5. Diagnosis

5.1 BACKGROUNDER TO EUS

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

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

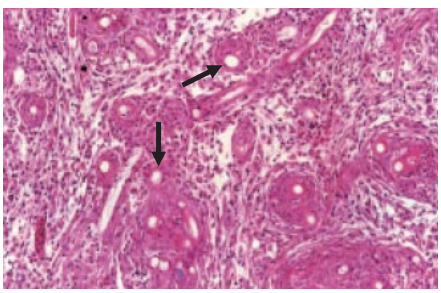
Various studies have listed a number of risk factors (see Table 4). These include temperature, rainfall and related water quality, flooding, soil and sediment characteristics. It is likely that there are a diverse group of biotic and abiotic agents/

PLATE 8

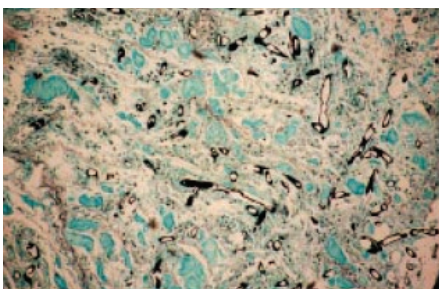
EUS-infected fish from the Philippines, Japan and Australia

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

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

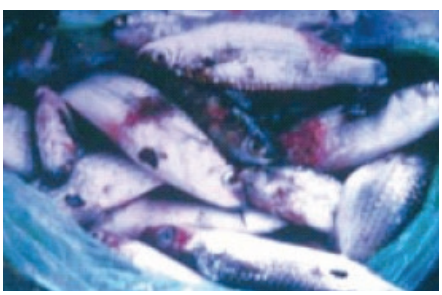


Typical severe mycotic granulomas (black arrows) from muscle section of EUS infected snakehead in the Philippines (1985) (H&E stain)



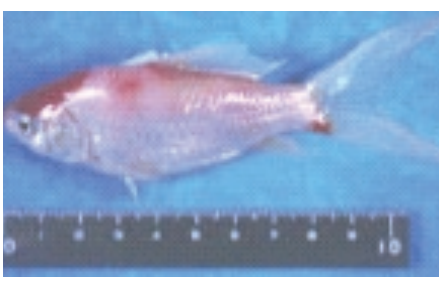
Mycotic granulomas showing fungal hyphae (stained black) using Grocott's silver stain

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



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

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



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

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

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

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

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

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

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

5.2 CONFIRMATION BASED ON INTERNATIONALLY ACCEPTED METHODS FOR EUS DIAGNOSIS

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

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

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

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

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

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