SUMMARY AND CONCLUSIONS
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A general survey of fish market at Amravati was carried out to know the intensity of EUS affected fishes coming in the market. It was observed that the fisherman brought the EUS affected fishes from nearby lakes Wadali and Malkhed.

Physico-chemical and biological parameters of Wadali and Malkhed lake waters were investigated and it was found that, during winter season these two lakes have water with low alkalinity, hardness and chloride concentration. The temperature was also found to be a fluctuating parameter.

Nine pathogenic species of bacteria were identified from Wadali lake water and 7 different species from Malkhed lake water.

In Malkhed lake Staphylococcus were found to be dominating with their absence only during May and June. Similarly, Aeromonas hydrophila and Streptococcus were present throughout the year except during April and May. Alcaligenes sp. were recorded in Malkhed lake water which were not found in Wadali lake water. Most of these bacteria were also isolated from the EUS affected Channa punctatus and Clarias batrachus collected from these lakes.

The water from the lakes under investigation was investigated to know the fungal flora. The fungi which were isolated were Saprolegnia parasitica, Aphanomyces invadans, Achyla sp. and Aspergillus sp. These fungi were also isolated from the EUS affected fishes.
62.79% to 78.33% *Channa punctatus* were EUS affected from Malkhed lake during winter season. Similarly, 47.22% to 56.52% *Clarias batrachus* exhibited EUS infection in Malkhed lake.

All EUS affected fishes exhibited three distinct stages during the EUS infection.

Stage - I  - Red spot is seen  
Stage - II  - Red ring with a white patch in the centre  
Stage - III  - The area of red ring became necrosed with puffy appearance with ulcer and haemorrhage. Multiple ulcers were seen on the body in advanced stage.

The healthy fishes were administered with the ulcerated tissue from the EUS affected fish and were studied after 7, 14 and 21 days of challenge infection. These fishes also showed the three stages of the disease.  

Histological studies of the skin of EUS affected fishes showed initial epidermal loss exposing the dermis, degeneration of muscles with infiltration of mixed inflammatory cells. Fungal hyphae were observed penetrating deep into the body muscles.

In EUS affected fishes the liver cells exhibited irregular cordal arrangement and vacuolated. In advanced stage of the disease, the hepatic cells appeared shrunked with fungal infiltration.

In EUS infected *Channa punctatus*, a well developed encapsulated granuloma in the gill lamellae was developed which appeared nodular with characteristic focal granular granulomas. These were composed of a diffuse distribution of reticular endothelial cells and macrophages.

The kidney of EUS affected fishes exhibited tubular necrosis, interstitial lymphocyte infiltration, haemorrhage and well developed inter-renal tissue. Glomeruli appeared shrunked.
Summary and Conclusions

In all the electron microscopic observations, the tissue cells of EUS affected fishes showed viruses like Parovirus and Paramyxovirus even at a red spot stage. Distortion of endoplasmic reticulum and breakdown of mitochondrial cristae were the common observations.

Serum enzymes like SGOT, SGPT, acid phosphatase and alkaline phosphatase were found to be elevated significantly (p<0.01) in all the EUS affected fishes under investigation.

All the EUS affected fishes exhibited decreased nutritive value with significantly lowered muscle proteins, cholesterol and glycogen. Moderate (p<0.05) to highly significant (p<0.01) elevation in acid phosphatase, alkaline phosphatase, GOT and GPT was recorded in liver and muscle tissues of all EUS affected fishes.

Serum total protein level in EUS affected fishes was declined significantly with decreased A : G ratio.

Significantly lowered Hb concentration, haematocrit values and RBC counts were recorded in both naturally and artificially infected Channa punctatus and Clarias batrachus. Neutrophils and Monocytes were depleted. However, these changes are associated with rise in average lymphocytes, eosinophils and basophils. Increased number of lymphocytes, in EUS infected fishes may be because of stimulation of viral, bacterial and fungal pathogens and their secreted toxins. The haematological observations can be taken up as the indicator of viral, bacterial and fungal infection.

The increase in the eosinophil count in response to pathogens observed might have been resulted into inflammation leading to ulcers on the fish body. Similarly after 14 days of challenge infection decline in agranulocytes indicated a stage of immunosuppression resulting into severe infection.

The antigenic proteins from all the bacterial and fungal pathogens were searched out from NCBI and their antigenicity was predicted by using the tools from mifoundation and it was found that the antigenic proteins from the EUS
Summary and Conclusions

Pathogens are having propensity above one. *Saprolegnia parasitica* was found to have 18 antigenic epitopes in its cystine protease protein. Lipase like protein from *Aeromonas* possesses 36 antigenic epitopes.

Antigen-antibody reactions in EUS infected fishes were confirmed by radial immunodiffusion test and it was found to be positively exponential with the three stages recognized.

EM section of ulcerated skin part from a red spot had showed a presence of virus indicates virus as an initial pathogen which is responsible for EUS outbreak.

Thus, it can be said that EUS infection follows the phylogenetic evolution in life in true sense i.e RNA virus → Bacterial cell → Fungus. It is also suggested that Viruses being crystalline in nature have cellular penetrating power. This natural tendency of virus itself indicates that viruses are the only agents which can act as the initial causative agents for EUS.

EUS has thus, a potential to financially decimate those who rely on fishing for income. In addition and perhaps more importantly, EUS outbreaks have threaten food security and subsequently physical health, of all those who are dependent on fish, as fish is an important source of animal protein for people in many countries of South and South-East Asia. EUS also has the potential to pose a threat to aquatic biodiversity leading to irreversible ecological damage.

Thus, from the present investigation, steps in occurrence of EUS can be summarized as given below. It indicates that EUS is a human creation due to release of pollutants in the nature which are affecting the basic environmental parameters like temperature, salinity, DO and pH affecting the protective mucus layer of the fishes making them vulnerable to the attack of virus followed by bacterial and fungal infection.
Summary and Conclusions

The probable path of EUS

Post rainy season

Water parameters fluctuate

(Temperature ↓, pH ↓, DO↑,
Alkalinity ↓, Hardness ↓,
Chlorides↓, Pollutants↑)

Run off

Stressed fish

Immunosuppression

Loss of mucus and scales

Bacterial and fungal attack

Red spot appear on the body at the point of infection

Bacterial and fungal attack↑

Scale loss, degeneration of epidermis with exposure of
dermis, inflammation and appearance of ring

Bacteria and fungi invade deeper in fish tissue

Tissue necrosis, Muscle necrosis, infiltration of Lymphocytes,
Granulomatous growth, Antigen - Antibody reactions, Proliferation of
lymphocytes leads to more damage to fish tissue leading to ulcers (EUS)

Death of the fish (Economic loss)

Loss to aquatic biodiversity

irreversible ecological damage