UTAH DIVISION OF WILDLIFE RESOURCES
FISHERIES EXPERIMENT STATION
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LOGAN, UT 84321

The Ichthyogram

VOLUME 19, NUMBER 3/4

DECEMBER 2008

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EGG DISINFECTION WITH COMBINATIONS OF HYDROGEN PEROXIDE AND POVIDONE IODINE.

Bacterial growth on eggs can compromise survival and can potentially introduce pathogenic species of bacteria to hatcheries when eggs are imported from outside sources. Experiments here at FES have shown that treatment with 100 ppm iodine for 15 min, while significantly reducing bacterial abundance, can leave eggs with numerous bacteria. Subsequent research has indicated that hydrogen peroxide is safe on eyed eggs (Wagner et al. 2008), though follow-up tests have indicated that buffering is needed to maintain pH in softer water applications. In an attempt to provide a disinfection protocol that is safe for eggs yet completely kills external bacteria, we tested the combination of both hydrogen peroxide and povidone iodine (Argentyne®) which is the subject of this article.

Methods

Triploid rainbow trout eggs at the eyed stage of development were treated with combinations of iodine and hydrogen peroxide or each chemical individually. Treatments were (1) 2,000 mg/L iodine for 10 min, then 3.0% buffered hydrogen peroxide for 1 min, (2) 3.0% hydrogen peroxide for 1 min, then 2,000 mg/L iodine for 10 min, (3) 3.0% hydrogen peroxide for 1 min, (4) 2,000 mg/L iodine for 10 min (5) 1.5% hydrogen peroxide for 2 min, and (6) untreated eggs (no chemical treatment, but similarly handled). The chemical solutions were 12 L, prepared in plastic pails. For each of the hydrogen peroxide treatments, the solutions were buffered by the addition of 1.32 g/L of baking soda (sodium bicarbonate). Eggs were treated on 18 September 2008 by dipping a net with 90 ml (3 oz) of eggs in the solution. For the combination treatments, the eggs were rinsed between the two chemical exposures by dipping the net into a bucket of clean hatchery well water. Given the low volume of eggs, the same solution was used for each of three replicates, but new solutions were prepared for each treatment.

After the duration of chemical exposure, the eggs were rinsed with sterile hatchery water, 15-20 eggs were transferred to sterile beakers, and the remaining eggs were transferred to an egg incubation tray. Dead eggs were enumerated and removed every 2-3 days. Hatching occurred 6 days after the chemical treatments. Percent hatch was derived from the number of fry obtained divided by the initial number of eggs, times 100. On 2 October 2008, 8 d after hatching, the deformed fry were removed and all surviving fry were hand counted. The percentage of crippled fry was expressed as the number of deformed fry removed divided the number of fry that hatched, times 100.

The eggs in the beaker were transferred individually with sterile forceps to a test tube with 2 ml of sterile peptone salt diluent solution (Barnes et al. 2005). The tube was agitated with a vortex mixer for 2 min and 100 ul was transferred to a trypticase soy agar (TSA) Petri dish. Another 100 ul was transferred to a Petri dish with enhanced Ordahl's agar with the antibiotic tobramycin (EOT; 50 ul of 100 mg/ml stock solution per liter of media; Kumagai 2004). A sterile spreader and spinning plate table were used to distribute the shaken solution onto the plate. The plate was wrapped in laboratory film and incubated at 15°C. Counts of colony forming units (CFUs) on both media (TSA and EOT) were made 2, 4, 6, 9 and 12 d after inoculation. If plates had too many CFUs to count accurately, the plate was labeled as "too numerous to count"(TNC). To get an estimate of the total number of bacteria on each treated egg, the CFU plate counts were multiplied by 20.

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Results

Egg survival

There were significant differences in survival of the eggs among the various chemical treatments ($P < 0.001$; Table 1). The combination of 3% hydrogen peroxide for 1 min followed by 2,000 mg/L iodine proved to be highly lethal (0.2% hatch), with only a few eggs surviving the first 48 hr after treatment. Paradoxically, if the order of the combination treatment was reversed, the hatching success (61.8%) was not statistically different from the untreated controls (62.3%). If eggs were exposed to 2,000 mg/L iodine alone, survival was also not significantly different from controls. Survival to hatch was slightly, but significantly, lower in the 3% hydrogen peroxide treatment (57.6%) than controls. However, the percent hatch for eggs exposed to 1.5% hydrogen peroxide for 2 min (60.9%) did not significantly differ from controls. The percentage of crippled fry ranged from 7.8 to 20.0% and did not significantly differ among treatments ($P = 0.85$). The 20% cripple rate was based on the 5 fry surviving in a single replicate of the hydrogen peroxide to iodine treatment. No fry survived in the other replicates of that treatment.

Table 1. Comparison of the percentage of eggs that hatched and the percentage of crippled fry among triploid rainbow trout eggs exposed to combinations of hydrogen peroxide and iodine or each chemical alone. Means (± SD, $n = 3$) within a column that are not significantly different from one another are followed by a common letter.

Treatment	Hatch (%)	Crippled (%)
2,000 mg/L Iodine for 10 min	59.6 ± 1.2 bc	7.9 ± 1.0 a
3% Hydrogen peroxide for 1 min	57.6 ± 4.9 b	8.7 ± 2.3 a
2,000 mg/L Iodine for 10 min to 3% hydrogen peroxide for 1 min	61.8 ± 0.9 c	7.9 ± 0.8 a
3% Hydrogen peroxide for 1 min to 2,000 mg/L iodine for 10 min	0.2 ± 0.4 a	20.0 ± 34.6 a
1.5% hydrogen peroxide for 2 min	60.9 ± 2.2 bc	8.9 ± 0.8 a
Untreated control	62.3 ± 0.8 c	7.8 ± 0.5 a

Bacteria and fungi control

Overall there were few CFUs, with only one plate noted as ‘too numerous to count’ in the untreated control, Replicate 3. For samples on TSA media, using the 30 eggs pooled across replicates, there were significantly more CFUs in the untreated controls than in any of the chemical treatments, which did not significantly differ from each other (Table 2). For samples on EOT media, the loglinear tests indicated an interaction between replicate and treatment. Within Replicate 1, there were no significant differences among treatments in CFU frequency ($P = 0.91$), but there were in replicates 2 ($P = 0.01$) and 3 ($P < 0.001$). Further analysis of Replicate 2 indicated that CFU frequencies for eggs treated with chemicals did not significantly differ from controls, but did vary among chemical treatments (Table 3). No CFUs were observed on eggs treated with 3% hydrogen peroxide or the iodine-hydrogen peroxide combination, whereas 50% of eggs treated with 2,000 mg/L iodine had CFUs; the other treatments were between these ranges. For Replicate 3, there were significantly more CFUs on the untreated controls than in the 3% hydrogen peroxide, iodine-hydrogen, or 1.5% hydrogen peroxide treatments. CFUs frequencies in the 2,000 mg/L iodine and hydrogen peroxide-iodine treatments did not differ significantly from controls.

Table 2. Percentage of eggs (n = 30) in one of three categories of colony-forming units (CFUs) abundance for eggs exposed to iodine or hydrogen peroxide treatments and plated on trypticase soy agar (TSA). Treatments that are not significantly different from each other share a common letter in the significance column.

Treatment	Statistical significance	TSA		
		No CFUs	1 to 10 CFUs	>10 CFUs
2,000 mg/L Iodine for 10 min	b	93.3	6.7	0.0
3% Hydrogen peroxide for 1 min	b	93.3	6.7	0.0
2,000 mg/L Iodine for 10 min to 3% hydrogen peroxide for 1 min	b	100.0	0.0	0.0
3% Hydrogen peroxide for 1 min to 2,000 mg/L iodine for 10 min	b	100.0	0.0	0.0
1.5% hydrogen peroxide for 2 min	b	86.7	10.0	3.3
Untreated control	a	50.0	36.7	13.3

Discussion

The egg survival results indicated that the combination of hydrogen peroxide and iodine can be highly lethal if hydrogen peroxide is applied first. For reasons unknown, the reverse order provided good survival. The single chemical treatments all provided similar survival results, though 3% hydrogen peroxide for 1 min did result in slightly lower egg survival (92% of untreated control). However, 1.5% hydrogen peroxide for 2 min, which had the same dose-duration total, did not significantly reduce egg survival. For control of bacteria, the statistical tests indicated that little difference between chemical treatments, though in some EOT replicates, more growth was noted on untreated controls and 2,000 ppm iodine treatments. Some of the growth observed was fungal and possibly derived from aerosol contamination during the plating process. Overall, there were very few colonies in any of the treatments. This was in contrast to earlier studies in which untreated controls were typically “too numerous to count”. Perhaps the iodine treatment (100 ppm for 15 min) for disinfection of the eyed eggs upon arrival at the Fisheries Experiment Station, less than 24 h before the test, significantly reduced abundance and insufficient time in the incubation trays had elapsed for bacterial colonization.

In summary, the results indicated that combination treatments are possible if iodine is applied first, though the risks of mistakes in the order of treatment may preclude implementation. Results from the 1.5% hydrogen peroxide treatment indicate that it is a viable option, achieving the same result at a lower cost than chemical combinations or 3% hydrogen peroxide.

Eric Wagner

Table 3. Frequencies of colony-forming units (CFUs) for eggs exposed to iodine or hydrogen peroxide treatments and plated on enhanced Ordahl's with tobramycin (EOT). Treatments that are not significantly different from each other are noted with a common letter in the significance column.

Replicate Treatment	Statistical significance	EOT No CFUs	EOT 1 to 10 CFUs	EOT >10 CFUs
1				
2,000 mg/L Iodine for 10 min	a	6	4	0
3% Hydrogen peroxide for 1 min	a	6	4	0
2,000 mg/L Iodine for 10 min to 3% hydrogen peroxide for 1 min	a	6	4	0
3% Hydrogen peroxide for 1 min to 2,000 mg/L iodine for 10 min	a	7	3	0
1.5% hydrogen peroxide for 2 min	a	8	2	0
Untreated control	a	7	3	0
2				
2,000 mg/L Iodine for 10 min	c	5	5	0
3% Hydrogen peroxide for 1 min	a	10	0	0
2,000 mg/L Iodine for 10 min to 3% hydrogen peroxide for 1 min	a	10	0	0
3% Hydrogen peroxide for 1 min to 2,000 mg/L iodine for 10 min	ab	9	1	0
1.5% hydrogen peroxide for 2 min	bc	7	3	0
Untreated control	abc	8	2	0
3				
2,000 mg/L Iodine for 10 min	a	4	6	0
3% Hydrogen peroxide for 1 min	b	10	0	0
2,000 mg/L Iodine for 10 min to 3% hydrogen peroxide for 1 min	b	10	0	0
3% Hydrogen peroxide for 1 min to 2,000 mg/L iodine for 10 min	a	6	4	0
1.5% Hydrogen peroxide for 2 min	b	10	0	0
Untreated control	a	3	6	1

2008 June Sucker Egg Take

In June of 2008 the Fisheries Experiment Station in Logan, Utah conducted its annual spawning of the June sucker (*Chasmistes liorus*). Seventy-nine females were injected and of those, 34 gave eggs (43%). Sixty-seven males were injected and the milt from 32 was used to fertilize the eggs given. The remaining 35 males went unused. The average fecundity rate of the females was 6728 eggs.

The brood stock is kept in 63°F water from mid June through the end of November. To induce spawning they are moved to 56°F water at the first of December through mid June. The days preceding spawning, the suckers are separated by sex and injected with HCG (human chorionic gonadotropin) to help induce spawning.

The females are injected for 3 consecutive days at 400 IU/kg of body weight the 1st day, 750 IU/kg of body weight the 2nd day, and 1000 IU/kg of body weight the 3rd day. After a resting period of one day, they are checked on the 5th and 6th days to see if they are ovulating. If so, a male is selected to make a cross. Males are injected on the 3rd day with 500 IU/kg and allowed a day of rest which allows them to be ready to spawn along with the females on the 5th and 6th days. Starting on May 31st the females were injected with their first dosage and continued through June 2nd. The males were injected with their single dose on June 2nd and both males and females were allowed to rest on June 3rd.

On June 4th FES staff started spawning the June suckers. Females were selected and checked to see if they would give eggs and if they were ovulating, a male was selected to create a correct cross. This process was repeated the following day until all injected fish were checked. Those females that did not ovulate or partially ovulated on the 4th were checked again on June 5th. Due to an insufficient number of eggs taken, it was decided to advance with another round of injections and spawning.

The second round of injections began on June 9th and followed the same process as above, which set the second spawn date for June 13th. Those females that did not ovulate or partially ovulated on the 13th were checked again on June 14th. The 2008 June sucker egg take ended on June 14th.

A total of 264,076 eggs were taken in 30 different crosses. Approximately 35,315 eggs were sent to Bozeman for a diet study which left 228,761 eggs at FES. Of these 228,761 eggs it is estimated 130,987 hatched and went on feed. One lot of eggs totaling 9,840 did not hatch due to unknown circumstances. Excluding this batch of eggs from the calculations, 59.8% hatched and went to feed. Including this batch of eggs into the calculations resulted in 57.2% hatching and going to feed.

Table 1. The age, sex, and average weights of the June suckers used in the 2008 spawning.

Females		Males	
AGE	**AVG LBS**	**AGE**	**AVG LBS**
5	1.16	5	0.94
6	1.44	6	0.92
7	1.34	7	0.88
12	1.54	12	0.93
13	1.69	13	1.20
14	1.43		
15	1.80		
16	1.88	16	1.03
18	1.74	18	1.22

Nine different year classes of suckers were used in this spawning which includes 5, 6, 7, 12, 13, 14, 15, 16, and 18 year old fish.

Once eggs were spawned they were allowed to water harden for one hour before they were moved. Eggs were inventoried to get a number of eggs per ounce and total number They were dipped in a solution of bentonite to reduce adhesiveness.

Eggs were then placed into McDonald-type hatching jars. Water at 65°F (18.3°C) flowed through the jars

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to rotate the eggs. The eggs were subjected to a 10 minute bath of a 1000 ppm solution of formalin once a day until eyeup. This was done to kill any fungi or bacteria that may be on the eggs. Once eyed up, it took approximately 2 days for the eggs to start hatching.

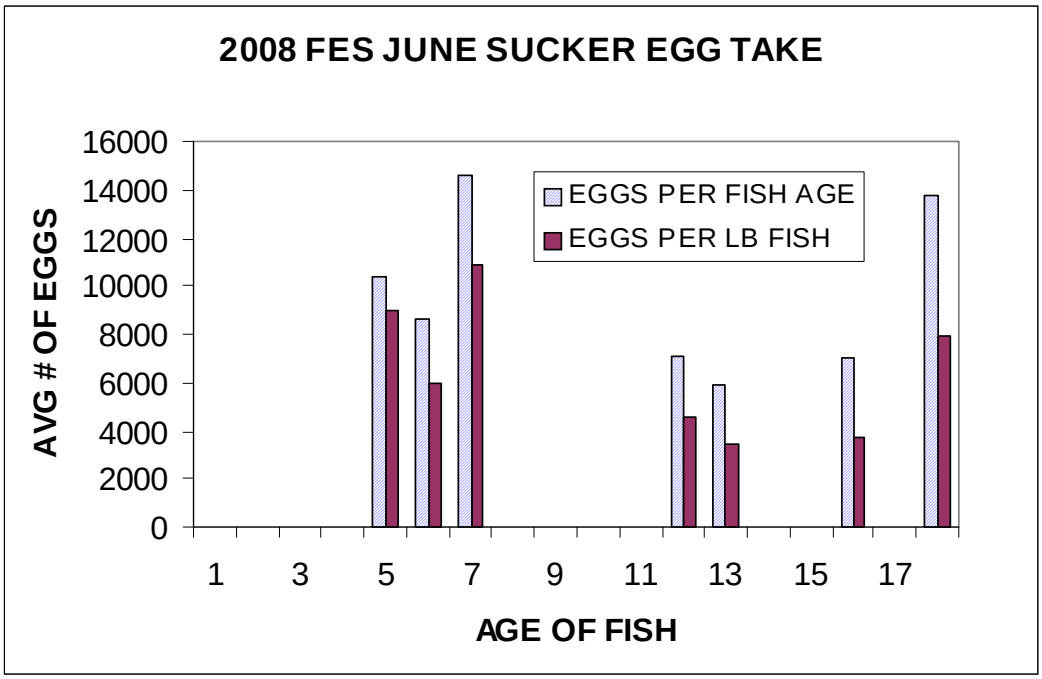

Figure 1. Age of the June suckers compared to the number of eggs they gave. The graph also shows the number of eggs per pound of fish per age.

Overall the 2008 June sucker egg take was a success. The target number of eggs was met and exceeded, and the hatch rate was acceptable to produce a sufficient number of fish. As of September 30, 2008 approximately 98,419 (excluding fish no longer at the facility) of the fish that went on feed were still alive, giving us an approximate mortality rate of 19.9%.

Travis Dees

New Faces at FES

We would like to welcome **Chris Heck** as the new virologist at the Fisheries Experiment Station. After several weeks of searching we were able to find a biologist to fill the position vacated by David Thompson, who recently moved to Hood River, Washington. Chris is a microbiologist with well over 15 years of experience performing laboratory research. He has a Bachelor's degree in Soil Science and Microbiology from the University of Minnesota and a Masters degree from Western State College in Gunnison, Colorado where he conducted research on the purification and characterization of isoenzymes involved in plant development. Chris is a long time Logan resident that has gained much of his resume experience working at Utah State University. Chris's extensive experience in microbiological and molecular biological lab techniques along with his practical experience with laboratory equipment will be a valuable asset in the growing fish health program at FES. Outside of his studies, Chris has a passion for back-country skiing, rock climbing, and road biking.

Electrode Erosion; A Major Factor Influencing the Performance of Electric New Zealand Mud Snail Barriers

Introduction

New Zealand mud snails (*Potamopyrgus antipodarum*; syn. *Hydrobia jenkinsi*; NZMS) were first identified in the Utah Division of Wildlife Resource's Loa State Fish Hatchery in November 2007. Currently, a major effort is underway to determine the best method of eradicating NZMS from Loa and to research methods that can be used to prevent NZMS from infesting other hatcheries. Electric barriers have shown promise at impeding the upstream movement of NZMS (Oplinger 2008). To date, small-scale electrical tests have identified the tolerance of NZMS to electrical currents (Oplinger 2008). Unfortunately, larger-scale experiments conducted with barriers constructed with copper pipe have been less successful. One such barrier was installed in a raceway at Loa. Within one month of installation, severe corrosion built-up on this barrier. As this corrosion accrued, a > 75% reduction in electric current flow occurred. This corrosion allowed NZMS to cross the barrier. Ultimately, this corrosion also led to barrier disintegration.

Since it appears that electrode corrosion potentially has a significant effect on the performance of electric NZMS barriers, the purpose of this study was to investigate whether alternative materials and electrode designs can be used to limit corrosion. Herein, we studied the corrosion and electrical performance of electrodes constructed of one of three metals (copper, aluminum, and stainless steel) that were subjected to both direct and alternating current. In addition, we determined the performance of three stainless steel alloys (304, 316, and 416) when subjected to direct current.

Methods

For the three metal DC and AC current experiments, 6.3 mm diameter copper, aluminum, and stainless steel rods were purchased from a local hardware store. These rods were cut into 150 mm lengths. A rubber band was wrapped around each 150 mm length, 25 mm from an end. Then, holes were drilled into two sheets of plexiglass and these sheets were suspended parallel to the water surface in two hatchery troughs. The flow through these troughs was set at 20 L/min. The cut metal lengths were then inserted into the holes on these sheets, with the rubber bands serving as stoppers preventing the metal lengths from falling through the holes into the trough. Thus, the electrodes were suspended perpendicular to the water surface. The "long" section (125 mm) of each metal length was suspended in the water while the "short" section (25 mm) stuck out above the water surface. The holes were drilled into the plexiglass sheet in such a manner that the metal lengths were arranged in pairs. Each metal length in a pair was separated by 25 mm and there was a minimum of 200 mm of separation between pairs. Both metal lengths (henceforth electrodes) in each pair were constructed from the same metal. A total of six pairs of electrodes of each metal type were constructed. Three electrode pairs of each metal were randomly placed on each plexiglass sheet. Three randomly selected electrodes of each metal were electrified during the experiment whereas the other three were not electrified and served as controls. All of the electrified electrodes were wired in series with copper wire. In the DC experiment, 12 V of electricity was supplied to the electrodes using a DC power supply (BK Precision Model 1715). In the AC experiment, 10 V of electricity was supplied to the electrodes using an AC power supply (Pragmatic Model 3305A). The DC experiment with the three stainless steel alloys (304, 316, and 416) was conducted in the same manner as the three metal DC experiment. However, in the stainless steel alloy experiment, 9.5 mm diameter rods were used and these rods were mounted to a single sheet of plywood that was suspended above an outside raceway.

Once a week during these experiments, the electrical current flowing through each electrode pair was measured using an ammeter (Radio Shack Model 22-811). Also, the electrodes were removed from the holding sheets and weighed (electrodes were weighed as a pair). The electrodes were always returned to their original holes in the holding sheets and the same directional current (+ or -) was applied to each electrode throughout the experiment. After 28 d, the experiment was concluded. We performed a repeated-measures ANOVA (SAS

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1998) to compare changes in mass and electrical current among metals (or alloys) through time. For analysis, the data was converted into percent change in mass or electrical current. A Tukey's multiple comparison test was used to investigate significant effects in our statistical model. Separate analyses were performed for the three metal DC, AC and stainless steel alloy experiments. Results were considered statistically significant at $P < 0.05$.

Results and Discussion

For all three experiments, the loss of mass among the control electrodes was insignificant (all $P > 0.90$) indicating that any loss of electrode mass during the experiment was a result of the effect of the electricity. Therefore, the control electrodes were not included in these analyses. Overall, significant electrode erosion and current loss occurred among the electrodes subjected to DC current in the three metal experiment (Figures 1 and 2). The rate of mass loss varied significantly among metals (Metal x Time; $F_{8,24} = 3.20$, $P = 0.01$). Initially, the aluminum electrodes lost mass more rapidly than the other metals. Regardless, after 28 d, both the aluminum (-21.3 ± 0.4%, mean ± SE) and copper (-21.4 ± 2.5%) electrodes experienced a comparable percentage of mass loss. The stainless steel electrodes were the most resilient, but still lost significant mass (-11.8 ± 4.8%). The percentage of current loss during the experiment varied among metals ($F_{2,6} = 7.13$, $P = 0.03$) with a significant metal x time interaction ($F_{8,24} = 6.94$, $P < 0.01$). An almost complete loss of current occurred among the stainless steel electrodes (-95.4 ± 2.3%). Meanwhile, a lesser percentage of current loss occurred among the copper (-83.1 ± 4.1%) and aluminum (-76.4 ± 5.7%) electrodes. Overall, the loss of electrode mass and current during the DC experiment was substantial and demonstrates that NZMS barriers utilizing DC current are likely to fail shortly after installation.

In contrast, mass loss was reduced among the electrodes subjected to AC current. We observed no significant change in electrode mass or current in the AC experiment across time (both $P > 0.20$; Figure 2). Regardless, we observed a trend towards decreased current with large standard errors (percent change in current at 28 d; aluminum: -43.0 ± 41.6%, copper: -23.5 ± 65.6%, stainless steel: -18.6 ± 36.5%). It is not clear why the currents in the AC experiment were so variable. However, given that we observed little corrosion of the electrodes in the AC experiment, we feel that these fluctuations may be more of a relict of the limitations of the design of a small scale experiment. We feel that it is likely that AC current may be more stable in a raceway sized barrier. Based on these results, it appears that any of these three metals may be suitable for use in an AC barrier. However, due to the fluctuations in current observed, we feel that AC barriers should be designed to accommodate more electrical current than is necessary to impede the upstream movement of NZMS. Previous studies have identified the tolerance of NZMS to DC current (Oplinger 2008). NZMS tolerances to AC current, however, have not been researched and may differ from the identified DC tolerances. Future research will attempt to identify the tolerance of NZMS to AC current and will test the efficacy of a large-scale AC barrier.

Finally, in the DC experiment where the performance of three stainless steel alloys was compared, electrode mass and current changed with time (Figure 2, mass: $F_{4,24} = 183.07$, $P < 0.01$, current: $F_{4,24} = 8.99$, $P < 0.01$). The rate of change of mass and current differed among alloys (alloy x time interaction, mass: $F_{8,24} = 191.60$, $P < 0.01$, current: $F_{8,24} = 2.99$, $P = 0.02$). These results, however, were driven by significant corrosion among the alloy 416 electrodes. The percent change in mass among the alloy 304 and 316 electrodes was less than 0.2 percent. In contrast, the mass of the alloy 416 electrodes decreased by 13.1 ± 0.5%. Also at the end of the experiment, the electrical current flowing through the alloy 416 electrodes decreased by 64.4 ± 0.5%. Unfortunately, at points during the experiment, the current loss among the alloy 304 and 316 electrodes was significant. Twenty-one days after initiation, the current loss in the alloy 304 electrodes was 18.6 ± 5.5%. Meanwhile, the current loss in the alloy 316 electrodes at 21 d was 35.7 ± 26.3%. Interestingly, the current flowing through the alloy 304 and 316 electrodes increased between 21 and 28 d after initiation and current loss among both alloys at the end of the experiment was less than ten percent.

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These results suggest that electrodes constructed with stainless steel or electrified with AC current should accrue the least corrosion and therefore have the most desirable properties for NZMS barrier construction. However, electrode performance differs among stainless steel alloys. It appears that minimal corrosion accumulates on stainless steel alloys 304 and 316, but, significant corrosion develops on alloy 416. Unfortunately, regardless of current (DC or AC) or stainless steel alloy, it appears that current loss occurs over time. Thus, this current loss should be accounted for in barrier design. In the next couple of months, researchers at FES will investigate the tolerance of NZMS to AC current and intend on installing a large-scale AC barrier at Loa. The results of these tolerance and large-scale experiments will be presented in future Ichthyogram articles.

Randy Oplinger and Eric Wagner

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Figure 1: Picture showing the corrosion incurred during 28 d of exposure to 12.0 V of DC current. Stainless steel (top), aluminum (middle), and copper (bottom) electrodes are shown. The electrodes on the left were electrified whereas those on the right were non-electrified control electrodes.

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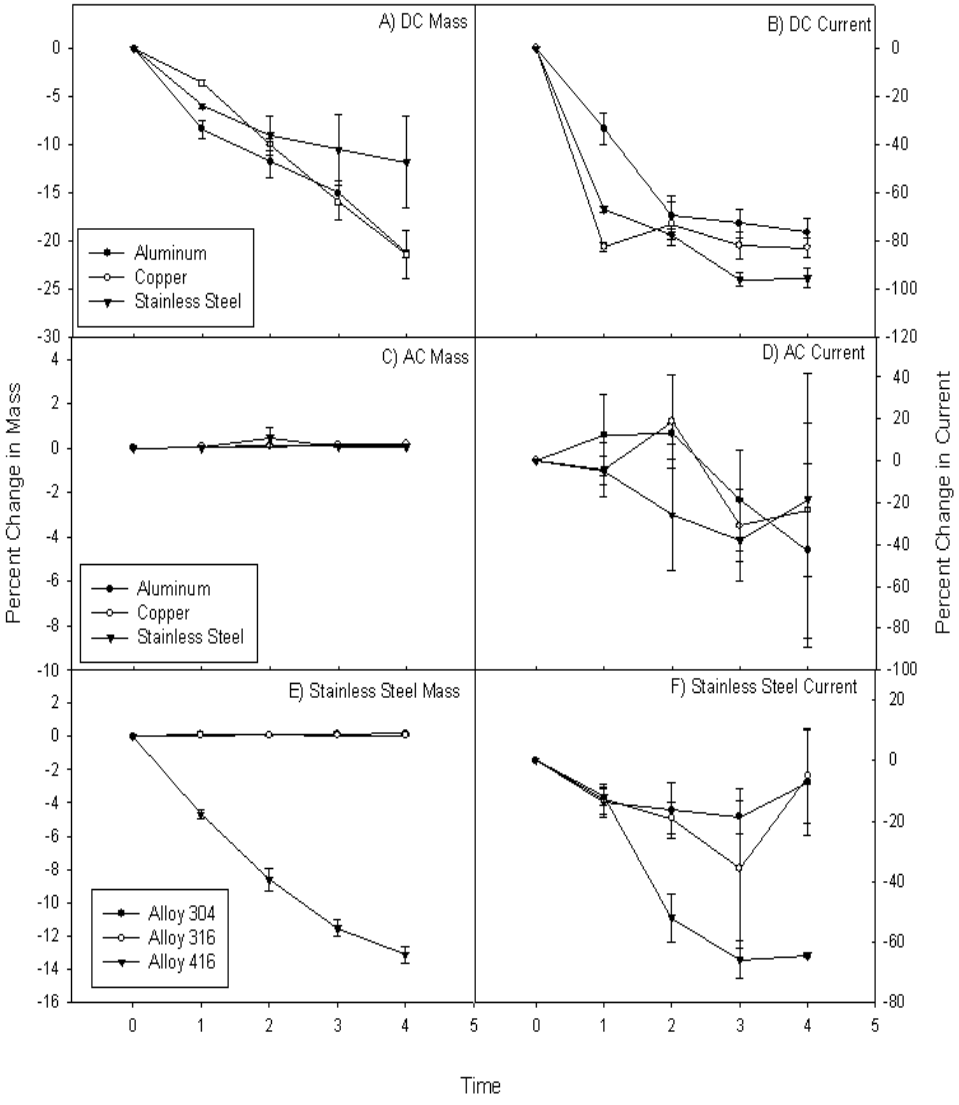

Figure 2: Multi-panel graph comparing the performance of aluminum, copper, and stainless steel electrodes subjected to DC (panels A and B) or AC (panels C and D) current through time. The performance of three stainless steel alloys (304, 316, and 416) subjected to DC current is also shown (panels E and F). The panels on the left side of the figure (A, C, and E) show the percent change in mass among the electrodes through time. The panels on the right side of the graph (B, D, and E) show the percent change in electrical current through time.