3. Methodology: field observations and laboratory examinations

3.1 GENERAL PLANNING OF THE TASK FORCE WORK WITH LOCAL COUNTERPARTS

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

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

3.2 FISH SAMPLING

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

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

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

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

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

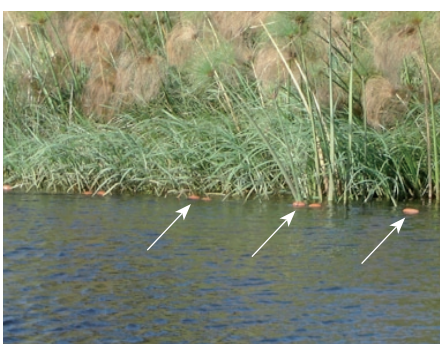
PLATE 2

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

(All photos courtesy of M.B. Reantaso)

Gillnet

Scoopnet



Gillnet in the water

Scoopnet

PLATE 3

Make-shift laboratory in the premises of the hotel

(All photos courtesy of M.B. Reantaso)

S. Kanchanakhan preparing materials for collecting samples for laboratory examination

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

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

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

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

3.3 COLLECTION OF SAMPLES FOR LABORATORY ANALYSIS

3.3.1 Gross clinical signs

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

3.3.2 Parasitology

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

3.3.3 Bacteriology

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

3.3.4 Mycology

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

3.3.5 Virology

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

3.3.6 Histopathology

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