**Introduction**

“The ‘optimal’ diet for laboratory fish is one that efficiently promotes definition and stability in nutritional profile, biosecurity, and maximal performance (growth, survival, and reproduction)” (Lawrence et al. 2015). *Mysis* have been the standard breeding and adult growth supplement for the cavefish facility at Stowers, despite a lack of nutritional content and increased biosecurity risk.

*Nutrient Profile*When comparing frozen *Mysis* to Gemma 800, a significant difference appears in the protein content as well as the lipid levels.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Product | Size (mm) | Protein (%) | Lipids (%) | Ash (%) | Fiber (%) | Fatty Acid n-3 (%) | Moisture (%) |
| Frozen *Mysis* - Hikari | ~6.0-13 | 11.9 | 2.3 | N/A | 0.5 | 1.4 | 84.5 |
| Gemma 800 | 0.8-1.5 | 57 | 15 | 10.5 | 0.2 | 3 | N/A |

Due to the energy demand required for growth and breeding, protein and lipid levels should be at their peak during these phases. Additionally, according to Harper and Lawrence (2012), there are key nutrients that should be considered for breeding, regardless of species, to produce high quality embryos. These include n-3 and n-6 Fatty Acids, Vitamin A, Vitamin C, and Carotenoids.

*Biosecurity*  
The Hikari brand *Mysis* shrimp, harvested from the Okanagan lake, are 3-step sterilized and claimed free of harmful parasites and bacteria. However, this product has still included fish carcasses, plastics, and other unidentifiable detritus placing its biosecurity in question. Even when flash frozen, some invasive species, like bryozoans, still survive. According to Hengherr et al. (2011), some species of bryozoans are capable of surviving both desiccation and subzero temperatures.   
  
Additionally, Wang et al. (2015) indicates a possible transmission of a novel pathogenic bacteria, *Vibrio* *fortis*, through a *Mysis* diet into seahorses, causing enteritis. This genus of bacteria has also been found in teleosts, indicating a possibility for transmission through the *Mysis* diet. Additionally, many species of *Vibrio* are zoonotic, further adding to the facility’s biosecurity risk.

*Maximal Performance*

Using Neon Tetras as a comparison for *Astyanax mexicanus*, Sealey et al. (2009) recommends a diet containing at least 45% crude protein for best mass gain. Sabina et al. (2016) states that Neon Tetras perform well at lipid levels ranging from 10-15%, with a peak at 12.3%. This not only promotes the highest growth rate and mass gain, but also increases the food conversion efficiency ratio and protein efficiency ratio. While specific studies comparing growth rates using *Mysis* could not be identified, based on the nutrient profile of frozen *Mysis*, this feed appears to be extremely nutrient poor for the growth of *A. mexicanus.*

If these factors are what determines an optimal diet for laboratory fish, then an alternative to frozen *Mysis* should be studied. As a natural resource, *Mysis* is costly, variable, and can be contaminated. The following pilot proposal will focus on and attempt to identify the maximal performance of a new feeding regime for *A.* *mexicanus* compared with frozen *Mysis*.

**Objective**

The experiment will be conducted in two separate phases.

The objectives are to [1] raise *A.* *mexicanus* on an alternative feed and compare the growth rate and survivability to fish raised on the traditional *Mysis* feed; and [2] compare the embryo fecundity of adult broodstock raised using this alternative feed.

As far as this study is concerned, there have been no previous experiments attempting to measure the difference in growth rate, survivability, and fecundity between Gemma 800 and *Mysis*.

**Fish Stocks/ Groups**

Fish will come from the following Stocks:

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Stock #** | **Strain** | **# Females** | **# Males** | **# Unsexed** | **# Total** |
| 3.XXX.0 | Surface (M) |  |  | 160 | 160 |
| 4.XXX.0 | Pachon |  |  | 160 | 160 |

If necessary, the experiments for 3.XXX.0 and 4.XXX.0 can be performed at different times, as their results are independent.

Fish for experiment 1 will then be continued into experiment 2 using the same groups for continuity. Fish may be redistributed within their groups to create similar sex ratios for each tank.

There is no provision for replacement in case of morbidity/mortality. Moribund fish will be euthanized. Mortalities will be removed. All known losses will be recorded.

**Housing and Feeding -General**

For experiment 1, Sub-Adult (100 dpf) *A. mexicanus* (male and female) will be reared on system in 3 L tanks as normal until they reach 180 dpf, where they will be transferred to 10 L tanks for the remainder of the study (365 dpf). They will be housed in densities of up to 20 fish per tank.

For experiment 2, Adult (365 dpf) *A. mexicanus* (male and female) will be housed in 16 or 38 L tanks on system depending on availability. They will be housed in densities of up to 14 fish per tank, reflective of the current breeding paradigm. The additional 6 fish per tank in experiment 1 will be used in exp. 2 to standardize the breeding ratio among tanks and groups. Any unused fish will be removed from the experiment once breeding ratios of approximately 1:1 (male: female) are confirmed.

Groups will be treated equally. Tanks will not be exchanged unless required. Baffle clearing will be kept to a minimum to reduce stress. Procedures performed with one group will be repeated with the other groups to provide equal treatments.

To reduce variables in our dataset, all three diet groups must be taken from the same breeding clutch. However, if space is limited, the comparisons between surface and cave morphs may occur at different times.

For experiment 1, fish will be fed a modified version of HUS 65 Astyanax Feeding 06 depending on their group as follows:

Control Diet – CD

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | **8:00-10:00 AM** | **11:00 AM -1:00 PM** | **2:00 - 4:00 PM** |  |
| **Life Stage** | **Symbol** | **Feed 1** | **Feed 2** | **Feed 3** | **~age** |
| **Sub Adult (S)** |  | ***Mysis* Shrimp** | **Gemma 500** | **Gemma 500** | ~100 - 179 dpf |
|
| **Mixed Maturity (MM)** |  | ***Mysis* Shrimp** | **Gemma 800** | **Gemma 800** | ~180 - 364 dpf |

Gemma Only – GO

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | **8:00-10:00 AM** | **11:00 AM -1:00 PM** | **2:00 - 4:00 PM** |  |
| **Life Stage** | **Symbol** | **Feed 1** | **Feed 2** | **Feed 3** | **~age** |
| **Sub Adult (S)** |  | **Gemma 500** | **Gemma 500** | **Gemma 500** | ~100 - 179 dpf |
|
| **Mixed Maturity (MM)** |  | **Gemma 800** | **Gemma 800** | **Gemma 800** | ~180 - 364 dpf |

For experiment 2, fish will be fed a modified version of HUS 65 Astyanax Feeding 06 depending on their group and breeding week as follows:

Non-Breeding Week: Monday - Sunday

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | **8:00-10:00 AM** | **11:00 AM -1:00 PM** | **2:00 - 4:00 PM** |  |
| **Life Stage** | **Symbol** | **Feed 1** | **Feed 2** | **Feed 3** | **~age** |
| **Adult (A)** |  | **Experimental Feed\*** | **Gemma 800** | **Gemma 800** | >365 dpf |
|

*\*Experimental Feed refers to each of the different groups and their respective changes to the feeding paradigm: Mysis or Gemma 800.*

Breeding Week: Monday - Friday

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | **8:00-10:00 AM** | **11:00 AM -1:00 PM** | **2:00 - 4:00 PM** |  |
| **Life Stage** | **Symbol** | **Feed 1** | **Feed 2** | **Feed 3** | **~age** |
| **Adult (A)** |  | **No Feed** | **Gemma 800** | **No Feed** | >365 dpf |
|

Breeding Week: Saturday & Sunday

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | | **8:00-10:00 AM** | **11:00 AM -1:00 PM** | **2:00 - 4:00 PM** |  |
| **Life Stage** | **Symbol** | **Feed 1** | **Feed 2** | **Feed 3** | **~age** |
| **Adult (A)** |  | **Experimental Feed\*** | **No Feed** | **No Feed** | >365 dpf |

**Method**

*General Guidelines*

* Measurements will be taken at approximately the same time of day (13 00 -14 00 h) to eliminate introducing error.
* The second feed will be withheld on the day of weighing and measuring to reduce mass discrepancies due to gut loading.

*Experiment 1: Growth and Survival*

* At the start of the experiment, the fish will be selected and split into groups using simple randomization until each tank has 20 fish.
* Tanks will all be housed on the same rack, where possible, to reduce water quality variability.
* Fish will be placed on the designated feeding regime for the designated period during this growth trial.
* There will be 4 replicate tanks for each treatment group.
* There will be 4 replicate control tanks.
* Fish will be anesthetized during weighing and measuring to reduce stress and variability.

*Experiment 2: Fecundity*

* Fish will be sexed and VIE tagged prior to the experiment. From there they will be redistributed within their groups into standard sex ratios for each tank until each tank has 14 fish and a 1:1 ratio.
* Males and females will be allocated using simple randomization to reduce bias.
* There will be 4 replicate tanks for each treatment group.
* There will be 4 replicate controls tanks.
* All tanks will be placed on the same breeding schedule. They will be bred once every four weeks, per standard operating procedure.
* Embryos will be collected using facility standard breeding traps.
* This study will continue for up to one year and will be evaluated after each breeding event.
* Total ova and fertilized embryos will be counted at time of collection. Survival will be monitored until 5 dpf and embryos will be counted before being discarded at 5 dpf.
* Photos will be taken of each dish daily for further analysis and confirmation.
* Embryos produced can be claimed by the lab prior to discarding.

**Measure**

Census: The fish will be physically counted on days post fertilization: 100, 142, 184, 226, 268, 310, 352, and 365. Losses (mortalities and moribund fish) will be recorded in the study workbook.

Mass and Length: Fish will be anesthetized and measured with a ruler to determine the standard length in millimeters (nearest half-mm) of each fish for each group. Each fish will then be placed onto a weigh boat that has been zeroed out on a balance. Mass will be taken in milligrams. The fish may be weighed or measured for length in either order. Data will be collected and logged in the assay workbook.

Each fish will be weighed and measured on day 100, 140, 180, 220, 260, 300, 340, and 365 following TEC 66 Astyanax Weigh and Measure 00. An analytical balance will be used for this procedure.

Sex Identification: Each fish will have their sex identified on day 365 and a VIE tag inserted to identify each fish’s sex.

Embryo Output: Photos will be analyzed using ImageJ software to record the overall number of embryos produced per tank.

Embryo Quality: Photos will be analyzed using ImageJ software to record the overall fertilized embryos produced per tank.

Embryo Size: Photos will be analyzed using ImageJ software to measure the diameter of the embryos upon collection.

Larval Survival Rate: At 5 dpf a final count of larvae will be measured per tank and divided by the total fertilized embryos in each dish at time of collection.

**Data**

Data will be analyzed by the team and then sent to a biostatistician for statistical analysis if necessary to determine significances in and between groups for: survival census, growth (condition factor) rates, standard length, mass, incidences of late onset morbidity, fecundity, and sex ratios (where possible).

**Recordkeeping**

All study data will be recorded in the study workbook:

\\ion\Reptile-Aquatics\Cavefish\Projects\*Mysis* Production\Pilot Project Proposal\*Mysis* Pilot Workbook.xlsx

As well, the OneNote Alternative Feeds section can be accessed within the Cavefish\_u Notebook.

**Veterinary**

The environmental conditions and densities are consistent with those typical in the facility. We do not expect higher losses due to these conditions. The veterinarian was consulted during the formation of this pilot. The veterinarian will be kept appraised of the pilot progress.

**References**

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