Kootenai Tribal Burbot Project Report 2010

A program funded by the Kootenai Tribe of Idaho

Report submitted to the Kootenai Tribe of Idaho
Fish and Wildlife Department
P.O. Box 1269
Bonners Ferry, Idaho 83805

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November, 2010

The Kootenai Tribal burbot project has been ongoing since fall 2001. Efforts began with broodstock and field gamete collections conducted by the Kootenai Tribe of Idaho and the British Columbia Ministry of Environment. Subsequently, adult fish and gametes were transported to the Kootenai Tribal fish hatchery where spawning, egg incubation and larval rearing were successful; however, no juveniles were produced due to time and rearing equipment limitations. In 2003, efforts shifted to the University of Idaho's Aquaculture Research Institute in Moscow (UI-ARI). Initial efforts at UI-ARI established the feasibility of captive burbot production through a series of empirical life-stage specific studies examining spawning, semen cryopreservation, egg incubation, and larval and juvenile feeding. After reliable culture techniques were established in 2006, specific pathogen susceptibility and carrier status of burbot was determined through a series of replicated challenge studies. The life cycle of burbot under controlled conditions was closed in 2008 when F1 progeny from 2004 brood year successfully spawned in captivity, eggs were fertilized and F2 progeny began feeding on artificial diets. With culture techniques, disease susceptibility and pathogen screening tools developed, preparations were initiated by the Kootenai Valley Resource Initiative (KVRI) Burbot Conservation Subcommittee to begin experimental releases of cultured burbot.

The success of this project's initial developments has enabled F1 progeny from wild broodstock to be cultured and released experimentally into the Kootenai River Subbasin including Canadian and US waters to support conservation efforts. Multiple releases of age 0, 1, 2, and 3 year old burbot occurred in 2009 and 2010. Radio telemetry data showed that survival of age 2 and 3 year old burbot was high (≥94%) post release in 2009 and these fish utilized a range of habitats from the north arm of Kootenay Lake, BC Canada to Montana, USA (Neufeld et al. *Accepted 2010*). More recently, in August 2010, age 1, 2 and 3 year old burbot were sonic tagged (Vemco™ V9, Amirix Systems Inc. Halifax, Nova Scotia, Canada) and/or PIT tagged (Destron Fearing™, St. Paul, MN, USA) and released in the Moyie River and Boundary Creek in Idaho, USA. Additionally, in November 2010, age 0 and 1 year old burbot were released in Canadian and US waters for the second time. Some of the research completed in 2010 included evaluations of temperature related larval and juvenile growth, replicated extensive and semi-intensive rearing studies, and extended (up to 1 year) visible implant elastomer (VIE; Northwest Marine Technology, Shaw Island, WA, USA) tag retention observations.

Specific 2010 project objectives identified by the KVRI Burbot Conservation Subcommittee included:

1. Verify the feasibility of spawning brood fish in their natural environment (on-site)
2. Further develop captive brood fish spawning, egg incubation and early life rearing tactics
3. Replicate extensive and semi-intensive rearing studies including:
    a. Cage
    b. Farm pond
    c. Semi-intensive outdoor tank culture
4. Further develop tagging methods based on 2008-10 observations
    a. New graduate student is working to develop novel methods for tagging age 0 burbot
5. Certify specific pathogen free status of UI-ARI burbot production facilities
6. Secure necessary permitting and continue burbot releases in Canadian and US waters

Additional research efforts were conducted in 2010 beyond the specific 2010 KVRI project objectives. These included:

7. Characterize egg maturation in-vivo to develop a visual index of ova development
8. Evaluate fertilization success of freshly expressed "dry" ova and ova allowed to water harden over time. More specifically:
    a. Fertilize dry ova stored for 0, 6 and 12 h
    b. Fertilize ova in water at 0, 15, 30, 45 and 60 min
9. Evaluate the effects of 0, 50, 100, 250 and 500 ppm hydrogen peroxide (H_2O_2) on larval and juvenile burbot survival. These trials included:
    a. Pre-mouth larvae
    b. Larvae feeding on rotifers
    c. Larvae feeding on artemia
    d. Juveniles feeding on artificial diets
10. Develop and test grading methods to reduce cannibalism of age 0 burbot. More specifically:
    a. Evaluate the effect of grading larvae actively feeding on live feeds
    b. Evaluate the effect of grading larvae during the dry feed transition phase
11. Characterize larval deformity of wild and captive held F1 progeny

The characterization of in-vivo burbot ova maturation will be used to develop a visual index to reference during on-site and captive spawning and examining fertilization of dry and wet ova is expected to refine fertilization success. Use of H_2O_2 will be used to further optimize early life rearing by improving water quality and combating external bacterial and fungal problems that arise. Grading methods and devices developed in 2010 will be used to decrease cannibalism at certain life stages when cannibalism is common. Overall, there were 15 specific research projects conducted in 2010 (Table 1).

Table 1. Research efforts (excluding stocking/monitoring efforts) scheduled for 2010; including agency, eggs/fish used, life stage of fish under study and current status of research projects.

Research Project	Agency	Eggs/Fish	Fish life stage	Status
Extensive rearing-farm pond	KTOI/IDFG	2000	45 d live feeds	Complete
Extensive rearing-cage culture	IDFG	500	45 d live feeds	Complete
Semi-intensive stocking density study 2	UI-ARI	12000	45 d live feeds	Complete
Larval temperature related growth 3	UI-ARI	0	44 d live feeds	Complete, did not repeat 2010
Juvenile temperature related growth 2	UI-ARI	0	190 d dry diet feeding	Complete, did not repeat 2010
Egg maturation study	UI-ARI	10000	Egg/Embryo	Complete
Larval H_2O_2 exposures (3)	UI-ARI	2250	On live and dry feeds	Complete
Juvenile H_2O_2 exposure	UI-ARI	300	Full feed transition	Complete
Cannibalism study 1	UI-ARI	900	Live feed	Complete
Cannibalism study 2	UI-ARI	450	Dry feed transition	Complete
SPF Disease sampling	UI-ARI	60	Full feed transition	Complete
Tagging studies (2): including PIT, VIE, freeze brand, fin clip	UI-ARI	600	Full feed transition	Studies in progress

KTOI-Kootenai Tribe of Idaho, IDFG-Idaho Department of Fish and Game, UI-ARI-University of Idaho Aquaculture Research Institute

Spawning

On-site artificial spawning of burbot occurred on Moyie Lake, BC Canada in mid-February 2010. A total of 18 egg lots were transported to the UI-ARI for incubation. All eggs were water hardened in 25 ppm Iodophore fish egg disinfectant (Western Chemical Inc. Ferndale, WA, USA) for 30 min. Although efforts were successful, a wide range in egg fertilization and embryo survival occurred. Transport was lengthy (approximately 6 hours, 240 miles), the majority of brood fish were not anesthetized, no hormone treatments were given, and gamete quality was not verified prior to general artificial spawning methods. Female brood fish were observed expressing ova of varying consistency and color. Many females collected on the spawning grounds did not express ova while males caught at the same time consistently expressed milt. When a ripe female was caught, multiple (2-5) males were used per female to circumvent the potential of using a poor quality male's milt. Sperm motility was not examined. Overall, egg viability ranged 0-92% at 48 h post-fertilization post transport. In response to these observations, in-vivo ova maturation was characterized (Figure 1) and fertilization of freshly expressed "dry" ova and ova allowed to water harden before fertilization were examined over time (Figure 2, Figure 3). Future on-site gamete collections are expected to improve by using the egg maturation index developed in 2010 and we recommend verifying sperm motility prior to artificial spawning.

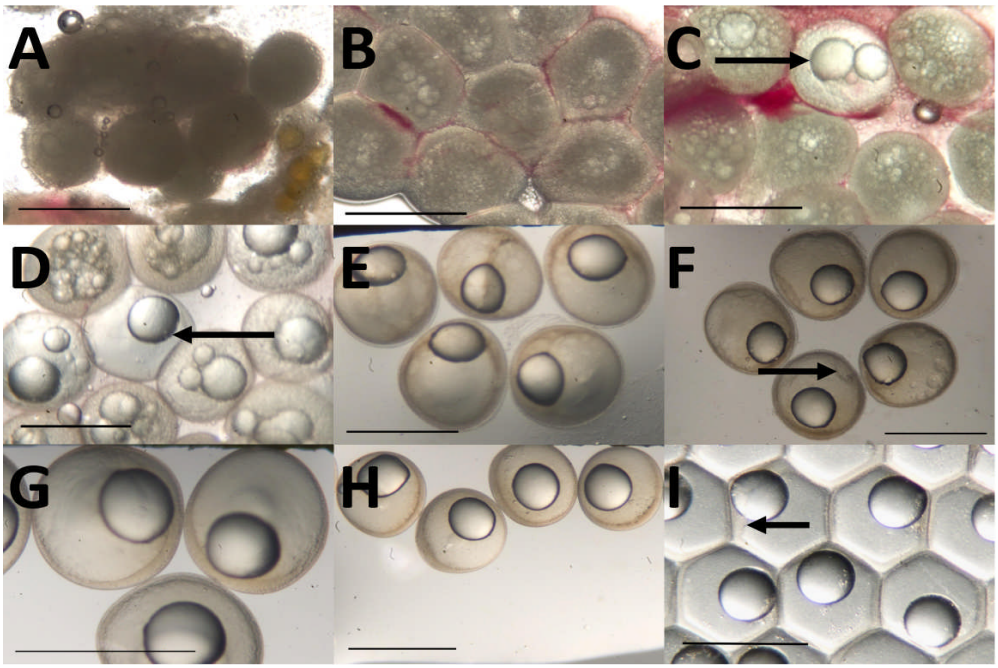

Figure 1. Burbot egg development, scale bars = 1.0mm. A) 32 days pre-release, B) 11 days pre-release, C) 9 days pre-release, arrow egg clearing and polarization of oil globule, D) 4 days pre-release, arrow to developed egg, transparent yolk, polarized oil globule and observable vitelline membrane, E) 2 days pre-release (preserved), F) 1 day pre-release (preserved), arrow pointing to germinal disk, G) 3 hours pre-release (preserved), H) time of release (preserved), I) 1 day overripe, arrow breakdown at the inner surface of the chorion (Draft image series courtesy of J. Foltz, UI-ARI).

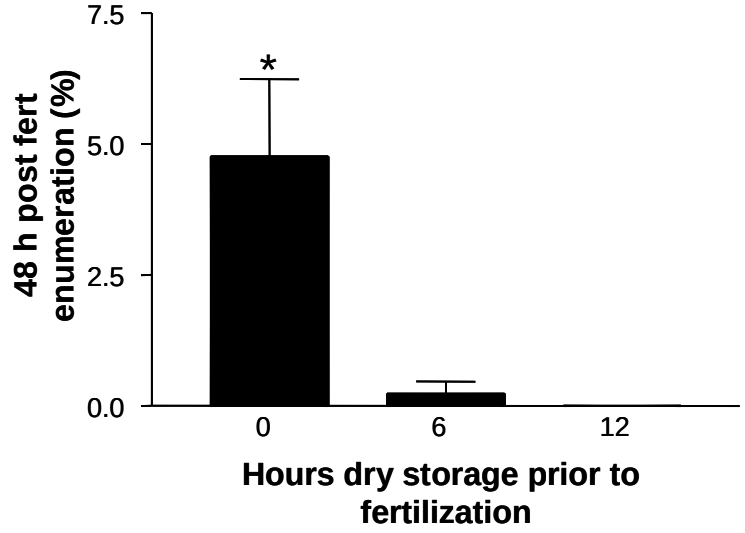

Figure 2. Fertilization success of burbot ova following periods of dry storage.

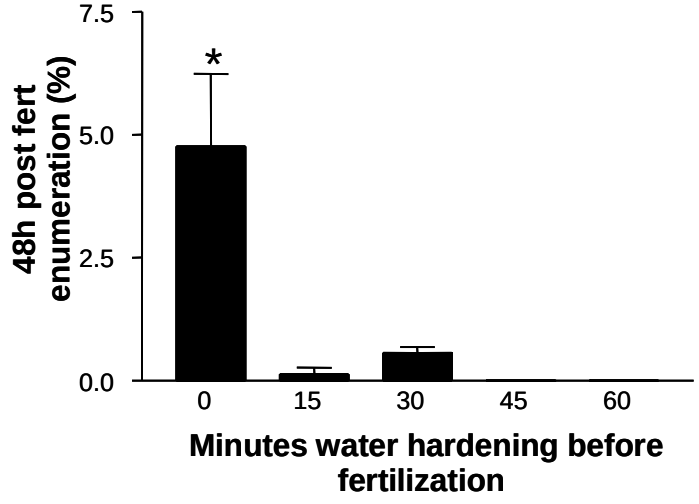

Figure 3. Fertilization success of burbot ova following periods of water hardening.

Captive spawning of Moyie Lake brood fish captured in 2006 and 2007 and held at UI-ARI, was successful with 10 of 10 female burbot releasing eggs during the 2010 spawning season. Egg viability at 48 h post-fertilization varied from 31-92%. Additionally, 2 of 3 captive brood fish from Duncan Reservoir and 1 of 2 UI-ARI F1 (2004 brood year) brood fish released eggs. All Duncan origin and UI-ARI F2 eggs were discarded because rearing Moyie Lake origin fish was considered a priority for experimental releases. Overall, 31 distinct spawning and/or egg collection events were documented during 2010.

Egg collection

Incubation capacity was met in 2010. An estimated total of 17,040,532 burbot eggs were collected during 2010. Approximately 54% of these eggs were viable at 48 h post-fertilization and were incubated through hatch. On-site egg collection yielded an estimated 7,724,888 eggs with a mean viability of 48% for a total of 3,032,143 viable eggs collected on-site and subsequently transported to UI-ARI. Captive egg collections included an estimated total of 9,315,644 eggs with a mean viability of 64% for a total of 5,944,684 eggs collected from captive brood fish. The combined estimated total of viable eggs collected and incubated through hatch was 8,976,827. Approximately 8,063,705 eggs were culled due to low viability.

Larval and juvenile production

Larval production levels were enumerated at the time of initial live feed introduction (mid-April) and when family groups could be observed feeding on live prey (mid-May). Juvenile production levels were measured after experimental demands were met and all remaining fish were fully transitioned to artificial diets (Otohime Hirame diets, Aquatic Ecosystems, Apopka, FL, USA). An estimated 6,445,086 larvae were introduced to live prey and approximately one month later an estimated 87,023 larvae (approximately 1.3 %) were successfully feeding on live prey (cultured Rotifers; *B. plicitilis*). High levels of mortality typically occur and are expected during this phase. Cannibalism was observed in all rearing tanks. Since the inception of this project high levels of deformity and mass larval mortality has occurred during this transition period.

Larval burbot deformities were characterized and enumerated in 2010. On average about 35% over all family groups were deformed, ranging from approximately 20-75% (Figure 4) regardless of broodstock origin (wild or captive held). Deformities included one or more of the following: vertebral (scoliosis, lordosis, star gaze), bent/folded fins, head/mouth/eye malformations, abdominal distension and air bladder malformation (Figure 4). It is possible that such deformity levels may be normal. However, intrinsic nutritional, developmental and aquaculture related factors (e.g. rearing density and system designs) may have contributed to the observed deformities. Future work should continue to document deformity levels of larvae.

By September 2,761 intensive reared juveniles had been successfully transitioned to artificial diets. This level of juvenile production, along with the 306 extensive farm pond and cage reared juveniles, was adequate to meet 2010 experimental release plans, initiate new research, and was an improvement over 2009 production levels (Figure 5).

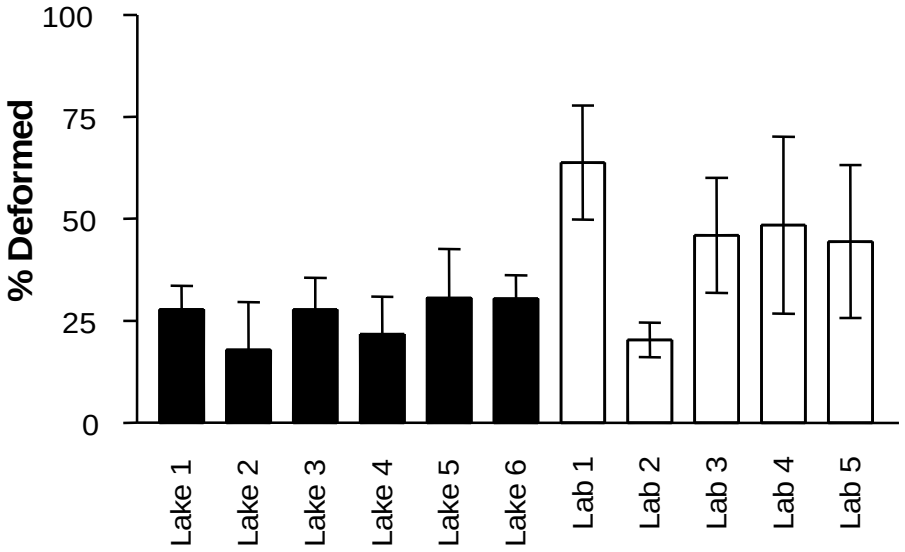

Figure 4. Estimated deformities by family group on 4/23/10.

2010 experimental burbot releases

Burbot stocking for 2010 began at the end of July with net pen harvests and was followed by farm pond trapping, which occurred until the first week of October. Net pens and the farm pond contributed 206 and 100 age 0 fish respectively. IDFG and KTOI tagged and released the net pen fish including: 50 fish stocked to Snow Creek, 106 fish stocked to Deep Creek and the remaining fish were released into the Kootenai River near the mouth of Deep Creek by KTOI. Farm pond age 0 fish were tagged with fluorescent orange VIE proximal to their right side pectoral fin and net pen fish were tagged with the same color VIE proximal to their left side pectoral fin.

UI-ARI and KTOI released 563 intensively cultured burbot in August and UI-ARI, KTOI, IDFG, USFWS and BCMoE released an additional 1,291 intensively cultured burbot in November. UI-ARI and the BCMoE surgically implanted 40 age 1, 2, and 3 year old burbot with sonic tags and PIT tagged 523 age 1 fish for the August releases in Idaho. These fish were evenly divided and stocked at two locations (Moyie River and Boundary Creek). The second release in November included age 0 and 1 year old burbot. For the Canadian release, 15 age 1 burbot were surgically implanted with sonic tags, marked with VIE fluorescent green proximal to their left side pectoral fin (VIE tag data 28-Oct-2010) and PIT tagged. These fish along with 400 age 0 burbot tagged with VIE fluorescent green proximal to their right side pectoral fin were released in the Goat River, BC Canada. The remaining 876 age 0 burbot were also tagged with VIE fluorescent green proximal to their right side pectoral fin and subsequently released in Idaho including 400 stocked into Deep Ck. and 476 stocked into Boundary Ck. In total, 2,110 age 0 and 1 year old burbot were released in 2010 (Figure 5). All F1 progeny released in 2010 were progeny of Moyie Lake brood fish.

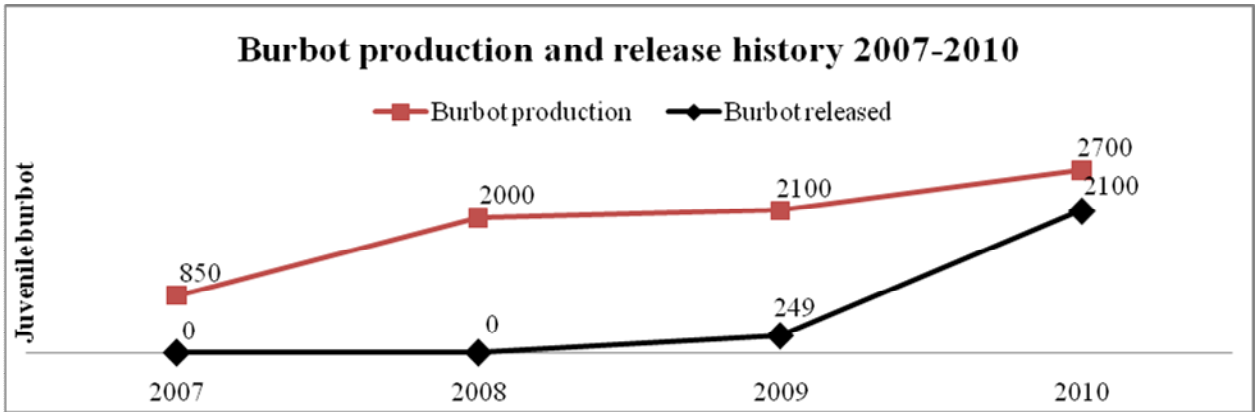

Figure 5. Juvenile burbot aquaculture production and experimental release history from 2007-2010.

Summary

All production and research objectives identified by the KVRI Burbot Conservation Subcommittee in 2010 were successfully met. On-site egg collections produced adequate numbers of eggs to meet production and stocking objectives, and demonstrated our ability to successfully use this method to meet future egg needs. Captive brood fish spawning, egg incubation, and early life rearing strategies were improved by reducing handling of brood fish, incorporating H_2O_2 treatments, grading out cannibalistic cohorts, and incorporating live feed injection to provide constant availability of live feed for burbot larvae during the mass mortality phase. All extensive rearing studies were successful including: net pens, a farm pond and semi-intensive outdoor tank culture at the UI-ARI. Tagging methods used in 2008 and 2009 were repeated with high (≥95%) tag retention and survival at the time of release in 2010. However, tag location and/or VIE color were changed in 2010 to distinguish release groups and distinguish extensive and intensive rearing methods. Regarding the VIE tag retention observational

study, tag readability was faint on approximately 17% of the fish making the tag hard to find. All VIE tags required an ultraviolet light to be visible (NMT's VI light; Shaw Island, Washington, USA). New graduate student research was initiated in 2010 to investigate novel tagging methods for tagging and marking small (≤60mm) age 0 burbot.

Specific pathogen free screening was completed and UI-ARI was certified disease free 14-September-2010 by the Idaho Fish Health Center (Appendix 1). Burbot releases during July through September 2010 have exceeded 2009 release numbers (Figure 5) and the second set of releases in November further increased 2010 release numbers (Appendix 2).

Additional research projects carried out in 2010, beyond the specific objectives identified by the KVRI Burbot Culture Subcommittee, were also successful. Egg maturation in-vivo was characterized and a picture set was created for reference during on-site and captive spawning to stage ova maturity. Additionally, fertilization success of dry and water hardening ova over time indicated that ova should be fertilized <15 min after collection to prevent significant decreases in fertilization success. Both the egg maturation and fertilization studies are in preparation for publication. Furthermore, the effects of 0, 50, 100, 250, 500 ppm H_2O_2 on larval and juvenile burbot were also successfully tested on four distinct life stages in separate experimental trials. The results of these trials showed varying levels of survival by life stage. Generally, pre-mouth larval survival was not significantly affected by ≤250 ppm H_2O_2. Thereafter, larval and juvenile survival was not significantly affected by ≤100 ppm H_2O_2. Future production will incorporate H_2O_2 concentrations from 100-250 ppm to improve water quality and control external bacterial and fungal problems that arise. Larval and juvenile H_2O_2 exposure trials will also be submitted for publication in primary literature.

Publications

Several scientific, peer-reviewed manuscripts, articles, and reports were generated during 2010, by UI-ARI staff and collaborators, including:

1. Foltz, J.R., N.R. Jensen, M.P. Polinski, S.C. Ireland & K.D. Cain. Characteristics of burbot (*Lota lota*) egg development and fertilization success (*In Preparation*)
2. Jensen, N., S. Ireland, M. Neufeld, P. Anders, R. Jones, V. Paragamian, and K. Cain. 2010. Hatchery reared burbot released for the first time in British Columbia, Canada, and Idaho, USA. American Fisheries Society Fish Culture Section, Winter 2010 Newsletter, Pages 11-12.
3. Jensen, N.R., P. J. Anders, C. A. Hoffman, L. S. Porter, S. C. Ireland, and K.D. Cain. Performance and macronutrient composition of age 0 burbot (*Lota lota*) fed four diet treatments. (*Submitted to North American Journal of Aquaculture, 11/10*).
4. Neufeld, M., K. Cain, N. Jensen, S. Ireland, V. Paragamian. Movement of Lake Origin Burbot Reared in a Hatchery Environment and Released into a Large River. North American Journal of Fish Management (*Accepted 2010*)
5. Polinski, M., N. Jensen, J. Foltz, S. Ireland and K. Cain. Effects of Hydrogen Peroxide on four distinct early life stages of burbot. (*In preparation*)

Acknowledgments

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References

Neufeld, M., K. Cain, N. Jensen, S. Ireland, V. Paragamian. *Accepted 2010*. Movement of Lake Origin Burbot Reared in a Hatchery Environment and Released into a Large River. North American Journal of Fish Management.

Appendix 1. Pathogen free certification 2010-11. Sample 04-Aug-2010, confirmed 14-Sep-2010.

DEPARTMENT OF THE INTERIOR
U.S. Fish and Wildlife Service

FISH HEALTH INSPECTION REPORT

This report is NOT evidence of future disease status. To determine current status, contact inspecting biologist below.

Name of Fish Source	Address or Location of Fish Source	Name of Owner or Manager ☒	Inspection Dates / Classification
Aquaculture Research Institute Case# 10-205	University of Idaho Poultry Hall, bldg 2260 Moscow, ID 83844	Ken Cain/Nathan Jensen	This 8/04/10 — No Pathogens 9/01/09 — No Pathogens Prior No prior inspections

FISH EXAMINED

Species[1]	Lot Number	Age[2]	Number/lot	Obtained as Eggs(E) or Fish(F) FROM:	Pathogens Inspected for and Results[3] BF	BR	BK	SW	VE	VH	VP	VC	A	B
Burbot	1	juv	800	(E) Own Broodstock	60	60	60		60	60	60			
					-	-	-		-	-	-			

Type of Fish Examined
☒ Hatchery ☐ Feral
Salmonid X Non-salmonid

Types of Water Supply
Spring ☐ Well
☐ Stream ☐ Impoundment
☐ Enclosed ☐ Free of fish

Inspecting Biologist Signature
Marilyn Blair
Title 50 Inspector

Concurred (signature & title)

Remarks: All fish were sampled 0n 8/4/10. Virology results by inoculation of 5-pool tissues homogenates onto virus-sensitive CHSE-214 and EPC cell cultures; plaque assays were monitored for at least 21 days. BKD by DFAT, other bacteriology by plating on TSA

Inspecting Biologist Address
Idaho Fish Health Center
PO Box 272
Orofino, ID 83544
208 476-9500

1- Use standard FWS abbreviations (see back of page)
2- For hatchery fish give age in months; for feral fish use symbols: e=eggs or fry; f=fingerlings; y=yearlings; b=older fish
3- See list of pathogen abbreviations on back of page; findings reported as $\frac{\text{number examined}}{\text{results}}$ where -=negative and +=positive; other pathogens listed in remarks.

Form 3-226(8/82) U OF IBURBOT09

Appendix 2. 2010 burbot release summary.

Release date(s)	Release location	Release number	External Markings	Tags∗	Mark / Tag location(s)	Stock origin / age at time of release	Rearing location(s) / type of rearing	Responsible Agencies®
08/10/2010	Boundary Ck., ID, US	261	No	PIT	Dorsal	Moyie Lake, Duncan Res. / 1	UI-ARI / intensive	UI-ARI, KTOI
08/10/2010	Boundary Ck., ID, US	20: Nine 1,2 yr Two 3 yr	No	PIT, V9	PIT,V9s in peritoneum, 2 PIT tags in 2,3 yr fish	Moyie Lake, Duncan Res. / 1,2,3	UI-ARI / intensive	UI-ARI, KTOI
08/10/2010	Moyie R., ID, US	262	No	PIT	Dorsal	Moyie Lake, Duncan Res. / 1	UI-ARI / intensive	UI-ARI, KTOI
08/10/2010	Moyie R., ID, US	20: Eight 1 yr Nine 2 yr Three 3 yr	No	PIT, V9	PIT,V9s in peritoneum, 2 PIT tags in 2,3 yr	Moyie Lake, Duncan Res. / 1,2,3	UI-ARI / intensive	UI-ARI, KTOI
07/2010	Snow Ck., ID, US	50	No	VIE	Orange left pec fin	Moyie Lake / 0	UI-ARI, Cow Pond / intensive, cage	UI-ARI, IDFG, KTOI
07/2010	Deep Ck., ID, US	106	No	VIE	Orange left pec fin	Moyie Lake / 0	UI-ARI, Cow Pond / intensive, cage	UI-ARI, IDFG, KTOI
07/2010-10/2010	Kootenai R., Deep Ck. mouth, ID, US	100	No	VIE	Orange right pec fin	Moyie Lake / 0	UI-ARI, Cow Pond / intensive, pond	UI-ARI, IDFG, KTOI
11/3/2010	Kootenai R., Deep Ck. mouth, ID, US	400	No	VIE	Green right pec fin	Moyie Lake / 0	UI-ARI / intensive	UI-ARI, USFWS, IDFG
11/3/2010	Boundary Ck., ID, US	476	No	VIE	Green right pec fin	Moyie Lake / 0	UI-ARI / intensive	UI-ARI, USFWS, IDFG
11/3/2010	Goat R., BC, Canada	400	No	VIE	Green right pec fin	Moyie Lake / 0	UI-ARI / intensive	UI-ARI, KTOI, BCMoE
11/3/2010	Goat R., BC, Canada	15	No	VIE, PIT, V9	VIE Green left pec fin; PIT,V9s in peritoneum	Moyie Lake / 1	UI-ARI / intensive	UI-ARI, KTOI, BCMoE

∗PIT-Passive Integrated Transponder; V9-Vemco™ ultrasonic transmitter; VIE-Visible Implant Elastomer

®UI-ARI-University of Idaho Aquaculture Research Institute; KTFH-Kootenai Tribal Fish Hatchery; KTOI-Kootenai Tribe of Idaho; IDFG-Idaho Department of Fish and Game; USFWS-United States Fish and Wildlife Service; BCMoE-British Columbia Ministry of Environment