

The Ichthyogram

VOLUME 17, NUMBER 3

SEPTEMBER 2006

A New Home for FES June Sucker

The June sucker (*Chasmistes liorus*) is an endangered species endemic to Utah Lake, Utah. The June sucker responsibilities at the Fisheries Experiment Station are increasing significantly and the demands for rearing more June suckers for stocking back into Utah Lake continue. Due to a lack of warm water in our present facility, the fish grow at a rate of 3"/year. The 65°F water that these fish are being raised on is a constant stressor on the June sucker and this increases the possibility of disease outbreaks in the hatchery.



A new 42' x 120' recirculation facility, was completed in July 2006 at FES to allow us to culture the fish on warmer water. The new facility will allow us to raise the June sucker in 72°F - 78°F water, which should greatly increase growth rates, survival and hopefully reduce diseases. We hope to rear at least 33,000 fish to 8" annually (5900) pounds and then stock them into Utah Lake and refuge sites such as the Rosebud Ponds, Red Butte Reservoir and Camp Creek Reservoir.

The operation and maintenance of the project is being funded by the Department of the Interior. We would like to thank PRAqua, JUB Engineers and Bailey Construction for the technical work they did on the project.

New Face at FES

Chad Hill recently accepted the assistant hatchery supervisor position at FES. This is a new position that was created to help handle the additional workload of the newly constructed recirculation facility. He comes to FES from the Fountain Green Fish Hatchery where he worked for over two years. A native of Washington State, Chad worked for the Washington Department of Ecology, the Bureau of Land Management, and the Washington Department of Fish and Wildlife prior to coming to work for the DWR. Chad received his B.S. in Conservation Biology from Brigham Young University in 2002. He also earned a minor in Geographic Information Systems and played the baritone in BYU's marching band for four years.



Chad has been interested in fish culture since the age of nine. He got his first aquarium at the age of ten and has been raising tropical fish ever since. The elementary school he attended hatched hundreds of Coho salmon fry each year and released them into the stream behind the school. This experience, along with numerous field trips to local salmon hatcheries, cemented in him the desire to work with fish as a career. His family still thinks he's just playing around and should get a real job.

Chad's interests include fishing and sailing. He is a self-described football nerd. He's been known to pour over football stats and obsesses over his fantasy football teams.

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EVALUATION OF DIETS FOR REARING JUVENILE NORTHERN LEATHERSIDE

The northern leatherside (*Lepidomeda copei*) is a small cyprinid native to streams and rivers in the Bear River drainage in the Bonneville Basin and the upper Snake River drainage of western North America (Sigler and Sigler 1987; Johnson et al. 2004). The Utah Division of Wildlife Resources is currently developing a conservation strategy for this species, and a draft has been written (Miller 2004). One of the strategy's objectives is to "identify and implement research questions that meet management needs for northern and southern leatherside chub and use the findings to better meet the goal and objectives of this conservation strategy." Development of techniques for captive breeding was identified as a need within this objective. Reduced numbers of natural populations suggest that artificial propagation is needed to help recovery efforts via supplementation and expansion, thereby reducing the potential for local or widespread extinctions.

Initial research on artificial propagation produced encouraging results as a small number of wild adults reproduced in a hatchery setting (Wagner et al. 2005b). Juveniles from the study proved easy to rear, although the fish needed an ontogeny of diets similar to that of the least chub owing to their small size at hatch (Wagner et al. 2005a). After swim-up, larvae were fed a commercial powder feed (Hatchfry Encapsulon Grade 0, Argent Laboratories, Redmond, Washington), followed by brine shrimp (*Artemia*) nauplii. After approximately a month, a ground commercial feed (TetraMin®) used to feed adult fish was fed in conjunction with brine shrimp for approximately one month before being fed exclusively on the ground commercial feed.

The juvenile northern leathersides appeared to survive and grow well on this ontogeny of diets for most cohorts. However, in some cohorts, deformities in the form of distended abdomens and spinal deformation (Figure 1) occurred at a rate of 12 – 18% when feed was switched exclusively to the ground commercial feed. The onset of the deformities was halted when brine shrimp nauplii were reincorporated into the diets, suggesting that the deformities were caused by the feed.

We hypothesized that the exclusive diet of ground commercial feed was initiated too early in the development of the juveniles. To test this hypothesis, we initiated juveniles onto the exclusive ground commercial feed diet at weekly intervals to determine at approximately what age fish no longer developed mortalities.

Methods

Juvenile northern leatherside were obtained from reproductive studies in 2006. Juveniles were produced by brood stock from Deadman Creek, Bear River drainage, Utah, held at 24°C. Eggs were collected on 7 April 2006, and had hatched by 9 April. Larvae were maintained on a diet of brine shrimp nauplii. At 17 days post hatch, 25 fish were randomly distributed into 12 4.4-L Tupperware containers. Digital images were taken of 20 fish from each tank, and total length (TL) was measured using digital imaging software (Adobe Photoshop 5.5). Fish averaged 11.3 mm TL, and were not significantly different in size among tanks ($P = 0.083$).

Beginning at 24 days post hatch, one tank per week was switched to the ground commercial feed (425 – 600 μm). Fish in each tank were fed equal biomass of brine shrimp nauplii or ground commercial feed each day. For the first 6 weeks, fish were fed 4 times per day, 40 mg per feeding. To increase the ration after 6 weeks, an extra feeding was added (5X/day, 40 mg per feeding) for the remainder of the study. Brine shrimp nauplii were fed via frozen ice cubes (2.5 mL volume). Dry weight (103°C) of ice cube samples was used to estimate the biomass of the brine shrimp nauplii. Deformities and mortalities were recorded daily. At 94 days post hatch (10 tanks on commercial feed for at least a week), the study was ended. Final total lengths (TL) were measured using digital images of the fish and digital imaging software. Final weights for fish within each tank were obtained by placing all remaining fish into a pre-weighed beaker of water, measuring the increase in total weight, and dividing by the number of fish. Care was taken to avoid introducing water with the fish.

Differences in final TL were determined using a one-way analysis of variance. Post-hoc comparisons were

conducted with the Tukey Test. The relationship between final weight and the number of weeks fed the ground commercial feed was determined using linear and multiple regression analysis. All statistical analyses were conducted using SPSS version 7.0 (SPSS 1996) and a significance level of 0.05.

Results and Discussion

The juvenile northern leatherside did not develop deformities in this study, and survival was high and similar between all tanks (Table 1). Fish that had been fed the commercial feed starting at 38, 52, and 59 days post hatch were significantly greater in length than one of two tanks that was only fed brine shrimp nauplii; the difference in length was 3 mm (Table 1). The relationship between final weight and the number of weeks fed the commercial feed was best represented by a quadratic equation ($\text{weight} = 152.015 + 26.113 * \text{weeks on feed} - 2.067 * (\text{weeks on feed})^2$; $P < 0.001$; $r^2 = 0.894$; Figure 2). The higher growth observed in the dry feed groups was likely due to an error in brine shrimp biomass estimation we discovered later; the brine shrimp were not rinsed free of the salt, so roughly half the biomass was salt. Nonetheless, the study was useful for demonstrating the lack of deformities and good growth and survival of leatherside fry switched to dry feeds when reared at 23-24°C.

Aquaculture of northern leathersides is currently used as a research tool to help understand the life history of this species and to develop culture techniques should aquaculture become necessary to preserve the species. Because production fish will be used for recovery efforts via supplementation and expansion, the fish should be of good quality to maximize likelihood of survival to maturation and reproduction at introduction sites. The deformities developed in juveniles during the 2005 production studies reduced the number of viable fish that would have survived if stocked into their natural environment. The deformities appeared to be induced by the type of feed, an occurrence not uncommon in aquaculture (Hansen 2003). However, the results of this study indicated that the juveniles would not develop deformities if fed the ground commercial feed as early as 24 days post hatch.

The deformities observed in 2005 may have been a combined effect of feed and temperature; fish that developed deformities were held at 18°C, whereas this study was conducted at 24°C. Because no deformities occurred in this study, juvenile northern leatherside from reproduction studies held at both temperatures were fed the ground commercial feed when fish were older than 5 weeks. While no fish held at the warmer temperature developed deformities, fish at the colder temperature did, indicating that the feed and colder temperature combined cause the deformities in these young fish. Fish held at 18°C or colder temperatures should not be fed the ground commercial feed until they reach older ages. However, juvenile growth is maximized at approximately 24°C (Billman, unpublished data), and future culture techniques should utilize water at or near this temperature for grow-out of the juvenile fish. The lack of deformities in this study indicated that the ground commercial feed is a viable diet for juvenile northern leathersides reared in water that is approximately 24°C. Based on the relationship between weight gain and time on feed, we recommend that juvenile northern leathersides be initiated on the ground commercial feed between 38 – 59 days post hatch.

References

- Hansen, E. 2003. Evaluation of feed regimes for rearing June sucker (*Chasmistes liorus*). Ichthyogram 14 (1):1,6-7.
- Johnson, J.B., T.E. Dowling, and M.C. Belk. 2004. Neglected taxonomy of rare desert fishes: congruent evidence for two species of leatherside chub. Systematic Biology 53:841-855.
- Miller, P. 2004. Conservation strategy for the leatherside chub (*Snyderichthys copei*: Cyprinidae, formerly *Gila copei*). Utah Div. Wildlife Resources, Salt Lake City, Utah.
- Sigler, W.F., and J.W. Sigler. 1987. Fishes of the Great Basin, a natural history. University of Nevada Press, Reno.
- SPSS Inc. 1996. SPSS® base 7.0 for Windows™ user's guide. SPSS Inc., Chicago, IL.
- Wagner, E. J., E. J. Billman, and R. E. Arndt. 2005a. Least chub: recovery through research. Publication number 05-34. Utah Division of Wildlife Resources, Salt Lake City, Utah.
- Wagner, E., E. Billman, R. Arndt, and M. Harvey. 2005b. Leatherside chub life history and behavior: discovery through captive breeding. Final report to the Utah Reclamation, Mitigation, and Conservation Commission. Utah Division of Wildlife Resources, Salt Lake City, Utah.

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Table 1. Mean total length (mm), deformities (%), and survival (%) of juvenile northern leathersides that were transitioned from brine shrimp nauplii to a ground commercial feed at weekly intervals. Significant differences among means within a column are noted with different subscript letters.

Time on commercial feed (weeks)	Mean total length (mm)	Deformities (%)	Survival (%)
0	27.8 e	0	96
0	28.4 de	0	100
1	28.6 cde	0	100
2	30.0 abcde	0	92
3	29.6 abcde	0	100
4	29.1 bcde	0	100
5	30.8 abcd	0	100
6	30.7 abcd	0	92
7	30.2 abcde	0	100
8	30.8 abcd	0	92
9	28.9 bcde	0	100
10	28.8 bcde	0	100



Figure 1.—Deformities (distended abdomens and spinal deformities) that occurred in juvenile northern leatherside.

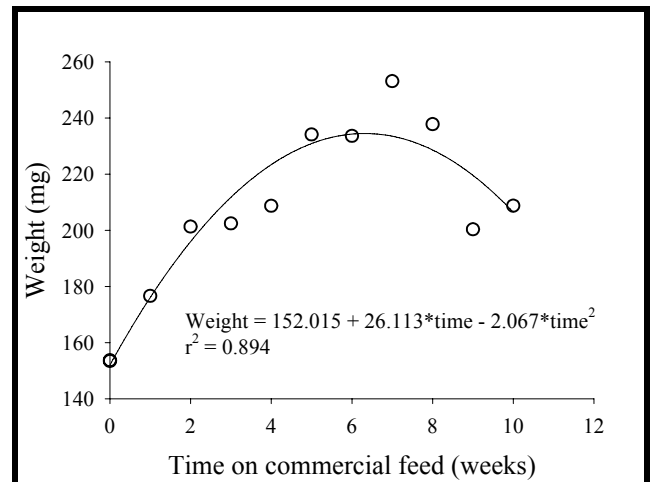


Figure 2.— Weight of juvenile northern leatherside in relation to the amount of time they were fed a ground commercial flake feed versus the early rearing diet of brine shrimp nauplii. Each circle represents the mean individual weight gain of fish for each tank at each weekly interval for onset of the commercial feed. The correlation coefficient of the regression is presented.

Evaluation of Probiotics for Prophylactic Treatment of Rainbow Trout Eggs and Fry and Fingerling Feeding

It has been theorized and to some extent determined, that the bacterial microflora within a batch of eggs, can have a strong negative impact on survival (Sauter et al. 1987, Barker et al. 1989). In addition, Barnes et al. (2000) noted an increase in bacteria from the top of a vertical incubator to the bottom. For these reasons and also for the control of aquatic fungi, the incubation of salmonid eggs generally involves the daily administration of a 1,667-ppm formalin treatment. Formalin treatments improve egg survival, but we have wondered if the reduction in bacteria abundance on the eggs might provide an opportunity for recolonization by even less desirable bacteria. Pathogenic bacteria such as *Flavobacterium psychrophilum* may be more opportunistic than other less pathogenic or even beneficial bacteria. It would be advantageous to develop a technique of controlling or eliminating the 'bad' bacteria, while at the same time encouraging the 'good'.

In recent years the term probiotics has been used in relationship to human and livestock nutrition. Probiotics, by definition, are live feed additives that positively influence the organism that consumes it (Irianto and Austin 2002). Among fish, for example, probiotic bacteria may improve gut microflora to improve digestive efficiency (Irianto and Austin 2002) and early fry survival (Carnevali et al. 2004). The beneficial probiotic bacteria may out-compete pathogenic bacteria for nutrients and space, thus displacing them.

In a sequence of three tests, we tested probiotics for their ability to enhance the survival of eggs, fry and fingerlings of rainbow trout. The first phase focused on whether or not a common probiotic (*Lactobacillus*) source could be used to colonize the exterior of eggs and whether this treatment enhanced egg survival. The second phase analyzed the effect these same bacteria might have on growth and survival of first-feeding rainbow trout. In the final phase, a secondary source of *Lactobacillus* was used as a feed ingredient in fingerling diets also to determine its effect on growth and survival of the fish.

Probiotic effects on eggs

For the first portion of the study, freshly fertilized eggs were transported 6 hr and placed into individual upwelling-type jar incubators from fertilization through eye-up at densities of 1,000 eggs per jar (temperature = 13-14°C). At eye-up the eggs were mechanically "bumped" and transferred to individual trays within a vertical incubator. During both periods of egg development, three treatments, with three replicates, were administered as prophylactic treatments:

T1 = standard 15-minute formalin drip (1,667 ppm).

T2 = 1667 ppm formalin treatment followed by a 30-minute drip of *Lactobacillus* (2.8×10^9 cfu)

T3 = *Lactobacillus* only, once a day.

Egg and fry survival in the first two phases of this trial were within normal ranges and did not significantly differ among treatments. Egg eye-up for all three treatments was all better than 80% with a low of 82% eye-up for the formalin treatment and a high of 86% for the formalin+probiotic treatment (Table 1). Egg hatch ranged from 93% for the formalin treatment to 99% for both the probiotic and formalin+probiotic treatments (Table 1). These differences were also not significant. During the culture of eggs in both jars and trays, no fungus was evident among any of the treatments. Regardless of these results, the two variants of the probiotic treatment were not effective at improving egg survival

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Table 1. Mean (\pm SD) values of performance exhibited by eggs prophylactically treated with formalin alone, formalin + probiotic, or probiotic alone. The treatments were administered from fertilization through to hatch. Total colony forming units (CFU) derived from the egg's surface immediately prior to hatch are also included. Mean values that have a different letter are significantly different from each other ($P \leq 0.05$).

Treatment	Fish Performance			Bacteriology
	Eye-up %	Hatch %	Deformed %	Total CFU
Formalin	82.0 \pm 4.6	93.2 \pm 4.6	1.3 \pm 0.0	37 \pm 38 a
Formalin + probiotic	86.1 \pm 0.3	98.9 \pm 0.2	1.3 \pm 0.4	2807 \pm 4591 b
Probiotic	84.8 \pm 0.3	99.0 \pm 0.0	1.0 \pm 0.2	5125 \pm 5020 c

although there was a general trend of improved eye-up and hatch within the two probiotic treatments.

The bacteriology work resulted in significantly lower total colony forming units (CFUs), 37, for the formalin treatment. The CFU count for the formalin+probiotic treatments, 2807, was significantly higher than counts for the formalin eggs, but less than the probiotic treatment (5125 CFUs).

Probiotic effects on first-feeding fry

Treatments in this phase were 1) controls derived from the formalin only treatment in the previous test and 2) the probiotic treatment, consisting of three tanks of fry derived from the *Lactobacillus* only treatment. The probiotic test tanks were given a daily drip treatment of *Lactobacillus*, identical in concentration to the egg treatment, while the controls received no treatment.

Survival and growth of fish during the 25 day, indoor, fry portion of the trial was high, with 99% survival for the control treatments and 98% for the probiotic. Final fish weights for both treatments averaged 0.7 g/fish. During this phase, both treatments were fed an identical grower ration, with the only difference between the two groups being the daily drip of *Lactobacillus* into the probiotic tanks. The results indicated that the probiotic treatment had no significant deleterious effects, but since control survival was so high, no benefit could be demonstrated either.

Probiotic effects on larger fry

For the third portion of this study, the fry used previously within the formalin and *Lactobacillus* treatments were transferred outdoors into 75 L circular tanks. Three tanks were designated as probiotic tanks (fry originated from *Lactobacillus* treatment), and three tanks were designated as controls (fry originated from formalin treatment). The control fish were fed a commercial diet and the probiotic fish were fed the same diet top-dressed with *Lactobacillus* premixed with herring oil. The control diets were processed identically, without the addition of *Lactobacillus*. The fish were hand-fed to apparent satiation three times daily during the workweek, and once daily on the weekend.

Sub-samples of all three batches of diets were assayed for viability of *Lactobacillus*. For this, 100 μ L liquefied feed sample was dispensed onto MRS agar plates and later observed for bacterial colony growth. For determination of the colonization of the gut by *Lactobacillus*, the fish were sampled according the protocol of Galindo (2004).

There were no significant differences between the control and *Lactobacillus* treatments with respect to growth or survival over the 47-day trial (Table 2). The probiotic diet was identical to that used in a previous test with cutthroat trout in which fish fed the probiotic diet grew and survived better than controls (Arndt and Wagner 2006). Probiotics improved cutthroat trout survival, in part because there was ‘room for improvement’, while that was not the case with the rainbow trout.

Feed analysis and gut samples indicated viable *Lactobacillus* bacteria were successfully incorporated onto feed particles and into the intestine. The results from this trial indicate *Lactobacillus* may not benefit rainbow trout, especially where they are experiencing high survival rates anyway. However, previous research (Arndt and Wagner 2006) indicated the feeding of top-dressed probiotic feeds to cutthroat improved fingerling survival.

Table 2. Mean (\pm SD) values of A) fry performance when drip treated prophylactically with *Lactobacillus* or nothing (control), and B) when fingerling rainbow trout were fed either a standard grower diet (control) or a diet top-dressed with *Lactobacillus* (probiotic).

Treatment	Fry		Fingerling	
	Final fish weight (g/fish)	Survival %	Final fish weight (g/fish)	Survival %
Control	0.68 \pm 0.00	99.2 \pm 1.0	3.1 \pm 0.1	98.0 \pm 0.9
Probiotic	0.67 \pm 0.00	97.7 \pm 1.6	3.0 \pm 0.2	94.2 \pm 2.5

References

- Arndt, R. E., and E. J. Wagner. 2006. Enriched artemia and probiotic diets improve survival of Colorado River cutthroat trout larvae and fry. *N. Am. J. Aquacult.* (In Press).
- Barker, G. A., S. N. Smith, and N. R. Bromage. 1989. The bacterial flora of rainbow trout, *Salmo gairdneri* Richardson, and brown trout, *Salmo trutta* L., eggs and its relationship to developmental success. *J. Fish Dis.* 12:281-293.
- Barnes, M. E., A. C. Gabel, and R. J. Cordes. 2000. Bacterial populations during rainbow trout egg culture in vertical-flow tray incubators. *N. Am. J. Aquacult.* 62:48-53.
- Carnaveli, O., M. C. Zamponi, R. Sulpizio, A. Rollo, M. Nardi, C. Orpianesi, S. Silvi, M. Caggiano, A. M. Polzonetti, and A. Cresci. 2004. Administration of probiotic strain to improve sea bream wellness during development. *Aquacult. Int.* 12:377-386.
- Galindo, B. G. 2004. *Lactobacillus plantarum* 44A as a live feed supplement for freshwater fish. Ph.D thesis Wageningen Univ., Wageningen, The Netherlands.
- Gildberg, A., A. Johansen, and J. Bøgwald. 1995. Growth and survival of Atlantic salmon (*Salmo salar*) fry given diets supplemented with fish protein hydrolysate and lactic acid bacteria during a challenge trial with *Aeromonas salmonicida*. *Aquacult.* 138:23-34.
- Irianto, A., and B. Austin. 2002. Probiotics in aquaculture. *J. Fish Dis.* 25:633-642.
- Sauter, R. W., C. Williams, E. A. Meyer, B. Celnik, J. L. Banks, and D. A. Leith. 1987. A study of bacteria present within unfertilized salmon eggs at the time of spawning and their possible relation to early lifestage disease. *J. Fish Dis.* 10:193-203.

Ronney Arndt and Eric Wagner

***Myxobolus cerebralis* Found In Bear Lake**

The Technical Service team at Utah's Fisheries Experiment Station (FES), in conjunction with Division of Wildlife Resource (DWR) biologists, have recently discovered the whirling disease parasite, *Myxobolus cerebralis*, in fish collected from Bear Lake. Since the parasite's original detection in Utah in 1991, the organism has spread to many areas within the state. Although widely distributed, the latest discovery represents the first of its kind within Bear Lake.

Bear Lake is located in the Bear River drainage along the Utah-Idaho border. The lake receives water from five major tributaries which include Swan Creek, Big Spring Creek, South Eden Creek and North Eden Creek in northern Utah and St. Charles Creek in southern Idaho. An additional tributary to the system is the Bear River, which has a seasonal connection to the Bear River National Wildlife Refuge along the north end of the lake. Although naturally reproducing trout populations exist within the lake, efforts to supplement and further facilitate the sport fish program by stocking cutthroat trout in the system have been underway for sometime. In accordance with this program, the Utah DWR operates and maintains a spawning trap at the confluence of Swan Creek along the west end of the lake. As adults mature and migrate to Swan Creek, these fish are captured, spawned, and fertilized eggs are distributed to a number of Utah's state hatcheries. Following a short rearing period, fish are returned to system as catchable adults.

Since fertilized eggs from this location are incorporated into Utah's culture system, fish from the lake must be examined and certified annually as pathogen free prior to the transfer of fertilized eggs to a state hatchery. In accordance with this policy, 60 fish including 59 cutthroat and 1 brown trout, were gathered from the lake using a combination of gill nets and electro-fishing equipment this past spring. Samples from these fish were examined at FES; spores morphologically consistent with *M. cerebralis* were isolated from the brown trout using pepsin-trypsin digest methods. Spores were later confirmed to be those of the whirling disease parasite using nested polymerase chain reaction analysis. No spores were found in any of the cutthroat trout.

The presence of a trout infected with *M. cerebralis* in Bear Lake is interesting, especially since brown trout are rarely seen in the system and decades of testing on cutthroat trout suggest the parasite is not established in the lake. Since brown trout have not been stocked into the system and no natural reproducing populations are known to exist in the lake, it has been suggested that these fish may have come from outside the lake's boundaries. Such a scenario would include movement of fish from the Bear River, which is a tributary where the parasite is known to be established.

Future plans for the lake and surrounding tributaries include a combined survey effort by Utah, Idaho and Wyoming resource agencies to identify where this fish may have originated. The immediate concern for Bear Lake is how a finding in the system will influence the current sport fish program. Under Utah's current fish health policy, movement of live fish from Bear Lake will be restricted, but since the parasite is not transferred from mother to progeny, the lake can still be used as an eggs source to supplement the current sport fish program. Although this latest finding is concerning, all evidence suggest the organism is not well established in the system and that Bear Lake can be certified in the future for the transfer of fertilized fish eggs into Utah's culture system.

Wade Cavender

Utah Division of Wildlife Resources
Fisheries Experiment Station
1465 West 200 North
Logan, UT 84321

Phone: 435-752-1066
Fax: 435-752-6977
Email: chriswilson@utah.gov

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

Editor:

Chris Wilson (chriswilson@utah.gov)

Contributors:

Ronney Arndt (ronneyarndt@utah.gov)

Eric Wagner (ericwagner@utah.gov)

Wade Cavender (wadecavender@utah.gov)

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