

The Ichthyogram

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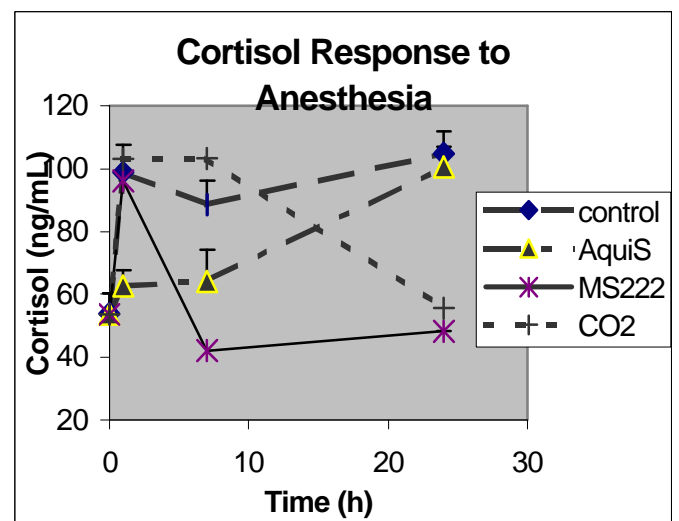
COMPARISON OF THE STRESS RESPONSE OF RAINBOW TROUT TO ANESTHESIA BY AQUI-S, CO₂, OR MS-222

The stress response of rainbow trout broodstock to anesthesia by MS-222 (tricaine methanesulfonate), AQUI-S⁷ (Aqui-S Ltd, New Zealand) and carbon dioxide gas was measured by assaying plasma chloride, glucose, and cortisol. Concentrations of 60 mg/L MS-222, 40 mg/L AQUI-S, and 220 to 275 mg/L carbon dioxide were based on preliminary tests and chosen to standardize induction time among anesthetics. Plasma cortisol concentrations generally indicated that anesthesia after crowding and netting did not eliminate the stress response, but did speed up return to baseline levels compared to controls. The recovery pattern differed among the three anesthetics. Fish anesthetized with AQUI-S had significantly reduced cortisol concentrations at 1 or 7 h post-immersion, but was elevated at 24 h. MS-222 and CO₂ treated fish returned to baseline levels within 7 and 24 h, respectively, whereas controls remained elevated at 24 h.

Recovery time was significantly longer for fish anesthetized by AQUI-S (370 sec) than the either CO₂ or MS-222 (192-199 sec). Gender had no effect on recovery time, nor did blood collection. The survival of eggs to the eyed stage and to hatch, from

anesthetized females and either anesthetized males or un-anesthetized males, did not differ from un-anesthetized controls. No delayed mortality was observed for any of the fish handled and bled for the test. Results indicated that MS-222, AQUI-S, or CO₂ were all suitable for brood anesthesia, but the longer recovery time and lower cost for AQUI-S may make it more useful than the alternatives. None of the anesthetics wholly controlled the stress response during a typical spawning process, but they helped reduce the duration of the stress response without compromising egg viability.

Eric Wagner



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Envirochek® Filter for TAM Filtration

The Envirochek filter is a commercially produced (Pall-Gelman Co.) cartridge filter with a nominal pore size of 1 μm . It is used routinely for sampling of the human parasites *Giardia* and *Cryptosporidium*. Of interest in this study was the ability of this device to filter triactinomyxons (TAMs), the infective stage of *Myxobolus cerebralis*, the causative agent of whirling disease.

About 55,500 TAMs obtained from Utah State University cultures were added to 38 L of hatchery well water. A submersible centrifugal pump was used and a valve used to control flow, which averaged 0.93 L/min during the 41 min filtration time. The hoses were flushed and emptied into the cartridge upon emptying the plastic tank. Phosphate-buffered saline (PBS, 110 mL) was added to the cartridge, which was subsequently shaken for 5 min laterally to loosen TAMs from the membrane. Next, 2 mL of this liquid was put into each of 7 test tubes and stained with 10 μL of crystal violet. Two slides of 85 μL each were examined for TAMs for each tube. Recovery averaged only 0.33%.

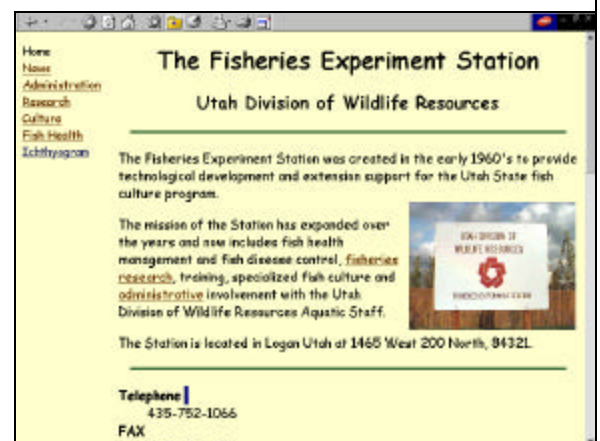
A test was conducted to isolate the possible effect of shaking in PBS on TAM recovery. In a test tube, 2 mL of TAM stock and 2 mL of PBS were mixed and shaken for 5 min. Recovery in this sample was about 85%, indicating that shaking in PBS was not a major source of TAM loss in the filter. In another follow-up test, 30,720 TAMs were added to a new filter, as well as PBS, and shaken for 5 min. This avoided the pump, valve, and hoses of the first test. Six slides of 100 μL each were examined from the 140 mL recovered from the cartridge. TAM recovery was 60.0%, indicating that significant losses were occurring without even applying pressure. Also it was apparent that the pump, pressure through the cartridge, and other factors were also contributing to TAM loss. The recovery rates were considered low enough that these filters are not recommended for TAM filtration.



FES Website Finds a New Home

The website of the Fisheries Experiment Station has found a new home with a domain name all its own. Effective September 1, the website can be found at <http://www.udwfes.org/>.

Changing the site will allow for much quicker updates of the site. The electronic form of the Ichthyogram will also be available online at this site as before.



EFFECTS OF SALINITY ON OLIGOCHAETE WORMS WITHIN AND WITHOUT SEDIMENT

The treatment of aquaculture ponds infected with *Myxobolus cerebralis* (whirling disease) is of interest, especially at Utah's Midway Hatchery which became contaminated with the parasite this year. In 1983, Maria Markiw and Ken Wolf, U.S. Fish and Wildlife Service, demonstrated that an oligochaete worm *Tubifex tubifex* is the alternate host of the parasite. To better understand what factors may affect worm survival in ponds, lab tests were conducted to determine the tolerance of oligochaetes (especially *T. tubifex*) to high temperature and salinity. All tests were conducted with worms collected from the Logan River where *T. tubifex* and *Limnodrilus hoffmeisteri* have been previously identified. Although *M. cerebralis* has been found in the Logan River drainage, the cultures used for the tests did not produce TAMs. The temperature tests are still ongoing, but the salinity data is summarized here.

Preliminary tests were conducted using test tubes without sediment, exposing 5 worms in each tube to various combinations of temperature and salinity. In 10 min exposures, temperatures of 30, 40, and 55EC resulted in average ($n = 3$) mortalities of 0, 89, and 100%, respectively. In a single 100 min exposure test, mortality at 35EC averaged 48%. With sediment added, 100 min exposure to 30EC did not result in any mortality. Salinities of 10, 15, 20, and 25‰ resulted in mortalities of 0, 33, 100, and 100%, respectively, in 10 min exposures at 18EC without sediment. With 2 cm of washed and autoclaved sand added to the tubes, the worms were protected from the effects of high salinity; salinities of 60‰ resulted in only 42% mortality after a 10 min exposure. However, by increasing the exposure time to 100 min, 10‰ was sufficient to kill all the worms.

Subsequent salinity and temperature tests were conducted using larger containers (250 mL Erlenmyer flasks or beakers) and

more worms per container (30). Each container had 2 cm of mud which had been filtered and autoclaved. After each exposure, worms were recovered on a sieve that was rinsed to remove the fine silt. The amount of time given for worms to acclimate to the mud was a concern, so salinity tests were conducted at acclimation times of 10 min and 24 h.

For the acclimation tests, worms that had been kept at 9EC were exposed for 100 min to 30EC or a combination of this temperature and salinities of 30, 40, 50, and 95‰. When tested separately for each salinity with *t*-tests, longer acclimation did not significantly reduce mortality. However, when the data were pooled across all salinities, mortality was significantly ($P = 0.053$) reduced at 24 hr (14.3% vs 8.1%) compared to the shorter acclimation treatment (24.2% vs 9.7%). Salinity of up to 95‰ combined with temperatures of 30EC did not kill all the worms in 100 min exposures (41% survival). One group exposed to 95‰ for 24 h also had an average survival rate of 39%.

It was apparent that salinities of up to 95‰ were insufficient for killing 100% of worms in as little as 2 cm of mud. It is likely that other chemicals would have a similar problem in reaching worms buried in sediment. Chapman et al. (Aquat. Toxicol. 2:47, 1982) noted that the LC_{50} for salinity for *T. tubifex* in 96 h tests was 9‰ without sediment and 14‰ in 5 mm sediment. The longer exposure and shorter sediment depth would explain most of the differences between this study and theirs.

Eric Wagner, Quinn Cannon, and Ronney Arndt

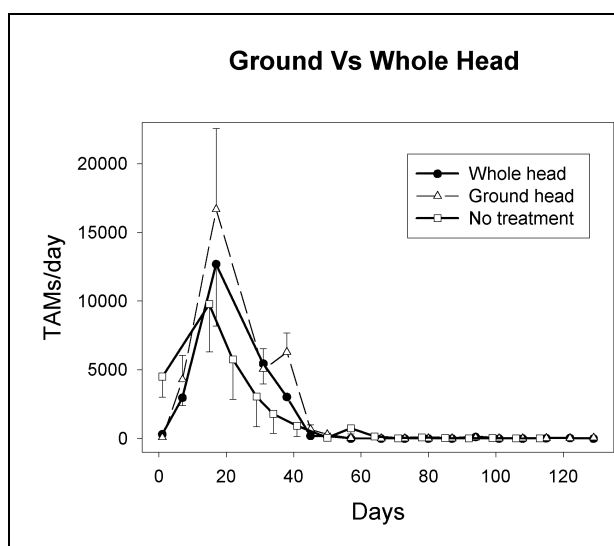
COMPARISON OF TAM PRODUCTION FROM *TUBIFEX TUBIFEX* CULTURES FED INFECTED GROUND OR WHOLE FISH HEADS

Whirling disease is caused by *Myxobolus cerebralis* and has an aquatic worm y (*Tubifex tubifex*) as an alternate host. The worm may feed on dead fish that are infected with *M. cerebralis* spores. The spores transform into the infective triactinomyxon (TAM) stage within *T. tubifex* after a period about 3.5 months.

Production of the TAMs in worm cultures is necessary to conduct research on this stage of the life cycle. For worm cultures kept at the Fisheries Experiment Station, water is exchanged in all of the cultures three times a week. Previously, *M. cerebralis* spores in the form of ground fish heads were added to the worm cultures in an attempt to increase TAM production. Generally, after 81-83 days at 15EC (Gilbert and Granath 1998), TAM production begins in infected worms. However, we have not observed the increase in production in our cultures after this period. It is hypothesized that many of the spores that were added may have been siphoned, or poured out during water exchanges.

An experiment was conducted to determine the differences in TAM production by *T. tubifex* cultures that were fed *M. cerebralis* spores in the form of either ground fish heads, or whole fish heads. The objective was to determine if adding spores in the form of whole fish heads to worm cultures is a solution to the apparent problem of losing spores during water exchange. Infected oligochaete worms were collected from a private fish farm in Paradise, Utah, on February 15, 2000. 70 g of worms were put into each of six containers containing hatchery well water and small amounts of organic matter. Worms were kept in a refrigerator at 14EC. Additional worms were collected on February 22 and two more cultures of 70 g each were started as a control group (no spores given). Whole heads or 4-7 g of ground heads, infected with *M. cerebralis* spores, were first added to cultures on March 10, again on March 31, and then once a month. Both spore sources were frozen prior to use. Water in the cultures was exchanged with fresh well water three times a week. TAMs were harvested and counted one to two times per week. Also, cultures were weighed periodically to track the survival of the worms.

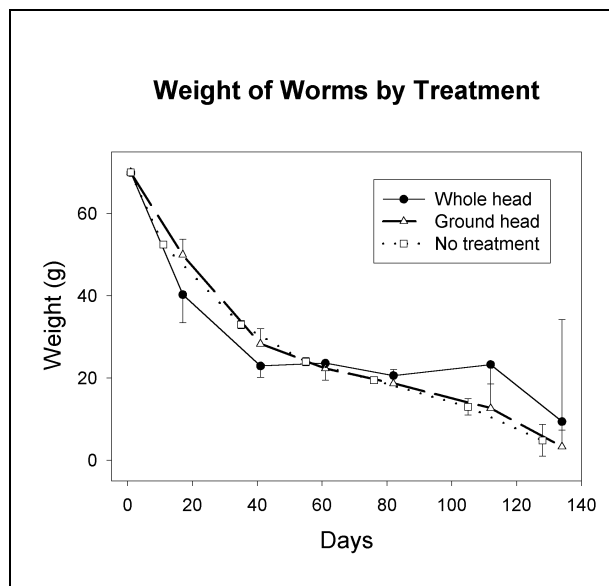
Each culture produced the greatest number of TAMs between day 14 and day 20, with the ground head treatment averaging the highest and the control group averaging the lowest (Figure 1). From this point on, the number of TAMs produced by each culture steadily declined and leveled out at zero on about day 60 and remained at zero for the remainder of the treatment period. An increase in TAM production did not occur during the 100 day period following the climax of production.



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In addition to a decrease in TAM production, the weights of the worm cultures dropped steadily (Figure 2). The average weight of the worms in each treatment started at 70 g and plummeted to a meager 5-18 g.

The experiment was ended on June 30, 2000 because of the drop in the number of worms and the resulting lack of TAMs produced. It could not be determined if the spore treatments had any significant effects on the TAM production because the worms may have died before the additional spores were consumed and transformed into TAMs. Furthermore, it has not been determined why the number of worms declined so dramatically. A follow up study comparing infected worms versus uninfected worms is necessary to determine if the loss of worm biomass is attributable to infection or merely attrition in the worm population without recruitment.



Quinn Cannon, Mark Smith, Ronney Arndt, and Eric Wagner

GOODBYE TO NELMA

Another important milestone was recently passed at FES with the retirement of **Nelma Gates**. Nelma Gates started working at the Fisheries Experiment Station in 1968 as a clerk/typist and over the years developed skills (many of them self-taught) in histology and clinical pathology preparation. She was later promoted to wildlife technician. Her histology work has been crucial in helping to diagnose whirling disease and solving other fish disease problems over the years. She helped raise awareness of chemical safety and helped provide FES and state hatcheries with vital information in that endeavor. Her keen eye for detail enabled her to work on database entry and provide legal records of fish health inspections and whirling disease surveys. Her work at FES has been as valuable as any biologist and she is recognized throughout the station and DWR for her innovation, service ethic and willingness to go the extra mile.



The FES Staff posed for a photo at Ron Goede's retirement party: (L-R) Mark Smith, Eric Wagner, Chris Wilson, Doug Routledge, Janet Baker, Ron Goede, Nelma Gates, Robert Montgomery, Kent Thompson and Roger Mellenthin. Not present: Ronney Arndt, Ernie Dean. Photo by Ron Goede

Nelma was also noted for her coining of innovative pathologic terms, such as "yucky-poo". Perhaps her most famous quote in describing work was "I thought I was having a warm, fuzzy feeling, but it turned out to be a hairball!" Nelma plans to spend her retirement doing crafts and traveling frequently to visit and spoil her grandchildren properly.

SAMPLE VOLUME, MESH SIZE, AND 24 H DELAYED SAMPLING EFFECTS ON TRIACTINOMYXON RECOVERY

Sample volume and mesh size

Research conducted at the FES during 1999 indicated that certain aquatic animals such as amphipods, ostracods and daphnia may prey upon triactinomyxons (TAMs) in a laboratory setting. This research may have important implications in the lessening of whirling disease's impact. For example, if a large population of daphnia is present in an infected reservoir, it may be possible to predict the number of TAMs preyed upon by daphnia and model the potential for disease severity. To better understand this relationship it was necessary to scale our research up to water volumes that would be more representative of the wild. The previous tests were conducted in volumes as small as 1 mL. Preliminary work conducted several years ago indicated that as the volume of water filtered increased, the percentage of TAMs recovered dropped dramatically. So, to better understand what volume of water to conduct future TAM predation work in, it was necessary to determine what optimal volume of water we could filter to still expect a reasonable TAM recovery, but still adequately represent conditions in the wild. Filter mesh size may also significantly influence the ability to recover TAMs from a solution. The standard mesh size used for field work is 20 Fm, but questions have been raised about the potential for TAMs to get through openings of that size. A series of tests were therefore conducted where volume and filter mesh size varied in an attempt to determine that optimal volume.

The first test involved filtering volumes of 46.5, 93, 186, and 372 L (12, 24, 48, and 96 gal) that had been spiked with TAMs from FES worm cultures. Approximately 1,332 TAMs were diluted into 15 L of pond water at our field site (Paradise Springs Ranch, Paradise, Utah). The water was then poured into a plastic tub fitted with

outlet valves so flow could be controlled. A 200 Fm pre-filter was located on top of the tub to filter the additional water added from each test volume. All water exited the control valves and was filtered through 20 Fm Nitex mesh filters. Two replicates were run for each test volume. Retentate from each test was placed into a resealable plastic bag and fixed with formalin. The samples were then examined for TAMs using the protocol of Thompson and Nehring (1998, p.177-180, Whirling Disease Symposium: Research in Progress). No TAMs were found in any of the volumes tested.

There was one difference in technique between what we did and the protocol of Thompson and Nehring (1998). Our filters were constructed from 6-12 in dia. PVC pipe with the Nitex mesh placed across the inside of a short section of the pipe which was then sealed in place with silicon. In a follow-up to the above test we decided to compare our PVC pipe filter with a design more closely related to that used by Thompson and Nehring (1998), a slanted sheet of mesh material. For our design, a piece of 20 Fm Nitex mesh was fitted to a window screen frame. One liter of TAM-spiked water (2,244 TAMs) was then filtered through both filter types. Recoveries for the pipe filter averaged 55 " 22% and 16 " 24% for the slant filter.

From these tests it was obvious that at large filtering volumes, TAM recovery approached zero, and that our filter design performed at least as well as the slant filter. However in order to conduct continued TAM predation work, a volume had to be chosen that allowed for enough TAMs to be recovered in order to make any inferences about predation effects on TAMs.

For the next series of tests it was decided

to start at smaller volumes of water and work up from there. Four volumes of water were tested: 2, 4, 6, and 8 L. For each of these volumes, water samples were filtered through either 10 or 20 Fm mesh filters constructed with Nitex mesh and 15 cm dia. PVC pipe. For the actual tests the water was placed into glass Erlenmeyer flasks for the 2-6 L tests and 19 L plastic buckets for the 8 L tests. The water was then spiked with the concentrated TAMs. For all tests TAMs were collected from FES cultures and well water samples were spiked with a like concentration of TAMs (. 13,000). After a 5-10 min dispersal time the water was filtered through one of the two filter sizes and concentrated to 15-30 mL. From this sample five subsamples were removed, stained with crystal violet, placed on a slide (50FI) and read under the microscope.

percentage would continue to decrease.

24 h Delayed Sampling

The research described above indicated that 2-6 L volumes would be adequate for using in TAMpredation work. It also indicated that there was not an increase in TAM recovery when a filter mesh of 10 Fm was used compared to 20 Fm. However some preliminary tests indicted that recovery was very low when a volume of 4 L was spiked with TAMs, and left for 24 h prior to filtering. The 24 h time was used because that was the duration of previous TAM predation work. Because of this loss of TAMs over time, a series of tests were conducted to determine what variables might be influencing recovery over time. These variables included water temperature, aeration, water volume, and

V

Volume (L)				
Filter size (Fm)	2	4	6	8
10	80 " 26	80 " 15	74 " 5	54 " 17
20	62 " 6	69 " 45	96 " 8	40 " 32

Table 1. TAM recovery from 2, 4, 6, and 8 L samples filtered through either 10 or 20 Fm filters.

From the number of TAMs counted per slide, the volume of filtrate, and the initial TAM concentration, TAM recovery was calculated. All treatments were run in duplicate over a two week period.

The results from this series of tests indicated no significant differences between treatments although there appeared to be a decrease in recovery as the volume increased (Table 1). Two-way ANOVA revealed no significant influence of volume, filter, or interactions between the two. Replicates among the various treatments tended to have large standard deviations. At 8 L it appeared that the trend was towards a decrease in recovery and is likely that at larger volumes the recovery

presence of predators.

The first sequence of tests were conducted in glass Erlenmeyer flasks using 4 L well water spiked with approximately 13,000 TAMs per container. After 21-24 h, water was filtered through a 20 Fm filter. From this retentate, five subsamples were examined and percent recovery was calculated as discussed previously. All treatments were run in duplicate over a three week period.

The first test evaluated TAM recovery after 24 h at room temperature (30EC) with aeration. The second follow-up test consisted of treatments of 9E C with

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aeration and 9E C without aeration. No significant differences were found between treatments although the 9E C treatment with aeration and without had slightly better recoveries, 24% and 17%, than the room temperature one, 9%. Overall, the recoveries were much lower than those experienced during the previous work with filtering volume and filter mesh size which ranged from 40-96% recovery.

One theory to explain the low recovery was the presence of protozoa and small crustaceans that were collected along with the TAMs from the original worm cultures. The presence of these potential predators is thought to significantly reduce TAM numbers over a period of time. This could explain the recovery differences between this study and the previous one involving volume and filter size which were conducted over minutes rather than hours.

To remedy this problem steps were taken pre-clean the TAMs immediately after collection from the worm cultures. Dr. Don Roberts, Utah State University Biology Dept., who is also involved in whirling disease research, suggested allowing the debris and protozoans to settle out of the TAM stock solution for 20 min prior to using them.

This procedure was evaluated in a further study. TAMs were collected from the FES cultures which was then split into two vials. One vial was allowed to sediment for 20 min in a glass cone, and the other was not. TAM counts and protozoan/crustacean counts were made from both vials immediately prior to use. The sedimentation process did not negatively influence TAM numbers in the two stock solutions, but it dramatically reduced the number of predators. Numbers were 52,700 for the stock solution that was not sedimented, and were only 11,700 for the sedimented solution. TAMs (10,000) from both groups were then placed into small Erlenmeyer

flasks containing 1 L of well water. After 24 h, samples were taken and slides read according to the protocol previously mentioned. The results indicated no significant differences between treatments with the average recovery from the sedimented TAMs being 87% and from the non-sedimented TAMs 70%. These results more closely reflect those found during the previous work with volume and filter size and were a substantial improvement on the other 24 h recovery work.

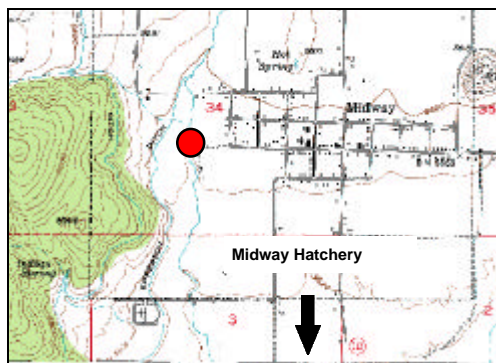
A further study was conducted to determine if recovery rates were similar between 1 and 4 L volumes using sedimented TAMs over 24 h. TAMs were cleaned as discussed above and then 1 and 4 L flasks (in duplicate) were spiked with an estimated 13,451 TAMs per flask. An estimate of protozoan numbers in the stock solution was also made by counting their numbers on 4 slides of 50 FL each. An estimated 25,373 protozoa were present in each container. Slide counts (4 slides for each of two replicates) and recovery calculations indicated that TAM recovery in the 1.0 L treatment averaged 62 " 6% and 47 " 2% for the 4 L treatment. This difference was not significant ($P = 0.333$). By cleaning the TAMs with sedimentation prior to use and by possibly using smaller volumes such as 1 L, we can now further pursue the line of TAM predation work originally set out for.

Ronney Arndt

NEW FINDINGS OF WHIRLING DISEASE PARASITE IN UTAH

Recent editions of the *Ichthyogram* carried reports of preliminary evidence of the whirling disease parasite, *Myxobolus cerebralis* at Midway hatchery in earthen systems at the low end of the hatchery system as detected by polymerase chain reaction (PCR) . Continuing surveys have demonstrated the parasite in wild fish adjacent to Midway hatchery in Summit county as well one other area in the state.

Young of the year brown trout have tested positive for both spores and by PCR in **Snake Creek**, a tiny tributary to the Provo River which runs west of Midway hatchery, but combines with the effluent from the hatchery (see map). Adult fish tested negative by both methods. It is believed that this is a recent infection, like Midway hatchery. The open water connection between the creek and the Provo river suggests both the creek and the hatchery may have become infected from a common source.



Map of Midway area showing site (red dot) of positive fish sampled from Snake Creek.

Also positive was **Geyser Creek**, upstream from the previously positive sites on Geyser Ditch. The latter site was found positive presumably from infected rainbow trout stocked into Buckeye Reservoir by the state of Colorado. UDWR biologists had hoped Geyser Creek was negative and had planned to raise a barrier to prevent the movement of infected fish upstream.

COMING SOON TO A COMPUTER NEAR YOU: ITS AUSUM FOR EXCEL!

Due to the widespread use of the AUTOPSY-BASED FISH HEALTH/CONDITION ASSESSMENT SYSTEM (AUSUM) both in and out of Utah, and the possibility of the UDWR switching to Microsoft products, a MS EXCEL version of the AUSUM program has been developed. The new "AUSUM EXCEL version 10/2000" consists of three worksheets accessible by tabs. The first tab is a title page with information about the program. The second tab is a data sheet very similar to the data sheet filled out when doing the HCP (health condition profile) necropsies. In this sheet, information is entered in as on the necropsies data sheet. It also includes three new areas: the deformity index, the skin lesion index and the fin deformity index. Ron Goede developed these three indices (See *Ichthyogram*, Volume 6, #3). Each fish is either given an N for normal in each index or abbreviations of deformities are listed. The third tab is the summary of necropsies. This page summarizes the raw data much the same way as the old program, but it has a few new features. In addition to the mean, standard deviation, and coefficient of variance we have added maximum value and minimum value columns. In the section titled "values as percent of total sample" we have added the three deformity indices. This section reads the deformities listed in the data sheet and converts them to a simple composite index and sums the binomial nominal variables, then divides them by the number of fish sampled, the mean number of deformities and the percent of possible deformities are reported. The debugging process should be finished by Oct 1st 2000 and it will be posted on the network G: drive for UDWR employees to try and review. Please send any questions/comments to: **Roger Mellenthin**: nrdwr.rmellent@state.ut.us.

The Ichthyogram is a quarterly publication of the Fisheries Experiment Station, Utah Division of Wildlife Resources, Logan Utah 84321.

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