Chapter 1 Preventive Medicine/Overview

Preventive medicine at the decentralized aquatic zebrafish laboratories located University of Washington Health Science Building H221, J083b and G617 are of up most concern to investigators, researchers, and staff that care for the fish. Preventive medicine encompasses many factors including maintenance of a healthy environment, adequate feed, proper record keeping, management of disease risk factors, disease surveillance and strict control over fish importation.

1.0 Colony Monitoring Procedures

Water quality and source are integral components of fish health because they are the main cause or source of aquatic pathogens. Essential water quality parameters are recorded and reviewed twice daily by trained personnel. Other important water quality parameters are monitored weekly. Germicidal ultraviolet disinfection of water is continuous on all fish system as is other water filtration methods. Any new water brought into the laboratories is derived from a reverse osmosis process that creates nearly pure water (98%). Fish tanks and equipment are disinfected or sterilized before use, and cleaned regularly there after. All the laboratories are maintained at a temperature of 28 ºC and kept on a 14 light, 10 dark light cycle both environmental factors are considered optimal for zebrafish (*Danio rerio*). For more information environment see **Chapter 5**.

Disease Surveillance

All fish are examined daily for disease by trained staff who are familiar with the most common diseases inflicting zebrafish such as *Oodinium pillularis* (velvet disease), *Mycobacterium marinum* (fish tuberculosis), and intestinal *Capillaria* (nematode parasite). Symptoms of these diseases or any other aberrant behavior are reported immediately to Veterinarian Services. For more details on fish disease see **Chapter 7**.

Alarms

Both H221 and J083B facilities have Sensaphones that provide 24 hour monitoring of air temperature (and thus water temperature), and floor water sensors that can detect fish system flooding. If an abnormal condition exists the Sensaphone will call preprogrammed phone numbers alerting staff. Most fish system have float switches that are capable of turning off system pumps in case of low water levels to prevent deadly gas bubble disease. Larger fish systems also have auto-fill systems that prevent in most cases low water levels. Water towers have high and low level alarms to prevent pump damage.

1.1 Feed

Adult fish are fed twice daily a diet of pelletized commercial fish food Nutra Plus Mash or Nutra Plus #0 obtained from Moore-Clark a division of Skretting International, Vancouver, British Columbia. This feed is supplemented with the live food *Artemia franciscana* that is cultured onsite and fed concurrently with pelletized food. Artemia cysts for culture are obtained from Argent Chemical Laboratories, Redmond, Washington. Young fish are fed three times a day a mixture of 1-part dry micro fine Spirulina powder from Earthrise Nutritionals, Petaluma, California and 1-part Argent artificial plankton. Their mainstay diet is live ciliates *Paramecium multimicronucleatum* that are cultured on site from wheat seed medium. Additionally, fish 10 days and older

are fed a small amount of artemia. Young fish remain on this diet until they can accept the adult diet. For details on feeding see **Chapters 4 and 8**.

1.2 Record Keeping

Population and Mortality Reports

Population and mortality reports for fish facilities H221, J083b, and G617 are compiled monthly at the request of the University of Washington attending Veterinarian and reported to Veterinary Services. Fish health inspections are performed daily by trained facility staff daily and any mortalities found are recorded in a log to facilitate this reporting.

Zebrafish Database

To keep track of the fish the facility maintains a computerized database of all fish in the laboratories. Information contained in the database includes: location (tank no#), user, status (dead or alive), fish genetic identification, birthday, and current age. This information is then referenced with a sequential computer generated stock number. The stock number is then recorded on water-resistant labeling tape and placed on all fish tanks containing that particular fish line. **Every line of fish and needs a stock number.**

To obtain a stock number go to the computer in H225. On the desktop open the FileMaker Pro Zebrafish Database. There are 3 view modes available in the file browse, list, and preview. You can toggle between the views in the upper left corner. Click on data entry and it will open up to a form. Click on generate a new stock number. You will get a new and unique stock number and be prompted to fill out the form. Use the pull down menu and select the facility and tank number(s) where the fish are located. Fill out the parental genetic identification, date of birth, user name (researcher), and the number of fish. Record the stock number on your tank label. Also in the database there are links to the mutant allele and the old zebrafish database (1996-2004).

Colony Monitoring Records

An electronic fish system maintenance database is also maintained for each individual recirculating fish system. Each laboratory has a weekly animal care log with a separate feeding checklist that is filled out and reviewed daily. The log contains but is not limited to a detailed accounting of fish tank cleaning, and disinfection of equipment. Water temperature, pH, and conductivity on all fish systems are recorded twice a day on environmental datasheets. More detailed water analysis including: ammonia, nitrite, nitrate, alkalinity, hardness, and dissolved oxygen are performed weekly and recorded in a supplemental animal and room care log. Environmental datasheets, weekly animal and room care logs with supplementals, mortalities logs and feeding checklists are stored onsite in labeled binders for at least 7 years or from their inception. Computerized databases are backed up onto a separate storage device monthly and hardcopies are produced biannually and stored onsite.

1.3 Sanitation

Fish tanks are inspected daily and spot cleaned when necessary. All tanks are disinfected monthly. During the disinfection process tanks and accessories are thoroughly scrubbed

down with a sponge, rinsed with reverse osmosis (R.O.) water, and immersed in a bleach bath (6.8% sodium hypchlorite/20 gallons R.O. water) for at least one hour. Tanks are then removed from the bleach bath and thoroughly washed off with R.O. water then left to dry on wire racks. Tanks are then reassembled and the date of disinfection is recorded on labeling tape on the bottom left corner of the tank face. In addition, fish tank lids and shelves if applicable are disinfected daily with isopropyl. Floors are disinfected 3 times a weeks with a weak bleach solution and any standing water on the floor is quickly mopped up. Spawning tanks used in fish breeding are autoclaved-sterilized after every use. Water recirculating fish systems have weekly maintenance on filtration systems. Maintenance typically includes disinfection of all micron fiber filters by bleach bath solution and dechlorinated with sodium thiosulfate, inspection of ultraviolet disinfection units, and replacement or replenishment of filtering substrates. For more details on these protocols see **Chapter 8**.

1.4 Fish Importation

Adult zebrafish and embryos previously housed in another fish facility including our own specially designed isolated quarantine laboratory (G617) may not be introduced into the H225 and J083b zebrafish facilities with out consent of the laboratory manager and/or authorization from the principal investigator and must be screened for disease by Veterinary Services. A typical screen includes histological examination and diagnosis. The fish must be found free of harmful pathogens, specifically those associated with Zebrafish (*Danio rerio*). Once cleared by Veterinary Services the fish can be spawned (breed) and embryos bleach disinfected. Only then can the disinfected embryos enter the main colony. For more details on quarantine procedures see **Chapter 9**.