

3 / 1 / 16 13

SAFS OA Beodock Epigenetics Project

NOAA Manchester

Roberts Boetz Putnam

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2

04 March 16 HP

pH probe calibration  
 Mettler D6115 SC pH probe

Fisher Scientific accurate pH meter 15  
 reads to 0.1 mV

calibrate to NBS buffers 4, 7, 10

slope 58.06 eft 98.1

3.999 zero point 0.7 mV

7.052

10.126

pH probe calibration in Tns Batch 14Feb16 SR

| <u>Temp</u> °C | <u>mV</u> | <u>Temp</u> °C | <u>mV</u> | <u>Temp</u> °C | <u>mV</u> |
|----------------|-----------|----------------|-----------|----------------|-----------|
| 14.75          | -82.5     | 13.63          | -84.0     | 10.40          | -88.3     |
| 15.02          | -81.0     | 12.92          | -85.0     | 11.05          | -87.5     |
| 17.21          | -79.2     | 12.46          | -85.6     | 11.55          | -86.8     |
| 18.18          | -77.9     | 9.90           | -89.0     | 11.80          | -86.4     |
| 19.07          | -76.7     | 10.10          | -88.7     | 12.20          | -85.9     |

Tank T °C mV  
 header 2 14.16 -61.7

header 1 13.83 33.6

Tank T °C mV Time 13:30

tank S - H2 13.14.02 -52.3

tank 1 - H1 13.99 -31.3

3

4 March 16 HTP

4

| Part | Time  | Tank | T °C     | PLTMV | Sal  |
|------|-------|------|----------|-------|------|
|      | 14:00 | 1    | 14.00    | -53.1 | 26.8 |
|      |       | 2    | 13.99    | -53.0 | 26.9 |
|      |       | 3    | 13.97    | -52.9 | 26.5 |
|      |       | 4    | 13.14.01 | -31.7 | 26.8 |
|      |       | 5    | 13.94    | -31.1 | 26.9 |
|      | ↓     | 6    | 13.99    | 31.3  | 26.9 |
|      | 14:30 | H1   | 13.87    | -31.3 | 27.1 |
|      | 14:30 | H2   | 13.81    | -54.2 | 27.1 |

opened H1 bleed valve a bit to match  
H2 flow rate

- Using 1085 nmol kg<sub>SW</sub><sup>-1</sup> for TA calculated  
PCO<sub>2</sub> ~ 540 ± 1460 for Amb & Hyh  
respectively

Temp probe calibration  
Digital outdoor temperature sensor  
THP-POT-SEN - AUFETCH  
-40 °C to 85 °C operating range  
± 0.125 ° every 2 seconds

| Probe           | Temp                                | AUTECH °C | Temp certified vwr °C | offset |
|-----------------|-------------------------------------|-----------|-----------------------|--------|
| 1               | 13.87                               | 13.96     | 13.96                 | 0.3    |
| 2               | 13.75                               | 13.96     | 13.96                 | 0.1    |
| 3               | 14.00                               | 13.96     | 13.96                 | 0.1    |
| 4               | 14.00                               | 13.96     | 13.96                 | 0.1    |
| 5               | 13.93                               | 13.96     | 13.96                 | 0.1    |
| 6               | 14.06                               | 13.96     | 13.96                 | 0.1    |
| Header 1        | 13.51                               | 13.51     | 13.51                 | 0.1    |
| Header 2        | 13.51                               | 13.51     | 13.51                 | 0.1    |
| Reservoir       | 13.51                               | 13.51     | 13.51                 | 0.1    |
| AUTECH car only | be adjusted by 0.1 °C so 0.1 offset |           |                       |        |

4 March 14 HTP 17:00

Held all treatment tank probes in tank 2

Held all reservoir and header probes in tank 1  
will record data over the weekend for batch  
sets

5-6 March 16 HTP

watched temp & pH data online, both are stable  
over the weekend

07 March 16 HTP

08:45 - moved temperature probes back to each  
individual tank

pH TDS calibration

| mv    | Temp'c |
|-------|--------|
| -80.9 | 10.09  |
| -88.7 | 10.35  |
| -88.0 | 10.89  |
| -86.8 | 11.77  |
| -86.0 | 12.28  |
| -85.0 | 13.02  |
| -84.5 | 13.41  |
| -84.0 | 13.78  |
| -83.4 | 14.28  |
| -82.7 | 14.79  |

March 16 Apr

- March 10 1991
  - removed all water from system
  - drained all tanks
  - rinsed water & Freshwater probe to control feed probes
  - calibrated salinity probes

27.1 @ 12

27.1 @ 12.5  
- for all drafters in  
- checked temp & pH  
- bucket of seawater  
- temp = 12.49°C

|      |       |     |    |    |
|------|-------|-----|----|----|
| 9:21 | b'g't | h'g | H1 | H2 |
| S:21 | o't,t | D3  | Z  | #  |
| S:21 | t,o   | D3  | H2 | #  |
| S:21 | t,g   | H1  | H2 | #  |
| 12:4 |       |     |    |    |

No connections needed

2021 injection  
on 10/20/2021  
and hatched  
on 10/20/2021

Set pump to auto valve @ 12.5 c.f.c

Header + row + column = Header | relay  
Header + treatment = Header | relay  
Header + report = Header | relay

sank among filters in freshwater overnight

settler  
spills out H<sub>2</sub>O  
loss of H<sub>2</sub>O  
settler  
connected feed lines  
add back tank  
and bubbler for feed

| Date<br>March 16 | Time<br>09:15 | Tank | pH mV | Temp'c | Salinity | Flow |
|------------------|---------------|------|-------|--------|----------|------|
|                  |               | 1    | -52.8 | 12.09  | 27.4     |      |
|                  |               | 2    | -53.0 | 12.05  | 27.4     |      |
|                  |               | 3    | -52.8 | 12.14  | 27.4     |      |
|                  |               | 4    | -31.6 | 12.03  | 27.4     |      |
|                  |               | 5    | -32.7 | 12.18  | 27.4     |      |
|                  |               | 6    | -33.4 | 12.15  | 27.4     |      |
|                  |               | H1   | -31.3 | 11.56  | 27.5     |      |
|                  |               |      |       | 11.55  | 27.6     |      |
|                  |               | 1    | 09:40 | H2     | -52.9    |      |

placed salinity relay 4 in tank 6  
 placed durafet 4 in tank 5

placed salinity relay 3 in tank 3  
 placed durafet 3 in tank 3

- cracked open co2 valve

Relay 1 cycle time 30sec dose 2sec

→ would recommend adding the inline Reg

Randomly assigned tanks to treatments

| Amb | High         |
|-----|--------------|
| 3   |              |
| 6   | 0.55 salinst |
| 2   | 4            |

Sxt @ 18:45 tanks and dosing

8 March 1964

Oct. 11 - \$7.50

overshoot CO<sub>2</sub> with loose aggregate

- Oct. 10 - 10,000  
- installed regulator for CO<sub>2</sub>  
- installed header tank 2 ft add reservoir  
- opened header tank  
- Vactor truck brought up  
- set regulator at 30 Psi.  
- labeled bottles for sun cleaning collection

| Date    | Time  | Bottle # | Tank # | pH           | Temp. | Salinity |
|---------|-------|----------|--------|--------------|-------|----------|
|         |       |          |        | sampler posn |       |          |
| 8 March | 10:15 | 13       | Head 1 | -13.7        | 13.73 | 27.4     |
|         |       | 14       | "      | "            | "     | "        |
|         | 10:23 | 15       | Head 2 | -53.6        | 13.68 | 27.6     |
|         |       | 16       | H2     |              |       |          |
|         | 10:27 | 7        | Tank 4 | -22.6        | 14.11 | 27.5     |
|         |       | 8        |        |              |       |          |
|         | 10:32 | 9        | Tank 5 | -22.0        | 14.10 | 27.5     |
|         |       | 10       |        |              |       |          |
|         | 10:37 | 11       | Tank 6 | -53.3        | 14.08 | 27.5     |
|         |       | 12       |        |              |       |          |
|         | 10:42 | 1        | Tank 1 | -19.7        | 14.05 | 27.5     |
|         |       | 2        |        |              |       |          |
|         | 10:47 | 3        | Tank 2 | -53.0        | 14.06 | 27.5     |
|         |       | 4        |        |              |       |          |
|         | 10:51 | 2        |        |              |       |          |
|         | 5     |          |        |              |       |          |
|         | 6     |          |        |              |       |          |
|         |       |          | Tank 3 | -52.8        | 14.14 | 27.5     |
|         |       |          |        |              |       |          |
| 10:55   | 11    |          |        |              |       |          |

12:00 Algal feed mix

Sgal 609 + Sgal CGW added to feed tank

13:00 6 containers of beadduck pooled & allocated equal volumes to all tanks

initial counts in 200 $\mu$ L

S aliquot counts

Total volume: 11.3862 L

| Well | # | Initial Count | Added Seawater and<br>Formalin 10% |
|------|---|---------------|------------------------------------|
| 1    | 1 | 113           |                                    |
| 2    | 2 | 119           |                                    |
| 3    | 3 | 124           |                                    |
| 4    | 4 | 126           |                                    |
| 5    | 5 | 126           |                                    |

Ave: ~122 remL: 608 total count: 6.90M

Sampling ~10k indv., 16.4 mL from total vol.

| Sample | Morph | Number | Notes |
|--------|-------|--------|-------|
| 1      | M     | 1000   | EPI   |
| 2      | M     | 1000   | EPI   |
| 3      | M     | 1000   | EPI   |
| 4      | M     | 1000   | EPI   |

3 ranges in EtOH (1mL)

add 1mL of RNA later to 1g2

In freezer @ 14:30

RNA water stored @ 4°C in Hatchery

Formalin stored in 4°C in Rick's lab

shap frozen stored in -80°C in Rick's lab

## 8 March 6 HP

15:30

- cell counts in treatment tanks
- removed 1.5ml of tank water through a  $60\mu\text{m}$  filter

- Added ~5gai of CGW #609 to feed tank
- changed to 130 rpm on feed pump

| tank | cont 1 | cont 2 | cont 3 | cont 4 |
|------|--------|--------|--------|--------|
| 1    | 75     | 84     | 97     | 87     |
| 2    | 106    | 114    |        |        |
| 3    | 113    | 115    |        |        |
| 4    | 112    | 93     |        |        |
| 5    | 103    | 115    |        |        |
| 6    | 115    | 84     |        |        |

$$3 \times 3 \times 0.1 = \text{mm}^3 = \text{mL} = 0.9 \mu\text{L}$$

100 $\mu\text{L}$

$$75 \text{ cells}/10.9 \mu\text{L} \times 1.5 \mu\text{L} \times 1000$$

$$(83.3 \text{ cells}/\mu\text{L})$$

16:00

- changed & rinsed all bano filters
- checked all drippers

8 March 16 @P 18:30 settings

- $\text{pCO}_2$  70 sec cycle 1 sec dosing while regulator is set ~10 psi
- water auto valve set to auto turn to maintain 12°C
- feed tank dosing rate 130SPM

9 March 16 HP

09:30 - Added signal of Cow #609 to the  
feed tank

- Checked drippers for flow in each tank

pH ms calibration 10:20

| milliV | Temp °C |
|--------|---------|
| -88.2  | 12.06   |
| -87.8  | 12.39   |
| -87.4  | 12.76   |
| -86.8  | 13.15   |
| -86.4  | 13.46   |
| -85.9  | 13.81   |
| -85.4  | 14.14   |
| -85.1  | 14.46   |
| -84.4  | 14.96   |
| -84.1  | 15.16   |



March 6 1898

| Cell Count              | Count 1 | Count 2 | $\bar{x}$ |
|-------------------------|---------|---------|-----------|
| Count 1                 | 119     | 140     | 134.5     |
| Count 2                 | 159     | 157     | 158       |
| Total                   | 278     | 297     | 287.5     |
| Cells / mm <sup>2</sup> | 165555  | 167777  | 166666.5  |

2.5 + 2.5 )  
2.5  
X 2.5  
12.5  
+ 12.5  
25.0

15 som  
11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99

Tube list

exp 3/10

111 15

Tube labels

| Date     | EPI      | M  | Date       |
|----------|----------|----|------------|
| 3/8      | 1        | 1  | 3/8        |
|          | 2        | 2  |            |
|          | 3        | 3  |            |
|          | 4        | 4  |            |
| 3/10     | 5 tank   | 5  |            |
|          | 6 tank2  | 6  | 3/10       |
|          | 7 tanks3 | 7  |            |
|          | 8 tanks3 | 8  |            |
|          | 9 tank4  | 9  |            |
|          | 10 tank4 | 10 |            |
| 11 tanks | 11       |    | cells 3/12 |
| 12 tanks | 12       |    |            |
| 13 tanks | 13       |    |            |
| 14 tank  | 14       |    |            |
| 15 tank  | 15       |    |            |
| 16 tank2 | 16       |    |            |
|          | 17       |    |            |
|          | 18       |    |            |
|          | 19       |    |            |
|          | 20       |    |            |
|          | 21       |    |            |
|          | 22       |    |            |
|          | 23       | T  |            |

21

10 March 16 AM

08:15 - checked water level, dripper flow  
temp & pH

09:30 - calibrated plt probe Tns 2/14/16 tube 3/9/16

| <u>MV</u> | <u>Temp</u> °C |
|-----------|----------------|
| -85.3     | 14.89          |
| -86.6     | 13.86          |
| -87.1     | 13.40          |
| -87.3     | 13.20          |
| -87.5     | 12.97          |
| -88.0     | 12.73          |
| -86.8     | 13.65          |
| -86.2     | 14.04          |
| -85.7     | 14.42          |

+2W SALT

SALT

193

| <u>Date</u> | <u>Time</u> | <u>Bottle</u> | <u>tank</u> | <u>pH</u> | <u>Temp</u> | <u>Sat.</u> |
|-------------|-------------|---------------|-------------|-----------|-------------|-------------|
| 3/10        | 10:15       | 37            | TANK 1      | -29.0     | 14.18       | 27.4        |
|             |             | 38            | "           | "         | "           | 3           |
|             | 10:20       | 39            | TANK 2      | -55.7     | 13.99       | 27.2        |
|             |             | 40            |             | -55.7     | 13.99       | 27.2        |
|             | 10:26       | 41            | TANK 3      | -55.6     | 13.98       | 27.2        |
|             |             | 42            | "           | "         | "           | "           |
|             | 10:31       | 43            | TANK 4      | -28.6     | 13.87       | 27.2        |
|             |             | 44            | "           | "         | "           | "           |
|             | 10:35       | 45            | TANK 5      | -28.4     | 13.96       | 27.2        |
|             |             | 46            | "           | "         | "           | "           |
|             | 10:38       | 47            | TANK 6      | -55.3     | 14.01       | 27.2        |
|             |             | 48            | "           | "         | "           | "           |
|             | 10:42       | 33            | Header 1    | -29.1     | 13.26       | 27.2        |
|             |             | 34            | "           | "         | "           | "           |
|             | 10:44       | 35            | Header 2    | -55.9     | 13.23       | 27.2        |
|             |             | 36            | "           | "         | "           | "           |

HgCl<sub>2</sub> & TSMC added @ 10:52

Draffet & Salinity moved from tank 3 to  
tank 2 @ 13:15 3/10/16

Larval collection

10 March 16 H.P.

11:45 Tank 1 dropped into 500ml  
plugged and removed 100 $\mu$ l  $\times$  3 counts  
removed sample and added 1ml EtOH

| Tank  | well 1 | well 2 | well 3 | $\bar{x}$ | A<br>$\frac{A}{D}$ | P<br>$\frac{A+D}{2}$ | A<br>$\frac{A+D}{2}$ | P<br>$\frac{A+D}{2}$ | cells/ml  | $\frac{\text{cells}}{10\text{L}}$ |
|-------|--------|--------|--------|-----------|--------------------|----------------------|----------------------|----------------------|-----------|-----------------------------------|
| 1     | 208    | 2      | 224    | 2         | 209                | 2                    | 219.7                | 2                    | 2127 / ml | 4.7                               |
| 12:45 | 2      | 228    | 1      | 184       | 3                  | 188.                 | 0                    | 200                  | 1.3       | 200                               |
| 13:20 | 3      | 198    | 188    | 0.3       | 190                | 1                    | 231                  | 2                    | 206.3     | 4.8                               |
| 13:55 | 4      | 207    | 0      | 222       | 0                  | 199                  | 2                    | 209.3                | 6         | 2093                              |
| 14:15 | 5      | 148    | 0      | 199       | 0                  | 180                  | 0                    | 175.6                | 0         | 1757                              |
| 14:45 | 6      | 192    | 0      | 182       | 0                  | 197                  | 0                    | 190.3                | 0         | 1903                              |
|       |        |        |        |           |                    |                      |                      |                      |           | 5.3                               |

- add 1ml of EtOH, ringdry / ml, renew / 0.5ml
- ✓ - RNA later  $\pm$  snap freeze 16:30
- ✓ - formalin fix 18:30
- ✓ - cell counts
- ✓ - algal feed 7 gal CGW + 7 gal TDSO @ 17:15
- ✓ - filter rinse (in line) - 19:45
- ✓ - tank upper check - 19:45
- ✓ - pH check 19:45
- ✓ - temp check 19:45
- ✓ - pump rate 105 rpm 19:45

| task | count 1 | count 2 | $\bar{x}$ | cells/ml | 1.5ml sample                    |
|------|---------|---------|-----------|----------|---------------------------------|
| 1    | 113     | 150     | 131.5     | 146,111  | Counted 9.0x10 <sup>-4</sup> ml |
| 2    | 117     | 123     | 120       | 133,333  |                                 |
| 3    | 128     | 134     | 131       | 145,555  |                                 |
| 4    | 136     | 146     | 126       | 140,000  |                                 |
| 5    | 133     | 127     | 130       | 144,444  |                                 |
| 6    | 153     | 139     |           |          |                                 |

with us for genomics & morphometrics

| Suspended<br>Vol mL | EPI tube | In tube    | RNA later | -80 |
|---------------------|----------|------------|-----------|-----|
| 4.7<br>500          | 5, 15    | 6, 7, 8    | 5         | 15  |
| 5<br>520            | 6, 16    | 9, 10, 11  | 6         | 16  |
| 5.8<br>500          | 7, 8     | 12, 13, 14 | 7         | 8   |
| 7<br>500            | 9, 10    | 15, 16, 17 | 9         | 10  |
| 7.3<br>500          | 11, 12   | 18, 19, 20 | "         | 12  |
|                     | 13, 14   | 21, 22, 23 | 13        | 14  |

\* Had 10 drop tanks / # 2 twice as  
only one molecular sample was  
collected in the initial drop

[Draft 4 \* Salinity 4 in tank 4

Draft 3 \* Salinity 3 in tank 2

(→ 15:00 on 10 March 18

11 March 16

- 10:00 - replaced temp probe in tank 1, was sitting in beaker on counter  
10:15 - added ~5gai of Cuv + 5gai 60g  
- noticed larvae in surface tension in tanks, rinsed them down  
: tank 2, tank 3, tank 5, tank 6  
- tank 3 probe getting pulled out by magnet or card, secured card  
14:10 - rinsed, vortex rinse, changed baggies  
14:15 - rinsed valve filters & replaced  
14:20 - turned algae feed rate to 90 rpm  
17:35 - daily checklist

11 March 16 MS Buffer calibration 2/11/16 small tube

| sal   | Temp °C |
|-------|---------|
| -90.3 | 11.50   |
| -89.6 | 12.07   |
| -88.5 | 12.75   |
| -87.6 | 13.28   |
| -86.6 | 13.68   |
| -86.0 | 14.54   |
| -85.4 | 15.01   |

16:30 - checked man under scope  
at 2nd level very each time  
@ Seminars & spoke

12 March 16

Heated water pump went off in the night  
The reservoir tank dropped ~1 ft. / no issues  
with pH or temp

pH probe calibration

| mv    | Temp: °C |
|-------|----------|
| -89.4 | 12.78    |
| -89.0 | 13.09    |
| -88.7 | 13.36    |
| -88.1 | 13.76    |
| -87.5 | 14.16    |
| -87.0 | 14.50    |
| -86.5 | 14.86    |
| -86.0 | 15.17    |

Added Sgal of C50 + Sgal of GSW

- 11:05 dropping & sampling  
- range, vertex, nose bbl w/ 100um & 100um filter  
and saucer and siphon  
- attach sw line  
- drop tank while rinsing sides & bang  
- pour through  
- 100 → 160 rinse  
- 60 rinse into tipur into 500ml  
- cont 100ml w/ plunger  
- sample 2x 10,000 ml

12.15 hrs 00 yesterday samples

cell counts - Sampled 3/m<sup>-2</sup> counted 3/13

12 March 16 ttp

tank dropping & Sampling

24

| TIME  | TANK | A   | D  | A   | D  | PP [alluvial] | ml for 10K |
|-------|------|-----|----|-----|----|---------------|------------|
| 12:25 | 3    | 161 | 23 | 179 | 29 | 153           | 26 164.3   |
| 13:25 | 2    | 161 | 21 | 144 | 38 | 149           | 29 151.3   |
| 14:30 | 1    | 129 | 36 | 160 | 23 | 138           | 15 142.3   |
| 15:35 | 4    | 183 | 13 | 171 | 9  | 186           | 11 180.1   |
| 16:55 | 5    | 160 | 29 | 141 | 19 | 150           | 20 150.3   |
| 18:00 | 6    | 177 | 27 | 174 | 29 | 174           | 31 150.3   |
|       |      |     |    |     |    |               | 1720       |

Wt Duraset 3 in tank 6  
Wt Duraset 4 in tank 1

1 1 25

| <u>Individual</u> | <u>EPI #</u> | <u>Morph Type</u> | <u>RNAmate</u> | <u>#</u> |
|-------------------|--------------|-------------------|----------------|----------|
| 500               | 17, 18       | 33, 34, 35        | 17             | 18       |
| 500               | 19, 20       | 36, 37, 38        | 19             | 20       |
| 500               | 21, 22       | 39, 40, 41        | 21             | 22       |
| 500               | 23, 24       | 30, 31, 32        | 23             | 24       |
| 500               | 25, 26       | 27, 28, 29        | 25             | 26       |
| 500               | 27, 28       | 24, 25, 26        | 27             | 28       |

13 March 16 Daylight Savings  
11:15 - checked system, all is well temp & stacko

| mV    | Temp °C |
|-------|---------|
| -93.6 | 9.73    |
| -92.4 | 10.58   |
| -91.2 | 11.45   |
| -90.6 | 11.89   |
| -89.8 | 12.46   |
| -89.2 | 12.90   |
| -88.6 | 13.38   |
| -88.3 | 13.65   |
| -87.1 | 14.02   |
| -86.4 | 14.98   |

### cell counts

| Tank | cont | count | $\bar{x}$ | cells/ml |
|------|------|-------|-----------|----------|
| 1    | 31   | 35    | 33        | 36,606   |
| 2    | 42   | 32    | 37        | 41,111   |
| 3    | 47   | 48    | 47.5      | 52,777   |
| 4    | 28   | 31    | 33.5      | 37,222   |
| 5    | 37   | 38    | 37.5      | 41,666   |
| 6    | 51   | 47    |           |          |

16:45 mixed culture  
17:00 added Sgal 60g + Sgal CGW  
17:15 mixed feed pump up to 100 rpm  
water in

| Date  | Time  | Bottle | Tank  | Temp  | pH    | Salinity | cells |
|-------|-------|--------|-------|-------|-------|----------|-------|
| 3/13  | 12:45 | 81     | H1    | 13.33 | -29.2 | 27.4     | X     |
|       | "     | 82     | H1    | "     | "     | "        | X     |
|       | 12:50 | 83     | H2    | 13.34 | -58.7 | 27.4     | X     |
|       | 84    | H2     |       | "     | "     | "        | X     |
|       | 12:57 | 91     | T4    | 13.89 | -29.9 | 27.4     | ✓     |
|       | 92    | T4     |       | "     | "     | "        | "     |
| 3.02  | 93    | T5     | 13.89 | -30.0 | 27.5  | ✓        |       |
|       | 94    | T5     |       | "     | "     | "        | "     |
| 13:14 | 85    | T1     | 13.89 | -30.4 | 27.5  | ✓        |       |
|       | 86    | T1     |       | "     | "     | "        | "     |
| 13:20 | 87    | T2     | 13.90 | -58.5 | 27.4  | ✓        |       |
|       | 88    | T2     |       | "     | "     | "        | "     |
|       | 13:25 | 89     | T3    | 13.95 | -58.5 | 27.4     | ✓     |
|       | 90    | T3     |       | "     | "     | "        | "     |

14 March 16 hr

- pump mapped overnight
- temp low @ 09:15

Pt probe calibration  
3/14/16 ms bottle batch 4/14/16 10:30

| mV    | Temp C |
|-------|--------|
| -94.8 | 1.32   |
| -93.7 | 0.20   |
| -92.5 | 11.07  |
| -91.4 | 11.74  |
| -90.7 | 12.31  |
| -89.9 | 12.92  |
| -89.1 | 13.45  |
| -88.3 | 14.01  |
| -87.5 | 14.73  |
| -86.4 | 15.55  |

| Time  | Tank | A D | A D | A D | A D | cells/ml | ml/g |
|-------|------|-----|-----|-----|-----|----------|------|
| 13:00 | T4   | 194 | 10  | 126 | 14  | 150      | 17   |
| 14:15 | +S   | 88  | 31  | 82  | 26  | 115      | 44   |
| 15:35 | T6   | 67  | 88  | 76  | 86  | 63       | 84   |
| 17:00 | T3   | 45  | 38  | 54  | 124 | 68.7     | 86   |
| 18:30 | T2   | 33  | 132 | 27  | 159 | 26       | 138  |
| 19:30 | T1   | 46  | 141 | 25  | 123 | 31       | 126  |
|       |      |     |     |     |     | 102      | 160  |
|       |      |     |     |     |     |          | 7.9  |

- 5 gal 60g 5 gal COW 21:00
- cleared seed tank 21:00
- culate explosion in ambient treatment with high mortality

| Date      | Time          | Bottle   | Tank      | Temp  | pH    | Sal   | Cells |
|-----------|---------------|----------|-----------|-------|-------|-------|-------|
| 3/4       | 10:31         | 97       | H1        | 13.11 | -31.0 | 27.43 | NA    |
|           |               | 98       | H1        | "     | "     | "     | NA    |
|           | 10:35         | 99       | H2        | 13.10 | -57.6 | 27.4  | NA    |
|           |               | 100      | H2        | "     | "     | "     | "     |
|           | 10:40         | 103      | T2        | 13.31 | -57.6 | 27.3  | ✓     |
|           |               | 104      | T2        | "     | "     | "     | "     |
|           | 10:46         | 101      | T1        | 13.30 | -30.3 | 27.3  | ✓     |
|           |               | 102      | T1        | "     | "     | "     | "     |
|           | 10:55         | 105      | T3        | 13.39 | -57.3 | 27.3  | ✓     |
|           |               | 106      | T3        | "     | "     | "     | "     |
|           | 10:58         | 107      | T4        | 13.26 | -29.7 | 27.3  | ✓     |
|           |               | 108      | T4        | "     | "     | "     | "     |
|           | 11:05         | 109      | T5        | 13.35 | -29.9 | 27.3  | ✓     |
|           |               | 110      | T5        | "     | "     | "     | "     |
|           | 11:15         | 111      | T6        | 13.49 | -57.2 | 27.3  | ✓     |
|           |               | 112      | T6        | "     | "     | "     | "     |
|           | fixed @ 11:45 |          |           |       |       |       |       |
| m   resus | # EPI         | # Morph  | RNT later | -80   |       |       |       |
| 500       | 29,30         | 42,43,44 | 29        | 30    |       |       |       |
| 500       | 31,32         | 45,46,47 | 31        | 32    |       |       |       |
| 500       | 33,34         | 48,49,50 | 33        | 34    |       |       |       |
| 500       | 35,36         | 51,52,53 | 35        | 36    |       |       |       |
| 500       | 37,38         | 54,55,56 | 37        | 38    |       |       |       |
| 500       | 39,40         | 57,58,59 | 39        | 40    |       |       |       |

15 March 16 hr

09:15 - added 5 gal 60g + 8 gal COW  
0.2 ml subsamples from 800 ml

| Tank           | A       | D       | A       | D       | A       | D       |
|----------------|---------|---------|---------|---------|---------|---------|
| High           | 215     | 215     | 236     | 83      | 228     | 62      |
| Flow rate / ml | 113 l   |
| ml / tank      | 150,800 | 150,800 | 150,800 | 150,800 | 150,800 | 150,800 |
| total          | 908,800 | 908,800 | 908,800 | 908,800 | 908,800 | 908,800 |
| each           | 133 ml  |

11:00 - 15:00 - dropped & cleaned all tanks  
pooled tanks 1, 4, 5 (high)

② Direct 4 # Salinity 4 in tank's  
B.15 Durafet 3 # Sahmty 3 in tank 6

### 16 March 16 hr

| Time  | Tank  | Temp  | PT    | Salt |
|-------|-------|-------|-------|------|
| 9:30  | 11    | 24.78 | -28.6 | 27.3 |
| 12    | 14.64 | -11.6 | 27.3  |      |
| 13    | 14.11 | -57.7 | 27.3  |      |
| 14    | 14.06 | -28.5 | 27.3  |      |
| 15    | 14.00 | -57.7 | 27.3  |      |
| 19:55 | 16    | 14.28 | -11.0 | 27.3 |

tank 5 flow split for seed tank  
tank 6 flow split for seed tank

16 March 16:10

- 9:15 - cleaned tanks  
 - prepped for sand tanks

6 tanks

~~45~~ numbers per tank

Sampled for genetic

@ 18:15

| tank | # seed | sampled   | 4              |
|------|--------|-----------|----------------|
| 11   | 44     | EPI       | 40, 42, 43, 44 |
| 12   | 44     | RNA later | -80            |
| 13   | 44     |           |                |
| 14   | 44     |           |                |
| 15   | 42     |           |                |
| 16   | 44     |           |                |

dippers set to 2gph

Feed line 1 = 100SPM  
 2 = 20SPM @ 20:00

Treatment setup

|    | Amb | Low       |
|----|-----|-----------|
| 13 | 14  |           |
| 12 | 15  | Amb       |
| 11 | 16  | Super Low |

Front

(H2)

(H1)

(R)

16 March 16 tpp 16 March 16 tpp

- Initiated juvenile experiment with 3 pH levels
- and 2 rep tanks per level
- Installed 6 flat + tanks with 1 inch of sand from Taylor

17 March 16 tpp

- added Sgal 60g + Sgal 60g to feed tank

- 1st tank temps for seed

pH tris calibration

| Tank | Temp °C | MV    | Temp °C |
|------|---------|-------|---------|
| 11   | 13.42   | -95.1 | 9.01    |
| 12   | 13.43   | -94.0 | 9.81    |
| 13   | 13.44   | -93.3 | 10.25   |
| 14   | 13.43   | -92.6 | 10.87   |
| 15   | 13.41   | -91.9 | 11.47   |
| 16   | 13.44   | -91.1 | 11.90   |
|      |         | -90.6 | 12.36   |
|      |         | -90.0 | 12.80   |
|      |         | -89.1 | 13.47   |
|      |         | -88.2 | 14.21   |
|      |         | -87.5 | 14.67   |
|      |         | -87.1 | 14.96   |

turned pump 2 down to 10 spm @ 13:00  
turned pump 2 down to 2 spm @ 18:00

| Date<br>3/17 | Time     | Bottle | Tank | Temp  | pH    | sal  | cols |
|--------------|----------|--------|------|-------|-------|------|------|
|              | 14:13:05 | 123    | T4   | 13.80 | -29.5 | 27.4 | ✓    |
|              | 124      | T4     | "    | "     | "     | "    | "    |
|              | 13:20    | 125    | TS   | 13.84 | -30.4 | 27.4 | ✓    |
|              |          | 126    | TS   | "     | "     | "    | "    |
|              | 13:28    | 127    | T6   | 13.87 | -30.1 | 27.5 | ✓    |
|              |          | 128    | T6   | "     | "     | "    | "    |
|              | 13:32    | 117    | T1   | 13.86 | -30.1 | 27.5 | ✓    |
|              |          | 118    | T1   | "     | "     | "    | "    |
|              | 13:40    | 119    | T2   | 13.81 | -9.5  | 27.4 | ✓    |
|              |          | 120    | T2   | "     | "     | "    | "    |
|              | 13:45    | 121    | T3   | 13.92 | -9.8  | 27.4 | ✓    |
|              |          | 122    | T3   | "     | "     | "    | "    |
|              | 13:50    | 113    | H1   | 13.24 | -27.9 | 27.3 | NA   |
|              |          | 114    | H1   | "     | "     | "    | NA   |
|              | 13:54    | 115    | H2   | 13.27 | -9.0  | 27.4 | NA   |
|              |          | 116    | H2   | "     | "     | "    | NA   |
|              | 13:57    | 139    | T16  | 14.07 | -8.3  | 27.4 | ✓    |
|              |          | 140    | T16  | "     | "     | "    | "    |
|              | 14:10    | 137    | T15  | 14.08 | -57.7 | 27.3 | ✓    |
|              |          | 138    | T15  | "     | "     | "    | "    |
|              | 14:15    | 135    | T14  | 14.00 | -28.7 | 27.4 | ✓    |
|              |          | 136    | T14  | "     | "     | "    | "    |
|              | 14:17    | 133    | T13  | 14.06 | -58.0 | 27.4 | ✓    |
|              |          | 134    | T13  | "     | "     | "    | "    |
|              | 14:23    | 131    | T12  | 14.12 | -10.0 | 27.4 | ✓    |
|              |          | 132    | T12  | "     | "     | "    | "    |
|              | 14:25    | 129    | T11  | 13.86 | -29.2 | 27.4 | ✓    |
|              |          | 130    | T11  | "     | "     | "    | "    |

fixed @ 15:05

| Tank | cell counts in 1.5ml samples | count 0.0009 ml |
|------|------------------------------|-----------------|
| 1    | 53                           | 58,333          |
| 2    | 51                           | 65,000          |
| 3    | 40                           | 45,000          |
| 4    | 46                           | 40.5            |
| 5    | 41                           | 43.5            |
| 6    | 56                           | 51,667          |
| 11   | 77                           | 60.5            |
| 12   | 88                           | 67,222          |
| 13   | 240                          | 71,666          |
| 14   | 71                           | 71,111          |
| 15   | 245                          | 286,667         |
| 16   | 51                           | 63,333          |

18:00 - added 2.5gal 6G W & 2.5gal 609

+ 2.3

18:30 sampled seed Iggeduck per tube

| Tank | ERL tube | PVA later | 80 |
|------|----------|-----------|----|
| 11   | 45 46    | 45        | 46 |
| 12   | 47 48    | 47        | 48 |
| 13   | 49 50    | 49        | 50 |
| 14   | 51 52    | 51        | 52 |
| 15   | 53 54    | 53        | 54 |
| 16   | 55 56    | 55        | 56 |

fixed & run C 19:15

18 March 16 H.P.  
picked up ~6mll ~~geoduck~~ & Taylor  
@ 08:30

~~Trapping~~

~~chow~~

10:40 - 11:40

12 - 8 drop sample clean

~~count & add~~

8-10

11:15

- drop tanks & place in 6 beakers - 14:00
- clear all & fill and allocate 1 bucket per tank of new larvae.

- 15:00 collected 200 $\mu$ l from 250 ml beakers

OC - trial #2 larvae

| Tank Count | A    | D    | A  | D    | A  | D    | A    | D     | Avg   | ml    |
|------------|------|------|----|------|----|------|------|-------|-------|-------|
| 1          | 24   | 23.0 | 17 | 18.7 | 2  | 20.4 | 20.7 | 20.7  | 103.5 | 96.6  |
| 2          | 64   | 68   | 64 | 61   | 35 | 84   | 54   | 71    | 270   | 37    |
| 3          | 46.6 | 46.8 | 7  | 54.8 | 7  | 40   | 64   | 46.7  | 79.3  | 233.5 |
| 4          | 15   | 24   | 22 | 23.2 | 19 | 20.7 | 18.7 | 217.6 | 93.5  | 106.9 |
| S          | 18   | 19.0 | 12 | 20.8 | 12 | 20.1 | 14   | 199.7 | 70    | 142.9 |
| b          | 42   | 112  | 45 | 102  | 43 | 89   | 43.3 | 101   | 216.7 | 46    |

- added 1 bucket of new larvae per tank for trial 3

| #<br>Treat | Tank | Alive | Tank | EPI<br>EPI later<br>-80 | EPI<br>cells/ml<br>for 10K | Morph    |
|------------|------|-------|------|-------------------------|----------------------------|----------|
| 7.3        | 1    | 20.7  | 1    | 57                      | 58                         | 60,61,62 |
| 7.3        | 4    | 18.7  | 2    | 59                      | 60                         | 63,64,65 |
| 7.3        | 5    | 14    | 3    | 61                      | 62                         | 66,67,68 |
| 7.0        | 2    | 54.0  | 4    | 63                      | 64                         | 69,70,71 |
| 7.0        | 3    | 46.7  | 5    | 65                      | 66                         | 72,73,74 |
| 7.0        | 6    | 43.3  | 6    | 67                      | 68                         | 75,76,77 |

18 March 16 ttp

| Type      | Sample |
|-----------|--------|
| -80       | EP169  |
| -80       | 70     |
| -80       | 71     |
| R.M.Later | 73     |
| -80       | 74     |
| -80       | 75     |

Super low (2, 3, 6)  
Super low (2, 3, 6)  
low (14) used up all of S on previous page  
To new batch from Taylor 18 March 16

Fed Sagal Cow + Sagal 60g  
changed Sun and Sun inline filters  
washed vango and trial Lamb remaining larvae  
- turned fed pump 1 to 90 rpm  
- turned fed pump 2 to 45 rpm

19 March 16 ttp

| MY    | Temp C |
|-------|--------|
| -94.4 | 94.4   |
| -93.4 | 94.4   |
| -92.6 | 94.5   |
| -92.1 | 94.7   |
| -91.5 | 94.7   |
| -90.8 | 94.7   |
| -90.0 | 94.6   |
| -89.5 | 94.7   |
| -89.0 | 94.7   |
| -88.6 | 94.7   |
| -87.6 | 94.7   |

19 March 1640ft bottle

Date Time Bottle Temp ptt Sal

3/19 12:30 H1 141 13.31 -28.5 25.8

12:30 H1 142 " " "

12:33 H2 143 13.31 -28.8.0 25.8

H2 144 " " "

12:35 T4 1521 13.91 -28.8 25.8 ✓

T4 1522 13.91 " " "

12:46 TS 153 13.93 -29.0 25.8 ✓

TS 154 " " " "

12:58 T4 155 14.01 -28.7.8 25.7 ✓

T6 156 " " " "

13:03 T1 145 13.95 -28.8 25.7 ✓

T1 146 " " " "

13:07 T2 147 13.98 -8.1 25.7 ✓

T2 148 " " " "

13:13 T3 149 14.02 -8.0 25.7 ✓

T3 150 " " " "

13:20 T11 157 13.84 -28.2 25.8 ✓

T11 158 " " " "

13:25 T12 159 13.83 -7.0 25.9 ✓

T12 160 " " " "

13:29 T13 161 13.84 -56.8 26.1 ✓

T13 162 " " " "

13:31 T14 163 13.84 -28.7 25.8 ✓

T14 164 " " " "

13:35 T15 165 13.84 -57.1 26.2 ✓

T15 166 " " " "

T16 167 13.86 -7.8 25.8

fixed @ 14:00

38

|      | 1.5m samples | cont'd 0.0009m |
|------|--------------|----------------|
| Tank | cont 1       | cont 2         |
| 1    | 48           | 53,333         |
| 2    | 58           | 121,60.5       |
| 3    | 72           | 67,222         |
| 4    | 61           | 59             |
| 5    | 49           | 65,555         |
| 6    | 66           | 54.5           |
| 7    | 50           | 60,555         |
| 8    | 56           | 53             |
| 9    | 49           | 58,888         |
| 10   | 44           | 44,444         |
| 11   | 49           | 48,888         |
| 12   | 44           | 48,888         |
| 13   | 44           | 44,444         |
| 14   | 49           | 49,444         |
| 15   | 49           | 53.5           |
| 16   | 50           | 58             |
| 17   | 40,000       | 50,555         |
| 18   | 36           | 45.5           |
| 19   | 49           | 49,444         |
| 20   | 50,000       | 55,555         |
| 21   | 58           | 53.5           |
| 22   | 50           | 58             |
| 23   | 49           | 49,444         |
| 24   | 44           | 44,444         |
| 25   | 49           | 49,444         |
| 26   | 44           | 44,444         |
| 27   | 49           | 49,444         |
| 28   | 44           | 44,444         |
| 29   | 49           | 49,444         |
| 30   | 44           | 44,444         |
| 31   | 49           | 49,444         |
| 32   | 44           | 44,444         |
| 33   | 49           | 49,444         |
| 34   | 44           | 44,444         |
| 35   | 49           | 49,444         |
| 36   | 44           | 44,444         |
| 37   | 49           | 49,444         |
| 38   | 44           | 44,444         |
| 39   | 49           | 49,444         |
| 40   | 44           | 44,444         |
| 41   | 49           | 49,444         |
| 42   | 44           | 44,444         |
| 43   | 49           | 49,444         |
| 44   | 44           | 44,444         |
| 45   | 49           | 49,444         |
| 46   | 44           | 44,444         |
| 47   | 49           | 49,444         |
| 48   | 44           | 44,444         |
| 49   | 49           | 49,444         |
| 50   | 44           | 44,444         |
| 51   | 49           | 49,444         |
| 52   | 44           | 44,444         |
| 53   | 49           | 49,444         |
| 54   | 44           | 44,444         |
| 55   | 49           | 49,444         |
| 56   | 44           | 44,444         |
| 57   | 49           | 49,444         |
| 58   | 44           | 44,444         |
| 59   | 49           | 49,444         |
| 60   | 44           | 44,444         |
| 61   | 49           | 49,444         |
| 62   | 44           | 44,444         |
| 63   | 49           | 49,444         |
| 64   | 44           | 44,444         |
| 65   | 49           | 49,444         |
| 66   | 44           | 44,444         |
| 67   | 49           | 49,444         |
| 68   | 44           | 44,444         |
| 69   | 49           | 49,444         |
| 70   | 44           | 44,444         |
| 71   | 49           | 49,444         |
| 72   | 44           | 44,444         |
| 73   | 49           | 49,444         |
| 74   | 44           | 44,444         |
| 75   | 49           | 49,444         |
| 76   | 44           | 44,444         |
| 77   | 49           | 49,444         |
| 78   | 44           | 44,444         |
| 79   | 49           | 49,444         |
| 80   | 44           | 44,444         |
| 81   | 49           | 49,444         |
| 82   | 44           | 44,444         |
| 83   | 49           | 49,444         |
| 84   | 44           | 44,444         |
| 85   | 49           | 49,444         |
| 86   | 44           | 44,444         |
| 87   | 49           | 49,444         |
| 88   | 44           | 44,444         |
| 89   | 49           | 49,444         |
| 90   | 44           | 44,444         |
| 91   | 49           | 49,444         |
| 92   | 44           | 44,444         |
| 93   | 49           | 49,444         |
| 94   | 44           | 44,444         |
| 95   | 49           | 49,444         |
| 96   | 44           | 44,444         |
| 97   | 49           | 49,444         |
| 98   | 44           | 44,444         |
| 99   | 49           | 49,444         |
| 100  | 44           | 44,444         |
| 101  | 49           | 49,444         |
| 102  | 44           | 44,444         |
| 103  | 49           | 49,444         |
| 104  | 44           | 44,444         |
| 105  | 49           | 49,444         |
| 106  | 44           | 44,444         |
| 107  | 49           | 49,444         |
| 108  | 44           | 44,444         |
| 109  | 49           | 49,444         |
| 110  | 44           | 44,444         |
| 111  | 49           | 49,444         |
| 112  | 44           | 44,444         |
| 113  | 49           | 49,444         |
| 114  | 44           | 44,444         |
| 115  | 49           | 49,444         |
| 116  | 44           | 44,444         |
| 117  | 49           | 49,444         |
| 118  | 44           | 44,444         |
| 119  | 49           | 49,444         |
| 120  | 44           | 44,444         |
| 121  | 49           | 49,444         |
| 122  | 44           | 44,444         |
| 123  | 49           | 49,444         |
| 124  | 44           | 44,444         |
| 125  | 49           | 49,444         |
| 126  | 44           | 44,444         |
| 127  | 49           | 49,444         |
| 128  | 44           | 44,444         |
| 129  | 49           | 49,444         |
| 130  | 44           | 44,444         |
| 131  | 49           | 49,444         |
| 132  | 44           | 44,444         |
| 133  | 49           | 49,444         |
| 134  | 44           | 44,444         |
| 135  | 49           | 49,444         |
| 136  | 44           | 44,444         |
| 137  | 49           | 49,444         |
| 138  | 44           | 44,444         |
| 139  | 49           | 49,444         |
| 140  | 44           | 44,444         |
| 141  | 49           | 49,444         |
| 142  | 44           | 44,444         |
| 143  | 49           | 49,444         |
| 144  | 44           | 44,444         |
| 145  | 49           | 49,444         |
| 146  | 44           | 44,444         |
| 147  | 49           | 49,444         |
| 148  | 44           | 44,444         |
| 149  | 49           | 49,444         |
| 150  | 44           | 44,444         |
| 151  | 49           | 49,444         |
| 152  | 44           | 44,444         |
| 153  | 49           | 49,444         |
| 154  | 44           | 44,444         |
| 155  | 49           | 49,444         |
| 156  | 44           | 44,444         |
| 157  | 49           | 49,444         |
| 158  | 44           | 44,444         |
| 159  | 49           | 49,444         |
| 160  | 44           | 44,444         |
| 161  | 49           | 49,444         |
| 162  | 44           | 44,444         |
| 163  | 49           | 49,444         |
| 164  | 44           | 44,444         |
| 165  | 49           | 49,444         |
| 166  | 44           | 44,444         |
| 167  | 49           | 49,444         |
| 168  | 44           | 44,444         |
| 169  | 49           | 49,444         |
| 170  | 44           | 44,444         |
| 171  | 49           | 49,444         |
| 172  | 44           | 44,444         |
| 173  | 49           | 49,444         |
| 174  | 44           | 44,444         |
| 175  | 49           | 49,444         |
| 176  | 44           | 44,444         |
| 177  | 49           | 49,444         |
| 178  | 44           | 44,444         |
| 179  | 49           | 49,444         |
| 180  | 44           | 44,444         |
| 181  | 49           | 49,444         |
| 182  | 44           | 44,444         |
| 183  | 49           | 49,444         |
| 184  | 44           | 44,444         |
| 185  | 49           | 49,444         |
| 186  | 44           | 44,444         |
| 187  | 49           | 49,444         |
| 188  | 44           | 44,444         |
| 189  | 49           | 49,444         |
| 190  | 44           | 44,444         |
| 191  | 49           | 49,444         |
| 192  | 44           | 44,444         |
| 193  | 49           | 49,444         |
| 194  | 44           | 44,444         |
| 195  | 49           | 49,444         |
| 196  | 44           | 44,444         |
| 197  | 49           | 49,444         |
| 198  | 44           | 44,444         |
| 199  | 49           | 49,444         |
| 200  | 44           | 44,444         |
| 201  | 49           | 49,444         |
| 202  | 44           | 44,444         |
| 203  | 49           | 49,444         |
| 204  | 44           | 44,444         |
| 205  | 49           | 49,444         |
| 206  | 44           | 44,444         |
| 207  | 49           | 49,444         |
| 208  | 44           | 44,444         |
| 209  | 49           | 49,444         |
| 210  | 44           | 44,444         |
| 211  | 49           | 49,444         |
| 212  | 44           | 44,444         |
| 213  | 49           | 49,444         |
| 214  | 44           | 44,444         |
| 215  | 49           | 49,444         |
| 216  | 44           | 44,444         |
| 217  | 49           | 49,444         |
| 218  | 44           | 44,444         |
| 219  | 49           | 49,444         |
| 220  | 44           | 44,444         |
| 221  | 49           | 49,444         |
| 222  | 44           | 44,444         |
| 223  | 49           | 49,444         |
| 224  | 44           | 44,444         |
| 225  | 49           | 49,444         |
| 226  | 44           | 44,444         |
| 227  | 49           | 49,444         |
| 228  | 44           | 44,444         |
| 229  | 49           | 49,444         |
| 230  | 44           | 44,444         |
| 231  | 49           | 49,444         |
| 232  | 44           | 44,444         |
| 233  | 49           | 49,444         |
| 234  | 44           | 44,444         |
| 235  | 49           | 49,444         |
| 236  | 44           | 44,444         |
| 237  | 49           | 49,444         |
| 238  | 44           | 44,444         |
| 239  | 49           | 49,444         |
| 240  | 44           | 44,444         |
| 241  | 49           | 49,444         |
| 242  | 44           | 44,444         |
| 243  | 49           | 49,444         |
| 244  | 44           | 44,444         |
| 245  | 49           | 49,444         |
| 246  | 44           | 44,444         |
| 247  | 49           | 49,444         |
| 248  | 44           | 44,444         |
| 249  | 49           | 49,444         |
| 250  | 44           | 44,444         |
| 251  | 49           | 49,444         |
| 252  | 44           | 44,444         |
| 253  | 49           | 49,444         |
| 254  | 44           | 44,444         |
| 255  | 49           | 49,444         |
| 256  | 44           | 44,444         |
| 257  | 49           | 49,444         |

|   |   |   |   |   |
|---|---|---|---|---|
| 9 | 5 | 4 | 3 | 7 |
| 6 | 2 | 8 | 1 | 5 |
| 7 | 3 | 9 | 4 | 6 |
| 8 | 1 | 5 | 2 | 3 |
| 9 | 4 | 6 | 7 | 8 |

6.45 cleaned bands  
7.00 added 1 gal. water  
7.30 fed them \$4.00  
8.00 checked pens

20 March 16 190  
10:30 added Sgal 66W and Sgal 609 to feed tank

pH calibration w/ 7ns standard

| <u>MV</u> | <u>Temp.</u> | <u>pH</u> | <u>5 min</u> | <u>1 min</u> | <u>1 min</u> |
|-----------|--------------|-----------|--------------|--------------|--------------|
| -95.2     | 9.68         | 41        | 5.94         | 1.84         | 1.44         |
| -94.5     | 10.21        |           |              |              |              |
| -94.0     | 10.58        |           |              |              |              |
| -93.2     | 11.23        |           |              |              |              |
| -92.2     | 11.95        |           |              |              |              |
| -91.4     | 12.48        |           |              |              |              |
| -90.5     | 13.19        |           |              |              |              |
| -89.6     | 13.88        |           |              |              |              |
| -89.0     | 14.29        |           |              |              |              |
| -88.7     | 14.58        |           |              |              |              |

Date Time Tank Bottle Temp. pH Sal Cells

| 3/20 | 12:10 | H1   | 16.9  | 13.30 | -26.9 | 26.2 | NA |
|------|-------|------|-------|-------|-------|------|----|
|      |       | H1   | 17.0  | "     | "     | "    | NA |
|      |       | H2   | 17.1  | 13.31 | -5.6  | 26.2 | NA |
|      |       | H2   | 17.2  | "     | "     | "    | NA |
|      | 12:12 | T2   | 17.1  |       |       |      |    |
|      | 12:16 | T3   | 17.7  | 13.85 | -8.0  | 26.2 | ✓  |
|      | 12:24 | T2   | 17.5  | 13.88 | -8.0  | 26.2 | ✓  |
|      | 12:28 | T1   | 17.3  | 13.90 | -29.4 | 26.2 | ✓  |
|      | 12:32 | T4   | 17.9  | 13.87 | -29.1 | 26.1 | ✓  |
|      | 12:35 | T5   | 18.0  | "     | "     | "    | "  |
|      | T5    | 18.2 | "     | "     | "     | "    | "  |
|      | T6    | 18.3 | 13.95 | -8.3  | 26.1  | ✓    |    |
|      | T6    | 18.4 | "     | "     | "     | "    | "  |

20 March 16 HP # SR

| Time  | Tank | A   | D  | A   | D  | A   | D  | $\frac{\text{cells/ml}}{876.7}$ | ML<br>LOCK |
|-------|------|-----|----|-----|----|-----|----|---------------------------------|------------|
| 13:00 | T1   | 173 | 12 | 167 | 9  | 186 | 6  | 1753                            | 11.4       |
|       | T2   | 116 | 03 | 127 | 01 | 135 | 03 | 126                             | 630        |
|       | T3   | 158 | 02 | 149 | 02 | 140 | 02 | 148.3                           | 741.6      |
|       | T4   | 135 | 5  | 128 | 3  | 132 | 1  | 131.7                           | 659.3      |
|       | T5   | 144 | 1  | 143 | 1  | 144 | 2  | 143.6                           | 718        |
| 17:00 | T6   | 152 | 3  | 146 | 2  | 147 | 3  | 148.3                           | 741.6      |
|       |      |     |    |     |    |     |    |                                 | 13.5       |

- counted 0.2 ml
- added 2 gal C6W # 2 gal 609
- mixed bags on old tank (and old)
- rinsed in line filters
- checked drippers & flow
- checked juveniles
- stored samples @ 4°C until later -80 # 4°C
- in formalin

| <u>resuspended</u> | <u>Epi tubett</u> | <u>Morph Dubett</u> | <u>RNA later</u> | <u>-80</u> |
|--------------------|-------------------|---------------------|------------------|------------|
| 800                | 76, 77            | 78, 79, 80          | 76               | 77         |
| 800                | 78, 79            | 81, 82, 83          | 78               | 79         |
| 800                | 80, 81            | 81, 82, 83          | 80               | 81         |
| 800                | 82, 83            | 82, 83, 84          | 82               | 83         |
| 800                | 84, 85            | 90, 91, 92          | 84               | 85         |
| 800                | 86, 87            | 93, 94, 95          | 86               | 87         |

- Samples removed to 0.1ml and added 0.5ml of 10% buffered formalin

21 March 16 thp

09:15 - dredged pH & temp & samples & flow

| Date | Time  | Tank | Bottle | Temp  | pH    | sal  | cells |
|------|-------|------|--------|-------|-------|------|-------|
| 3/21 | 11:00 | H1   | 185    | 13.33 | -28.3 | 26.7 | NA    |
|      |       | H1   | 186    | "     | "     | "    | "     |
|      | 11:04 | H2   | 1867   | 13.31 | -6.9  | 26.8 | NA    |
|      |       | H2   | 1878   | "     | "     | "    | "     |
|      | 11:08 | T1   | 1889   | 13.97 | -30.1 | 26.8 | ✓     |
|      |       | T1   | 189190 | "     | "     | "    | "     |
|      | 11:19 | T2   | 191    | 14.04 | -8.6  | 26.6 | ✓     |
|      |       | T2   | 192    | "     | "     | "    | "     |
|      | 11:26 | T3   | 193    | 14.04 | -8.6  | 26.6 | ✓     |
|      |       | T3   | 194    | "     | "     | "    | "     |
|      | 11:30 | T4   | 195    | 13.98 | -29.9 | 26.6 | ✓     |
|      |       | T4   | 196    | "     | "     | "    | "     |
|      | 11:54 | T5   | 197    | 13.93 | -30.3 | 26.6 | ✓     |
|      |       | T5   | 198    | "     | "     | "    | "     |
|      | 11:59 | T6   | 199    | 14.08 | -8.6  | 26.6 | ✓     |
|      |       | T6   | 200    | "     | "     | "    | "     |
|      | 12:02 | T11  | 201    | 13.81 | -27.1 | 26.7 | ✓     |
|      |       | T11  | 202    | "     | "     | "    | "     |
|      | 12:08 | T12  | 203    | 13.80 | -5.5  | 26.7 | ✓     |
|      |       | T12  | 204    | "     | "     | "    | "     |
|      | 12:11 | T13  | 205    | 13.84 | -57.4 | 26.8 | ✓     |
|      |       | T13  | 206    | "     | "     | "    | "     |
|      | 12:15 | T14  | 207    | 13.82 | -27.7 | 26.7 | ✓     |
|      |       | T14  | 208    | "     | "     | "    | "     |
|      | 12:18 | T15  | 209    | 13.75 | -57.7 | 26.7 | ✓     |
|      |       | T15  | 210    | "     | "     | "    | "     |
|      | 12:21 | T16  | 211    | 13.84 | -7.3  | 26.7 | ✓     |
|      |       | T16  | 212    | "     | "     | "    | "     |

pH Tns calibration

| <u>mV</u> | <u>Temp °C</u> | <u>mV</u> | <u>Temp °C</u> |
|-----------|----------------|-----------|----------------|
| -92.1     | 11.8           | 42.       | -91.1          |
| -91.5     | 12.25          |           | -91.1          |
| -91.0     | 12.61          |           | -90.7          |
| -90.6     | 12.94          |           | -90.4          |
| -90.4     | 13.37          |           | -90.0          |
| -89.6     | 13.65          |           | -89.6          |
| -89.2     | 14.00          |           | -89.3          |
| -88.7     | 14.38          |           | -88.9          |
| -88.1     | 14.83          |           | -88.6          |

calibration 1  
tanks H1-H4

calibration 2  
tanks T5-T16

cell counts of 1.5 ml samples in 0.0009 ml of

the hemocytometer

| Date collected | Tank | C1 | C2 | X    | cells/ml |
|----------------|------|----|----|------|----------|
| 3/21           | 1    | 31 | 48 | 39.5 | 43,888   |
|                | 2    | 47 | 62 | 54.5 | 60,555   |
|                | 3    | 43 | 53 | 48   | 53,333   |
|                | 4    | 40 | 60 | 50   | 55,555   |
|                | 5    | 63 | 58 | 60.5 | 67,00222 |
|                | 6    | 58 | 55 | 56.5 | 62,778   |
|                | 11   | 58 | 54 | 56   | 62,222   |
|                | 12   | 56 | 70 | 63   | 70,000   |
|                | 13   | 54 | 36 | 45   | 50,000   |
|                | 14   | 47 | 66 | 56.5 | 67,778   |
|                | 15   | 47 | 53 | 50   | 55,555   |
|                | 16   | 74 | 54 | 64   | 71,111   |

10.35  
5 gal COW + 5 gal 609  
checked pH & temp & flow  
turned up fed pump 2 to 5SPM