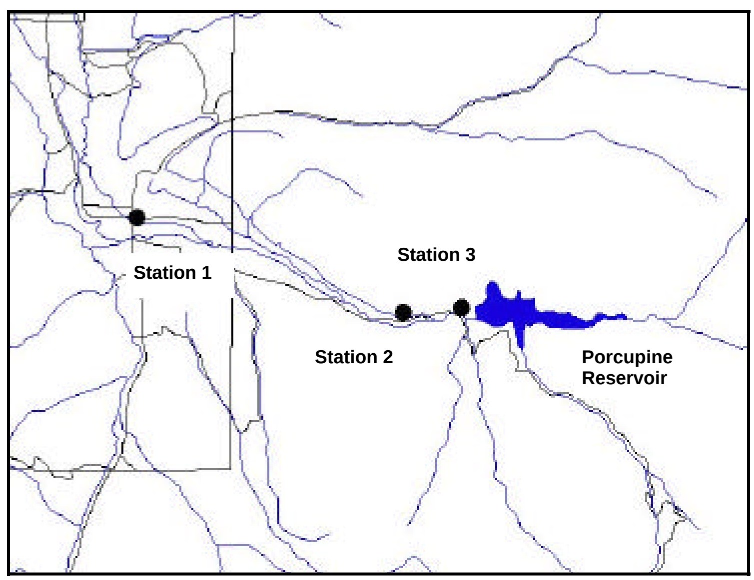
The Ichthyogram

December 2000 Volume 11 Issue 4

East Fork Little Bear River: 2000 Population Estimates

The parasite *Myxobolus cerebralis* was discovered in 1993 in the East Fork Little Bear River drainage. Its impacts on the salmonid populations are of interest, as well as impacts of recent construction at Porcupine Dam and low flows during this especially dry year. In addition, cutthroat trout of three different strains (Bear Lake, southern Bonneville, and Yellowstone) had been stocked in May in an attempt to determine any differences in prevalence in a field setting. Population estimates in this stream have been conducted for several years, permitting some long term perspectives on impacts. In September 2000, additional sampling was conducted to monitor possible changes.

Two-pass electrofishing was used to capture all fish possible in a 100 m reach. The three reaches sampled in 1997 and 1998 were sampled again on 19 September 2000. Station 1 was the reach furthest downstream, just above the Liberty Road bridge in Avon. The middle reach (Station 2) was in the area that the Division of Wildlife reconstructed to return the stream to a normal meander, and the uppermost reach (Station 3) was between the reconstructed area and Porcupine Dam.



Map of Little Bear River, showing sampling sites.

As in past years, the catch consisted primarily of brown

(Continued on page 2)

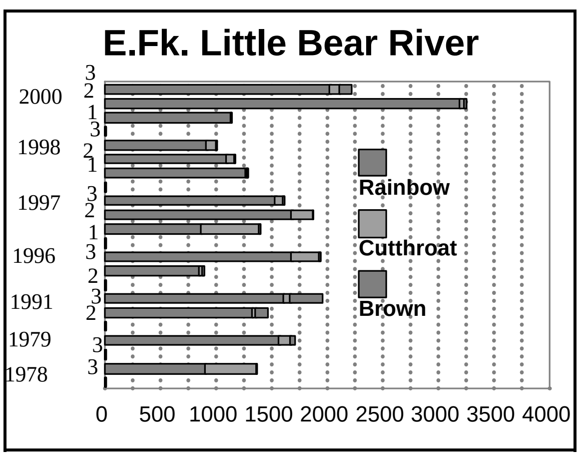
inside...

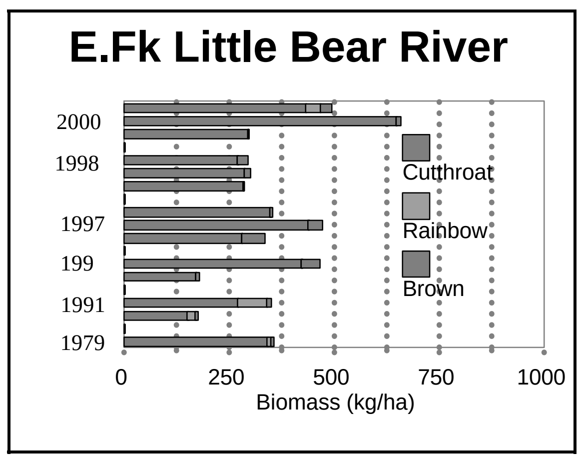
(Continued from page 1)

trout, data for which are summarized in Figures 1 and 2. Cutthroat were found in low numbers, primarily in the two upstream reaches. These were four survivors of the 2000 stocking (2 Bear Lake and 2 southern Bonneville, identified by fin clips) plus other cutthroat trout with no fin clips that were presumably of natural origin. These averaged 241 to 260 mm in total length. Sloped head deformities seen in 1998 were found only in a single cutthroat trout. Blacktail, another clinical sign of whirling disease, was found in one brown trout young-of-the-year.

Cutthroat trout showing classic slopehead deformity.
Photo by Chris Wilson

Despite, the drought, whirling disease, and dam construction upstream in the winter of 1998, brown trout are doing better than ever. The area rehabilitated by Kent Summers and his crew had an especially high number of fish this year. The drought likely drew fish from less suitable habitat into this area where better pools and cover were available, possibly skewing the data. Nonetheless, the contrast of the rehabilitated section with the other sections indicates the potential for habitat improvement to make real impacts on number and biomass of fish. *Eric Wagner*





EXTRUSION OF POLAR FILAMENTS IN *MYXOBOLUS CEREBRALIS* ACTINOSPORES

The life cycle of *Myxobolus cerebralis* includes the actinospore called a triactinomyxon or tam. The tam is released into the water from the worm host. In order to complete the life cycle the tam must find a salmonid fish, attach itself and inject its spores into the fish. Tams attach to the fish host using three polar filaments. The polar filaments are tiny hair-like threads that, before firing, are tightly coiled within cells called polar capsules. The polar capsules are located at the distal end of the style that contains the tams spores. When the polar capsules receive certain chemical and/or physical cues that it has come in contact with a fish, the polar filaments are rapidly released from the capsules. This attaches the tam to the fish, permitting the sporozoites within to emigrate to the fish host.

Understanding the cues that fire polar filaments in tams may be useful in the control of whirling disease. If it is possible to prematurely fire the polar filaments, we may in effect be able to disarm the tam making it unable to attach to the fish and continue the life cycle. However at this point it is unknown whether tam attachment is the only way in which sporozoites may enter a fish.

Experimental Discharge

Discharge of the polar filaments of tams was attempted using a variety of methods that worked for cnidae discharge. In one series of tests, the effect of pH on polar filament extrusion was examined. In another, various salts and concentrations were evaluated. In a third series of tests, the effects of electricity on polar filament extrusion was examined.

pH

Acid pH was made using HCl and basic pH using KOH. Acids and bases were diluted with de-ionized water (DI) so that when combined with equal volumes off tam stock solution (pH 7.5 to 8.0) on slides, the resulting pH experienced by tams could be calculated. After mixing tam and altered pH solutions on a slide, it was immediately cover slipped and read. Time required for reading one slide averaged 3.5 min. Observed tams were separated into 3 categories; Fired, Unfired and Empty.   Tams with at least one of the three polar filaments fired was categorized as Fired. Unfired tams were those that retained all filaments within capsules. The Empty category was created for tams that had no polar capsules.

The ranges of pH tested were 3.8 to 1.1 and 11.7 to 12.9. Discharge of tam polar filaments increased towards both extremes of the pH range (Figure 1), though the percentage discharged was greater at the basic end of the spectrum. Results indicated that discharge increased at pH values at or below 3.8 or above 11.7.

An additional test was conducted with H_2SO_4 to determine if hydrogen ion concentration alone was responsible or if certain anions favored discharge more than others. Results from this test showed no difference from those obtained using HCl.

Salts

The effects of various chloride salts (NaCl, KCl, CaCl, NH_4Cl, MgCl) on polar filament discharge were tested at concentrations ranging from 3.1 to 100 **l** . In addition, other salts were evaluated for a narrower range of concentrations to determine if certain anions or cations were better discharge agents than others. Salts were evaluated by combining salt stock solution in known concentration with tam stock solution on glass slides. Recorded

(Continued on page 4)

(Continued from page 3)
salt concentrations reflect actual concentration experienced after mixing on slide. Slides were covered, read and recorded as noted above.

Comparison of discharge among the chloride salts indicated that some cations were significantly more effective than others (Figure 2). K^+ tended to have higher discharge percentages than the other ions, though the difference was significant only at 50%. NH_4^+ had significantly lower discharge percentages at 6.2 and 12.5 I than for K^+, Mg^{2+} or Ca^{2+}. Comparison of $NaPO_4$ and KPO_4 resulted in no significant difference in the percentage discharged (*t*-test, P $ 0.12). Generally, as salt concentration increased, discharge percentage increased, peaking at 71 I for 100 I KCl. Most of the salts induced discharge, indicating that osmotic differences in general can induce discharge. Discharge differences among anions were also significant. Comparison of KCl, KI, and KPO_4 indicated that Cl^- was significantly more effective (*P* # 0.02) at both 6.2 (45.6% discharge) and 12.5 I (57.8%) than the other anions. Comparison of NaCl and $NaPO_4$ at 12.5 I also indicated chloride ion was more effective (*P* = 0.02).

Electricity

Direct electrical current was also examined as a method for firing tam polar filaments. We used a Electro Square Porator, model T 820 manufactured by BTX, San Diego, CA. This electoporator was designed to administer short, high voltage pulses of electricity to a cell for the purpose of injecting extracellular material such as DNA into a living cell through the pores temporarily created. For these tests, 100Fl samples of fresh tam stock solution were put into a 1mm wide chamber through which the electricity was pulsed. Voltage (1 or 3 kV), pulse length (up to 99 Fsec) and the number of pulses (1 to 25) were then manipulated to evaluate effects on tam polar filament discharge and tam survival. After electrical stimulation, tam solution was transferred to microscope slides and categorized as noted above.

Higher voltages increased the number of polar filaments fired up to 1kV. Between 1kV and the machine maximum of 3kV, little difference was observed. Higher pulse lengths significantly increased the percentage of tams firing polar filaments (Figure 3). At 1kV, a 1 Fsec pulse resulted in about 18% of tams firing filaments. When this was increased to a 99 Fsec pulse (electroporator maximum), 85% of tams observed had filaments fired. Increasing the number of pulses significantly increased the number of tams firing polar filaments as well (Figure 3). At 99 Fsec and 3kV, a single pulse fired 78% of tams, while 25 identical pulses resulted in 98% of tams with fired filaments.

Survival of discharged sporozooites was of interest, applying to possible use for in-vitro tissue culture. Using vital stains (propidium iodide and fluorescein diacetate), survival was severely compromised by use of KCl. Additionally with electroporation there was a significant correlation (p=0.00, r^2=0.91) between the percentage of tams with fired polar filaments and the percentage of dead tams (Figure 4).

Summary

Discharge of polar filaments was produced by extreme pH, osmotic changes produced by salts and electroporation. Chloride and potassium ions were more effective in firing of polar filaments than other ions tested. It is possible that these play some role in firing of polar filaments when in contact with the fish host. However, KCl was shown to be lethal to spores in concentrations that produced significant firing of polar filaments, which would not be expected by a natural trigger. High pH values were particularly effective in firing polar filaments, with all tams firing at pH 12.9. Previous work at this lab has shown high pH to be lethal to tams, with 50% of tams dead after 1hr at pH 10. In light of this it is likely that the majority of tams firing after exposure to high pH in our experiments did not contain viable spores. Because of the extreme values required for firing polar filaments, pH alone doesn=t appear to hold any promise for management applications. Electroporation proved effective in firing tam polar filaments as well as killing sporozoites within the tam. Because of its rapid effect and potential lack of lasting water quality impacts, further research with electricity may be fruitful for treatment of hatchery water supplies.

Mark Smith

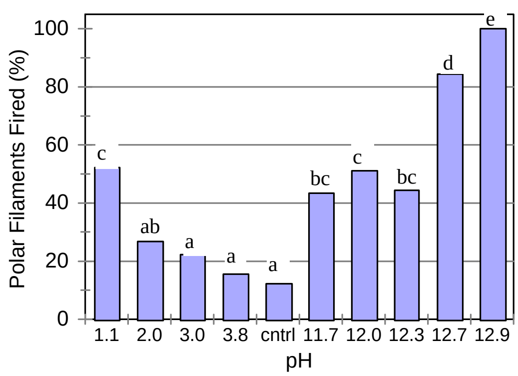


Figure 1. The results of high and low pH on *Myxobolus cerebralis* actinospore polar filaments. Reported as mean percent of tams with fired polar filaments.

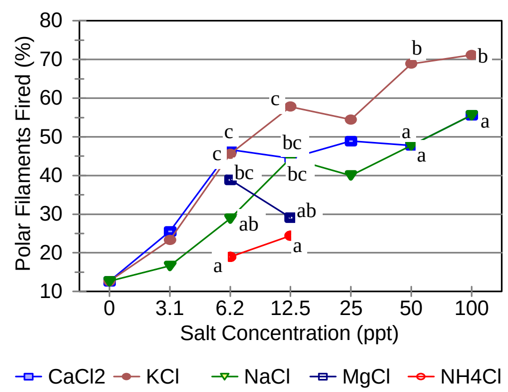


Figure 2. Effectiveness of chloride salts in firing tam polar filaments. Reported as mean percent of tams with fired polar filaments " SD. Significant differences (*P*# 0.05) between salts are noted by a different letter.

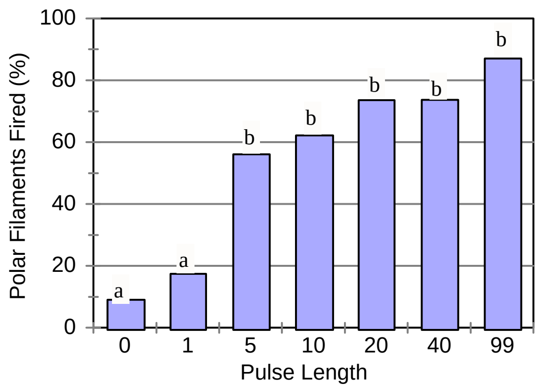


Figure 3. The firing of tam polar filaments using electroporation. Experiment conducted using 1, 1kV pulse at various pulse lengths. Reported as mean percent of tams with fired polar filaments " SD. Significant differences (*P*# 0.05) between treatments are noted by a different letter.

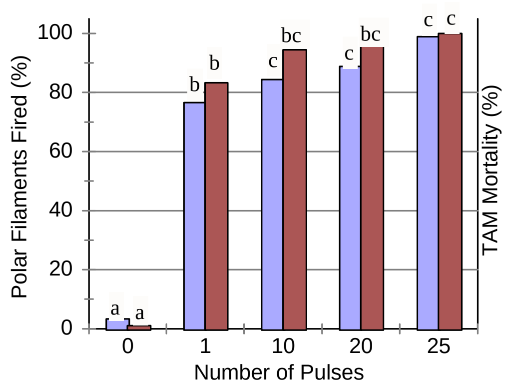


Figure 4. The effect of multiple electrical pulses on the viability and firing of polar filaments in tams. Experiment conducted at 3kV and pulse length of 99 Fsec with 0 to 25 pulses. Results expressed as mean percentage of dead tams (dark bar) and mean percentage of tams with fired polar filaments (lighter bar). Significant differences (*P*# 0.05) among the number of pulses are noted by a different letter.

Influence of Flow Dynamics and Raceway Substrate on Fin Erosion

Work conducted previously at the Fisheries Experiment Station (FES) has focused on gravel-substrate raceways and their influence on fin erosion. With early designs the gravel was placed loosely on the raceway bottom but this was plagued by detrital accumulation in the gravel. Follow-up studies looked at lifting the gravel off the raceway via a false floor through which detritus would flow, or by actually affixing the gravel to plastic sheets which then lay on the raceway floor. All designs contributed positively to fin condition, but were not configured to be realistically applied to a hatchery setting.

To improve on the later design where gravel was affixed to plastic sheets, it was decided to incorporate a cross-flow system with the gravel substrate. With a cross-flow design, water enters and exits through a manifold system set up parallel to the raceway length. This design was chosen based on the theory that water flowing uniformly across the gravel substrate over a short distance (across the raceway as opposed to down its length) would enhance waste removal. The benefits of the cross-flow design include: self cleaning characteristics, decreased water residence time, improved feed conversions, and more even distribution of dissolved oxygen.

When rearing rainbow trout in cross-flow, plug-flow, and circular tanks, Ross et al. (1995 Aquacultural Engineering 14:29-47) found that fish were more evenly distributed in a cross-flow raceway compared to a plug-flow and that fish tended to avoid each other more in the cross-flow design. Whether the fish were distributing themselves more evenly due to the uniform oxygen level or if it was based on various orientation potentials relative to current compared to a plug-flow raceway was not determined. But it does seem likely that due to the circular flow pattern along the length of a raceway, in a cross-flow design there would be more uniform distribution, less concentration of fish, and possibly less aggression and better fin condition.

There were two purposes to this trial. First, to continue to improve the gravel-substrate design in order to make it a realistic alternative for large-scale aquaculture and to determine if the cross-flow design improved waste removal from the gravel substrate. Second, to determine whether or not a cross-flow raceway design distributes fish such that aggression and dominance were broken down and therefore fin erosion decreased.

Rainbow trout of the Sand Creek strain were stocked into nine separate raceways at the FES at 1,000 fish per raceway (4.5 g/fish). Three raceways were designed as cross-flow systems with a concrete substrate, three were cross-flow systems with a gravel substrate, and three were left untreated as controls. The initial density index for all raceways was 0.22 and went as high as 0.40 (700-2,100 fish/m^3) for several raceways. Midway through the 123 day study, water flow was increased from 15 to 30 gpm/raceway, fish densities were also reduced by increasing the occupied raceway length. At that point additional water delivery and effluent pipes were added to the cross-flow raceways. The flow indices used during the study ranged from 0.24 at the beginning to 0.53 at the end of the study.

To evaluate fish performance and fin condition raceways were inventoried monthly for weight gain, and fin measurements were made from 10 fish per raceway at approximately the same time. Necropsies were performed according to the Health Condition Profile (HCP) methodology on ten fish from each raceway and fin measurements made from 20 fish per raceway at the study conclusion. Video observations were also made on days 85-86 to document the fish distribution patterns within the treatment raceways. No attempts were made to quantify orientation or aggression between fish, but anecdotal information was collected regarding fish orientation with respect to current and each other.

For the cross-flow raceways, 5.1 cm PVC pipe was used for both the water delivery and effluent pipes. Both influent and effluent pipes lay at opposing ends of the bottom of the raceway at the corner formed by the floor and wall. A dam board was placed at the head of the raceway immediately below the LHO unit through which the water delivery pipe passed. This forced all water through the delivery pipe. A dam board at the raceway tail with the effluent pipe passing through it worked in a similar manner to discharge water. Slots (20mm x 3mm) were made along the length of the pipe every 30mm. Over time, detritus began to clog some of the inflow and effluent pipes so a twice weekly pipe cleaning regimen was initiated during which a brush attached to a section of garden hose was inserted throughout the length of the pipes to clean out debris and unplug the slots. The backing material used for the gravel substrate was a prismatic plastic material normally used as coverings for fluorescent light fixtures. The panels were first sanded, then coated with a layer of the epoxy after which gravel was placed on the resin to a depth of a single height of gravel. The average size of the gravel was 11.9 mm (mean axis) with a range of 6.6-17.2 mm.

The combination of gravel substrate and cross-flow design had a beneficial impact on fish performance. Growth and feed conversions were significantly better for the cross-flow/gravel fish compared to the controls. Indicators of fish performance were also better for the cross-flow fish compared to the controls but these relationships were not significant (Table 1).
The decreased growth among the controls may have been caused by gas saturation within the control raceways. Total percent saturation averaged for the controls was 106% on Day 86 and 108% on Day 123. For the same two days saturation values averaged for both cross-flow treatments averaged 101% and 104%. It is possible the control fish were exposed to chronic supersaturation approaching acute and that therefore growth may have been negatively affected due to the additional stress. However this acute exposure is likely to have occurred only during the final month of the study and growth was already less (*P*=0.068) for the controls prior to that time.

Table 1. Hatchery performance of rainbow trout reared in control, cross-flow, or cross-flow/gravel raceways. Mean ("SD) values with a different letter are significantly different ($\underline{P}$ # 0.05).

	control	**cross-flow**	**cross-flow/gravel**
Final fish weight (g/fish)[1]	53.4 ± 1.9b	56.2 ± 2.9ab	61.1 ± 0.6a
Specific growth rate (%)[2]	1.99 ± 0.03b	2.03 ± 0.04ab	2.10 ± 0.01a
Condition factor[3]	1.19 ± 0.10	1.20 ± 0.09	1.23 ± 0.09
Feed conversion ratio	1.21 ± 0.03b	1.17 ± 0.06ab	1.08 ± 0.01a
Cumulative mortality (%)	1.0 ± 0.4	2.0 ± 0.8	0.9 ± 0.4
Fat index	3.4 ± 0.5	3.3 ± 0.4	3.3 ± 0.5
Fin Index	0.9 ± 0.7b	0.4 ±0.7a	0.7 ±0.9ab

[1]Initial fish weight = 4.54 g/fish
[2]Specific growth rate = $[(\ln weight_2 - \ln weight_1) / (\# \text{ of days})] \times 100$
[3]Condition factor = $[weight / (length)^3] \times 10^5$

(Continued on page 8)

(Continued from page 7)

Fin erosion data collected throughout the study indicated a trend of less fin erosion among the cross-flow raceways, gravel or not, compared to the controls (Table 2). Early in the study both types of cross-flow raceways produced fish with fins superior to the controls, but by Day 62 all fins measured were superior for the cross-flow/gravel treatment compared to the other two groups. By Day 90 this trend had lessened in magnitude, but in general the cross-flow/ gravel fish had better fins than the other two groups. By Day 123 the cross-flow fish had better caudal, anal, and pelvic fish than the controls or cross-flow/gravel fish indicating a switch in treatment affects on fin erosion.

Why this shift from improved fin condition among fish in the cross-flow/gravel raceways to the cross-flow fish occurred is unknown. Video observations of the raceway types revealed no differences between the two cross-flow treatments with respect to fish orientation. This would suggest that fish in both cross-flow treatments were subject to the same distribution patterns, aggression levels and possible affects on fin condition. At the study conclusion caudal, anal, and pelvic fins were not different between the controls and cross-flow/gravels fish, but were better for the cross-flow. This indicates that at that age there was some aspect of the cross-flow design alone that was contributing to improved fin condition in the absence of gravel. This may not be an especially strong relationship because pectoral fins at the same time were superior for both crossBflow testaments relative to the controls. However the mean fin index values from the HCP (possible range from 0, no erosion, to 2, erosion with hemorrhaging) were slightly better for the cross-flow compared to the cross-flow/gravel, although not significantly so. The index values do not measure the actual fin length, but the presence of active fin erosion. The higher value among the cross-flow/gravel fish indicates that more active erosion was occurring compared to the cross-flow fish.

Table 2. Relative fin index measurements of rainbow trout reared in control, cross-flow, or cross-flow/gravel raceways. Mean values with a different letter are significantly different (*P* # 0.05).

	Dorsal	**Caudal**	**Anal**	**L. pelv**	**R. pelv**	**L. pect**	**R. pect**
Day 62							
control	4.1 b	10.8 c	7.7 c	9.0 b	8.9 b	7.5 b	7.3 b
x-flow	4.2 b	11.4 b	8.4 b	9.2 b	9.2 b	8.2 b	8.4 b
x-flow/grvl	5.6 a	14.0 a	10.6 a	11.6 a	11.6 a	11.2 a	11.7 a
Day 90							
control	4.2	11.2 b	8.0 b	9.4 c	9.4 b	7.2 b	7.3 b
x-flow	4.2	11.9 a	8.6 ab	9.9 b	10.1 a	8.4 ab	8.2 b
x-flow/grvl	4.5	12.3 a	9.1 a	10.8 a	10.8 a	10.1 a	10.8 a
Day 123							
control	3.6	10.5 b	7.9 b	9.0 b	8.9 b	5.5 b	5.6 b
x-flow	3.8	11.6 a	9.3 a	10.4 a	10.4 a	8.0 a	7.9 a
x-flow/grvl	3.6	10.2 b	8.4 b	9.2 b	9.2 b	7.9 a	8.2 a

(Continued from page 8)

By incorporating the gravel into the cross-flow design we were attempting to improve fin condition by using a gravel substrate and at the same time take advantage of the flow dynamics of the cross-flow system as a means of more easily removing waste particles from the gravel substrate. This design did work although there was a higher quantity of sediment and algal growth associated with the area around the pipes than was found in the plug-flow raceways. This load of organic material did not adversely affect the treatment fish because no impacts of raceway design were revealed in data from the HCP=s or fish mortalities. The growth of algae and associated aquatic invertebrates that were found in the gravel raceway substrates may have been prey items, providing the fish with a supplemental food source; a food source that may have provided additional minerals or micronutrients in quantities to positively impact fin condition.

Lellis and Barrows (1997 Aquaculture 156:229-240) 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 more than the fish meal-based diet, which contained higher levels of iron, calcium and phosphorus. This line of reasoning may be in part substantiated by the results from previous work at the FES where we reared fish in coated raceways with smooth walls or rough raceway walls. The results revealed better fin condition among the control fish in the concrete raceways compared to the fish in the coated raceways. It is possible that the resin coated raceway walls were so smooth they did not allow colonization of algae and aquatic invertebrates which may have served as supplemental food items and possibly improved fin condition.

Ronney Arndt, Eric Wagner, Doug Routledge, Roger Mellenthin

Changing of the Guard

As often happens, the Fisheries Experiment Station sees a lot of folks coming and going in and out of the program. January will see the departure of **Mark Smith** to Laramie Wyoming, where he will enter graduate school at the University of Wyoming under Dr. Wayne Hubert. Mark has worked intermittently at FES for 4 years as part of the research team.

Replacing Mark in research will be **Robert Montgomery**, who has worked the past two years in the parasitology laboratory and field inspections under Technical Services. Robert also plans to start his graduate program at Utah State University this year.

Returning to FES after a two year stint in graduate school will be **Art Butts**. Art is finishing up his masters degree, which focused on the impacts of whirling disease on kokanee salmon at Porcupine reservoir. Art will be working in the parasitology laboratory, focusing on the whirling disease survey and wild broodstock inspections.

Best wishes to these three gentlemen in their new career positions!

The Ichthyogram is a quarterly publication of the Fisheries Experiment Station, Utah Division of Wildlife Resources, Logan Utah 84321.
Editor:
Chris Wilson (nrdwr.cwilson@state.ut.us)
Contributors:
Eric Wagner (nrdwr.ewagner@state.ut.us)
Ronnie Arndt (nrdwr.rarndt@state.ut.us)
Mark Smith
This newsletter is distributed electronically at http://www.udwrfes.org/ichthyog.htm or is printed on recycled paper.

Send comments or change of address to:
EDITOR, The Ichthyogram
1465 West 200 North, Logan, UT 84321

STATE OF UTAH
NATURAL RESOURCES
Wildlife Resources

The Ichthyogram

Fisheries Experiment Station
1465 West 200 North
Logan, UT 84321
http://www.udwrfes.org/