



AFRICAN LUNGFISH, *Protopterus* sp. STANDARD OPERATING PROCEDURES



Revised: December 2005

HOUSING:

Lungfish may be held in a variety of tanks as long as they meet the following criteria:

- 1) The tank size is suitable to hold one lungfish (one 100g fish in approx 8L of water).
- 2) Lungfish are housed separately to prevent aggression between fish.
- 3) A lid as well as outflow covers are provided to prevent escapees.
- 4) An air space **MUST** be provided in the tank to prevent drowning.

ENVIRONMENTAL CONDITIONS:

■Light: Lights are kept at a minimum to simulate natural lighting conditions. Photoperiod may be determined by the individual researcher (12 hours light/12 hours dark is standard).

■Air temperature: Is maintained at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

■Water: Animals are kept in a recirculating well water system (maintained at ambient temperature). The system should be equipped with at least one filter, so that each tank receives a continuous flow of clean filtered water.

■Aeration: No aeration is required since they breathe air.

WATER QUALITY: When the room is first put into use, water quality testing should be carried out on a regular basis, until a stable state is reached. Further testing will become necessary as the rooms biomass increases (growth or greater numbers of fish). Testing should be done at approximately the same time of the day, as diurnal fluctuations do occur in the production of nitrogenous wastes and the utilization of oxygen.

Tests should include dissolved oxygen, pH, ammonia, nitrite, nitrate. The first two tests can be carried out using hand held metres. These metres can be located either in the lab or the Aqualab Office. Aqualab has a Hach DR2000 portable spectrophotometer for the analysis of nitrogenous compounds. Procedures for the use of this instrument may be found in the Dry Lab (room 166).

In a room with a functioning biofilter and adequate water replacement, ammonia and nitrite should be near zero, with nitrate levels below 10 mg/L. pH should be between 6.5 and 8. Other tests that could be performed are copper, hardness, total suspended solids and phosphate, however these tests are generally not necessary in this system.

FEEDING: Fish are fed three times a week a diet of frozen bloodworms or krill (or a suitable substitute). Prior to feeding, leftover food must be removed by suctioning it out of each tank. Excess food left in the system will result in clogged filters, cloudy water and increased levels of nitrogenous waste. Fish food is stored in the freezer. Since all animals are housed separately daily monitoring of feeding will occur and will be recorded as each animal consumes All, Some or None of their food. Any animal which does not appear to be eating sufficiently will be offered a different diet (such as live blackworms). Lungfish will be weighed monthly to ensure that they are not losing weight and to monitor growth.

SANITATION: Disinfectants or detergents are not used in the routine cleaning of tanks. Tanks are scrubbed with brushes or abrasive pads and clean water to remove accumulations of algae, faeces, bacteria and light build-ups of calcium.

- daily - All tanks must be inspected daily to ensure proper waste removal and water quality and to ensure that outflow screens are not clogged.
- monthly - All tanks must be partially drained so the sides and bottoms can be well scrubbed. Tanks are then rinsed and refilled with aged, room temperature water.
- net care - Nets are stored dry between uses and dipped for at least 10 minutes and rinsed between tanks in a solution of A-33™ at a concentration of 12mL/L or Westcodyne™ at a concentration of 25 mL/L.
- disinfection - At the completion of each experimental use of a room or tank the user is required to disinfect the room or tank. Failure to do this will result in Aqualab's staff being responsible for the disinfection of the room or tank. This will result in a bill to the primary researcher for time and materials. All tanks, lids, water hoses and outflows must be scrubbed and disinfected with A-33™ disinfectant at the manufacturers recommended concentration; Quatricide 20™ at a dilution of 2.5 mL/L. Calcium may also be removed by acid washing with a 50% solution of Airkem Brawn™ lime remover and descaler or 50% solution of Muriatic acid. The tanks must be properly rinsed afterward to ensure removal of excess disinfectant or acid.

Note: It is important to ensure that disinfected and rinse water is not mixed with system water. Contamination of system water may result in fish death as well as biofilter death.

ANIMAL IDENTIFICATION:

- Tanks are numbered for animal identification. A card identifying species, primary and associate researcher, and emergency contact person are to be posted on the room door.
- A record must be kept of species, supplier, numbers, arrival date and disposition.

Hagen Aqualab

Animal Utilization Record

Researcher:

Species	Nº	Arrival Date	AUP Nº	Supplier	Disposition
Lungfish	20	Sept 3, 2000	99R000	National University of Singapore	10 euthanized Sept 25, 2000
					10 euthanized Nov 2, 2000

VETERINARY CARE:

- **All mortalities in the Aqualab must be reported to Aqualab Staff.** Any animal that dies of unknown causes or is suspected of dying of a disease related problem must be bagged, tagged and taken immediately for a post mortem examination, the **results of which must be reported to Aqualab Staff.** It is of vital importance that PM's be done on animals that die of unknown causes in this facility. There are several users and an unknown infection has the potential to cause wide spread disease problems not only for the individual researcher but also to other users. Reports including diagnosis, numbers of mortalities, treatment and success or failure of treatment are required for all outbreaks of infection and disease.
- Dead fish are to be transported to dead stock containers located in the freezer using a bucket. (NO FISH ARE TO BE TRANSPORTED IN NET, DRIPPING WATER ON THE FLOOR).
- Surface scrapes of mortalities may be conducted if external parasites are suspected by either the primary researcher or OVC and the mucous viewed under a microscope.

Veterinary care is on a consultative basis only. Advice for the treatment of diseased fish may be sought from either

- **Dr. John Lumsden (X54519) in the OVC Fish Pathology Lab**
- **Dr. Roz Stevenson in the Fish Health Lab in Microbiology (X52517)**
- **Dr. Marcus Litman (X58856) the staff veterinarian.**

Prescriptions for the treatment of disease can be received from Dr. Lumsden or Dr. Litman.

ENVIRONMENTAL ENRICHMENT: Tanks are covered and lights are kept at a minimum to decrease ambient light levels thus providing more natural lighting conditions.

TECHNICAL PROCEDURES:

■ **Euthanasia:** Fish are to be euthanised by method of cervical dislocation. After the animal is dead all waste tissue must be disposed of in the dead stock containers in the freezer.

■ **Blood Sampling:** (See: Fish Blood Sampling SOP).

■ **Body weight:** changes may be monitored on a monthly basis. All fish should be weighed by placing them on a moist paper towel on an electronic balance.

WEEKEND RESPONSIBILITIES: Fish held in Aqualab must be monitored 7 days-a-week. Weekend and weekday tank care are similar. An individual in each lab must be identified to be on call to deal with extra-ordinary problems which might occur overnight or on weekends. Procedures for contacting the person(s) responsible is to be posted on the tank card or on a poster on the door.