The Ichthyogram

December 2001 **Volume 12 Issue 3-4**

June Suckers at the Fisheries Experiment Station

The year has been eventful for the June sucker program at the Fisheries Experiment Station (FES). We received eight new lots in the spring, provided fish for research on a variety of projects, started construction on an additional facility and have been preparing studies for when the new facility goes online.

We currently have 28 lots at the station from Utah State University (USU), Brigham Young University (BYU), the Provo River and the FES.

Table 1. June sucker lots

Year	# of Lots	Type of Lot	Source	Comments
1989	1		USU	Brood 1& 2*
1991	2		USU, BYU	Brood 1& 2*
1992	1		USU	Brood 1& 2*
1993	1		USU	Brood 1& 2*
1994	4	Family	USU	
1995	1	Family	USU	
1999	1	Family	Provo River	Light-trapped
2000	6	Family	Provo River	Received as eggs
	1	Family	Provo River	Light-trapped
	1	Sib-lot**	Provo River	Received as eggs
	1	Family	FES	Brood-stock mating
2001	6	Family	Provo River	Received as eggs
	2	Sib-lot**	Provo River	Received as eggs

***Broods 1 & 2 combined to form 1 family lot**

****Cross between hatchery fish and wild fish**

Due to lack of space, the June suckers are found in two locations at the FES, the facility constructed in 1991 and in the wet lab located on the main station. The use of the wet lab is temporary until the new facility is operating. Lots from 1999 and 2000 were moved to the wet lab at the end of April to open up space for the 2001 lots. Lots from 2001 were moved into the wet lab at the end of September for space

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requirements and the availability of warmer water at 66^{o} (F) compared to 60^{o} F. The lots were moved on to the main station after the fish health staff conducted the annual inspection certification.

The June suckers received in April and May 2001 were eggs from fish spawned at the Provo River. This year USU received all of the larval fish light trapped on the Provo River.

Effect of Iodine Treatment

Two lots of eggs were used to test the use of iodine for disinfection. Lot 010502SKJNPR03 was used in the first test. This lot was spawned over three days, but only the second and third days of spawning produced eggs for use. Only 808 eggs were fertilized on the second day of spawning, but the third day of spawning produced 5839 eggs. The eggs from the third spawn were split and 3391 of the eggs were treated in 100 ppm of iodine for 10 minutes (a standard method for disinfecting trout eggs), after which the eggs were netted into an eyeing jar setup in a tank separate from the rest of the lot due to space. The control group eggs and the eggs from the second spawn were setup separately in two other eyeing jars. Seven days after fertilization the control group began eyeing up as expected and began hatching two days later. The treatment group hatched seven days after fertilization and began developing eyes the next day. Lot 010515SKJNPR04 was the second lot used to test the iodine. The lot was split in two with 2020 eggs in the control group and 4000 eggs in the treatment group. The eggs were treated with iodine with the same procedure as the previous lot. Seven days after fertilization, both the control group and the treatment group hatched prior to developing eyes. The lots that hatched prior to developing eyes were in the same trough; other lots were hatched in the same trough without hatching prior to eye development. The lots not treated with iodine experienced losses due to fungus, whereas the treated lots did not have problems with fungus. The iodine decreased losses caused by fungus and with no known significant negative impacts, iodine will likely be used with additional testing in subsequent years.

Table 2. Survival of the June sucker 2001 year class

Lot #	# Eggs	# Fish @ Start*	% Hatch	# On Hand 12/1/01	% Survival After Start*	Comments
010424SKJNPR01	3308	1009	30.5%	300	29.7%	Fungus problems
010426SKJNPR02	1736	232	13.4%	165	71.1%	Majority lost to fungus.
010502SKJNPR03	6647	5655	85.1%	4860	85.9%	1/2 of the lot treated with iodine
010515SKJNPR04	6020	963	16.0%	126	13.1%	2/3 of the lot treated with iodine
010516SKJNPR05	35512	8164	23.0%	2151	26.3%	Fungus problems
010516SKJNPR06	14267	1741	12.2%	1160	66.6%	90% lost to fungus
010518SKJNPR07	1457	873	59.9%	356	40.8%	Sib-lot, Fungus problems
010518SKJNPR08	10869	997	9.2%	3	0.3%	Sib-lot, Fungus problems

*Start is considered first feeding

Transfers

Fish have been transferred from the FES for additional space and for various studies involving June suckers. In early April, fish from lot 000601SKJNPR07 were transferred to Wahweap due to space requirements. Through the summer, fish from this same lot from both FES and Wahweap were used in a graduate project for Utah State University being conducted by Kresta Davis-Butts at Mona Reservoir. They are also being used in a selenium study at Goshen Warm Springs, a potential site for an interim hatchery for June suckers. BYU researchers used fish from this year's egg take to compare morphological characteristics of hatchery versus wild raised fish.

New Facility Construction

Construction of the new June sucker facility began at the end of September and should finish in mid December. The facility consists of a 40' x 70' metal building (see photo) containing approximately 64 tanks, troughs and aquariums. A smaller building 7'x 7' will be used for quarantine, in particular the light-trapped larval fish from the Provo River. There will also be a head box housing the low-head oxygen unit manufactured by Water Management Technologies and a shelter for the liquid oxygen cylinders. The main building will have 15 troughs for feed and density studies allowing 5 treatments with 3 replicates per treatment. Twelve aquaria will be used for temperature studies and for culturing algae, rotifers, daphnia, brine shrimp and small lots of eggs and fish. Eight larger troughs will be used for hatching and raising new family lots. These lots will be transferred to three 88 ft^3 tanks for grow out until transfer to an interim facility. There are twenty 36 ft^3 circular tanks for holding family lots. Six 12 ft^3 tanks will be used for fish that have been sorted for spawning and possibly for smaller lots.

Studies for 2002

Current plans are to start a feed study as soon as the tanks are set up. This study will run until fish are available to start another feed study that begins at the initial feeding. During the next few months, trials on the culture of rotifers and brine shrimp will be done to determine procedures for production prior to the feed study beginning in May. Studies on induced spawning using human chorionic gonadotropin (HCG) are in the plans for the spring.

Eriek Hansen

Effect of sand size on filtration of *Myxobolus cerebralis* triactinomyxons

Triactinomyxons (tams) are the infective stage of the salmonid parasite *Myxobolus cerebralis* that causes whirling disease (Fig. 1). Removal of tams from hatchery water supplies is of interest to prevent the disease and infection. Tams typically measure about 125 µ long, but the style and processes are only about 10 µ wide so passage through a single plane (such as Nitex cloth) is conceivably easier than through the tortuous path of water through a three-dimensional media such as sand or beads. Tests here have indicated inefficient removal of tams using mesh sizes as small as 10-20 µ especially for larger volumes of water. Sand filtration may offer a possible means of preventing tams from reaching vulnerable fish. An initial laboratory test was conducted to determine what sized particles of sand would be best for tam filtration.

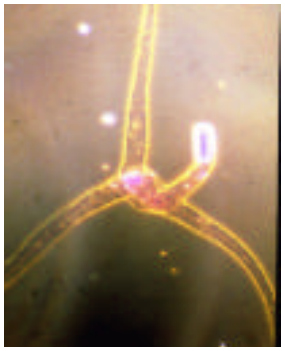

Figure 1. Triactinomyxon of *Myxobolus cerebralis*

A series of sieves was used to separate masonry grade sand purchased from a local landscaping supply business. The sieve sizes ranged from 180 µm to 2 mm mesh and provided sand of 12 different sizes (see side bar table).

Sand size range (µm)
180-211
212-249
250-299
300-354
355-424
425-499
500-599
600-709
710-849
850-999
1000-1999
>2000

Sand recovered on each of the sieves was kept separate for each recovery test in which tams were added to a small filter (Gelman filter funnel, 49 mm diameter) with either 2 or 4 cm of sand. Prior to testing, the sand in each filter was rinsed with hatchery well water until there was no turbidity in the filtrate. Tams harvested fresh from worm cultures were diluted in 200 mL of hatchery well water. This was carefully added to the filter to avoid disturbance of the sand layer. The total tams added varied among the tests due to availability, ranging from 10,226 to 66,400 per treatment. An additional 200 mL of well water with no tams was added as a rinse. The filtrate was subsequently filtered through a 20 µm mesh to concentrate the tams. A control group consisted of tams filtered through the Gelman funnel without sand present and recovered on the 20 µm mesh filter. Three slides of 50 ì L were made for each treatment counting the total number of tams. The filtration process was repeated twice for each sand depth (2 or 4 cm) and sand size.

Additional tests were conducted in which all sizes of sand combined (no sorting) were compared to filtration using sand for which particles less than 180 µm had been removed. The removal of the smaller sizes was of interest to improve the filtration speed. The same process described above was used for these tests except that 3 replicates were conducted instead of two.

Control recovery averaged 74 " 28.5% and 87.1 " 24.8% for the 2 and 4 cm depth tests, respectively. This indicated that some of the tams were lost in the process and recovery on the 20 µm mesh was not 100% efficient and highly variable, but sufficient to run the test. Use of sand significantly reduced the number of tams recovered from the filtrate ($P < 0.001$), with higher tam numbers in the filtrate noted for larger sand particle sizes (Fig. 2). The critical size at which no tams were found in the filtrate was 300 µm at 2 cm depth and 425 µm for 4 cm of sand depth. For sand of 500-599 µm diameter, 4 cm of sand was sufficient to reduce tam numbers by 99%.

Sand depth effects were significant: 4 cm of sand reduced tam numbers more than a sand depth of 2 cm when sizes of 500, 600, or 710 µm were used (Fig. 2). At 4 cm sand depth, no tams were recovered from filtrate when sand of up to 425 µm was used, whereas a few tams made it through the 2 cm depth for sand 355-425 and 425-500 µm. Greater depths would likely be more efficient in trapping tams, but at a cost to hydraulic head.

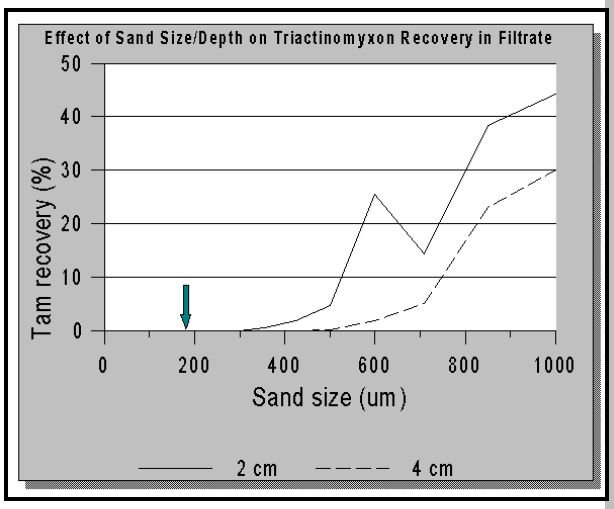

Figure 2. Effect of sand size and depth on triactinomyxon recovery in filtrate. Arrow indicates the smallest size tested

The results from this series of tests indicated that sand can filter out tams and indicated what target minimum sand sizes could be used. One sand size could be used for filtration, but this would require a substantial amount of sieving to produce enough of a given sand size to use on a production scale. Tests with all sand sizes combined indicated that 2 cm depth was not enough to remove all the tams (0.60 " 1.27% recovery), but a sand depth of 4 cm resulted in 0.0 " 0.0% recovery of tams in the filtrate. Using sand with particles >180 µmgave similar results: 0.18 " 0.53% and 0.0 " 0.0% recovery at 2 and 4 cm sand depth, respectively.

Removing the smallest particles (<180 µm) made a significant impact ($P < 0.001$) on water flow rates. For gravity flow through 2 cm of sand depth, the time required for 200 mL of water to pass through sand of all sizes averaged 155.0 " 35.6 sec ($n = 6$). Passage time was reduced to 32.5 " 1.0 sec when sand size of >180 µm was used. With 4 cm of sand depth, the same relationship was apparent: sand of >180 µm improved flow (39.0 " 7.7 sec) compared to sand of all sizes combined (160.5 " 18.7 sec, $n = 6$). Within a given sand size, there was no significant difference in filtration time between sand depths of 2 or 4 cm (*t*-test, $P > 0.05$).

The results from these tests were used to guide further testing. Still unknown are the effects of water volume (i.e., does more flow push the tams through the media?), backflushing (are tams given a opportunity to slip through at this time?), and bacterial biofilms that develop on the sand grains over time (greater trapping efficiency? reduced flows?). At the Midway State Fish Hatchery, Midway, Utah, a filter using sand >180 µm has been established to filter contaminated spring water. Sentinel fish have been exposed to the filtered water since hatching November 5th, 2001. Control fish are being exposed to the spring water without filtration. These will be sampled after 4 months of exposure. Additional tests are being conducted at the Fisheries Experiment Station using rainbow fry in aquaria exposed to water filtered by sand of either >180 µm or >300 µm(Figure 3). These tests are ongoing.

Figure 3. Sand filtration devices and aquaria used in the study

Eric Wagner

Myxobolus cerebralis Triactinomyxon Polar Filament Extrusion: Effects of Bovine Mucin, Ammonium Chloride, and Carbon Dioxide

Myxobolus cerebralis, the parasite that causes whirling disease in salmonids, features polar capsules that fire polar filaments coiled within it into the fish host. Premature firing of polar filaments may be a possible method of preventing the infection of fish by the triactinomyxon (tam) stage of the parasite. The mechanism of polar filament discharge is still not understood. Natural proteins such as proline or glutathione have failed to induce extrusion of polar filaments (Ichthyogram 12(2):5). Previous tests with fish mucus have indicated that mucus alone will cause only a small portion of polar capsules to fire (see Ichthyogram 12(1):6). For *Myxobolus cultus* actinospores, Yokoyama et al. (1995) was able to induce polar filament extrusion with bovine mucin. In an effort to determine other possible natural stimulants for polar filament discharge, the tests described below examined the effects of bovine mucin, ammonium chloride, and carbon dioxide. Ammonia and carbon dioxide are excretion products from fish that may occur at the water-fish interface.

Bovine mucin

A stock concentration of 2000 mg/L bovine mucin was made using hatchery well water. Control slides were made directly from a tam stock solution (tams in well water) concentrated from worm culture supernatant the day of the test. A total of 100 tams were examined using light microscopy to determine the number of tams with polar filaments discharged. If one or more filaments had fired, the tam was counted as discharged. Treatment slides were made by mixing equal amounts of tam stock and bovine mucin, achieving a 1000 mg/L concentration on the slide.

There were no significant differences ($P = 0.164$) in the percentage of tams with fired polar filaments between tams exposed to bovine mucin (14.7 " 8.3%) and controls (4.3 " 1.1).

Ammonium chloride

The effect of ammonium chloride on tam polar filament discharge was evaluated in 4 separate tests. In the first, two concentrations of NH_4Cl were compared to controls sampled directly from the tam stock harvested fresh from worm culture supernatant. For each concentration, 50 ì L of tam stock was mixed with 50 ì L of NH_4Cl, 50 ì L of propidium iodide (PI), and 50 ì L of fluorescein diacetate (FDA) on a microscope slide. The resulting mixtures were 310 and 31 mg/L NH_4Cl. A total of 100 tams were examined on each of 2-3 slides per treatment using the procedure noted above to quantify discharge. In addition, the viability of each tam was classified based on vital staining (PI/FDA): viable if it stained green, non-viable if it stained red, and possibly viable if it stained red and green. Tams that had no spore body present were classified as empty.

In the second test, concentrations of 3.8, 0.38, 0.038, and .0038 g/L NH_4Cl were compared to controls made directly from tam stock. A total of 100 tams were counted on each of three slides for each concentration, classifying the tam as discharged (1 or more polar filaments

fired), empty, or not discharged. Slides were examined by light microscopy as soon as the slides were made.

In the third test, a solution of 3.8 g/L NH_4Cl was tested at 3 different pH levels (9.17, 8.73, and 7.00) to determine the effect of ammonia. Higher pH is known to increase the percentage of ammonia relative to ammonium ion in solutions. The pH was adjusted with 1 N NaOH or 0.5 N H_2SO_4. The pH levels were the levels achieved after mixing on a slide. Actual pH measurements were made in test tubes where equal amounts (2 mL) of tam stock and NH_4Cl solution were mixed. The 4th test examined the effect of varying pH (9.2, 8.7, 7.0) at a lower NH_4Cl concentration (38 mg/L). The pH of the controls was 7.6. Discharge of polar filaments was recorded as noted above.

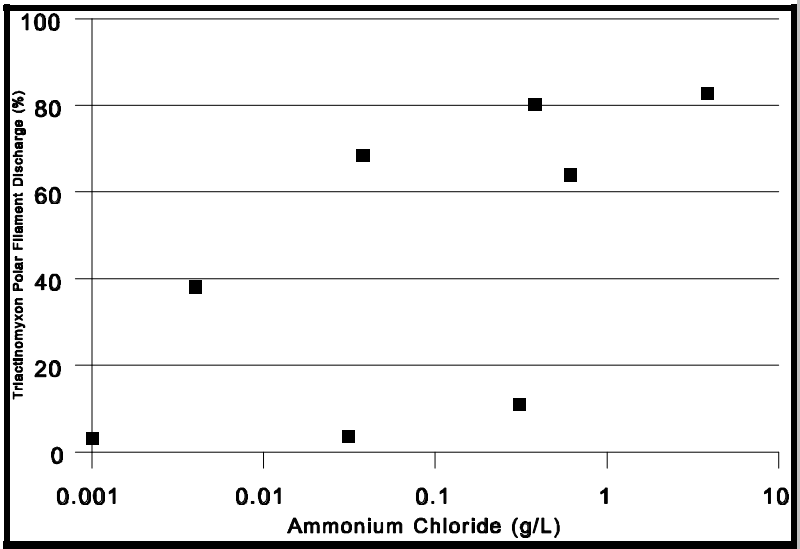

Figure 1. Effect of ammonium chloride concentrations on the discharge of polar filaments of the triactinomyxon stage of *Myxobolus cerebralis*.

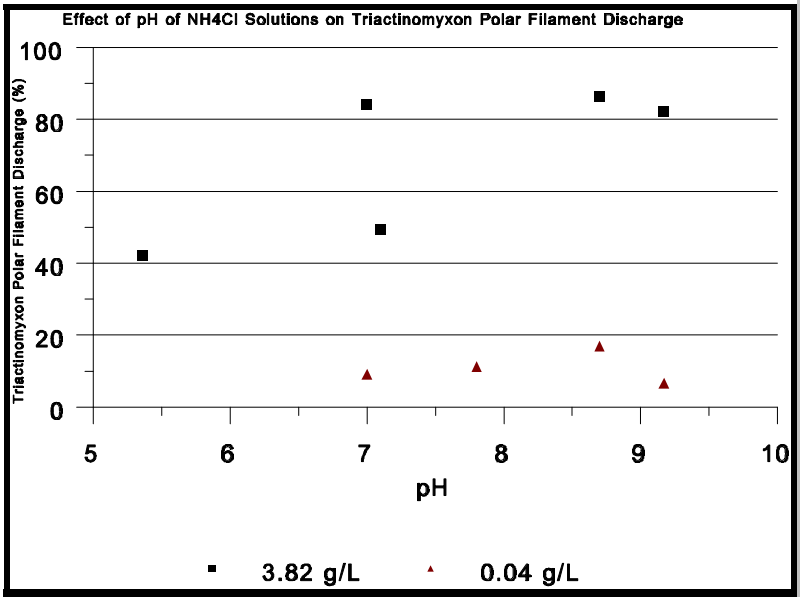

Figure 2. Effect of pH of two different ammonium chloride solutions on the percentage of *M. cerebralis* triactinomyxon polar filaments discharged.

In the first test, vital staining indicated that a high percentage of tams were viable (92.5%). The effect of NH_4Cl on discharge was minimal at concentrations of 31 mg/L (3.5 " 2.1% discharge) and 310 mg/L (11.0 " 8.5%), and did not significantly differ from controls (3.2 " 2.2%; $P > 0.105$, test 1 and 2 pooled, $n = 5$). As concentrations of NH_4Cl increased, discharge increased significantly ($P < 0.001$; Fig. 1). There were some incongruities in the results between the first two tests where lower concentrations in the second test stimulated polar filament discharge not observed in the first test. For example, 3.8 mg/L NH_4Cl induced 38.0 " 4.6% discharge compared to 3.5%

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discharge noted at 31 mg/L. The discrepancy may be due to different observers doing the tests or some other unknown factor.

The effects of ammonia (via changes in pH) on discharge were inconsistent, but significant (P = 0.012; Fig. 2). At a concentration of 38 mg/L NH_4Cl, discharge at a pH of 9.2, 7.0, and 7.8 did not significantly differ from controls. However, discharge at pH 8.7 (17.0 " 7.0%) was significantly higher than controls and the other pH levels tested. At the higher concentration of 3800 mg/L NH_4Cl, there was a significant increase in discharge relative to controls (P < 0.001). However the effect of pH was inconsistent as well. Discharge at pH 7.0 did not differ from discharge at pH 9.2 or 8.7. To test the correlation between discharge and pH of NH_4Cl solutions, least square regression was used. At 38 mg/L NH_4Cl, the correlation coefficient was only 0.026, and not significant. At 3800 mg/L, the correlation was significant (P = 0.021), but weak (r^2 = 0.250).

Carbon dioxide

Hatchery well water was aerated with carbon dioxide gas for 40 min. The CO_2 concentration in the stock solution measured 700 mg/L using a commercial test kit. Tams harvested the same day were mixed with an equal amount of the carbonated water (50 ì L) on microscope slides. Slide concentrations of CO_2 were 350 mg/L or 88 mg/L after mixing. Discharge percentages were determined as noted above for 100 tams on each of three slides per treatment. Control tams were taken directly from the tam stock solution.

Triactinomyxon polar filament discharge was not significantly affected by exposure to carbon dioxide concentrations of 88 mg/L (18.0 " 1.0% discharge) or 350 mg/L (20.0 " 12.5%). Control discharge averaged 13.7 " 1.5%.

The results above indicate that bovine mucin and carbon dioxide are not significant discharge inducing agents. The pH tests with ammonium chloride indicated that ammonia per se is likewise a poor agent. The high concentrations of ammonium chloride that induced discharge would be above concentrations normally encountered in typical fish culture or wild fish environs. The discharge effects observed were likely due to chloride ion, which was shown to be one of the more potent ions for discharge in previous tests (Ichthyogram 11(4):3). It appears that the mystery of polar filament discharge remains to be solved.

Eric Wagner

Literature cited

Yokoyama, H., K. Ogawa, and H. Wakabayashi. 1995. Chemoresponse of actinosporean spores of Myxobolus cultus to skin mucus of goldfish *Carrassius auratus*. Dis. Aquat. Org.21:7-11.

Changing of the Guard

The end of 2001 has seen several changes of personnel at the Fisheries Experiment Station and the Aquatic Section for the Division of Wildlife Resources. Retiring after 33 years of service is **Kent Thompson**, wildlife biologist at the Fisheries Experiment Station. Kent has worked for many years in the Technical Services Section, primarily in bacteriology and parasitology, which included testing many thousands of fish heads for the presence of the whirling disease parasite. Kent plans on spending his initial retirement working in his garden, doing genealogy research, tying flies and maybe even doing a little fishing.

Biologist Kent Thompson relaxes on his couch after retiring from the Division of Wildlife Resources after 33 years service

Also leaving FES for other opportunities in December were wildlife technicians **Brendon McGinn** and **Ryan Hillyard**.

Also retiring from the Aquatics section at other locations in recent months were **Blaine Hilton**, superintendant at Egan hatchery; **Paul Harmer**, superintendant at Glenwood hatchery; **Charlie Thompson**, aquatics manager for the Central region; **James Ivory**, assistant superintendant at Ft. Green hatchery and **Henn Gruenthal**, hatcheries development coordinator in the Salt Lake office. Our thanks go out to these guys for a job well done and best wishes for the future!

Starting in February at FES will be biologist **Mary George** (Technical Services) and technician **David Latremouille** (research). Look for more information on these two in the next edition.

Myxobolus cerebralis Found in Settling Pond Below Loa Hatchery

Pathologists at the Fisheries Experiment Station have confirmed the presence of the whirling disease parasite in feral rainbow trout found in a settling pond below the Loa State Fish Hatchery. Spores were initially found in samples collected in September, and the parasite confirmed in additional fish collected in November. Fish in the hatchery raceways were tested intensively three times in 2001, and have continued to test free of the parasite. The pond is separated by from the hatchery by screens and security fencing. In contrast to the hatchery, the settling pond is rich in organic matter and oligochaete worms, which act as the intermediate host for the parasite.

A response team was formed and came up with a plan to eliminate the settling pond, a retesting schedule for hatchery fish and a re-evaluation of the locations where fish from the hatchery are stocked. Loa fish have been primarily stocked into reservoirs and streams already positive for the parasite as well as lower elevation reservoirs with no reproducing salmonids. The parasite was first confirmed in Utah in Spring Creek, immediately below the settling pond in 1991.

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