The Ichthyogram

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NEWLY CREATED FISH HEALTH BOARD BEGINS WORK

Based on legislation passed in the last session of the Utah legislature, the newly created fish health board has been appointed and begun their work. H. B. 407, considered a compromise bill, called for the formation of a seven person policy making board, which will set fish health rules for the entire state. Fish health programs from both the Utah Dept. of Agriculture and Wildlife Resources will operate under these rules, while overseeing their legislatively mandated constituency.

Voting members are balanced between representatives from the Department of Agriculture, Wildlife Resources, private aquaculture and sportsmen. Agriculture is represented by Mike Marshall, state veterinarian and Kent Hauck, fish pathologist. Wildlife Resources is represented by Division director John Kimball and Ron Goede, director of the Fisheries Experiment Station. Grant White, president of the Utah Aquaculture Association represents private aquaculture and John Neuhold from the National Resource Board of Trout Unlimited represents sport fishing.

Joanna Endter-Wada from Utah State University was jointly appointed as the non-voting chairperson of the Board. Dr. Endter-Wada is an associate professor in forest resources in the College of Natural Resources, an adjunct in sociology department and has extensive experience in environmental policy and sociologic aspects of natural resource issues.

Since its formation, the Board has met three times and the meetings have been characterized as being very friendly, cooperative and productive. Policies on Board notification of prohibited pathogen findings and emergency response teams have been drafted and approved unanimously. The largest remaining tasks are to modify the existing fish health rule to bring it in compliance with the new law and review the prohibited pathogen list.

Progress of the Fish Health Board will be reviewed by an Interim Session committee of the State Legislature in November.

inside...

Effect of Serial Water Reuse on Cutthroat and Rainbow Trout

In aquaculture, water if often a limiting factor and fish culturists attempt to make the best use of the water they have. Recirculating systems with biofilters that recycle a high percentage of the water have been researched and developed. Reuse of water can also take place where water effluent from one raceway can be input into another downstream. This is generally referred to as serial reuse. In serial reuse, several water quality variables change simultaneously as one moves downstream; e.g., dissolved oxygen decreases, ammonia increases, and suspended solids increase. Water quality extremes have been studied in detail in laboratory assays and guidelines established for many fish species, but these studies generally examined the variables singly. However, a few studies have examined the effects of serial reuse. Negative effects were observed when dissolved oxygen dropped below 5 mg/L and un-ionized ammonia-nitrogen exceeded 0.0144 mg/L (total ammonia-nitrogen of 0.5 to 0.6 mg/L). With the advent of oxygen injection, oxygen need not be a limiting factor, permitting greater use of serial reuse.

In this study, oxygen was maintained at high levels by injection and water quality variables were measured monthly. This study was conducted to determine if rainbow trout were compromised by differences in water quality at their location in a serial reuse system. We also wanted to compare the response of rainbow and cutthroat trout to serial reuse water. Six concrete raceways received first use water and were each stocked with 3,970 rainbow trout of 4.1 g mean weight. The effluent from these raceways was pooled into a common headbox which delivered water to the next set of six raceways downstream. Three of these were stocked with 2,290 rainbow trout each and three were stocked with 2,290 cutthroat trout (mean weight 15.6 g).

Fish were fed a commercial ration to satiation, adjusting the ration from 4.4-5.5% to 1.9% of body weight over the course of the 134-day experiment. Raceways were cleaned twice a week and the waste from first use raceways permitted to drift to the second use raceways. In addition to monitoring standard performance variables, necropsies using Ron Goede's Health and Condition Profile system were also conducted twice during the experiment.

Water quality was monitored monthly. Incoming total gas saturation dropped during the study and was significantly higher in first- than second-use water for the first three months (107-108% vs 105-107%, respectively). Dissolved oxygen was significantly lower for fish receiving second-use water from day 31 onward. Effluent dissolved oxygen from the second use raceways did not drop below 4.1 mg/L and averaged 5.7 to 6.3 mg/L over the course of the study. Suspended solids increased significantly from a mean of 12.07 mg/L at the head of first use raceways to 12.70 mg/L at the head of the second use raceways, and further increased to 13.6 mg/L at the tail end of the second-use system. Un-ionized ammonia levels in effluent water were not significantly different between first and second use raceways except for day 87, when levels were significantly higher for second use water (9.9×10^{-4} mg/L) than first use (1.4×10^{-4} mg/L). Un-ionized ammonia levels did not exceed 0.0066 mg/L during the study. Turbidity, total alkalinity, total hardness, and temperature did not differ between first and second use raceways. Similarly, influent pH did not differ between the treatments except for day 60.

Percent mortality ranged from 1.2 to 4.3%

for rainbow and from 3.4 to 4.5% among the cutthroat trout raceways. Mortality did not differ significantly between rainbow trout exposed to first and second use water. Similarly, no significant difference was noted between rainbow and cutthroat trout exposed to second use water.

Cutthroat trout had significantly lower final feed conversion ratios than rainbow trout (1.16 vs. 1.23, $P = 0.023$). However, specific growth rates, mesenteric fat level (median of 3 vs.2), and condition factor (1.580 vs. 0.9063) of rainbow trout was significantly greater than that of cutthroat. Growth of the rainbows was also greater in second-use water than first-use; final weights averaged 62.9 g and 57.4 g, respectively. Presumably the difference was due to the abundance of insect life in the headbox between the two systems.

Hematocrit was significantly higher for rainbow than cutthroat trout (e.g., 35.5 vs. 29.0%), despite no significant differences in water quality between the two raceways. Hematocrit of rainbow trout in the final sample was significantly higher in second use (39.9%) than in first use water (37%). This indicated some adaptation to lower oxygen values by increasing the number of red blood cells. Differences in gill pathology based on gross observation indicated that rainbow trout gills in second use water were compromised, though the cause is not clear. Ammonia concentrations were well below levels known to induce pathologies.

Despite some gill pathology, production of serial reuse was successful for doubling production when supplemental oxygen was available and ammonia concentrations were kept below acceptable limits. Cutthroat trout and rainbow trout did equally well, although the gill damage

(Continued from page 11)

(*n*=12). Heat treatment killed 100% of TAMs at temperatures of 75 C or greater. Temperatures of 49 or 58 C produced some TAMs that stained both red and green; this raises the question "are these still viable?" If these are not viable, only 1% or less of the treated TAMs were viable (all green) at these two temperatures. Temperatures of 38 C or lower had increasing higher percentages of viable and possibly viable TAMs (Table 3).

Table 3. Mean percent viability of the triactinomxyon of *Myxobolus cerebralis* after heating to test temperature in a water bath for 5 min. Due to rounding, percentages across columns may not add to 100%.

Temperature	Percent dead (red)	Percent viable (green)	Percent possibly viable (red & green)
7 (control)	14.2	79.7	6.1
21	17.6	66.6	15.8
27	4.6	47.2	48.2
32	11.2	44.3	44.5
38	32.2	5.3	62.4
49	73.1	0.6	26.3
58	89.8	0.6	9.6
75	100.0	0.0	0.0

From these trials it appears that the infective stage of whirling disease is effectively killed when frozen or dried for a relatively short period of time. Temperatures above 75°C for at least 5 min were also effective. Pressure and temperatures of 58°C or lower reduced viability, but were not completely effective. Hopefully this information can be used to prevent the spread of whirling disease to areas where it still is not found. Eric Wagner and Mark Smith

EXPERIMENTAL USE OF HUMAN CHORIONIC GONADOTROPIN IN JUNE SUCKERS

The June sucker (*Chasmisties liorus)* is an endangered species endemic to Utah Lake. The June sucker was a critical food fish for the early settlers in Utah. Unique features of the June sucker include a subterminal mouth, smooth lips, and a cleft bottom lip with almost parallel sides to the wide gap. June suckers are often confused with the more abundant Utah sucker (*Catostomus ardens*), but upon closer examination the Utah sucker has a small wedge shaped gap and rough lips on the ventral mouth.

Propagation and population augmentation is part of the June sucker recovery plan. The Fisheries Experiment Station (FES) Logan, Utah has constructed an interim facility to hold June suckers for research and brood stock (see *Ichthyogram* Volume 2, #3). The Utah Division of Wildlife Resources plans to build a permanent facility to house these and other endangered fish. In 1991, the construction of the June Sucker interim facility was completed and the first year group of June suckers (1989 year class) was transferred in from Utah State University (USU). The fish from USU had been hatched from eggs collected during the June sucker spawn on the Provo River.

Currently, the FES facility has five year classes, three of which have shown signs of spawning preparation each Spring, yet few females ripen. Necropsies of natural mortality in May, June, July and even into August have shown developed ovaries in the females. A prominent sign of staging in the female June sucker is an enlarged vent. Male June suckers develop tubercles on their caudal fins, caudal peduncle, but mostly on their anal fin.

Fish reproductive timing is regulated by a neurohormonal mechanism in which environmental stimuli and genetically imprinted cycles trigger the release of gonadotropin-releasing hormone (GnRH) or gonadotropin-release inhibiting factor GRIF from the hypothalamus. The GnRH released from the hypothalamus triggers the pituitary to release gonadotropic hormones. The gonadotropic hormones cause the maturation of gametes in the gonads through progesterone or testosterone. In females, prostaglandin controls the rupture of the follicle and expulsion of the eggs. Hormonal specificity among vertebrates is low, the ratios of hormones is the greatest difference. The use of pituitary extracts, or gonadotropin from one species can effect the reproductive cycle of another species. The use of human chorionic gonadotropin (HCG) has long been know to induce spawning in many breeds of fish. HCG is extracted from a placenta or can be synthetically produced. HCG can be dried and stored for extended periods of time. HCG is not registered for use in fish by Food and Drug Administration.

In a preliminary research trial in June of 1998, five female June suckers received an 800 IU (international units) per pound of body weight intraperitoneal injection of HCG.

(Continued on page 5)

Seven male June suckers received a 400 IU/lb IP injection of HCG. For this trial the fish most likely to have mature gametes were chosen. Females were chosen by size and amount of swelling around the vent. Males were chosen by size. One day after injection, none of the five females gave eggs but three of the seven males gave increased volumes of milt. Four days after the injection two of the five females gave a small quantity of eggs (<200). The fish that were unaffected by the first injection (3 females, 4 males) received a second injection of HCG. Due to the limited amount of HCG obtained, only half of the original dose was administered. Five days later, no eggs or milt were collected. On a side note, one of the fish which had never been injected gave over 5,000 eggs and one fish that had given eggs five days earlier gave another small lot. The participating June suckers showed no side effects from the drugs, but experienced additional handling stress.

The results of the limited trial showed a tolerance for HCG but little/no favorable effects from the injection. Possible reasons for the failure include: timing of the injection, additional stress on the fish, dose too low, and other hormones may be limiting. The timing of the injection (June 15) was chosen to correspond with dates that ripe females had been found in previous years. Necropsies done on natural mortality showed developed ovaries in all dead females from these year classes. The necropsies have continued to show developed ovaries into August. The natural spawn takes place in late May or early June.

The inability to spawn may also be related to stress. The June suckers were evaluated the first part of May for deformities as part of a feed trial. The handling associated with this evaluation may have induced handling stress.

Successful spawning has been achieved in many species using HCG in a variety of dosages (Opuszynski and Shireman, 1995). In similar trials, grass carp were not successfully spawned on HCG alone but were successful when HCG was used in conjunction with carp pituitary extracts (Opuszynski and Shireman, 1995). In another trial Grass Carp were successfully spawned on high concentrations of HCG (Opuszynski and Shireman, 1995).

Further trials utilizing three modifications to these methods are being considered: higher doses of HCG, varied timing, and a follow up injection of carp pituitary extracts. White sucker (*Catostomus commersoni)* have been successfully spawned using 1,000 IU/kg IM injections for four consecutive days. The use of this method may prove successful in the June sucker. Injecting some fish each week through May, June, and July may show that 800 IU/KG is adequate if the timing is right.

Thanks go out to the USFWS for their assistance on this project.

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Opuszynski, K and Shireman, J. V. 1995. Herbivorous Fishes Culture and Use for Weed Management. CRC Press, Ann Arbor, Michigan

Influence of Rearing Conditions and Diet Type on Fin Erosion

Fin erosion can be a common occurrence among fish raised in modern, large-scale culture operations. Fish with eroded fins which are stocked into waters may not be aesthetically pleasing to anglers, may have impaired survival, and may be more prone to bacterial infections. It has been theorized that fin erosion is derived from aggression between fish, nutritional imbalances in feeds, or environmental factors inherent to a hatchery. In work conducted previously at the Fisheries Experiment Station (FES), it was demonstrated that rainbow trout (*Onchorhynchus mykiss*) and cutthroat trout (*Onchorhynchus clarki* Utah) raised in concrete raceways which contained a layer of cobble as substrate exhibited significantly less fin erosion than their counterparts raised in concrete bottomed raceways. An earlier inventory of Utah state hatcheries found that better fin condition was associated with fish which were raised in raceways and ponds that contained natural bottoms of mud or cobble.

Nutritional imbalance in fish feeds has also been implicated as a possible source of fin erosion. Lellis and Barrows (*Aquaculture* 156:229-240, 1997) demonstrated that steelhead trout fed a krill-based diet exhibited improved fin condition compared to fish fed a fish meal-based diet. They theorized that the krill-based diet, which contained naturally higher levels of copper, in some way improved the process of collagen formation in fin rays than the fish meal-based diet, which contained higher levels of iron, calcium and phosphorus. The purpose of this study was to test the hypothesis that raceway substrate (cobble vs. concrete) and dietary components improved the fin condition of rainbow trout.

Rainbow trout of the Sand Creek strain were raised indoors from swim-up for five weeks during which they were fed either a control diet or a test diet. After five weeks on their respective diet (0.9 g/fish) the fish were moved outside into concrete raceways. A total of six raceways were fitted with a false floor which was comprised of a layer of gravel supported by perforated aluminum under which a drainage system lay. The remaining six raceways were left untreated. For the series of false floor raceways three were fed the control diet (ctrl/ff) and three were fed the test diet (test/ff). For the untreated raceways three were fed the control diet (ctrl/rw) and three were fed the test diet (test/rw). The fish were stocked at densities of 1,200 fish per raceway.

The fish were hand fed either the control diet which was formulated to match a standard trout grower diet with fish meal as the primary protein source, or the test diet which contained krill meal as the primary protein source along with supplemental minerals thought to improve fin condition. Diets were made by Dr. Rick Barrows of the U. S. Fish and Wildlife Service at the Bozeman Fish Technology Center, Bozeman, MT. At the beginning of the study ,fish were fed a ration that was 4.2% of the fish body weight, and by the end this ration was adjusted to 2.5%. Fish were inventoried monthly for weight gain. Fin measurements, which were used to calculate relative fin index values, were made at the onset of the study, and then on weeks 7, 11, and 17. Necropsies were performed according to the Health Condition Profile (HCP) on ten fish per raceway (30 total per treatment) on weeks 11 and 17.

(Continued from page 6)

Water was supplied to the raceways by a well which had the following qualities: temperature = 13 °C, oxygen = 7.2 mg/l, alkalinity = 235 mg/l, hardness = 222 mg/l, pH = 7.2. Supplemental oxygen was supplied to the well water via liquid oxygen injected into sealed packed columns which fed into a common head box. Final water quality measurements showed all parameters within acceptable ranges for good trout growth. Dissolved oxygen concentrations were significantly lower, however, at the raceway tails for false floor treatments, 5.5 mg/l, compared to 6.5 mg/l for the control raceways.

The hatchery performance of the fish was significantly influenced by raceway and feed type. Final weights, feed conversion (FCR), and specific growth (SGR) rate ranked in order of performance were as follows: test/rw > test/ff > ctrl/rw > ctrl/ff (Table 1). For final weights and SGR there were significant diet and raceway effects, and for FCR there was a significant raceway effect. For each of the above parameters there were no significant diet x raceway effects. No significant differences were found between the treatments with respect to mortalities which averaged 2.2%.

Comparisons of relative fin lengths also revealed significant dietary and raceway effects. The initial fin measurements taken five weeks after first feeding indicated those fish on the test diet had better dorsal and pectoral fins than the control fish (Figure 1). By week seven, fish in the false floor raceways had significantly longer fins than fish in concrete bottom raceways; the test diet also resulted in longer fins for all but the ventral fins. Significant diet x raceway effects were also found for caudal and ventral fins.

Measurements made on week 11 also revealed significantly better fins among fish in false floor raceways compared to concrete raceways, and better dorsal, anal, and pectoral fins for fish fed the test diet compared to the control diet. Significant diet x raceway effects were also found for caudal and ventral fins. By the conclusion of the study (week 17), all fins measured, with the exception of the caudal, were significantly longer for fish on the test diet than the control diet. All fins of fish in the false floor raceways were significantly longer than those of counterparts in the concrete-bottomed raceways. Significant diet x raceway interactions were evident for all fins except the dorsal.

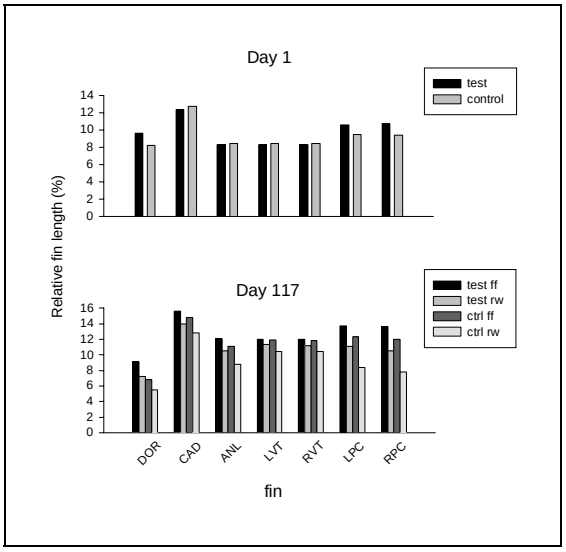

Figure 1. Comparison of relative fin length (% of total length) of rainbow trout fed a test diet and raised in untreated raceways (test rw) or raceways with false floors (test ff). And fish fed a control diet and raised in untreated raceways (ctrl rw) or raceways with false floors (ctrl ff). Day numbers indicate time of study in outdoor raceways. Fin abbreviations are DOR = dorsal, CAD = caudal, ANL = anal, LVT = left ventral, RVT = right ventral, LPC = left pectoral, and RPC = right pectoral.

Health Condition Profile information also indicated significant diet and raceway effects. Mesentery fat levels (ranked from 0, no fat, to 4, pyloric caeca covered) were significantly better for the test/rw (2.8), test/ff (2.7), and control/rw treatments (2.5) compared to the control/ff fish (1.8). By week 17, test/rw fish had

(Continued on page 8)

significantly better fat scores (3.2) than ctrl/ff fish (2.7) and ctrl/rw fish (2.9); test/ff treatment had an intermediate score (3.1). For week 11, mean fin index values (possible range from 0, no erosion, to 2, erosion with hemorrhaging) were best for the test/ff fish (0.1), followed by test/rw (0.2), ctrl/ff (0.4), and ctrl/rw (0.5). By week 17, fin index scores were significantly better the test/ff fish (0.4) followed by ctrl/ff (0.6), test/rw (0.8), and ctrl/rw (1.1).

In this study, for hatchery performance and fin condition, both test diet and substrate had a significant impact. Growth and fat accretion were best for the test/rw treatment and this may be, at least in part, a result of the false floor design. The design appeared to serve the function of allowing detritus to filter down through the cobble and out of the raceway via the drainage system. But there were several problems associated with the design. First, it did not allow for a true plug flow through the raceway because incoming water was being filtered out through the cobble and drainage system the entire length of the raceway. This was shown in the lower DO readings at the raceway tails which were 5.5 mg/l for the false floor raceways compared to 6.5 mg/l for the concrete-bottomed raceways. Second, the presence of the cobble may have prevented fish from eating feed that had settled to the raceway bottoms. Within the test and control dietary groups, final weights, feed conversions, and growth rates were better for those fish in concrete-bottomed raceways compared to false floor raceways. These differences in fish performance with respect to raceway type were arguably offset by the quality of fins obtained. By the end of the study fish fed the test diet in false floor raceways exhibited superior fin condition, however with the exception of the dorsal fin, fish fed the control diet in false floor raceways exhibited similar fin condition. Fish fed the test diet in concrete-bottomed raceways did have slightly better fins than those fed the control diet in concrete-bottomed raceways, although the differences were not as dramatic. For both cases, cobble as a

Table 1. Hatchery performance of rainbow trout fed a test diet and raised in untreated raceways (test rw) or raceways with false floors (test ff). And fish fed a control diet and raised in untreated raceways (ctrl rw) or raceways with false floors (ctrl ff). Values are means (± s.d.) of three replicate raceways of fish.

Diet/treatment	Final weights	FCR[1]	SGR[2]	Mortality (%)
Test/raceway	22.0 ± 0.5	0.84 ± 0.02	1.76 ± 0.01	2.3 ± 0.3
Test/false floor	20.7 ± 0.8	0.86 ± 0.03	1.72 ± 0.02	2.1 ± 0.2
Control/raceway	19.4 ± 0.9	0.89 ± 0.03	1.70 ± 0.03	2.1 ± 0.1
Control/ false floor	18.0 ± 0.8	0.91 ± 0.03	1.66 ± 0.02	2.2 ± 0.5
P > F Diet	0.00	0.03	0.00	0.93
Raceway	0.03	0.29	0.03	0.62
Diet X RW	0.96	0.81	0.84	0.51

[1] FCR = total g feed/total g weight gain
[2] SGR = ((ln final weight - ln initial weight)/length of study in days)*100

Lake Trout Brood at Egan Hatchery Now Producing

Concerns about shortages of splake for fisheries management needs prompted an effort to build a broodstock at the Egan State Hatchery. This year, the 4 year-old lake trout are producing eggs and splake production is no longer limited to whatever can be collected from the wild spawners at Fish Lake. The first eggs were taken September 15th and 68,704 splake eggs were produced (304 eggs/oz). A second egg take on September 23rd resulted in 137,115 splake eggs at 277 eggs/oz. An additional egg take on 5 Oct 98 resulted in 127,512 eggs (264/oz). An abundance of splake is anticipated. Fisheries managers can now consider splake for their waters without being limited by their numbers. The only bottleneck now is the rearing space in the hatchery system. Fisheries managers should make any requests for additional splake known to the fish culturists involved so that the appropriate number of broodstock are maintained. These numbers will be trimmed soon if there is no increase in splake quotas.

The average number of eggs per female was 1,115 for the first two takes and 1,315 eggs/female in the third. By comparison, a 1994 Federal Aid report by Louis Berg and Dale Hepworth noted 1,136 to 6,975 eggs/female of all ages at Fish Lake between 1973 and 1994.

Brood history

In 1994, 21,000 lake trout eggs were taken at Fish Lake (which has been annually tested and found free of pathogens) over the first 3 weeks of October and combined. These were eyed at the Fish Lake and transferred to the Kamas Hatchery for rearing at a warmer temperature. These were later transferred to the Fisheries Experiment Station (FES) (5504 fish at 519.7 fish/lb) on 3-22-95 for routine broodstock quarantine and faster growth. This first cohort was sent to Egan Hatchery in Feb/Mar 1997 (3970 fish at 0.86 fish/lb).

Eggs for two additional year-classes of broodstock were taken in subsequent years and sent to the FES. In October 1995, a total of 19,210 eggs were taken over a three week period in three separate lots of 9,971, 1,575, and 7,664 eggs respectively. Of these eggs, only 19.9% made it to initial feeding. Egan did not need this year class, so the fish were given to Idaho Fish and Game which stocked 1,876 fish into Lucky Peak Reservoir near Boise. In 1996, 4 lots of eggs were taken (4,816 on 10/8, 4,575 on 10/15, 9,471 on 10/23, and 11,593 on 11/05). In this cohort, 25.9% of the total eggs taken made it to first feeding. This cohort is still at the FES and will be transferred to Egan in February 1999.

Eric Wagner and Doug Routledge

TAM Vital Staining: Effects of Pressure, Drying, Freezing, and Elevated Temperature

The parasite that causes whirling disease in salmonids has a two stage life cycle. The myxosporean stage is a round shaped spore of about 8-9μm in diameter found in the cartilaginous tissues of infected salmonids. After the fish dies, this spore is consumed by the aquatic worm *Tubifex tubifex*. In the digestive tract of T. *tubifex* the actinosporean stage called a *Triactinomyxon* (TAM) develops. The TAM is anchor shaped, and within one arm or process is a spore body containing a number of sporoplasts that will infect a new salmonid host. TAMs may be the weak link in the life cycle due to its short life (up to 15 d, depending on temperature) and its greater fragility in comparison to the myxospore. Control strategies aimed at this stage may be fruitful.

Disinfection of equipment is an important control measure to prevent transfer of the disease to uninfected sites. In this article, tolerance of TAMs to freezing, desiccation, pressure, and high temperature are discussed.

Freezing

Live TAMs were obtained from the Biology Dept. at Utah State University. For evaluation of freezing, two 30μl samples of this solution were placed in vials and frozen for 105 minutes at -20 C. The solution was allowed to thaw and then stained with propidium iodide (30 μl of 52 mg/L solution) and fluorescein diacetate (30 μl of solution made of 100 μl FDA stock [5 mg/mL] in 8.3 mL hatchery well water). The vials were refrigerated in light proof containers (to avoid decay of stains caused by light) for 30-45 min to allow the staining to take place. These two stains are absorbed by the TAMs and with the aid of a fluorescence microscope, TAM spore bodies fluoresce either red (dead) or green (live). After the incubation period, each of the 90μl samples were split equally into two slides and read under blue light to observe the viability of the TAMs.

Freezing resulted in a marked decrease in TAM viability (Table 1). Unfrozen TAM stock solution had 46% non-viable, compared to 98-100% dead after freezing. A few TAMs had both red and green sporoplasts fluorescing, but these made up just 0 to 2% of the frozen samples. The true viability of these is unknown. None of the frozen samples contained TAMs that were entirely viable (fluoresced green).

Table 1. Number of triactinomyxons (TAMs) of *Myxobolus cerebralis* classified as viable, non-viable, or possibly viable, after freezing and vital staining.

Treatment	number of	Percent viable (green fluoresc-	Percent non-viable (red fluorescing)	Percent possibly viable (red+green fluorescing)
Freezing at -20 C				
Controls				

Drying

For the desiccation trial, 10 μl of the concentrated TAM stock solution was put on each of 6 slides

and dried at room temperature. Upon appearing dry, the slides were allowed to remain on the counter an additional 15 minutes. After this period the slides were stained with the fluorescing dyes previously mentioned, incubated 30 minutes at 7°C, and examined under blue epifluorescent light for viability of TAMs. An additional test was conducted in which TAMs were dried for 60 min.

Desiccation at room temperature effectively killed TAMs. As shown in Table 2, very few TAMs (0 to 1%) survived the 15 minute drying period to which they where exposed. Partially viable TAMs made up only 0 to 7% of the six samples. The longer exposure (1 h) killed 100% of the TAMs, making this and freezing both appear as though they are effective ways of killing this actinospore.

Table 2. Number of triactinomyxons of *Myxobolus cerebralis* classified as viable, non-viable, or possibly viable after desiccation for 15 or 60 min, as determined by vital staining.

Treatment	number of	Percent viable (green fluorescing)	Percent non-viable (red fluorescing)	Percent possibly viable (red+green fluorescing)
Drying 15 min				
6	107	0	93	7
Drying 60 min				
1	145	0	100	0
2	211	0	100	0
3	191	0	100	0
Control				
1	51	71	6	23
2	107	72	5	23
3	124	74	6	20

Pressure

A 3 ml vial was filled with a TAMs-in-well-water solution and covered with parafilm. This was clipped to a metal support that was lowered into a hydrostatic pressure chamber. Pressure of 9,000 psi was achieved after about 30 sec using a press. After 5 min, the pressure was immediately released. TAMs on 3 slides were examined for viability as noted above. Total TAMs per slide ranged from 126 to 148. The percentage of viable (green) TAMs ranged from 43 to 60%. This was a significant drop from the viability of controls (70-74%), but was not low enough to consider pressures of up to 9,000 psi effective for controlling whirling disease.

Temperature

For these tests, 500 μ of fresh TAMs were heated in microcentrifuge tubes in a water bath at the test temperature. For each temperature, three tubes were spaced on a plastic rack and submersed for 5 min. The tubes were then transferred to a water bath at room temperature to cool rapidly. Propidium iodide and fluorescein diacetate were added to the tubes (250 ul of each) and incubated for at least 45 min in a refridgerator. Samples of 150 ul from each tube were examined by epifluorescence for viability.

Results of the heat treatments are presented in Table 3. Viability of control TAMs averaged 79.7%

(Continued on page 3)

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