The Ichthyogram

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Large-Scale Production of Sterile Triploid Rainbow Trout Planned for Utah

In Utah, as in many states, the Division of Wildlife (DWR) serves as both a steward of the wildlife resources and a producer of additional fish and game via hatcheries and habitat management. Sometimes these roles of commodity versus stewardship are at odds. Such is the case where the DWR stocks exotic species such as rainbow trout to provide recreational fishing while attempting to conserve populations of native species such as the cutthroat trout. Cutthroat are the only trout species (other than mountain whitefish) that were here when white settlers first arrived. All others have been introduced, including the rainbow trout. Cutthroat trout readily hybridize with rainbow trout, diluting their genetic purity. This can make them less able to survive in the habitats they evolved in by altering factors such as spawning times, escape behavior, feeding habits, growth rates, disease tolerance, and cryptic coloration. However, rainbow trout are a great sport fish, displaying faster growth and more fight than cutthroat trout. Providing rainbow trout to anglers that have become accustomed to them is desirable, but so is the preservation of cutthroat trout, a fine sport fish itself. Also, cutthroat conservation is needed to prevent their listing as a threatened or endangered species.

With the advent of triploidy techniques. it is now possible to “have our cake and eat it too”. In this process, rainbow trout eggs are shocked with warm water shortly after fertilization. This results in 3 sets of chromosomes (triploid or 3N) instead of the usual two (diploid or 2N). The triploid is sterile and therefore can’t hybridize with the cutthroat trout.

The DWR fisheries managers have begun to use these sterile fish for stocking. The rainbow trout going into Strawberry Reservoir have been sterilized to protect the cutthroat trout there and maintain their fish-eating habits to help control chub and sucker populations. Recently, aquatics managers have indicated a desire to eventually convert all stocking of rainbow trout to sterile fish. To this end, key DWR personnel met recently to plan the first steps. It was decided to build on last year’s success with triploid production for Strawberry Reservoir (see previous issue) and heat shock all of the fall-spawning Sand Creek strain rainbow trout. This would

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permit an evaluation of the *variation* of triploidy rates with various parts of the spawning cycle, such as early versus peak or late spawners and differences between ages of females. Additional research will be conducted by Fisheries Experiment Station (FES) researchers to examine additional rainbow trout strains and develop heat shock protocols for lake and brook trout. FES personnel would also evaluate ploidy in the lots tested and develop possible techniques for ploidy assessment using a Coulter counter machine in the Salt Lake City DWR office. The lake trout experiments are being coordinated with personnel from Idaho Fish and Game which are interested in stocking about 50,000 sterile lake trout into Bear Lake, located on the Utah-Idaho border. Idaho's Grace Hatchery will be raising these fish after shocking the eggs at Egan.

There will be some additional labor needed to conduct these heat shocks on a production scale. This usually requires two other people during a spawn day, one monitoring times and transferring eggs and the other carrying eggs from the spawn trailer to the hatchery building and rinsing the eggs. During peak spawning periods, an additional person may be needed. Personnel within the hatchery system and elsewhere will be called upon to help. Training is planned for one of the early egg takes.

As time goes on, I'm convinced that the triploidy induction process will be streamlined by inventive culturists. The outcome will be a useful fish for fisheries managers that are trying to balance the need to protect remnant cutthroat populations and provide a fast growing sport fish. In larger lakes or reservoirs where fish have a chance to get to 3-4 years of age, triploids may also provide bigger fish for anglers. The sterile fish put the energy into growth, rather than gametes. Recent research indicates the triploids have slightly large red blood cells, but are otherwise very similar to a normal fish. Monitoring of the triploids produced will be desirable to determine how these fish perform relative to diploids in Utah. Research in Idaho and Alaska has indicated that the triploids survive and grow at similar rates to diploids, but other reports indicate some possible negative effects. Triploid males may pose a threat to cutthroat trout by attempting to spawn with females even though no sperm is ejected. This could be addressed by using all female triploids, but would require additional work and a sex-reversed broodstock. Hopefully these initial steps will help provide some data to evaluate the success of the triploid program.

by Eric Wagner

Effect of Electrically Generated Mixed Oxidant Solutions on *Myxobolus cerebralis* Triactinomyxon Viability

INTRODUCTION

Control of *Myxobolus cerebralis* in hatchery water supplies is needed to prevent whirling disease. Several hatcheries in the country, such as some state fish hatcheries in Wyoming, New Mexico, and Utah, have had to bury fish that were infected. If the water supply could be treated to prevent the infection of susceptible fish, this would be of great benefit. Currently, ultraviolet treatment is the method of choice for treating incoming water (Hoffman et al. 1974; Hedrick et al. 2000). However, this technology can be very expensive and often requires prefiltering.

Mixed oxidants have been tested for disinfection of *Cryptosporidium* and *Clostridium* (Venczel et al. 1997). The oxidants are electrically generated from a sodium chloride brine. The electrolysis of the brine solution results in a variety of reactive oxidants including chlorine (Cl_2) and hypochlorite (OCl). The mixed oxidant solution has proven more effective than free chlorine on the basis of equal weight per volume concentration of total oxidants (Venczel et al. 1997).

METHODS

A mixed oxidant generator was generously loaned for testing by the Miox Corporation. A solution of sodium chloride was pumped through the electrolytic cell to generate the mixed oxidant (miox) solution. This solution was assayed for chlorine concentration according to standard methods (APHA et al. 1989) using a 1:500 dilution of the miox stock solution and $KMnO_4$ for standards. A series of 11 tests were conducted. Due to the short shelf life of the miox solution, the miox stock solution was assayed before conducting each of the tests. Dilutions were made with de-ionized water to achieve target concentrations (after mixing on the slide) of 4 to 300 mg/L as chlorine. *Myxobolus cerebralis* triactinomyxons (tams) harvested from the water of tubificid worm cultures were concentrated by filtration through a 20 µm mesh. Equal amounts of tam stock solution (tams in well water) and miox solution were mixed in test tubes, adding tam stock first. After exposure times of 1, 5, or 10 min, the tube contents were poured into a 20 µm mesh filter and rinsed with 20 mL of hatchery well water. The retentate (50 µL) was transferred to microscope slides and the vital stains propidium iodide and fluorescein diacetate were added (50 µL each). These were incubated in dark chamber in a refrigerator for at least 45 min before examination by epifluorescence light microscopy. Red-staining tams were categorized as dead, green-stained were viable, and red and green stained tams were classified as possibly viable. The number of empty tams (no spore body present) was also recorded and included in the total. At least 40 to100 tams per slide were counted to determine the percentages and 2 to 3 slides counted per treatment.

RESULTS AND DISCUSSION

The results of the testing indicated that miox solutions were inactivating tams, although the variation was high. The tam mortality percentage increased with dose and duration of exposure (Table 1). In 1 min exposures, dosages of 300 mg/L were required to reduce viability rates to zero. Even at this dosage, 32.8% were still possibly viable according to the vital staining (Table 1). For 5 min exposures, the percentage of possibly viable tams after exposure to 300 mg/L dropped to 2.0%. In 10 min exposures, the longer contact time resulted in higher mortality at lower concentrations; e.g., 100% mortality at 22-50 mg/L.

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Table 1. Summary of vital staining results of *Myxobolus cerebralis* triactinomyxons exposed to various concentrations of an electrolytically generated mixed oxidant solution for 0 (control), 1, 5, or 10 min.

Exposure duration (min)	Mixed oxidant concentration (mg/L)	Viable (%)	Non-viable (%)	Possibly viable (%)	Empty (%)
0	0	90.8 ± 8.5	1.2 ± 2.4	5.4 ± 5.8	2.8 ± 2.3
1	4	21.0 ± 15.6	17.5 ± 6.4	60.5 ± 10.6	1.0 ± 1.4
	22	2.5 ± 0.7	9.0 ± 1.4	87.0 ± 2.8	1.5 ± 0.7
	50	0.5 ± 0.7	4.5 ± 2.1	93.5 ± 3.5	1.5 ± 0.7
	110	6.0 ± 9.5	32.8 ± 14.4	58.5 ± 14.5	2.7 ± 0.9
	185	4.4 ± 6.2	36.4 ± 25.3	53.4 ± 23.8	5.8 ± 4.1
	230	0.0 ± 0.0	52.1 ± 40.8	41.3 ± 42.4	6.6 ± 5.2
	300	0.0 ± 0.0	55.8 ± 16.7	32.8 ± 18.6	11.5 ± 2.4
5	20	1.0 ± 0.7	10.1 ± 6.5	85.8 ± 7.2	3.1 ± 2.7
	100	0.0 ± 0.0	85.9 ± 11.5	10.3 ± 13.9	3.8 ± 3.4
	200	0.2 ± 0.5	82.9 ± 6.9	10.3 ± 13.9	6.0 ± 5.6
	250	0.0 ± 0.0	78.0 ± 22.0	19.2 ± 23.5	2.8 ± 2.7
	300	0.0 ± 0.0	85.5 ± 0.7	2.0 ± 0.0	12.5 ± 0.7
10	5	0.0 ± 0.0	36.3 ± 1.5	63.7 ± 1.5	0.0±0.0
	22	0.0 ± 0.0	94.2 ± 5.3	0.0 ± 0.0	5.8 ± 5.3
	50	0.0 ± 0.0	98.2 ± 2.4	0.0 ± 0.0	3.5 ± 2.1

These doses are higher than that observed in previous tests with sodium hypochlorite (bleach) in which concentrations of 131 mg/L for 1 min was needed to inactivate tams (Wagner et al. 2001). In 10 min exposures, the lethal levels were more congruous between studies (>22-50 mg/L for miox solutions, compared to >13-26 mg/L for bleach). The longer exposure times needed could be a problem when applied to hatchery water supply treatment. The toxicity of the oxidants to fish may also be problematic, although activated carbon filters could be used to eliminate the mixed oxidants prior to the treated water reaching the fish. Testing by EVS Environmental Consultants (Camille Merchant, unpublished data) indicated that 96 h LC_{50} for chlorine bleach was 0.89 mg/L, whereas miox solutions of 1.0 mg/L were not toxic. Further testing is needed to determine the toxicity of mixed oxidant solutions to fish.

Eric Wagner

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Effect of Calcium Chelators, Proline, and Glutathione on Polar Filament Extrusion of Triactinomyxons of *Myxobolus cerebralis*

Calcium has been shown to play a key role in the discharge of inverted threads within specialized structures called cnidae of some members of the phylum Cnidaria (jellyfish, anemones, corals). Nematocysts are one of three types of cnidae, specialized for prey capture. In isolated nematocysts (i.e., those that are no longer housed within the parent cell called a cnidocyte), contrary to in situ nematocysts, removal of calcium has induced discharge. For example, Lubbock and Amos (1981) used potassium citrate to chelate calcium; the drop in calcium induced discharge in nematocysts isolated from anemones. This effect was reversible by the addition of $CaCl_2$, but not NaCl, KCl or $MgCl_2$. Salleo et al. (1983) observed that isolated nematocysts of *Pelagia noctiluca* pretreated with $CaCl_2$ were inhibited from discharging when exposed to trypsin, an effect not observed in controls or for nematocysts treated with $MgCl_2$. Hidaka and Mariscal (1988) noted similar results using isolated nematocysts from *Calliactis tricolor*, but discharge depended on the medium used for isolation; e.g., no discharge occurred in Ca^{2+}-free artificial seawater, but there was heavy discharge among nematocysts isolated with sodium citrate and subsequently treated with low-osmolarity solutions. They surmised that loss of calcium from the capsule augmented discharge.

The effect of calcium chelators on polar filament discharge in Myxozoa has not been studied. Though a few preliminary tests have been conducted in which the effects of electricity, pH extremes, and various salts on polar filament discharge have been observed here at the station, the natural mechanisms of discharge in general in Myxozoa are still a mystery.

Also of interest in this study was the effect of chemosensitizing agents. In sea anemones, two distinct classes of chemoreceptors are found on neighboring cells of cnidocytes that predispose cnidocytes to discharge nematocysts in the event of prey contact (Thorington and Hessinger 1988; Watson and Hessinger 1987). One class of chemoreceptor binds N-acetylated sugars and the other binds certain amino compounds, including proline (Watson and Roberts 1994). N-acetylated sugars, which occur as conjugates of the surface mucins or external chitin of prey, sensitize cnidocytes to discharge (Thorington and Hessinger 1988). Glycine and N-acetylneuraminic acid (NANA) have chemosensitized nematocyst discharge in the sea anemone *Aiptasia pallida* (Thorington and Hessinger 1998). Spirocyst discharge was also modulated by these same compounds as well as proline (Thorington and Hessinger 1990). Chemoreceptors for N-acetylated sugars may stimulate adenylate cyclase to sensitize and tune mechanoreceptors involved in initiating the discharge of nematocysts (Watson and Hessinger 1992).

This article summarizes the results of tests with two calcium chelators, sodium citrate and EGTA, that have been effective discharge agents for Cnidaria (Hidaka and Mariscal 1988). Also the effect of proline and the tripeptide glutathione were examined as well.

The concentrations of EGTA tested were 5 and 12.5 mM. This was the final concentration after adding equal parts triactinomyxon (tam) stock solution (tams concentrated in hatchery well water). For each concentration 3 slides were made and over 30 tams observed on each slide. Because the pH had to be adjusted to get EGTA to dissolve, a pH 9.35 treatment was also included as a pH control that exactly matched the EGTA pH. A single slide of 25 mM EGTA was also examined. The time from mixing to examining the slide

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ranged from 1 to 60 min. Slides were kept in a sealed box to prevent drying during this time.

Sodium citrate was examined at 10 and 25 mM concentrations, mixing and counting tams as noted above for EGTA. A sodium chloride treatment was also included as a control for the effects of Na^+ per se. The tam-stock control was the same as for the EGTA tests since the tests were conducted on the same day with the same tam stock solution. A single slide of 50 mM sodium citrate was examined as well.

For both chelators, a tam polar capsule was considered fired if one of the three capsules had fired. Tams that had no spore body present were classified as empty and counted in the total. These tams may have extruded sporozooites *and* the polar filaments.

Results indicated that EGTA at both 5 and 12.5 mM concentrations induced significantly higher rates of discharge than controls (Table 1). However, the discharge rate at 5 mM was not significantly different from the pH control. The highest discharge rate achieved was 34.4% at 12.5 mM, indicating a possible dose response. However, the single slide observed at 25 mM had only 12.5% discharge. If the percentage fired and the percentage of empty tams were combined, the analysis of variance indicated that 5 mM EGTA induced significantly higher rates of discharge (47.2 ± 6.5%) than the pH control (30.7 ± 11.7%) and tam-stock control (0.8 ± 1.6%). The combined discharge data for the 12.5 mM EGTA treatment (38.5 ± 12.6%) was not significantly different from the pH control or the 5 mM EGTA treatment.

Table 1. A comparison of the effects of EGTA (pH 9.35) with untreated controls and a pH control (NaOH) on the mean percentage of discharged polar filaments of the *Myxobolus cerebralis* triactinomyxon (tam). The mean percentage of tams in which the spore body is missing, i.e., 'empty' and the number of slides (each containing >30 tams) counted is also presented. A different letter follows means within a column that are significantly different.

Treatment	Discharged (%)	Empty (%)	Combined (%)	*n*
control	0.0 ± 0.0 a	0.8 ± 1.6 a	0.8 ± 1.6 a	4
pH 9.35 control	18.2 ± 9.3 b	12.5 ± 7.9 b	30.7 ± 11.7 b	4
EGTA 5 mM	22.7 ± 5.1 b	24.5 ± 7.6 c	47.2 ± 6.5 c	3
EGTA 12.5 mM	34.4 ±15.6 c	4.2 ± 3.6 ab	38.5 ± 12.6 bc	3

The results with sodium citrate indicated that the concentrations of 10 or 25 mM induced significantly higher rates of discharge than in the tam-stock control (Table 2). However, both treatments did not differ from the NaCl control, indicating that the presence of sodium ion was likely responsible for discharge, rather than the chelation of calcium ion. When the percent fired and percent empty categories were combined, the discharge comparison gave the same results.

Table 2. A comparison of the effects of sodium citrate with untreated controls and a Na^+ control (NaCl) on the mean percentage of discharged polar filaments of the *Myxobolus cerebralis* triactinomyxon (tam). The mean percentage of tams in which the spore body is missing, i.e., 'empty' and the number of slides (each containing >30 tams) counted is also presented. A different subscript letter follows means within a column that are significantly different.

Treatment	Discharged (%)	Empty (%)	Combined (%)	*n*
control	0.0 ± 0.0 a	0.8 ± 1.6 a	0.8 ± 1.6 a	4
NaCl 25 mM	30.2 ± 6.2 b	12.6 ± 0.0 b	36.4 ± 12.6 b	4
sodium citrate 10 mM	17.4 ± 19.9 b	8.6 ±12.1c	26.0 ± 32.0 c	2
sodium citrate 25 mM	20.1 ± 7.8 c	10.0 ± 1.5 ab	30.0 ± 6.4 bc	3

To determine if chemosensitization would trigger polar filament discharge in Myxozoa, triactinomyxons (tams) of *Myxobolus cerebralis* were exposed to the amino acid proline. A solution of 10^{-5} M was made using deionized water. The proline stock solution was mixed in equal parts (50 µL each) with tams from a concentrated stock solution harvested fresh from worm cultures kept at the lab. One slide was examined and only 2 of 30 polar filaments had discharged (6.7%). It was clear that proline was not a potent discharge agent for tams.

In another test, glutathione, a simple tripeptide, was mixed with freshly harvested tams to evaluate the effect of this compound on polar filament discharge. Final concentrations after mixing were either 0.5 M or 0.05 M in microcentrifuge tubes. After 10-15 min, two slides at each concentration were made by transferring 180 µL of this mixture. These were examined at 100-400X for polar filament discharge. If at least one of the polar capsules had discharged, the tam was counted as discharged. The number of tams on each slide ranged from 24 to 46. The discharge rate of tams exposed to 0.05 M glutathione ranged from 33.3 to 35.1% (mean of 34.2%). At the higher concentration (0.5 M) discharge ranged from 17.9 to 34.8% (mean of 26.3%).

A follow-up test was conducted in which 2 slides were coated with either 0.1 M or 1.0 M glutathione and tams (180 µL) added. The slides were incubated for about an hour at room temperature (20.5EC) in a covered chamber. The slides were examined for polar filament extrusion at 100-400x. In two control slides made of the tam stock solution, polar filament extrusion ranged from 20.0-25.0% (*n* = 20-25 tams per slide). On the 0.1 M slides, discharge ranged from 37.2 to 48.9%. On the 1.0 M slides, the glutathione was too thick to observe tams. Results indicated a minor effect of glutathione on discharge.

The effect of calcium ion does not appear to apply to Myxozoa, or at least *Myxobolus cerebralis*. It also does not apply to all cnidae. Salleo et al. (1990) noted that very little calcium was discharged from the nematocysts of *Calliactis parasitica*. These authors also found that Ca^{2+} and Mg^{2+} -free artificial seawater did not induce discharge of isolated nematocysts. Mariscal (1984) has similarly observed holotrichous isorhiza nematocysts of *Haliplanella luciae* that appear to lack calcium, suggesting that other ions and chemical messengers aid in triggering these.

The effect of proline observed in sea anemones was not observed in these tests. Since the effect of proline is upon supporting cells and not directly upon the cnidocyte, this mechanism may not have evolved in Myxozoa. Similarly, glutathione, which was a potent discharge agent for Hydra (Loomis and Lenhoff 1956), failed to evoke a similar response from tams. Further research is needed to understand the discharge mechanism of polar filament extrusion in Myxozoa.
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And the Folks Keep Comin' and Goin'!

The newest member of the Fisheries Experiment Station Crew is **Eriek Hansen**. Eriek is working as an AL wildlife technician, acting as chief caretaker for the June sucker culture facility.

Eriek is a graduate of the school of Wildlife & Fisheries at Utah State University. He earned a B.S. degree there in 2000. He hopes to eventually earn a masters degree in fisheries. Besides culturing fish, Eriek enjoys fishing and bowhunting. Welcome to FES!

Also, long time whirling disease biologist **Art Butts** will soon be leaving FES to work as a research biologist for Idaho Fish & Game at Nampa. Utah's loss will be Idaho's gain and Art's valuable efforts will be missed.

Good luck to both these guys in their new jobs!

Triactinomyxon Release and Worm Survival Among Mixed Species Oligochaetes Reared Under Low Oxygen Conditions

The threat of the whirling disease parasite *Myxobolus cerebralis* to wild and stocked trout in Utah has existed for a decade. However in the past several years the threat to state hatcheries has increased as the number of infected waters in proximity to hatcheries has grown. Several state hatcheries have used dirt ponds and raceways, which can be a good habitat for infected *Tubifex tubifex* worms and serve as a focal point of infection. Disinfection of such dirt systems may be very difficult , so research was undertaken to look at the effects of anoxic conditions on oligochaete survival and triactinomyxon (TAM) production among *T*. *tubifex*. It is possible that the development of TAMs, the infective stage to fish, could be affected such that their release by worms could be reduced or halted. If the parasite could be purged from the worm host under low oxygen conditions, then it may be a control strategy.

A substantial amount of research has been conducted on various water quality parameters and their effect on *T*. *tubifex* survival. It has been shown that *T*. *tubifex* actually survives better in low, compared to high, oxygenated water and can survive up to 296 days in oxygen free water. The survival of *T*. *tubifex* under anoxic conditions has been relatively well established. However, what has not been established is their survival under chronically low oxygen conditions, and the impact of those conditions on the release of TAMs from *M*. *cerebralis* infected worms. The purpose of this research was to determine the influence of low oxygen conditions on the survival of *T*. *tubifex* in mixed oligochaete populations, and the impact of those conditions on TAM release.

In the first of two tests, mixed species oligochaetes were placed into beakers that contained autoclaved silt and well water. The worms were from a culture collected from the Mt. Whitney State Fish Hatchery, Independence, CA. The culture had been actively producing *M*. *cerebralis* TAMs for several months. The worms were allowed to acclimate to the beakers for a period of 19 days under oxygenated conditions at 15E C. Four beakers were placed into a low oxygen chamber after the acclimation phase. The chamber was a plastic tub (7.8 L) that contained well water to a depth such that the beakers were covered by approximately 2 cm of water. Nitrogen gas was bubbled into the container by an air stone to lower the oxygen level. The target oxygen concentration was 1-2 mg/L. Each beaker was covered with a piece of 20 Fm Nitex mesh held in place with a rubber band. The beakers were covered to keep TAMs from drifting out of the individual beakers, but to allow for gaseous exchange. The remaining four control beakers were covered with mesh and placed into a similar tub that contained water oxygenated with an aquarium pump and air stone. All cultures were routinely checked for TAMs. The test was discontinued after 37 days of low oxygen conditions due to the apparent high mortality among the treatment worms, and also because of the decrease in TAM production between both the treatment and control worms.

In the second test, mixed species of TAM-producing oligochaetes collected from the East Fork of the Little Bear River (Cache County, Utah), were placed into similar beakers as Test 1, and allowed to acclimate for a period of 21 days. For this test the beakers were not covered with the 20 Fm Nitex mesh, but were left open to the water in the tub. This was done to address concerns about adequate gaseous exchange in the first test within the mesh-covered beakers. However, five days after the start of the test, worms began to climb out of all treatment beakers into the larger tub. To address this the loose worms were pooled together and divided equally among the three beakers. To avoid further problems nitrogen was bubbled to each treatment beaker individually, and each beaker was covered with a piece of clear kitchen plastic wrap held in place with a rubber band. The controls beakers were each aerated with an air stone after that point to maintain continuity. The test was conducted an additional 19 days under these conditions until it was ended due to a decrease in TAM production for both the treatments

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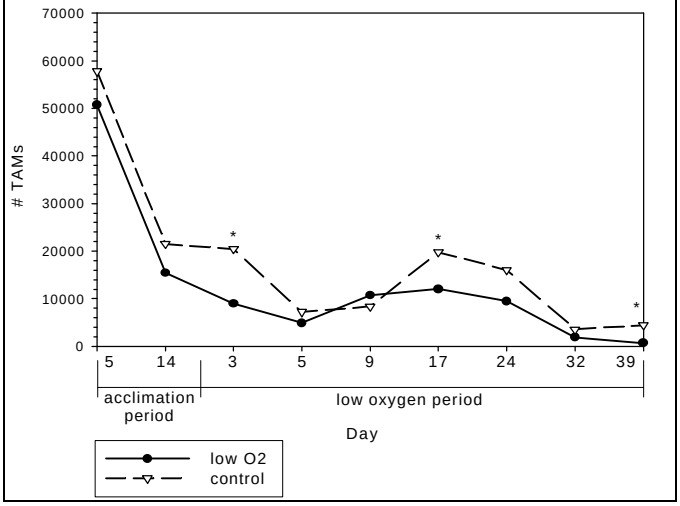
and controls. The acclimation and test period were conducted at 15E C. At the conclusion of the test, worms were washed from the silt, and counts were made on the number surviving. Samples were preserved in 10% buffered formalin followed by 70% alcohol for later species identification to check for differential mortality between the controls and the treatments. The length of the test was 21 days of acclimation and 24 days under low oxygen conditions.

For both tests, tams were collected and enumerated by drawing water from each beaker via a siphon onto a 20 Fm Nitex mesh filter. The material retained on the filter was then washed into 50 mL plastic test tubes and the total volume recorded. From each tube, three 50 FL sub samples were withdrawn and placed into individual glass test tubes. This quantity was then stained with crystal violet, prepared as a wet mount, TAM counts made, and total TAM production calculated.

Triactinomyxon (TAM) releases over the course of the acclimation and low oxygen phases of Test 1 showed a gradual decrease in production over time (Figure 1). During the acclimation period average TAM production was high for the first sample; 50,698 for the low oxygen treatment and 57,785 for the controls. By the second week of acclimation the TAM production had dropped to 21,510 for the controls and to 15,437 for the treatments. Following the acclimation period, when the worms were actually under low oxygen conditions, several significant differences were found in TAM production between the control and treatment groups (Figure 1). After the treatment worms had been in the low oxygen chamber for three days, TAM release was significantly higher among the controls (20,430) compared to the treatment worms (8,942). TAM release was also significantly higher for the control worms (19,792) after two weeks post acclimation compared to the treatment worms (12,018). The final TAM sample, five weeks post acclimation, was also significantly higher for the control worms (4,415) than the treatment worms (667).

Figure 1. Low Oxygen Test 1: Tam release from mixed species oligochaete cultures kept well oxygenated (8-9 mg/L) conditions for 19 days and then under either low oxygenated or well oxygenated conditions for an additional 37 days. An asterisk indicates a significant difference (P < 0.05) for a given sampling day.



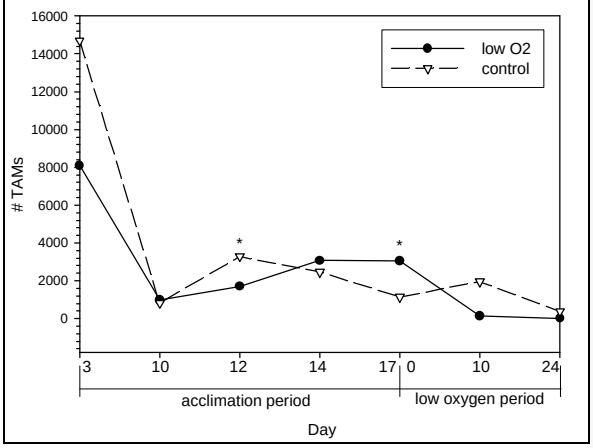
Visual observations of the treatment worms during the test phase indicated possible stress and elevated mortality in response to the low oxygen treatment. After the acclimation phase, most worms were buried in the silt and were not readily observable, however after four days in the low oxygen chamber, a large number of dead worms appeared on the surface of the silt. These mortalities continued for approximately one week, after which they stopped. Several mortalities were also found within the control beakers. No attempts were made to quantify the mortalities because of their decomposed state. Worm counts were made at the end of the test did indicate a significant difference in survival between controls and treatments. Within the treatments, worm survival averaged 17% compared to 69% for the controls. Oxygen measurements over the course of the test averaged 2.3 mg/L for the treatments and 8.8 mg/L for the controls.

The results from the second test revealed several significant differences in TAM production. However, they occurred during the acclimation phase prior to the treatment worms being placed in the low oxygen chamber. As with Test 1, TAM release decreased rapidly shortly after the worms were placed into the test beakers at the beginning of the acclimation phase (Figure 2).

The average for all beakers was 11,398 on day three of the acclimation phase, but by day 10 this had dropped to 901. Twelve days into the acclimation phase the control worms released significantly more TAMs (3280) than those designated as treatments (1,710). On the 17th day of acclimation the treatment worms released significantly more TAMs (3,060) than those designated as controls (1,140). The two TAM samples taken during the low oxygen exposure indicated no significant effect on TAM release.

Beginning on day 12 of the low oxygen phase, a large proportion of dead worms were found on the surface of the silt. As in Test 1, no attempt was made to quantify the worms due to their state of decomposition. Dead worms routinely appeared on the silt surface through day 19 after which we assumed the majority of treatment worms were dead because no new mortalities were seen, and live worms were not observed in the silt or by looking through the transparent sides of the beakers. Final worm counts made on the treatments revealed very low survival. An average of 35 worms were recovered from the low oxygen beakers, down from the initial 700, which resulted in a survival percentage of 5%. Final counts were not made on the controls. During the low oxygen phase of the study, oxygen concentrations averaged 1.0 " 1.5 mg/L for the treatment beakers and 8.0 for the controls.

Figure 2. Low Oxygen Test 2: TAM release from mixed species oligochaete cultures kept under well oxygenated (8-9 mg/L) conditions for 21 days and under either low oxygenated conditions or well oxygenated conditions for an additional 24 days. An asterisk indicates a significant difference for a given sampling day.



It is difficult to say, with a great deal of confidence, that rearing worms infected with *M*. *cerebralis*, under low oxygen conditions had an effect on TAM release. For both tests TAM release was already decreasing during the acclimation phase of the test for all cultures. During the low oxygen phase of Test 1, TAM release was significantly less for the treatments compared to the controls on three sampling dates. The first sampling date corresponds with the initial die-off observed among the treatments. It is intuitive to assume that TAM production would be less among a group of worms where a significant proportion of potentially TAM producing individuals had died. Whether or not there was a differentially high mortality among those individuals infected with *M*. *cerebralis* cannot be concluded. Although one could theorize that those infected individuals might be more susceptible to environmental stressors due to a degraded physiological state derived from the infection. TAM release was already at such a low point after the acclimation phase from Test 2 that it is difficult to make any conclusions about the effect of oxygen levels on TAM production.

From both tests we can conclude that mixed specie cultures of oligochaetes, containing *M*. *cerebralis* infected *T*. *tubifex*, experience a high level of mortality when exposed to oxygen concentrations of 1-2 mg/L. Treatments from both tests experienced mortality of more than 80% of the original worms. The method we used to lower oxygen, bubbling nitrogen gas, has been implicated in other studies as a cause of mortality as opposed to the low oxygen levels themselves. The continuous nitrogen bubbling may have negatively affected the physical state of the worms in some way. During both tests large scale mortality occurred during days 4-12 of low oxygen exposure.

Future tests may be required to address the issue of constant TAM release immediately prior to low oxygen exposure. Also due to worm mortality at the oxygen levels tested it may be necessary to test for the effects of higher oxygen concentrations on TAM release and possibly the role *M*. *cerebralis* infection plays on the ability of infected worms to survive low oxygen levels. **Ronney Arndt**

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