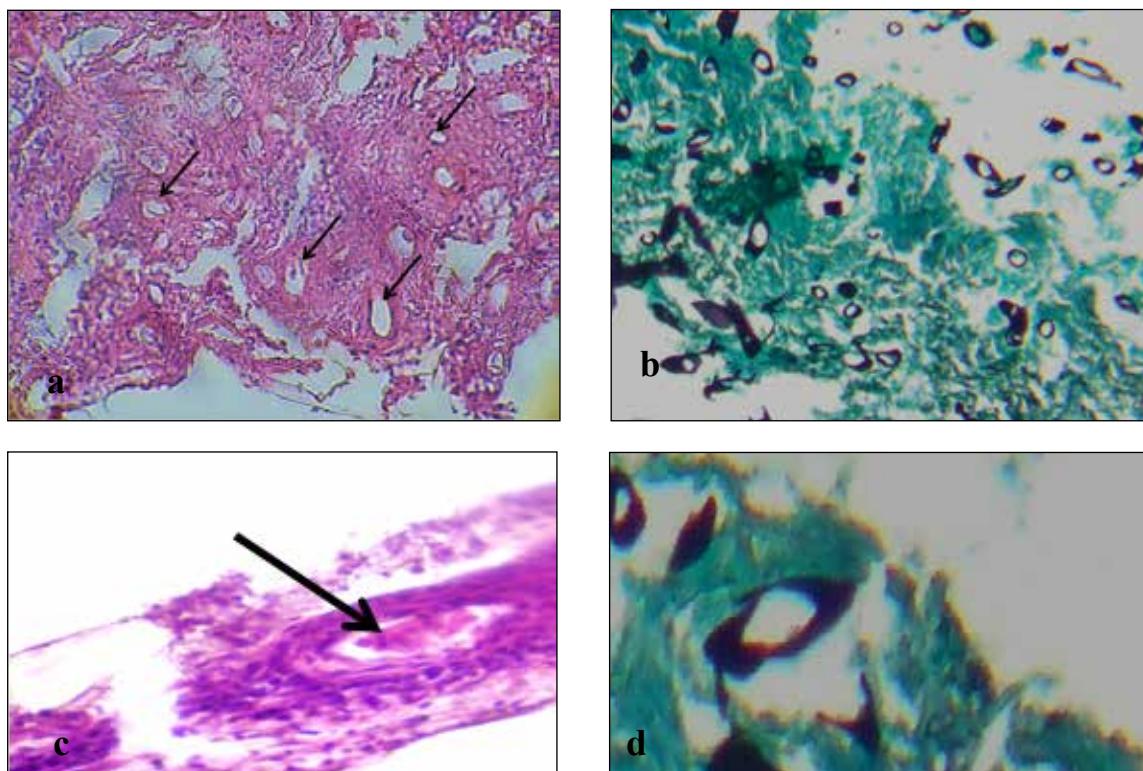


FIGURE 13 a) Photomicrograph demonstrating typical mycotic granulomas surrounding invasive oomycete hyphae (black arrows) below the dermis of an EUS-infected lungfish (*Protopterus annectens*) (H&E, $\times 100$); (b) A Grocott's stained slide ($\times 100$) showing black-staining oomycete cell walls in affected tissue from specimen GLF4; (c) Histopathology of EUS-infected *Clarias theodorae* (GCLG1) showing a typical mycotic granuloma surrounding invasive oomycete hyphae (black arrow) in the skin layer (H&E, $\times 400$); (d) Black-staining oomycete hyphae (Grocott's, $\times 400$).

4.5 Synthesis of reports

Fish showing sores and ulcers referable to EUS were reported in the Equateur Province of the Democratic Republic of the Congo in December 2014, with a record of heavy fish mortalities. The reports indicated that three marketable species were involved: *C. theodorae*, *P. obscura* and *P. annectens*. These species showed gross pathology similar to that reported for other fish species elsewhere (Das and Das, 1993; Callinan *et al.*, 1995; Lilley *et al.*, 1997; Blazer *et al.*, 2002; Baldock *et al.*, 2005; Andrew *et al.*, 2008; FAO, 2009; Huchzermeyer and Van der Waal, 2012; Songe *et. al.*, 2012). The critical gross pathology was represented by cutaneous lesions fitting the case definition of EUS – red spots, erosions, ulcers and wounds. Despite not directly observing any sick fish in their natural habitats, observations from the field defined a suitable environment for propagation of *A. invadans*. A body of water with low pH has been documented in other outbreaks as a significant predisposing factor for EUS outbreaks (Sammut, White and Melville, 1996; Baldock *et al.*, 2005; Choongo *et al.*, 2009).

Diagnosis of infection with *A. invadans* in clinically affected fish may be achieved by histopathology, oomycete isolation or polymerase chain reaction amplification (OIE, 2014a).

In this investigation, histopathology was used to demonstrate granulomas in *P. obscura* and in *P. annectens*. Furthermore, PCR confirmed the presence of genomic DNA of *A. invadans* in collected specimens.

The findings showed that environmental, climatic, water quality and human demographic conditions in the Congo River basin support the possibility of pandemic spread of the disease.

4.6 Validation of findings by the OIE Reference Laboratory for EUS

Ms Varinee Panyawachira, EUS Expert at the Aquatic Animal Health Research Institute (AAHRI), Bangkok, Thailand and OIE Reference Laboratory for EUS provided validation based on histopathology photos of EUS-affected fish. She indicated that photos clearly showed lesions typical of EUS. She further suggested the use of special stains (e.g. Grocott's or Uvitex stain) to demonstrate the fungal hyphae. Grocott's staining was performed at the University of Pretoria by Dr Johan Steyl.

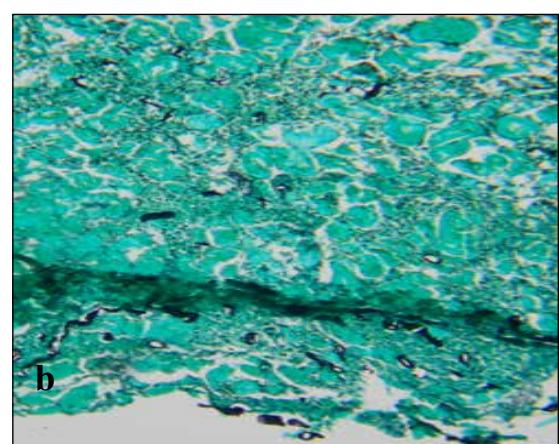
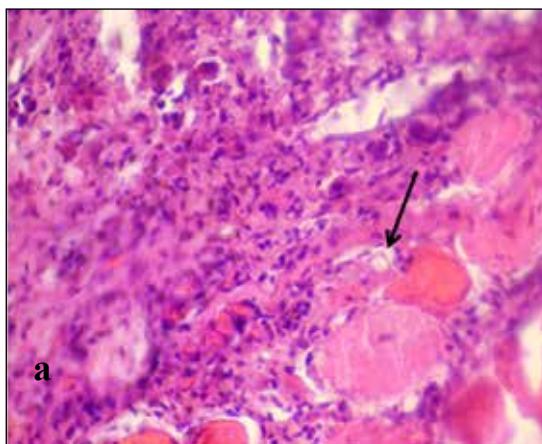


FIGURE 14 (a) Photomicrograph depicting a granulomatous inflammatory response surrounding necrotic muscle tissue in association with oomycete hyphae (arrow) in an EUS-infected snakehead, *Parachanna obscura*. (MPACH2). (H&E, $\times 100$). (b) Section from the same fish stained with Grocott's stain ($\times 100$) demonstrating dark-staining oomycete structures in the muscle tissue.

5. Conclusion

5.1 Conclusions based on field and laboratory investigations

Epizootic ulcerative syndrome caused by infection with *A. invadans* has been confirmed as the cause of the clinical signs observed in live wild-caught fish sampled during this disease investigation. The following parameters were used to confirm the disease: (1) the presence of characteristic hyperaemic and ulcerative dermal lesions; (2) presence of hyphae and sporogenesis on microscopic examination of wet-preparation tissue squashes; (3) presence of granulomatous myositis and myonecrosis on histological sections; (4) mycelial growth on culture media and (5) the identification of genomic DNA specific to *A. invadans* by polymerase chain reaction (PCR) analysis. Background information indicating an epizootic onset of disease with a high mortality rate at the onset of the dry season followed by a reduction in mortality but continued presence of diseased fish during the following three months is consistent with outbreaks of EUS elsewhere (Das and Das, 1993; Callinan *et al.*, 1995; Lilley *et al.*, 1997; Blazer *et al.*, 2002; Baldock *et al.*, 2005; Andrew *et al.*, 2008; FAO, 2009; Huchzermeyer and Van der Waal, 2012; Songe *et al.*, 2012). During outbreaks of EUS in other countries, low water pH and a drop in water temperature have been identified as the main predisposing factors favouring outbreaks of EUS in the rivers Libala, Loko and Mongala and their tributaries. The ubiquitous presence of forest streams and rivers with low pH (black waters) in the northwestern region (Equateur Province) of the Democratic Republic of the Congo supports observations made elsewhere including, in Africa, that low water pH favours infection with *A. invadans* and expression of disease (Baldock *et al.*, 2005; Choongo *et al.*, 2009). In countries where flood-plain fish have been affected by EUS, it is the seasonal flooding cycles that bring about pH changes in water covering inundated vegetation. Under such conditions, cases of EUS have been reported to occur when flood plains drain and when the onset of the dry season coincides with a drop in water temperature. As fish have a poikilothermic metabolism, it is believed that a drop in water temperature may result in a retarded immune responsiveness to challenges by infections of *A. invadans*. The streams and rivers affected by EUS in the northwest of the Democratic Republic of the Congo fall within the equatorial climatic belt and are not exposed to the seasonal temperature fluctuations experienced at greater latitudes. It therefore seems unlikely that influence of temperature on the immune responsiveness of the fish in this part of the country would have a significant influence on the manifestation of EUS outbreaks. It would appear more likely that the year-round presence of low water pH favours infectivity with *A. invadans* and that seasonal flooding cycles causing migration of fish into swampy areas will promote seasonal outbreaks of disease.

The genus *Aphanomyces* falls within the kingdom Straminopiles, together with diatoms, brown algae and golden-brown algae. Many of the Straminopiles are pathogens of plants and animals. A number of these have had devastating effects on agriculture and aquaculture throughout the world (Walker and Van West, 2007). These organisms are characterized by producing free-swimming spores or zoospores by which, in the case of *A. invadans*, the disease spreads from one host to the next. In contrast to free-living members of the Straminopiles, *A. invadans* is an obligate pathogen of fish. Zoospore formation is considered one the fastest developmental processes in nature and the rapid dispersal of free-swimming zoospores from infected hosts explains the epizootic spread of disease (Walker and Van West, 2007).

During this investigation, freshwater fish belonging to 12 families were inspected for evidence of EUS: Clariidae, Channidae, Protopteridae, Mormyridae, Schilbeidae, Cichlidae, Hepsetidae, Mochokidae, Osteoglossidae, Polypteridae, Distichodontidae and Characidae. Of these, members of the families Clariidae, Channidae, Protopteridae and Mormyridae were most severely affected by the disease. The habitat preference for well-vegetated backwaters of forest streams and floodplains that represent ideal conditions for infection with *A. invadans* explains the high prevalence of disease

among fish in these four families. Members of the Clariidae, Channidae and Protopteridae are of greatest concern regarding the spread of EUS, as fish in these families represent an important food commodity in the Democratic Republic of the Congo. All three families represent air-breathing fish, and marketable fish are transported to and from markets live, providing an effective pathway for pathogen transfer.

5.2 Conclusions based on available reports

The earliest report of an ulcerative disease in fish in the Democratic Republic of the Congo stems from a CVL report documenting lesions in fish from Kasangulu in 2013. It remains speculative whether the lesions mentioned in this report represented a case of EUS, but the report did mention presence of non-specified mycelia. The findings of the current field investigation corroborate concerns raised in earlier postings on the SARNISSA network. The time-line of disease observations mentioned in the SARNISSA postings coincides with descriptions provided by fishers and local officials of when diseased fish were first observed, and it is likely that these cases were representative of the current outbreak that has been confirmed as EUS. The disease usually starts with heavy mortalities and then subsides, as is the current situation in the areas visited by the Field Investigation Team and as confirmed by reports from local people met during the disease investigation.

Permissive factors that favour the propagation, infectivity and disease occurrence of EUS occur in the rivers and streams investigated in Equateur Province of the Democratic Republic of the Congo.

5.3 General diagnosis

The diagnosis of EUS in fish in Equateur Province of the Democratic Republic of the Congo has been confirmed using two recommended confirmatory tests (OIE, 2014a). These included demonstration of mycotic granulomas and oomycete structures through the microscopic examination of suitably stained histological sections, and confirmatory identification by PCR of infection with genomic DNA of *A. invadans*.

6. Recommendations

A preliminary report based on the findings of the Field Investigation Team was prepared and presented to FAODC for the Government of the Democratic Republic of the Congo on 19 March 2015 and recommended that urgent action be taken to limit the spread of the disease.

Based on the full findings and laboratory examination of specimens collected during the field investigation, the Field Investigation Team has confirmed a serious and epizootic outbreak of EUS (infection with *A. invadans*) in the Equateur Province of the Democratic Republic of the Congo. Environmental, climatic, water quality and human demographic conditions in the Congo River Basin support the possibility of pandemic spread of the disease.

There is no known protective vaccine nor effective chemotherapeuticant for EUS. Treatment of EUS in natural waterbodies is not possible. Practical control is limited to identifying and reducing pathways of spread.

The following recommendations are provided to address the risks of further disease spread in the Democratic Republic of the Congo:

6.1 Specific recommendations

- Immediate notification by the veterinary authority in the Democratic Republic of the Congo (Animal Health Services and Chief Veterinary Officer of the Democratic Republic of the Congo) to the World Organisation for Animal Health (OIE) of an outbreak of EUS (infection with *Aphanomyces invadans*) in the Libala, Loko and Mongala rivers and in their tributaries in Equateur Province of the Democratic Republic of the Congo.
- Active surveillance of fish markets should be initiated immediately with tracing of the actual source (origin) of all infected fish. Affected streams, rivers and villages need to be mapped through initiation of local surveillance programmes, and the spread of the disease outbreaks needs to be monitored. Surveillance teams need to be equipped with suitable sampling apparatus to allow collection of relevant environmental data. This should include use of digital multimeters capable of measuring pH, water temperature, dissolved oxygen, oxygen saturation and conductivity at river sampling sites.
- The movement of live fish between markets and between river systems in areas affected by EUS needs to be restricted (e.g. by quarantine and biosecurity measures); as well, a ban of fishing activities during EUS-outbreak seasons should be considered and alternative livelihood options for affected fishers should be determined.
- An initial national workshop for all personnel involved with fish and aquaculture activities needs to be urgently organized to initiate public awareness and extension programmes to raise understanding of the disease and measures required to reduce its impact (technical assistance).
- Funding needs to be urgently sourced to mobilize regional EUS expertise for a more in-depth epidemiological investigation of the extent of EUS in the Democratic Republic of the Congo and to provide training on EUS recognition, biology, diagnostics, pathology and surveillance procedures, emergency preparedness and contingency planning for both veterinary and fisheries officials and especially extension officers in the Democratic Republic of the Congo.
- The epidemiological investigation of the disease in the Democratic Republic of the Congo should be aimed at establishing the EUS status in the country (zones, farms etc.) and the critical areas near or along the border of affected areas in the Democratic Republic of the Congo or affected countries (e.g. the Republic of Zambia), the prevalence of EUS, the extent of natural waterbodies affected, the seasonality, the full range of species affected or susceptible (cultured and wild populations), potential carriers, risk factors and other pathways (e.g. fish markets, fish vendors) and the risk to inland fisheries and human food security. The outcome of such an investigation should promote the development of appropriate risk management responses for the Democratic Republic of the Congo.
- The laboratory facilities at CVL in Kinshasa need to be urgently strengthened to enable the laboratory to initiate and perform effective fish disease investigations. This will require implementation of the fish pathology laboratory that has been initiated by the European Union and the International Atomic Energy Agency of the United Nations (joint division FAO-IAEA).
- Capacity building for local staff, particularly veterinary, fisheries and aquaculture officials, is urgently needed to enable them to educate the populace (consuming public, capture fishers, fish farmers, fish vendors, sports fishers/anglers) on the significance of this

disease, the importance of implementing biosecurity measures and the need for vigilance in collecting relevant field information, so that they can provide appropriate advice and technical assistance (who to contact and what they need to do when some mortalities are observed):

- for consuming public: that the disease has no public health significance;
- for capture fishing communities: season/time of year the disease is likely to occur (heavy rainfall, flood events, low temperature season, e.g. when water temperature ranges between 18–25 °C), and reporting of any observed mortalities;
- for farming communities: not to culture susceptible species or to avoid farming susceptible species during the EUS season; implement farm level biosecurity; reporting of observed mortalities.

6.2 General recommendations

- *Aphanomyces invadans*, the causative agent of EUS is not known to be infectious to humans and warm-blooded animals, and poses no direct health risk to human populations. Fish suffering from EUS may have extensive changes to the muscles underlying visible lesions on the skin. The quality of such flesh is poor. This may be compounded by secondary bacterial invasion of the tissues rendering such fish unsuitable for human consumption. It is therefore recommended not to eat EUS-infected fish unless properly and thoroughly cooked. Such information needs to be conveyed urgently to human communities in affected areas.
- Dialogue on information-sharing systems about EUS status needs to be initiated. Neighbouring countries and a FAO Regional Technical Cooperation Programme for EUS covering Central African countries should be established to initiate a practical action plan for the region. This should include subregional disease surveillance, monitoring, preparedness and response programmes. Dissemination of the findings to countries of the region as part of an early warning of potential wider spread of the disease is necessary.
- Authorities managing natural waterbodies in the region should also be informed and considered as important stakeholders to address this EUS problem.
- Countries in the region, including the Democratic Republic of the Congo should be encouraged to formulate national aquatic biosecurity strategies as part of long-term plans.

7. The Way Forward

The current challenge is to formulate concrete and effective responses/actions by the Government of the Democratic Republic of the Congo, with the support of FAO, OIE and other relevant organizations and stakeholders to curtail the spread of the disease.

There is a high risk of spread to other African waterbodies from one lake or river system to another, endangering susceptible fish species as well as neighbouring countries. Factors include heavy rainfall and flooding, poor biosecurity, including movement of infected fish, as well as

natural spread by fish and birds. Members of the Clariidae, Channidae and Protopteridae are of greatest concern regarding the spread of EUS, as fish in these families represent an important food commodity in the Democratic Republic of the Congo. Additionally, all three families represent air-breathing fish, and marketable fish are transported to and from markets live, thus providing an effective pathway for pathogen transfer.

Strong and timely collaboration among different internal and external stakeholders is needed. Collaboration may begin with the fish farmers and the Democratic Republic of the Congo government officials having close contact with each other. A strong and open communication may lead to timely identification and control of the disease at its beginning stages. Ensuring that government officials' capacity to identify, monitor and manage aquatic animal diseases, particularly in dealing with the current and future outbreaks, will enhance the success of relevant interventions. It is also essential that dialogue among the Democratic Republic of the Congo, its neighbouring countries, FAO and OIE on the status of the current EUS outbreak continues with timely and relevant information shared. Lastly, it may involve a consultative process of establishing clear short- and medium-term guidelines and policies aimed at responding to current and future EUS outbreaks.

The work and accomplishments of the International Emergency Fish Disease Investigation Team provide an impetus for further support to improve aquatic biosecurity awareness in the country.

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Appendix 1

Members of the EUS Investigation Task Force

Fao Democratic Republic of the Congo

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Itinerary of the Field Investigation Team

Dates	Activities
14 March 2015 (Saturday)	Kinshasa to Gemena, local security briefing and courtesy meeting with local officials Sampling from a live fish market
15 March 2015 (Sunday)	Gemena to Karawa and Businga. Sampling at Libala River
16 March 2015 (Monday)	Sampling in Businga at Mongala River and live fish market Sampling at Loko River
17 March 2015 (Tuesday)	Sampling at Ubangi River, Gbadolite to Gemena Sampling at live fish market in Gemena

Appendix 3

Persons met during the Mission –

Districts of South and North Ubangi/Equateur Province

Name	Function
Persons met in Karawa	
Mathieu Dawili Mangbo	Administrative Chief of Karawa Territory
Kennedy Seterezola	Chief of the Immigration Service in Karawa
Florent Molene	Chief of the Documentation Service, Karawa
Jean Bosco Gila Gali	Inspector of Agriculture, Fishery and Husbandry
Felix Kanake Kogbo	Chief of the Veterinary Post
Nelson Mbele	Driver, Agriculture, Fishery and Husbandry Service
Afin Mangbo	Auxilliary veterinary agent (1)
Mabele Wele	Auxilliary veterinary agent (2)
Persons met in Businga	
Désiré Bangila	Territory Administrator
Roger Bakombe Nelly	Inspector of Agriculture, Fishery and Husbandry
Maurice Gbalanda	Auxilliary veterinary agent
Idirana Nzonde	Fisher
José Mpsange	Fisher
Mbomba Zipo	Fisher
Louisi Nyapenze	Fisher
Vital Selengbe Vungbo	Inspecteur de l'Agriculture
Persons met in Gbadolite	
Leonard Rethsazu	Chief , Provincial Division of Agriculture & Fishery
Vital Selengbe Vungbo	Inspector of Agriculture, Fishery & Husbandry North Ubangi District
Gelengbo Koto	Chief of the Fishery Service North Ubangi District
Persons met in Gemena	
Prudent Landon	FAO Focal point in Gemena District
Eulalie Lelo	Assistant FAO Focal point in Gemena
Eulalie Kashwantale	Chief Animal Production & Health – PARSSA (World Bank)
Guillaume Ngbanga	Inspector of Agriculture, Fishery & Husbandry, South Ubangi District

Persons met in Agripe Secteur Bodangabo

Lowa Jules Zabuso	Veterinaire
Toasele Vong	Agron
Yaango Dalanza	
Nzombi Nbango	
Kubu Demba	
Lio Sebutu	

Appendix 4

SARNISSA Communication and Postings

BOX 1. SARNISSA email message dated 08 January 2015

-----Original Message-----

From: Sarnissa-african-aquaculture [mailto:sarnissa-african-aquaculture-bounces@lists.stir.ac.uk] On Behalf Of William Leschen via Sarnissa-african-aquaculture

Sent: 08 January 2015 09:29 PM

To: sarnissa-african-aquaculture Mailing List; sarnissa-french-aquaculture Mailing List

Subject: FW: PHENOMENE DES POISSONS DANS NOTRE ZONE D'INTERVENTION(ILOKO-MOLEGBE)
RDC

BOX 2. Email message from Peter Corey to William Leschen dated 08 January 2015

-----Original Message-----

From: Peter Corey [mailto:peter.corey@ns.sympatico.ca]

Sent: 08 January 2015 18:44

To: William Leschen

Subject: FW: PHENOMENE DES POISSONS DANS NOTRE ZONE D'INTERVENTION(ILOKO-MOLEGBE)

Hello Will:

What's the possibility of this getting posted to the French forum? These photos are of a diseased snakehead. It would contribute to the recent discussion on fish disease found elsewhere on the Congo River.



Thanks,

Peter

Bonjour Will:

Quelle est la possibilité de cette se posté sur le forum français? Ces photos sont d'une snakehead malade. Elle contribuerait à la discussion récente sur les maladies des poissons trouvés ailleurs sur le fleuve Congo.

Merci,

Peter

BOX 3

Email message from Emmanuel Dole Nadonye to Peter Corey dated 08 January 2015

-----Original Message-----

From: Emmanuel Dole Nadonye [mailto:edolenadonye@gmail.com]

Sent: Thursday, January 08, 2015 2:23 PM

To: Peter Corey

Cc: jules ngate; zukujp; sheldon; Sheryl Noren

Subject: Fwd: PHENOMENE DES POISSONS DANS NOTRE ZONE D'INTERVENTION(ILOKO-MOLEGBE)

Bonjour Peter,

Je vous transmet la photo de ce poisson prise par docteur Doudou, médecin chef de Zone de santé de Loko sur trois position. Comme je vous ai dis ce jour là, le rayon de notre intervention est vraiment menacé par cette maladie et cela inquiète non seulement nos associations mais toute la population de ce rayon qui vive des poissons en cette période. Je vous informe que c'est toutes les espèces qui sont touchées par ce problème. La photo prise ici est celle d'*Ophicephalus* sp, que nous appelons MONGUSU que nous avons prise dans la rivière LOKAME a Loko.

Notre crainte est ce fléau ne puisse atteindre nos étangs expérimentaux au risque de compromettre notre le bon fonctionnement de notre projet.

Pouvez-vous prendre contact avec d'autres experts pour nous dire de quoi s'agit-elle si possible?

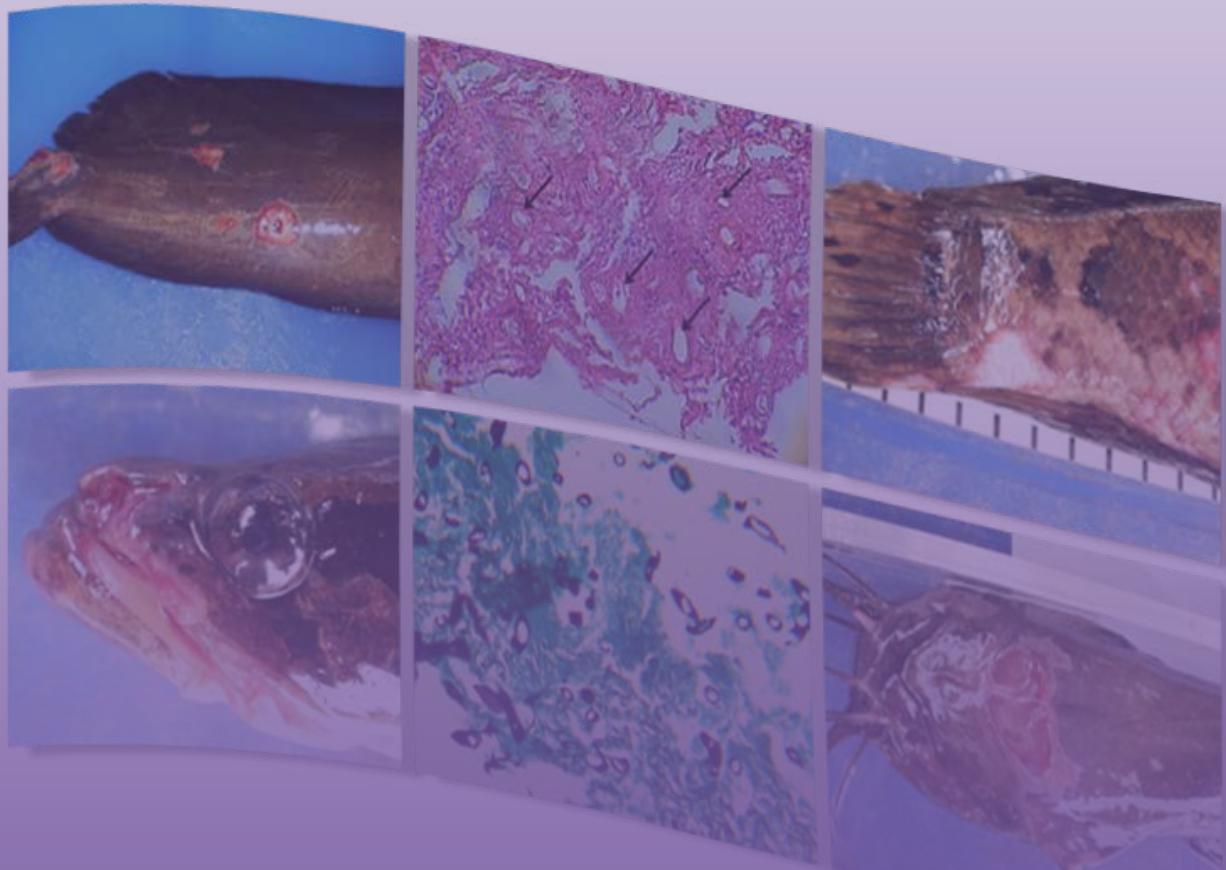
Dole Nadonye Emmanuel

Coordinateur Fish For Hope/CEUM

Hi Peter, I shall send you a photo of the fish taken by Dr. Doudou, Chief Medical Officer of Health Zone Loko three position. As I said to you that day, the radius of our intervention is really threatened by this disease and that worries not only our associations but the entire population this fish that lives in this area. I inform you that it's all the species that are affected by this issue. The picture taken here is that of *Ophicephalus* sp, which we call MONGUSU we took in LOKAME River Loko. Our fear is this plague cannot reach our experimental ponds at the risk of compromising our proper operation of our project. Can you make contact with other experts to tell us what was is possible cause or reason?

Dole Nadonye Emmanuel Fish coordinator for Hope/MUEC

This report describes the work carried out by the International Emergency Fish Diseases Investigation Mission on a Suspected Outbreak of Epizootic Ulcerative Syndrome (EUS) in the Democratic Republic of the Congo, organized and funded by the Food and Agriculture Organization of the United Nations (FAO) through the Special Fund for Emergency and Rehabilitation Activities and funds from the FAO Representation in the Democratic Republic of the Congo and jointly implemented with the Government of the Democratic Republic of the Congo. The EUS Investigation Task Force confirmed the presence of the epizootic ulcerative syndrome (EUS) using two OIE-recommended confirmatory tests, i.e.: (1) histopathology, which demonstrated granulomas in *Parachanna obscura* (snakehead), *Protopterus annectens* (lungfish) and *Clarias theodorae* (snake catfish) and (2) polymerase chain reaction (PCR), which confirmed the presence genomic DNA of *Aphanomyces invadans* in collected specimens. The EUS Investigation Task Force concluded that permissive factors that favoured the propagation, infectivity and disease occurrence of EUS occur in the rivers and streams investigated in the Equateur Province of the Democratic Republic of the Congo and that environmental, climatic, water quality and human demographic conditions in the Congo River basin support the possibility of pandemic spread of the disease.



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