

SOP #: BBC 104.01Title: SOP - Sailfin Molly (*Poecilia latipinna*) Husbandry

Approvals:

Director of Marine
Science Program

A handwritten signature in dark ink, appearing to be "Paul J. ...", written over a horizontal line.

Date:

10/10/13

Attending Veterinarian

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Date:

10/10/13

1. Purpose

1.1 Sailfin Molly (*Poecilia latipinna*) adults, and juveniles and used in my laboratory for studies of ecology. It is important that a steady supply of fish be available at all times for research purposes.

2. Responsibility

2.1 It is the responsibility of all personnel using sailfin mollies in research, teaching and testing to abide by this sop.

3. Definitions

4. Guidelines

4.1 Temperature range: These fish live in a wide range of temperatures in their natural range, which extends from the Rio Grande drainage in Mexico, along the coastal plain of the Gulf of Mexico, to southern North Carolina. In laboratory conditions, a desirable temperature range for stock tanks is 26-29°C. However, temperatures in experimental aquaria should be maintained as required for the experimental goals. See Trexler et al. (1990) for example.

4.2 Water Supply:

4.2.1 Large water holding tanks will be used to prepare water for sailfin mollies (*Poecilia latipinna*). Freshwater from the building supply will be used to maintain the water tanks with at least 200 gallons available at all times. After tank is filled, a

commercially available chlorine neutralizing treatment will be placed in the tank and the water will be aerated for at least 24 hours before use.

- 4.2.2 Salinity of the water will be established at the time of filling, either by addition of building sea water or commercially available sea salt mix. Stock salinity for sailfin mollies will be maintained at 3ppt, unless alternative salinities are desirable for experimental purposes. Stock salinity is variable and depends on the species and experimental protocol in place.

4.3 Water quality in the stock tank will be monitored on a weekly basis. Salinity and nitrogen levels will be checked and recorded on the tank log.

- 4.3.1 Salinity and conductivity will be checked by use of a refractometer or salinometer, whichever is available. Sailfin mollies live in waters from 0 ppt to 36ppt in their natural range, though they do best in waters with conductivity exceeding 350 uS conductivity. In stock tanks, salinities should be maintained between 2 and 6 ppt, depending on the salinities of waters where the fish were collected or salinities to which they have been acclimated in laboratory conditions.

- 4.3.2 Nitrogen levels will be estimated by a colormetric test obtained from commercial sources and will not be permitted to rise above 40 ppm. Should nitrogen levels reach this 40 ppm; the stock water will be drained and replaced with new water.

4.4 Aquaria:

- 4.4.1 Tanks Type: three types of aquaria may be maintained for culturing sailfin mollies: stock tanks where mixed groups of individuals are held for grow-out or between protocols; breeding tanks where females, possibly with males, are maintained to obtain offspring; and experimental aquaria.

- 4.4.1.1 Stock tanks: These tanks shall be maintained with no more than 3 adults or 10 juveniles per gallon of water. For example, a 20 gallon stock tank could hold 60 adults or 200 juvenile mollies; or a mixture. Large adults (>35mm standard length) should be stocked at lower density.

- 4.4.1.2 Breeding tanks: These tanks shall be maintained with one or more sexually mature females and up to 2 males per female in order to obtain offspring. A device producing hiding places for offspring will be included in each tank, for example a wool mop or a clump of natural vascular plants.

- 4.4.1.3 Experimental tanks: These tanks will hold fish in conditions delineated by the experimental protocol in use.

- 4.4.2 Water Quality in Aquaria: Water quality will be maintained by replacing 1/3 of the volume of the aquarium each week. Replacement water will be obtained from the

water-supply tank. Weekly water replacement can be substituted by water quality testing, both salinity and nitrogen. If water quality testing is employed, the results for each tank must be reported on the project log.

- 4.4.3 Lighting: All lights over the tanks will be maintained in a 12:12 (light:dark) cycle. Greer (1975) demonstrated that reproductive cycles of sailfin mollies require this photoperiod. Shorter daily lighting periods will lead to no reproduction in the laboratory.
- 4.4.4 Project Log: A maintenance log for each protocol will be maintained on a clipboard beside the aquaria and adjacent to the IACUC protocol permit. The log will include daily records of tank maintenance and notes about any observations made for specific tanks or conditions observed.
- 4.4.5 Daily Feeding: Fish in stock tanks and breeding tanks will be fed once daily an amount that can be consumed by the fish present within one hour. All food left in the tank after one hour should be removed with a fine-mesh dip net. Fish in experimental protocols will be fed the daily proscribed ration for their feeding treatment. Any excess food on the bottom of a tank will be removed by scooping with a fine-meshed dip net before more food is added. If appropriate for the experimental protocol, the amount of food should be adjusted appropriately if excess food is left for multiple days.
- 4.4.6 Weekend maintenance: All daily care for experimental fish and stock tanks should be maintained on Saturday and Sunday.
- 4.4.7 The level of aeration in each tank should be visually checked to maintain a moderate bubbling that is neither too lethargic to circulate the water in the tank nor too vigorous to force the fish to adopt swimming or hiding behavior to accommodate it. Sailfin mollies do not routinely inhabit fast-flowing water.
- 4.4.8 Daily Maintenance: Daily maintenance will include checking each aquarium and examining the fish for signs of disease, by both examination of the of each fish's appearance and behavior. All morphological or behavioral signs of changing health will be noted on the log and treatment actions will be instituted if necessary. See the SOP for disease treatment. Daily maintenance also includes checking tanks for neonates, when appropriate.
- 4.4.9 When live neonates appear in breeding tanks, they should be immediately removed by netting and be placed in separate tanks as required by the research protocol.
 - 4.4.9.1 Breeding/Neonates: Sailfin mollies are not generally cannibalistic and neonates can be left in stock tanks if cover is provided. At times females will have some or all neonates stillborn. In this case, all stillbirths are to be removed

immediately and discarded in a biohazard container provided in the aquarium room. It is common to find adult mollies mouthing neonates and some people conclude that the adults killed the offspring; however, this is not the case. Sailfin mollies are not anatomically able to eat fish as large as neonates (their teeth are inadequate and their gut has no defined stomach), so this behavior is similar to their scraping feeding observed commonly for feeding on bacterial slime and algae.

4.4.10 Health Assessment: healthy behavior of fish should include swimming in the water column with fins open frequently (not clamped). After a short acclimation period, sailfin mollies generally do not cower constantly behind structures in the tank and often adopt 'begging' behaviors looking for food; fish held in multiple specimen groups should interact with each other without bullying to the point of external damage.

4.4.11 Weekly Maintenance:

4.4.11.1 Once per week, algal growth, if present, should be scrubbed from the walls of the aquarium facing researchers. All walls should be scrubbed if this is appropriate for the experimental protocol. In stock and breeding tanks, sailfin mollies graze continually on walls of their tanks in a behavior commonly seen in nature that is a positive sign of health and should be permitted; it is not necessary to cultivate algae on the tank walls, however.

4.4.11.2 One-third of the tank water should be changed each week by draining with the laboratory siphon. Siphoning with a 'Python' device is desirable to combine with removal of solid wastes. The sponge filter in each tank should be shaken at this time to permit siphoning off any particulates, but the filter should remain submerged to maintain helpful bacterial cultures. The water drained should be replaced from the appropriate lab stock tank.

4.4.11.3 Sponge filters should be examined for accumulation of organic debris as part of weekly maintenance and this should be vacuumed off by the Python device if practical. In the case of a large accumulation of debris, the filter should be removed and rinsed in de-chlorinated water, then replaced in the tank. Sponge filters work by encouraging a biological filter and should not be 'disinfected' or vigorously rinsed except when being taken out of service.

4.4.11.4 Note: A dedicated set of nets and cleaning tools should be maintained for each set of tanks and disinfected in a chlorine bleach solution (5%) or KMNO₄ (1g/l) dip before use in other tanks.

4.4.11.4.1 Solutions:

4.4.11.4.1.1 Chlorine dip: Use Chlorox or similar bleach and mix in a 5% solution for a disinfectant. Disinfection is relatively quick in this solution; soaking for at least 30 minutes is recommended. After soaking, utensils should be allowed to air dry for at least 24 hours before using with fish or fish water. Fish are VERY sensitive to chlorine, but it is highly volatile and can be used safely with proper drying periods.

4.4.11.4.1.2 Potassium Permanganate dip: A dip of Potassium Permanganate can also be used for disinfecting lab utensils, including nets. A stock solution is maintained in a bucket where utensils are stored between use. In this case, all utensils must be rinsed before use in a tank. A stock solution is made by mixing 1 gram KMNO₄ crystals in 1 liter of water.

4.4.12 Measuring Sailfin Mollies:

4.4.12.1 Mollies over 21 days of age can be measured manually to obtain standard length in mm. Neonate mollies can be handled by use of a small scoop made by cutting a 2" x 1" rectangle of window screen and pinching one end (the 1" dimension) and stapling it. These scoops can be used to capture and move neonates without harm. Measurements and weighing should be done on petri or similar dishes (with lips to keep them for flipping out); neonates can be safely handled manually. After 21 days, sailfin mollies can be handled manually for measuring. Standard length can be measured on a petri dish with a 1mm x 1mm grid of graph paper fixed to the underside.

4.4.12.2 Anesthetizing mollies with MS-222 is possible for measurements; however, it can be harmful to the fish and requires them to be moved to a separate container with the solution.

4.4.12.3 Mollies quickly become behaviorally acclimatized to handling if done with care, and regain their orientation and begging behavior quickly after being returned to the tank. Our preferred treatment is to measure mollies without anesthesia unless they display agitation requiring it. All handling, including measurement, is done over the fish's home tank to minimize time out of the water and minimize the chance of dropping the fish on an inappropriate surface.

5. References

- 5.1 Grier, HJ. 1975. Aspects of germinal cyst and sperm development in *Poecilia latipinna* (teleostei-Poeciliidae). *Journal of Morphology* 146:229-249

5.2 Trexler, J. C. 1997. Resource availability and offspring provisioning: plasticity in embryo nourishment in sailfin mollies. *Ecology* 78:1370-1381

5.3 Trexler, J., J. Travis, and M. Trexler. 1990. Phenotypic plasticity in the sailfin molly (Pisces: Poeciliidae). II. Laboratory experiments. *Evolution* 44:157-167

AGE (Days)	Food (mg/day)	AGE (Days)	Food (mg/day)	AGE (Days)	Food (mg/day)	AGE (Days)	Food (mg/day)
1	10	22	50	43	100	64	100
2	10	23	50	44	100	65	100
3	10	24	60	45	100	66	100
4	15	25	60	46	100	67	150
5	15	26	65	47	100	68	150
6	15	27	75	48	100	69	150
7	15	28	75	49	100	70	150
8	20	29	75	50	100	71	150
9	20	30	75	51	100	72	150
10	25	31	75	52	100	73	150
11	25	32	75	53	100	74	150
12	25	33	100	54	100	75	150
13	30	34	100	55	100	76	150
14	30	35	100	56	100	77	150
15	30	36	100	57	100	78	150
16	40	37	100	58	100	79	150
17	40	38	100	59	100	80	150
18	50	39	100	60	100		
19	50	40	100	61	100		
20	50	41	100	62	100		
21	50	42	100	63	100		