

ANIMAL BEHAVIOR LAB
BALL STATE UNIVERSITY

00002

Institutional Animal Care and Use (IACUC) No.

1142896-1

Principal Investigator:

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In case of emergency, contact:

Dr. Jessica Ward

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Graduate Student

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Species/source/sex: *Pimephales promelas* (fathead minnow);
Environmental Testing and Consulting; males and females

Date of arrival: 05/15/18

Age: embryo, larva, juvenile, mature; 0 days post hatch – 6+ months

LABORATORY SPECIFIC STANDARD OPERATING PROCEDURES

Please fill out the form, save, and then print it on your local printer and place in your Chemical Hygiene Plan.

DATE: **05/15/18**

PRINCIPAL INVESTIGATOR: **Dr. Jessica Ward**

ROOM & BUILDING: **CL 226**

PHONE NUMBER: **(504) 941-0899**

Section 1: (Check One)

Process

Hazardous Chemical

Hazard Class

Section 2: Describe Process, Hazardous Chemical or Hazard Class.

β-N-methylamino-L-alanine (BMAA) -- neurotoxin that affects motor neurons.

Section 3: Potential Hazards.

Harmful if swallowed or inhaled.

Causes skin irritation.

Causes serious eye irritation.

Section 4: Personal Protective Equipment.

Wear eye shields and gloves.

Continued on page 2...

LABORATORY SPECIFIC STANDARD OPERATING PROCEDURE**Section 11: Protocol(s):**

BMAA ($\geq 99\%$ purity; Sigma-Aldrich, St. Louis, MO) is dissolved in Milli-Q ultrapure water to generate stock solutions before being serially diluted to produce the desired treatment solutions.

To create solutions (calculated for 100 uL stock in 3 L water)
625 ug/L stock: Weigh out 0.09375 g on scale, add to 5 mL Milli-Q ultrapure water.

125 ug/L stock (serial dilution): add 1 mL 625 stock to 4 mL Milli-Q ultrapure water.

25 ug/L stock: add 1 mL 125 stock to 4 mL Milli-Q ultrapure water.

5 ug/L stock: add 1 mL 25 stock to 4 mL Milli-Q ultrapure water.

1 ug/L stock: add 1 mL 5 stock to 4 mL Milli-Q ultrapure water.

Update 09/29/18 JW

To create solutions (calculated for 100 uL stock in 1 L water)

625 ug/L stock: Weigh out 0.03125 g on scale, add to 5 mL Milli-Q ultrapure water.

125 ug/L stock (serial dilution): add 1 mL 625 stock to 4 mL Milli-Q ultrapure water.

25 ug/L stock: add 1 mL 125 stock to 4 mL Milli-Q ultrapure water.

5 ug/L stock: add 1 mL 25 stock to 4 mL Milli-Q ultrapure water.

1 ug/L stock: add 1 mL 5 stock to 4 mL Milli-Q ultrapure water.

Vol of	mass	volume soln.	[stock]	[final] g/L
A	0.03125g	5 mL	6.25 g/L	
B	1 mL of A	4 mL		.625
C	1 mL of B	4 mL		.125
D	1 mL of C	4 mL		.25
E	1 mL of D	4 mL		5
				1

$$M_1 V_1 = M_2 V_2$$

$$(6.25)(x) = (125)(10 \text{ mL})$$

$$(6.25)(x) = (125)(2 \text{ mL})$$

$$x = 0.400$$

Dilution calculation ①

$$(x)(0.1 \text{ mL}) = (1000 \text{ mL})(6.25 \text{ mg/L})$$

$$x = 6250000 \frac{\text{mg}}{\text{L}}$$

$$\frac{6250}{\text{L}} \frac{\text{mg}}{\text{L}}$$

$$6.25 \text{ g/L}$$

$$6.25 \text{ mg/mL}$$

$$\frac{6.25 \text{ g}}{\text{L}} \cdot \frac{0.005 \text{ L}}{1} = 0.03125 \text{ g}$$

$$\frac{625 \text{ mg}}{\text{L}} \quad 6.25$$

$$100 \text{ mL in } \frac{1}{1000} \text{ L}$$

Need more
brine shrimp eggs

0.125 in 2 mL

+4 =

0.03125 in .5 mL

400 mL into 1600

100 mL into 400

200 mL into 800

3/27

0.004

BMAA/DABA motor performance experiment – Eggs and larvae care

Daily tasks for maintenance of the experiment

1. Label new clutch containers with **treatment, clutch number, date exposed, date tested** (21 days later)

For files/assays label as:

BMAA_treatment_ParentTank-letter clutch_Fish (ex: BMAA_0_2-7C_A)

2. Complete water exchanges in each jar daily

- Fill tubing with water and siphon out half of the jar water into a waste bucket.
- Use siphon to then add new water back into jar—fill to 700 mL level. **Only use aged, aerated water.**
 - For the H₂O treatment, just use aged water.
 - For the BMAA treatment, add 100 µL stock solution to 1 L of water in a beaker. Swirl until mixed and use that to refill jars.
 - **Use the water from the original clutch jar to fill individual fish containers.** This is done because fish kept individually still need to be exposed to conspecific pheromones.

3. Feed larvae (beginning after they have all hatched) brine shrimp.

In 6 well plate, individual fish should get ± 20 µL shrimp per feeding

4. Record lab activities in the lab book –fed, exchanges, whether eggs were brought up, what treatments they were put in, mortalities, when they are tested, etc.

STANDARD OPERATING PROCEDURE

EMBRYO ASSAY

7/18/2018

Materials: Petri dish Microscope and Camera Scooper

****This assay is to be taken on Day 5, this is 4 days after being laid. This is to maximize the exposure to treatment, but test the embryos before hatching****

Procedure:

1. GENTLY scoop eggs into the petri dish. This should be done carefully so that the eggs do not pop. Aim for taking data for at least 10 embryos, but if there are not a lot of eggs laid, in order not to destroy the eggs, use less for testing.
 2. Turn on microscope and camera. Select the TCapture application.
 3. Place the embryos so that they can be recorded, adjust the focus to make the embryo video clear. For personality assays, embryos should be individual.
 4. Select File Save -> browse for folder -> TCapture images -> BMAA Embryo ->treatment Name BMAA_treatment_clutch_name_video#
BMAA_0_2-7C_A_1

*note, if you need to take more than one video of a single fish, the last number will change

 5. Select Video to start recording. Allow to record for 60 seconds (1 minute), then stop.
 6. Open up the application HandBrake
 7. Select File and upload the video just recorded.
 8. Save As name BMAA_treatment_clutch_name_video#_CON
BMAA_0_2-7C_A_1_CON

*the CON stands for converted, because this application converts the file to another format so that the DanioScope can read and analyze it.

 9. Select Start Encode. This should take about 15 seconds. The file should now be an MF
 10. Open the application DanioScope 1

11. Open experiment BMAA_DABA_Embryos
12. Under the Acquisition tab select Activity.
13. Select Add Videos. Open the CONVERTED video.
14. Check video then start to make a circle around the embryo to detect.
15. In the pop-up type in what treatment and that it is 96 hours post fertilization (hpf)
16. Move the circles so that they are encircling the embryo. Make the outline as circular as possible to surround the egg.
17. Select Acquire (Acquire all is for multiple videos at once, however only do one video at a time). This should be completed immediately.
If the program says that it is processing, and does not give data, there are some troubleshooting options:
 - 1- Select Acquire again
 - 2- Move the outlines again
 - 3- Shutdown and open the application again
 - 4- Re-convert video using HandBrake again
 - 5- Re-record video and start again
18. Once the data has been correctly acquired, save Changes on the application and close.

SIGNATURE

DISCLOSED TO AND UNDERSTOOD BY

DATE

PROPRIETARY INFORMATION

Last edited 4/23/18

Swim Performance SOP

1. Fill each of the three petri dishes half full with aged water.
2. Put one fish from the same jar in each.
3. Place one dish on the light box and turn it on.
 - a. Make sure the lights in the room are off.
4. Open EthoVision XT 13.
5. Open "BMAA SwimPerformance.evxt under Open experiment.
6. Place a
7. In the left bar, click "Arena Settings," then select the most recent arena setting (highest number).
8. In the top menu, click "Setup," then "Arena Settings," then "Duplicate from."
 - a. You will duplicate it from the most recent arena setting and it will automatically name the new arena setting.
 - b. Click OK.
9. In the right Arena Settings menu near the bottom, there are three small pictures. Click the third to the right image which is the "Grab Background Image Button."
 - a. This will generate a video in the top left screen.
 - b. Adjust the petri dish that is on the light box so that it is in the middle of the camera.
 - c. Click "grab" in the "Grab Background Image" menu.
10. Adjust the arena settings to fit the current image.
 - a. The orange circle goes around the very outermost border of the dish.
 - b. The green should be an equal distance away from the orange circle all the way around the diameter.
 - c. The yellow should be an equal distance away from the green circle all the way around the diameter. You can use the three marks from the petri dish to help judge the distance.
 - d. The pink circle should be directly in the middle.
 - e. The yellow line should spread across the bottom of the dish.
11. Click on "Detection Settings" in the left menu and select the most recent detection setting.
 - a. After letting it sit for a few seconds, if all the yellow and red is on the fish, this detection setting can be used.
 - b. If this detecting is not picking up the fish or it is picking up other parts of the dish, a new detection setting must be made.
 - c. In the top menu, click "Setup," then "Detection Settings," then "New." A new detection setting will automatically be named. Click OK.
 - d. In the right menu, click "Automated Setup."
 - e. Move the mouse over the image to pause it when the fish is away from the wall
 - f. Draw a box around the fish's body, excluding the tail.
 - g. Make sure the setting is picking up only the fish and click "Yes."
12. Click "Trial List" in the left menu to set up a new trial.
 - a. For arena setting, select the most recent.

- b. Select "Trial Control Setting 1."
 - c. For detection setting, select the most recent.
 - d. For subject, list the environment type, fish parent, and which offspring was tested.
 - i. "H2O_14_A"
 - ii. This fish was in H2O, its parents were in tank 14, and it was the first fish tested (A, B, C...)
 - e. Put in the correct date and time.
13. In the left menu, select "Acquisition" after the fish has been on the light box for 3 minutes.
14. Wait for the program to detect the fish.
15. Press "Ready for Start." The timer will start but the trial will start once the first crosses into the middle most circle.
16. After 1 minute has passed after the fish cross into the middle circle, click the red circle to stop the video.
17. Repeat for each fish, adjusting the arena settings, detection settings (if needed) and the name of the fish.

Last edited 10/3/2018

Spinning Task SOP

1. Fill 1L glass beaker up to 700mL.
2. Place on stir plate with smallest stir bar.
3. Increase the speed of the stir bar to 150 rpm (the first notch on the plate).
4. Once the speed is steady, place the fish in the beaker. The fish will immediately start swimming against the current.
5. Record the time from when the fish is put in the beaker until it is swept away by the current (stop the clock).
6. Record the fish and time in the lab notebook as endurance time.
7. Give the fish 3 minutes to rest.
8. Repeat steps 1-5 and record the times as fatiguability.

TITLE

PROJECT

Continued from page

Personality CheatSheet

Ethovision dish

Day 14 58mm
21 58 mm] arena 9 or 11

49 58 mm

77 89 mm] arena 7

105 89 mm 138mm

133 138 mm] arena 10 or 8,s

161 138 mm

189 138 mm

Spinning Task

Day 77 500mL beaker @ 400mL @ 2 rpm

Day 105 500 mL beaker @ 500mL @ 3 rpm

Day 133 500mL beaker @ 500mL @ 3 rpm

Day 161 1L beaker @ 800mL @ 4 rpm

Day 189 1L beaker @ 800mL @ 5 rpm

TITLE

PROJECT

Continued from page

Preliminary tests done on DABA fish

7/19/18 2-7C 9 embryos moved to jars (0ug/L)
 5 (See other notebook)

7/24/18 2-4F 5 embryos moved to separate jar (1ug/L)
 (See other notebook)

10 8/3/18 fish were moved to 6-welled plates
 (6 in 2-7C + 2 in 2-4F)

8/9/18 Personality testing on 2-7C (Ethovision only)
 15 2-7C-a_fishA (21 days)

2-7C-a_fishB

2-7C-b-fishC

2-7C-C-fishD

2-7C-C-fishE

2-7C-C-fishF

20 8/10/18 Personality testing on 2-4F (Ethovision)
 2-4F-a_fishA (21 days)

2-4F-a_fishB

8/23/18 2-7C moved to jars

25 9/4/18 Personality testing on 2-7C

2-7C-a-fishA

(49 days) (ethovision)

2-7C-C-fishF

30 9/28/18 NEW Fish (Batch 3) arrived!

10/3/18 Personality testing on 2-7C (77 days)

2-7C-a-fishA

(Ethovision +
 spinning task)

2-7C-C-fishF

35 Embryos for 3-1Lc + 3-1LcB run + 6 fish

per jar Selected for personality trials

BMAA-D-3-1LcB-Ariel - popped egg

- Bo

- Cate

- Davy

- Ellen

- Fidge

BMAA - 0 - 3 - 16 - Amy
 ↓
 - Becky
 - Carly
 - DahlKemper
 - Emily
 - Franch

10/4/18

BMAA - 0 - 3 - 2 - Aley
 ↓
 Bailey
 Charlie
 Dirty Dan
 Elizabeth
 Fish

BMAA - 0 - 3 - 18 - Ally
 ↓
 - Bob
 Christy
 - Duce
 .ET
 - Freddy

BMAA - 0 - 3 - 14 - Aycerrah - popped
 ↓
 Billy
 Cara
 - Demi
 Eric
 - Farmer

Spinning task procedure tested

*@ end of this day, Data on the computer was lost
 now, videos online are more difficult to acquire

10/5 Danioscope + Tcapture not fully working

BMAA - 5 - 3 - 13 - Adam
 - Brady
 - Sydney
 - David
 - Eeyore
 - Frank

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SIGNATURE

DATE

Continued from page

BMAA-5-3-1-Ashley
- Brian
- Clarissa
- Delilah
- Eliza
- Fern

BMAA-5-3-3-Abigail
- Barney ✓
- Carrie ✓
- Dallas ✓
- Eli X [not acquired on
- Filmore X Danoscope

10/10/18

BMAA-25-3-7B-Ginny X
- Hermione X
- Igor X
- Lupin X
- Katie X
- James ✓

BMAA-25-3-18C-Minerva X
- Neville X
- Olivander X
- Peter X
- Rubeus X
- Quirell X

10/7/18

BMAA-25-3-12-Albus X
- Bartemius X
- Cornelius X
- Draco X
- Errol X
- Finnigan X

BMAA-25-3-13C-Marvolo ✓
- Norbert X
- Oliver X
- Percy ✓
- Quirinus X
- Ron X

Continued to page

SIGNATURE

DATE

Calibrate to 38.21 mm

Personality Assay 8/9/18

Trial 1 - Personality

2-7C-a-fishA → broken back upon transfer to arena

2-7C-a-fishB: 15 sec to center

2-7C-b-fishC ♂ in center

2-7C-c-fishD ~5 sec, meet twice during setup of trial
↳ intestines came out during transfer

2-7C-c-fishE 80 sec to center

2-7C-c-fishF 21 sec to center - possible tail injury

8/10/18

1-2-4F-a-fishA didn't go around the whole arena a full time before crossing into the middle @ 40 sec. more back & forth runs compared to circling

1-2-4F-a-fishb - 10 sec to center
for some reason, the video stopped @ 5 min 2 sec, so I took another video for 58 sec.

C Start - E + F weak responses

* Tummies all very full

2 no response C start

2 unmotivated to eat

→ may need larger arena
or enough for diet

acquire for 60
minutes still

9/4

Personality trial - 49 days

O-2-7C-fish A

no time til got in center of arena
possible bids to right side of arena

O-2-7C-fish F

5/7 seconds til in center

lots of standing

1.9% missed sample

approx. 21.07 mm

6mm
larger
arena

10/3

Personality trial - 77 days

O-2-7C-A-fish A

in center before acquiring

very little time spent in center zone

Spinning task - 1:38 sec 1st time - toss in
need to go pro [3 min break]

2nd time - capped @ 10:00 - slow ↑

→ body plane closer to edge, bottom,
top heavy

→ less rest period/ wasn't trying to have
bursts/go with current

→ learning curve? or cuz already
in H₂O

[3 min break]

3rd time - capped @ 10:00 min

O-2-7C-C-fish F - 29 sec til zone 2

1st 2:46

2nd 8:28

3rd 1:52

same

1 speeds

↳ 2nd speed

All C smallest stir bar

Pg 2

10/3

We are running embryos on
3-16B + 3-16. (10 eggs each jar)
Six embryos will be randomly
selected to be used for personality
trials.

They are exposed to 0mg/L BMAA.

3-16B had 5 embryos + 6 indiv embryos tested
BMAA - 0 - 3-16 - Ariel - 1 - popped egg

Bo - 1

Cate - 1

Daryl - 1

Ellen - 1

Fridge - 2

3-16. had 5 embryos + 6 indiv + tested

BMAA - 0 - 3-16 - Amy - 2

Becky - 1

Carl - 1

Dahkemper - 1

Emily - 1

Franch - 1

not fertilized

10/4 embryos 3-2, 3-8, 3-11, 3-18, 3-14

BMAA - 0 - 3-2 - Alex

Bailey

Charlie

Dirty Dan

Elizabeth

Fish

3-8 did not have enough for personality fish

BMAA-D-3-18 - Ally

- Bob
Christy

Duce

ET

Freddy

BMAA-D-3-14 - Azeemah ← X on Danioscope
popped + still analyzed

- Billy - weirdly shaped
- Cara

Demi

Eric - 3

Farmer - 2

500 mL beaker @ 400mL, small bar, sim size
1st RPM capped @ 40 Aug/L

500 mL beaker @ 500mL, small bar, sim size
@ 1st RPM, effect Aug/L ~ 27 days
@ 2nd rpm, 15 sec

→ 500 mL beaker @ 400mL, small bar, sim size
1st @ 1st rpm, 25 sec Aug/L ~ 77
2nd 20 sec
3rd 22 sec

500 mL beaker @ 400mL, small bar, smaller size
@ 1st rpm, 3:08 min:sec ~ 14 days

500 mL beaker @ 300mL, small bar, smaller size
1st @ 1st rpm, 12 sec Aug/L
2nd @ 1st rpm, 2:15 min:sec
3rd @ 1st rpm, 11.7 sec

500 mL beaker @ 300mL, small bar, sim/smaller
1st @ 1st rpm, 11.61 sec Aug/L ~ 30 days
2nd @ 1st rpm, 58.08 sec
3rd @ 1st rpm, 27.53 sec

500 mL beaker @ 500mL, ^{large} small bar, adult
@ 2nd rpm nonexistant
@ 1st rpm 9.03 sec

1000 mL beaker @ 500mL, large bar, adult
@ 1st rpm 5.01 sec

1000 mL beaker @ 800mL, large bar, adult
@ 1st rpm 13.56 sec

1000 mL beaker @ 500mL, small bar, adult
@ 1st rpm, 2nd, 3rd capped @ 2 min

500mL beaker, 400mL, small bar, sim size/larger

→ C 1st rpm 2:22.22 77
C 2nd rpm 1:46.07 25 mg/L
C 2nd rpm 3:15.51

500mL beaker 300 mL, small bar, small ~25 days

C 1st rpm 2:25.67 25 mg/L
C 1st rpm 54.70 sec

DABA - 0-2-7C-C Fish F

3 min
occlusion
3 min
rest

500mL beaker, 400mL, small bar, 78 days old
1st C 1st rpm 43.20 sec possibly earlier, hard to tell
2nd C 1st rpm 34.15 sec
3rd C 1st rpm 57.15 sec

DABA - 0-2-7C - A - Fish A

500mL beaker, 400mL, small bar, 78 days old
1st C 1st rpm capped C 10 min
2nd C 1st rpm 2.95 sec
3rd C 1st rpm 8:27:45

/ aquired
X video error

10/15

Danioscope & TC capture & working
BMAA - S-3-13 - Adam ✓

/ - Brady ✓
/ - Cydney ✓
/ - David ✓
/ - Eeyore ✓
/ - Frank ✓
/ - I ✓

→ BMAA - 1-3-13 B - X - con-real (X 40)
/ - 2 ✓ 6 eggs

→ BMAA - 1-3-14 B - X - con-real X
/ - 2 ✓ embryo 2 popped still aquired

BMAA - 5-3-1 - Ashley ✓

/ - Brian ✓

/ - Clarissa ✓

/ - Delilah ✓ con - 2 - con - 3 ✓

/ - Eliza - X - con - 2 - con - 3 ✓

/ - Fern ✓

/ - 1 4 embryos ✓

BMAA - 5-3-2 B - 1 - 5 embryos, 1 popped
/ - 2 ✓ 21 eggs so only ✓

37

3-186

BMAA - 5-3-3 - 1 ✓

/ - Abigail ✓

/ - Barkley ✓

/ - Carrie ✓

/ - Dallas X - con - 2 ✓

/ - Eli X - 2 B X

/ - Filmore X - con - 1 - con - 3 X

pg7

→ BMAA-1-3-18B-X - con X
↓ - Z-con 2 popped X

→ BMAA 1-3-7-V - con X
→ - Z-con X

] only 8 embryos
+ total on
tile

10/6/18

BMAA-25-3-7B-1 1 popped - took out
X 2 X + retook
video w/ new
embryos

- Ginny X
- Hermione X
- Igor X
- Lupin X
- Katie X
- James ✓

THEY ALL KEPT HATCHING

BMAA-25-3-19c-1 X

- Minerva X
- Neville X
- Olivander X
- Peter X
- Rubens X
- Quirell X

Reared
6/6
died
6/6
molted

BMAA-125-3-7C-1 X (5 eggs)
- 2 ✓ (1 egg)
- 3 X (2 eggs)

Pg 8

BMAA_125_3-18d - 1X (3 eggs)
- 2X (1 egg)
- 3X (5 eggs)
= 4X (6 eggs)

10/7/17

BMAA_25_3-12 - 1X

- Albus X
- Bartemius X
- Cornelius X
- Draco X
- Errol X
- Finnigan X

BMAA_25_3-13c - 1 ✓

- Marvolo ✓
- Norbert X
- Oliver X
- Percy ✓
- Quirinus X
- Ron X

Pg 9

10/10/18 BMAA-0-3-16B - Anel died.

BMAA-0-3-14-Azetnah] missing? empty
- Billy wells

BMAA-5-3-3 - Filmore died

10/10 embryos - supposed to be tested 10/9

BMAA-625-3-6A-1 2 embryos ✓
hatched after transfer

BMAA-625-3-11A unfertilized.

10/10 Embryos

BMAA-125-3-1B ✓ 5 embryos. 1 vid

BMAA-625-3-6B ✓ 2 embryos, 1 vid

BMAA-125-3-6C 1st video ✓ 2nd: not acquired

BMAA-625-3-13D ✓ ✓

10/10/18

BMAA-D-3-16B-Ariel died

BMAA-D-3-14-Azeemah] empty wells
- Billy

BMAA-5-3-3-Filmore died

Online personality datasheet made

10/11/18

BMAA-5-3-13-Eeyore had 2 fish in the well.

(One fish was moved to plate BMAA-5-3-3 (Filmore's old plate).
The fish that was moved is EeyoreII & the fish that is in the old plate is EeyoreI

10/12/18

Deaths :

BMAA-D-3-14-L Demi

BMAA-D-3-18-Bob

BMAA-D-3-16-Amy

BMAA-5-3-13-Adam

BMAA-5-3-13-Brady

BMAA-5-3-1-Charissa

BMAA-5-3-1-Eliza

BMAA-5-3-3-Eeyore II

BMAA-2S-3-18C-Quirrell

BMAA-2S-3-7B-Ian

BMAA-2S-3-12-Atlas

BMAA-2S-3-12-Draco

Embryos on 3-18E & 3-9A taken

BMAA-5-3-18E-Acorn X

- Blueberry ✓

- Cantaloupe ✓

- Dragonfruit X

- English Muffin X

- French Fry ✓

BMAA-S-3-9A-Aloysius	✓
- Bevi	✓
- Comet	✓
- Dog	✓
- Eve	✓
- Fountain	✓

BMAA-S-3-1BE-Gabby	✓
- Heath	✓
- Idiot	✓
- Juan	✓
- Kelley	✓
- Lauren	✓

BMAA-S-3-9A-Carret	X
- Hamlet	✓
- Izzy	X
- Jerry	✓
- Krieger	✓
- Lil Wayne	✓

10/13/18

Personality testing on 3-16 + 3-16B (14 days)

BMAA-O-3-16-Becky	- hard to find w/ Ethouision	(1st 13 sec)
- Carli	- 1st moved @ 3:04, stopped @ 4:34	1:14 sec
- Dahlkemper	- 1:08 moved, 2:15 @ center	~ 1:14 sec
- Emily	- 3:57 @ center	3.4% MS 1.1%
use 2nd at 3rd	3 Zvideos	Franch - 2:410 @ center Stopped @ 4:49?

BMAA-O-3-16B-Daryl	- 2:43 @ center	4:49 stopped in 2 videos
- Ellen	- long time to detect	
- Fridge	- 2:52 @ center	
- Cate	- never went to center	
- Bo	- long time to detect	- 2:52 @ center

embryos

BMAA-O-3-3B-Gunner	✓
- Hi	✓
- Igloo	✓
- Jess	✓
- Kite	✓
- Lamp	X

Continued to page

embryos BMAA-2-3-3B - Mandy ✓
 - Nancy - 2 ✓
 - Otis ✓
 - Philip ✓
 - QTIP ✓
 - Roy ✓

5

10

15

20

25

30

35

40

Deaths: BMAA-25-3-7B - Ginny → Water dried up
 BMAA-25-3-7B - James → Overnight
 BMAA-25-3-18C - Minerva
 BMAA-0-3-18 - Freddy
 BMAA-25-3-17 - Barkemus 10/12
 BMAA-5-3-3 - Barney
 BMAA-25-3-18C - Divander
 BMAA-25-3-12 - Errol
 BMAA-25-3-18C - Neville
 BMAA-0-3-16B - Davy
 BMAA-5-3-18C - Magolo

10/14/18

Deaths: BMAA-0-3-16B - Fido
 BMAA-0-3-14 - Farmer
 BMAA-0-3-16B - Cate
 BMAA-0-3-16 - Frank
 BMAA-0-3-16 - Family
 BMAA-0-3-16 - Dan Kemper
 BMAA-25-3-12 - Finnigan
 → embryos 3-7F 25 ug/L

Gillett - hatched but still ran ✓

Hat Trick ✓

I Love Lucy ✓

Jr. ✓

Kamall ✓

Lucy X

Death: BMAA-0-3-2 - Bailey

Personality testing on 3-2, 3-18, 3-14

BMAA-0-3-2 - Haley - lots of mount

- Charlie - 53% center

Dirty Dan - mount +1 1:31, 0% center at all

got sucked up in syringe → Elizabeth - 1104% center

Fish - 1.13% center

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BMAA - 0-3-14 - Cara = middle @ start + 116 then @ 1:44
 - Eric - 30 @ center, very active

BMAA - 0-3-18 - Ally - 6 in center

- Christy - 5 @ center

- Duce - 1:07 @ center, bumping & moving @ 5:54, 2 videos

- ET - 1:28 @ center

10/15/18

→ embryos 3-18 F 25 µg/L (BMAA - 25-3-18F)

Apple X

Dongle X

Banana X

Esmoralda ✓

Citrus ✓

Fly X

Marlin ✓

Pecan ✓

Nemo ✓

Quishy ✓

Octopus ✓

Ringo ✓

Squash X

Van ✓

Tobin ✓

Willy ✓

Ugly ✓

Xylophone ✓

Personality Testing

BMAA 5-3-3 - Abigail 1:41 @ center

- Carrie 1:57 @ center

- Dallas 2:39 @ center

- Eli 3:24 @ center

BMAA 5-3-1 - Ashley 2:08 @ center

- Brian 0:16 @ center - 2 videos, first stopped @ 3:54

- Delilah hard time to detect, replaced in plate & tried again later.

- Fern 0:12 sec @ center - long time to detect

BMAA 5-3-13 - Cydney 1:22 @ center

- David RIP

- Frank 0:47 @ center

- Eeyore in center right before start, 1:42 @ center

Death: BMAA - 5-3-13 - David

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