

3 / 1 / 16 13

SAFS OA Geoduck Epigenetics Project

NOAA Manchester

Roberts Boetz Putnam

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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 left 98.1  
 3.999 zero point 0.7 mV  
 7.052  
 10.126

pH probe calibration in Tns Batch 14Feb16 SR

Temp °C	mV	Temp °C	mV	Temp °C	mV
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

Header 2 14.16 -61.7  
 header 1 13.83 33.6

Tank T°C mV  
 tank S - H2 13.14.02 -52.3  
 tank 1 - H1 13.99 -31.3

Time 13:30

4 March 16 HTP

4

Part	Time	Tank	T °C	Pt1mV	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	13.94	-51.7	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 AUFETCH °C Temp certified v/wr °C offset  
 1 13.87 13.96  
 2 13.75 13.96  
 3 14.00 13.96  
 4 14.00 13.96  
 5 13.93 13.96  
 6 14.06 13.96  
 Header 1 13.51 0.1  
 Header 2 13.51 0.1  
 Reservoir 13.51 0.1  
 AUFETCH can 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 April

- flushed all tanks
  - drained all tanks
  - rinsed water bath probe to catch peak
  - calibrated salinity probes

27.1 @ 12°C

- 27.1 @ 12.5  
for all droplets in  
-  
checked temp & pH  
bucket of seawater = 12.4°C  
Temp = 12.4°C

9.21	b'g't	hD	H1	H2
9.5	ot't	D3	H2	H1
12.1	t'eo			
12.5	teg			
12.4	gtt			

No connections needed

Referred patient and turned over to  $\text{CO}_2$  Infection

Set Hemp to auto valve @ 12.5.c

Header + regular = Headers  
Header + 2PVL = Headers + regular  
Header + treatment = Headers + regular

sank bank filters in freshwater overnight

added bubbler for seed tank  
connected feed lines to H2O spitter

Date	Time	Tank	pH mV	Temp'c	Salinity	Flow
March 16	09:15	1	-52.8	12.09	27.4	
		2	-53.0	12.05	27.4	9-24.00
		3	-52.8	12.14	27.4	Water
		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	
		1	09:40	H2	-52.9	11.55
						27.6

placed salinity relay 4 in tank 6 & 5  
 placed durafet 4 in tank 6 & 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	0.85 Salinity
2	4

Sat @ 18:45 tanks and dosing

8 March 16 KTP

over 5 ha + CO<sub>2</sub> with  
loose aggregate

- 08.05.2010 - Achim

  - installed regulator for CO<sub>2</sub>
  - installed header tank 2 ft add new one
  - opened header tank
  - water turning pth up
  - set regulator to 30 Psi
  - labeled bottles for sun screen collection
  - sat

Date	Time	Bottle #	Tank #	pH	Temp.	Salinity
8 March	sampled from					
	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	Tank 3	-52.8	14.14	27.5
		5				
	6	3				

12:00 Algal feed mix

Sgal 609 + Sgal CGW added to feed tank

13:00 6 containers of beeduck 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 Formalin 10%
1	1	113	113
2	2	119	119
3	3	124	124
4	4	126	126
5	5	126	126

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

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

Sample	Morph	Sampling Tubes	Sample
1	M	EPI	1
2	M	EPI	2
3	M	EPI	3
4	M	EPI	4

In freezer @ 14:30  
3 ranges in EtOH (1mL)

add 1mL of PMA later to 1#2

stab 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 $\mu\text{l}$

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

$$(83.3 \text{ cells/ml})$$

16:00

- changed & rinsed all bongo filters
- checked all drippers

8 March 16 @ 18:30 settings

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

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

WV	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 16 1916

9 March 1913  
Dear friends

Cell Count B  
Cell Count C

Taw  
Cent  
lasi  
Ch

2  
1  
158  
151  
144

7  
11  
14  
15  
16

bill  
dull  
fuzz

5  
121  
511

9  
19

Centrifugal squares (9.0 x 10<sup>-13</sup> cm)

gal add 10%  
TSD 2.5 + 2.5  
2.5 + 2.5  
2.5 + 2.5

Winds still as cloudless & feet  
down turned over  
midday.

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Tube list

exp 3/10

111 15

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

cells 3/12

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

MV	Temp°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

16

Date	Time	Bottle	tank	pH	Temp	Sat.
3/10	10:15	37	Tank 1	-29.0	14.18	27.43
		38	"	"	"	"
	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

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

18

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 P	A D	A D	A P	cells/ml	cells/ml
12:45	2	228	1	184	3	188	0	200	1.3	200
13:20	3	198	88	0	3	190	1	231	2	206.3
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	1903	0	5.3

- add 1ml of EtOH, Rinsed w/ 1ml, then w/ 0.5ml
- ✓ - RNA later  $\pm$  snap freeze 16:30
- ✓ - formalin fix 18:30
- ✓ - cell counts
- ✓ - algal feed 7 gal CGW + 7 gal TNSO @ 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 9.0 $\times 10^{-4}$ 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 Vol mL	EPI tube	M tube	PNA water	-80
500	5, 15	6, 7, 8	5	15
520	6, 16	9, 10, 11	6	16
500	7, 8	12, 13, 14	7	8
500	9, 10	15, 16, 17	9	10, 11
500	11, 12	18, 19, 20	"	12, 13, 14
500	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

(→ 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 ~5g al of Cuv + 5g al 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 inline 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 | 14.10   |
| -86.0 | 14.54   |
| -85.4 | 15.01   |

1 / 1 21

Date 3/11	Time 12:00	Bottle 49	Tank Heater 1	Temp 13.34	pH 30.2	Sal 27.4
	12:07	S1	Heater 2	13.34	-57.5	27.5
		S2	Heater 2	"	"	"
	12:12	63	Tank 6	13.99	-56.6	27.5
		64	Tank 6	"	"	"
	12:17	61	Tanks	14.00	-30.5	1 27.5
	12:25	62	Tanks	"	"	"
	12:25	59	Tank 4	14.03	-30.2	27.5
		60	Tank 4	"	"	"
	12:30	55	Tank 2	14.00	-56.6	27.5
		56	Tank 2	"	"	"
	12:36	53	Tank 1	14.06	-30.3	27.5
		54	Tank 1	"	"	"
	12:42	57	Tank 3	14.05	-56.8	27.5
		58	Tank 3	"	"	"

- fixed w/ HgCl<sub>2</sub> @ 13:00

11 March 16 HgCl<sub>2</sub>

Cell counts 14.45 1.5 ml samples counted 9.0x10<sup>4</sup>

Tank	cont 1	cont 2	X	cells/ml
1	68	82	75	83,333
2	65	72	68.5	76,111
3	77	66	71.5	79,444
4	81	77	79	87,777
5	74	61	67.5	75,000
6	72	62	67	74,444

16:30 - checked larva under scope  
 collected 200 μl from each tank  
 locked @ swimming & sleep

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

ml	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 5gal of C50 & 5gal 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

Date 3/12	Time 9:37	Bottle 67	Tank H2	Temp 13.20	pH -58.2	sal 2627.4	Na 1.1
	9:43	65	H1	13.19	-27.9	27.4	"
	66	H1	"	"	"	"	"
	9:47	77	T5	13.86	-29.6	27.5	✓
	78	T5	"	"	"	"	*
9:54	79	T6	13.87	-57.7	27.5	✓	
	80	T6	"	"	"	"	"
10:00	75	T4	13.80	-29.5	27.5	✓	
	76	T4	"	"	"	"	"
10:04	69	T1	13.83	-29.7	27.5	✓	
	70	T1	"	"	"	"	"
10:07	71	T2	13.81	-57.6	27.5	✓	
	72	T2	"	"	"	"	"
10:14	73	T3	13.84	-57.6	27.5	✓	
	74	T3	"	"	"	"	"

Samples fixed @ 10:45

cell counts - sampled 3/12 counted 3/13

Tank	Contl	Cont2	$\bar{x}$	cells/ml
1	76	72	74	82,222
2	60	71	65.5	72,777
3	73	64	68.5	76,111
4	50	65	57.5	63,888
5	87	69	78	86,666
6	76	67	71.5	79,444

12 March 16 ttp

24 tank dropping & Sampling

TIME	TANK	A	D	A	D	PP [cell/ml]	ml for 10K
12:25	3	161	23	179	29	153	26 164.3 <sup>26</sup> 1643.3 6.1
13:25	2	161	21	144	38	149	29 151.3 <sup>27</sup> 1513.3 6.6
14:30	1	129	36	160	23	138	15 142.3 <sup>24.7</sup> 1423.3 7.0
15:35	4	183	13	171	9	186	11 180 11 1800 5.6
16:55	5	160	29	141	19	150	20 150.3 22.6 1503.3 6.7
18:00	6	177	27	174	29	174	31 175 29 1750 5.7

Wt Duraset 3 in tank 6

ST

08

Wt Duraset 4 in tank 1

PT

00:00

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 / Saving  
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 count

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

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	"	"	"	"	
13:02	93	T5	13.89	-30.0	27.5	✓	
	94	T5	"	"	"	"	
13:06	95	T6	13.96	-58.4	27.5	✓	
	96	T6	"	"	"	"	
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 1989

- 5:50 a.m. I dumped -  
+ pumped over night +

Pt probe calibration  
3141b test batch

3/4/10  
MS battle  
batch 2/4/10

Temp 13.2 Wet

72.1 72.0 71.9

- طلاق

-  
90.7  
12.31

- 899  
12.92

-89.1  
13.45

-88.3  
14.61

14.73  
-84.5

Log 55

0  
H  
K  
S

HT 0  
HT 1  
HT 2

St

60

31 Oct 1974

21 0

hill. — T. —

9a 609

Land speech

Mac Explains

John H. Morgan

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29

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				
500	31,32	45,46,47	31				
500	33,34	48,49,50	33				
500	35,36	51,52,53	35				
500	37,38	54,55,56	37				
500	39,40	57,58,59	39				

15 March 16 TR

09:15 - added 5 gal 60g + 8 gal COW  
0.2m<sup>2</sup> subsamples from 800ml

Tank	A	D	A	D	A	D	average estimatd
High	215.51	236	83	228	62	26.3	1131

line ~~effs/m<sup>2</sup>~~ = 1131  $\times$  800 ml = 908,800 total  
in 6 tanks  $\approx$  150,800/l tank 133 ml each

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 # Sahmtry 3 in tank 6

### 16 March 16 TR

Time	Tank	Temp	PT	Salt
9:30	11	214.78	-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 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 3/17	Time 14:13:05	Bottle 123	Tank T4	Temp. 13.80	pH -29.5	sal 27.4	colls ✓
		124	T4	"	"	"	"
	13:20	125	TS	13.84	-30.4	27.4	✓
		126	TS	"	"	"	"
	13:28	127	T6	13.87	-30.19	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

17 March 16 MP  
cell counts in 1.5ml samples 0.0009 ml

Tank	count	cells/ml
1	53	58,333
2	51	65,000
3	40	45,000
4	46	40,5
5	41	47
6	56	65
11	77	107
12	88	77
13	240	250
14	71	57
15	245	271
16	51	63
		92
		102,222
		71,666
		272,222
		71,111
		286,667
		63,333

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

Fe. 18

18:30 sampled seed Iggeduck per tube

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

fixed & sonde 19:15

18 March 16 HPO  
picked up ~6mL geoduck & Taylor  
@ 08:30

- Timing
  - ~~count~~ 10:40 - 11:40
  - drop 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 200µl from 250 mL beakers  
OC - trial #2 larvae

Treat	Tank	Alive	Arie		ml cells/ml for 10K
			A	D	
7.3	1	20.7	1	57	58
7.3	4	18.7	2	59	60,61,62
7.3	5	14	3	61	63,64,65
7.0	2	54.0	4	63	66,67,68
7.0	3	46.7	5	64	69,70,71
7.0	6	43.3	6	67	72,73,74

18 March 16 ttp

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

Fed Sagal Cow + Sagal 609

- changed Sun and Sun inline filters  
washed vango and trial Lamb remaining larvae  
- turned fed pump 1 to 90 rpm  
- turned feed pump 2 to 45 rpm

19 March 16 ttp

MY.	Temp. C
-94.4	94.4
-93.4	10.45
-92.6	10.97
-92.1	11.36
-91.5	11.81
-90.8	12.32
-90.0	12.96
-89.5	13.37
-89.0	13.82
-88.6	14.11
-87.6	14.89

19 March 1640ft bottle

Date Time Bottle Temp pH Sal cel's

<u>3/19</u>	<u>12:30</u>	<u>T1</u>	<u>141</u>	<u>13.31</u>	<u>-28.5</u>	<u>25.8</u>
		<u>T11</u>	<u>142</u>	<u>"</u>	<u>"</u>	<u>"</u>
	<u>12:33</u>	<u>T2</u>	<u>143</u>	<u>13.31</u>	<u>-28.0</u>	<u>25.8</u>
		<u>H2</u>	<u>144</u>	<u>"</u>	<u>"</u>	<u>"</u>
	<u>12:35</u>	<u>T4</u>	<u>152</u>	<u>13.91</u>	<u>-28.8</u>	<u>25.8</u>
		<u>T4</u>	<u>153</u>	<u>13.91</u>	<u>"</u>	<u>"</u>
	<u>12:46</u>	<u>TS</u>	<u>153</u>	<u>13.93</u>	<u>-29.0</u>	<u>25.8</u>
		<u>TS</u>	<u>154</u>	<u>"</u>	<u>"</u>	<u>"</u>
	<u>12:58</u>	<u>T4</u>	<u>155</u>	<u>14.01</u>	<u>-28.7.8</u>	<u>25.7</u>
		<u>T6</u>	<u>156</u>	<u>"</u>	<u>"</u>	<u>"</u>
	<u>13:03</u>	<u>T1</u>	<u>145</u>	<u>13.95</u>	<u>-28.8</u>	<u>25.7</u>
		<u>T1</u>	<u>146</u>	<u>"</u>	<u>"</u>	<u>"</u>
	<u>13:07</u>	<u>T2</u>	<u>147</u>	<u>13.98</u>	<u>-8.1</u>	<u>25.7</u>
		<u>T2</u>	<u>148</u>	<u>"</u>	<u>"</u>	<u>"</u>
	<u>13:13</u>	<u>T3</u>	<u>149</u>	<u>14.02</u>	<u>-8.0</u>	<u>25.7</u>
		<u>T3</u>	<u>150</u>	<u>"</u>	<u>"</u>	<u>"</u>
	<u>13:20</u>	<u>T11</u>	<u>157</u>	<u>13.84</u>	<u>-28.2</u>	<u>25.8</u>
		<u>T11</u>	<u>158</u>	<u>13.83</u>	<u>"</u>	<u>25.9</u>
	<u>13:25</u>	<u>T12</u>	<u>159</u>	<u>13.83</u>	<u>-7.0</u>	<u>25.9</u>
		<u>T12</u>	<u>160</u>	<u>"</u>	<u>"</u>	<u>"</u>
	<u>13:29</u>	<u>T13</u>	<u>161</u>	<u>13.84</u>	<u>-56.8</u>	<u>26.1</u>
		<u>T13</u>	<u>162</u>	<u>"</u>	<u>"</u>	<u>"</u>
	<u>13:31</u>	<u>T14</u>	<u>163</u>	<u>13.84</u>	<u>-28.7</u>	<u>25.8</u>
		<u>T14</u>	<u>164</u>	<u>"</u>	<u>"</u>	<u>"</u>
	<u>13:35</u>	<u>T15</u>	<u>165</u>	<u>13.84</u>	<u>-57.1</u>	<u>26.2</u>
		<u>T15</u>	<u>166</u>	<u>"</u>	<u>"</u>	<u>"</u>
		<u>T16</u>	<u>167</u>	<u>13.86</u>	<u>-7.8</u>	<u>25.8</u>
		<u>T16</u>	<u>168</u>			

fixed @ 14:00

19 March 16 trp 1.5ml samples counted 0.0009 ml  
Tank cont 1 cont 2 X cells/ml Date Collected 10/3  
 1 46 50 48 53,333 3/19  
 2 63 58 127,60.5 67,222 3/19  
 3 67 72 61.5 77,222  
 4 57 61 59 65,555  
 5 66 50 53 58,888  
 6 49 49 49 44 48,888  
 11 49 39 44 45.5 50,555  
 12 49 32 36 40,000  
 13 47 44 44 53.5 59,444  
 14 40 32 36 40,000  
 15 58 49 53.5 59,444  
 16 66 50 58 61,111  
 11  
 12  
 13  
 14  
 15  
 16

T

T

16:45 cleaned barrows  
 17:00 added 4 gal GFW & 4 gal 609 to feed tank  
 checked juveniles

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
		T3	17.7	13.85	-8.0	26.2	✓
		T3	17.8	"	"	"	"
		T2	17.5	13.88	-8.0	26.2	✓
		T2	17.6	"	"	"	"
		T1	17.3	13.90	-29.4	26.2	✓
		T1	17.4	"	"	"	"
		T4	17.9	13.87	-29.1	26.1	✓
		T4	18.0	"	"	"	"
		T5	18.1	13.90	-29.8	26.1	✓
		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	cells/ml	ML 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
	T6	152	3	146	2	147	3	148.3	741.6
17:00									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

suspended Epi tubett Morph Dubett RNA later -80  
 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 & bagnos & 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 TS-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

10.3  
5 gal cow + 5 gal 609  
checked pH & temp & flow  
turned up for pump 2 to 55 rpm