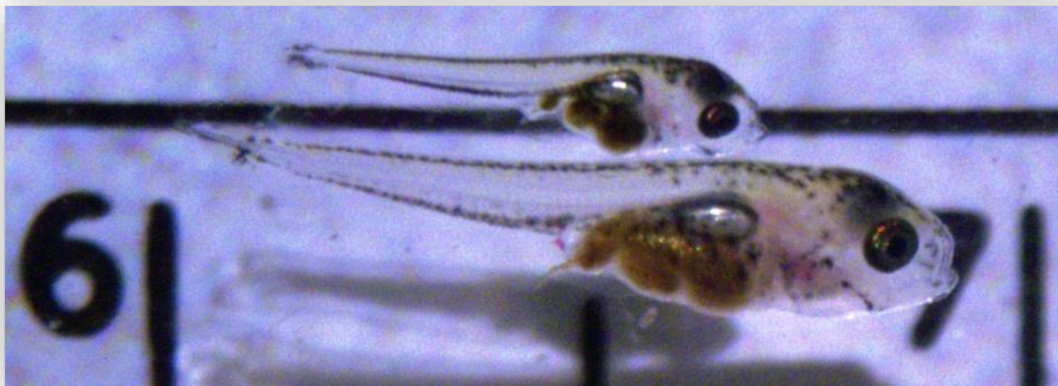
Hatchery Management Plan Supporting Lower Kootenai Burbot Restoration



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This document is intended to be an extension of the Burbot Conservation Strategy (KVRI 2005), an update of the Monitoring and Evaluation Plan for Lower Kootenai River Burbot (KTOI 2012a) produced during the NPCC Step 1-3 Master Plan process, which was required to secure funding for the Twin Rivers Tribal White Sturgeon and Burbot Hatchery, and an extension of previous Burbot working group 5-year Plans. The Kootenai Tribe of Idaho would like to acknowledge the previous version (KTOI 2012a) of this document was based on the monitoring and evaluation (M&E) plans created for the Chief Joseph Dam Hatchery Program by the Confederated Tribes of the Colville Reservation and their consultant, D.J. Warren & Associates Inc. (Colville Tribes 2009a, 2009b). We believe their approach, which provided a framework for implementing and evaluating hatchery and field monitoring and a process for multi-agency cooperation and management, is well suited to the Kootenai River Native Fish Conservation Aquaculture Program (KRNFCAP) and has greatly helped to ensure the program's successful implementation.

Cover Photo: University of Idaho-Aquaculture Research Institute

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PROGRAM SUMMARY

The overall goals of the Kootenai River Native Fish Conservation Aquaculture Program (KRNFCAP or Program) are to prevent extinction of the endangered White Sturgeon (*Acipenser transmontanus*) population and reintroduce Burbot (*Lota lota maculosa*), both of which are culturally significant resources to the Kootenai Tribe of Idaho (KTOI), related First Nations, and the citizens of the Kootenai Basin, by rebuilding a healthy age structure using conservation aquaculture best management practices and best available science. The Burbot program goals were to reintroduce Burbot into the Lower Kootenai River and Kootenay Lake, and rebuild the population using genetically similar stock from within the Basin (KVRI 2005, KTOI 2007, Ireland and Perry 2008, KTOI 2010, KTOI 2012a, KTOI 2012b, KTOI 2018b, and KTOI 2019b). Moyie Lake, British Columbia, was selected as the donor stock. The KTOI has successfully achieved these interim goals by collaborating with the University of Idaho Aquaculture Research Institute (UIARI), Idaho Department of Fish and Game (IDFG) *Project 198806500*, and BC Ministry (subcontracting through KTOI *198806400* and IDFG *198806500*). KTOI *Project 198806400* supported a long-standing research collaboration with UIARI; and after extensive trials from 2003-2008, the UIARI successfully cultured Burbot using methods that could be scaled for population restoration (Jensen et al. 2008a). UIARI successfully reared year-classes released from 2009-2014 to jump start population abundance and structure, and to provide sentinel fish to evaluate post-release survival and behavior.

KTOI constructed a new conservation aquaculture facility during 2013-2014 (Twin Rivers Tribal White Sturgeon and Burbot Hatchery), located at the confluence of the Kootenai and Moyie Rivers, near Moyie Springs, Idaho. The first large-scale Burbot rearing at the new facility occurred during 2015, and in subsequent years, significantly larger annual year-classes have been released into Idaho and British Columbia. Much of the restoration strategy has focused on rearing 4- to 6-month-old (post-hatch) juveniles for release. However, based upon positive research, monitoring, and evaluation (RM&E) results, the restoration strategy gradually diversified to releasing all major life stages from 0-6 months post-fertilization (e.g., eggs, larvae, juveniles). Also, beginning in 2017, the program developed a novel broodstock spawning strategy incorporating sexually mature hatchery-released Burbot captured from the Kootenai River in Idaho during IDFG winter hoop-net surveys. These adults volitionally spawn in-hatchery providing fertilized eggs for the recovery program. The two broodstock sources, the wild-origin Moyie Lake Donor Population and the hatchery-reared Kootenai River Population, provide a more reliable strategy to begin each rearing cycle with enough fertilized eggs to 1) release early life stages to identify causes of persistent recruitment failure and 2) meet annual juvenile release targets.

Since 2009, the program has released substantial numbers of hatchery-origin Burbot (Millions of the pre-feeding larvae (≤14 days post-hatch (dph), feeding larvae (15-59 dph), and juvenile (≥60 dph) life stages have been released)). Additionally, due to COVID-19 protocols during 2020, 27 million developing embryos were released into a variety of tributary and off-channel habitats as a large-scale experiment before “Stay-at-Home” coronavirus protocols were enacted, effectively ending Burbot rearing during 2020. Since 2009, >95% of Kootenai/y basin Burbot releases were produced at Twin Rivers Tribal White Sturgeon and Burbot Hatchery.

Post-release monitoring and evaluation (M&E) of hatchery fish is conducted by IDFG *Project 198806500*, KTOI *Project 198806400*, BC Ministry subcontracted by IDFG *Project 198806500* and KTOI *Project 198806400*, and Montana Fish Wildlife and Parks’ (MFWP) *Project 200600800* within their respective jurisdictions. The program has successfully rebuilt an adult spawning-stock exceeding the original conservation strategy goal of 17,500 sexually mature adults (Hardy et al. 2020). Hatchery Burbot have dispersed throughout the recovery area, which includes the Lower Kootenai River and Kootenay Lake (Stephenson et al. 2013; Hardy et al. 2015; Ross et al. 2018; Hardy et al. 2020). The restored population consists almost entirely of Burbot progeny from the Moyie Lake, BC, donor population broodstock (Ross et al 2018; Hardy et al. 2020). To date, hatchery reared Burbot have survived, grown, and matured; reversing extirpation, and are contributing a very low but quantifiable amount of natural recruitment (Ross et al. 2018; Hardy et al. 2020). The program has rebuilt a healthy age-class structure by spawning >2,000 adults and created >1,000 unique families.

However, after reporting the promising results-to-date above, this Plan also must address the fact that the ultimate goal of restoring a self-sustaining population has not been achieved (natural recruitment at self-sustaining levels without hatchery intervention). Hopefully, the strategies discussed and proposed herein will bridge recent successes to a future ecosystem supporting increasing natural recruitment to self-sustaining levels.

The spawning/breeding plan has and will continue to emphasize genetic management (KTOI 2018b; KTOI 2019b) as well as creating and maintaining “family groups” to fully utilize the power of Parental-Based Tagging (PBT). PBT genetic techniques were developed by IDFG Eagle Genetics Lab via *Project 198806500* and have been integrated into the Kootenai Tribe’s Native Fish Conservation Aquaculture Program *Project 198806400*. The first and most important step is to sample fin clips from all broodstock spawned. Second, progeny from all crosses or spawning adult groups are segregated during hatchery rearing. These unique “families” form “family groups” when combined (either at the time of hatch or during other life stages) and are released at specific times and places. Family groups are formed based on co-manager agreements, available rearing space, and fish health status. Tracking and recording the spawning crosses/groupings and any subsequent combinations into “family groups” must be accurate and complete. After release, when a hatchery reared Burbot is recaptured, a fin clip is collected and DNA is extracted and genotyped to determine parentage, matching the individual with its release location, date, and corresponding environmental conditions, which provides information on hatchery versus wild origin, year-class, and release-site survival without applying traditional tags. Another novel use of PBT has allowed for experimental release strategies of different life stages in habitat recovery areas. By releasing specific “family groups” at key times and places and monitoring ecological conditions at those respective times and places, studies investigating habitat-related recruitment failure may be undertaken while simultaneously rebuilding the population.

The KRNFCAP has released multiple life stages throughout the Lower Kootenai River and Lake Recovery Area in Idaho and British Columbia, with the majority released into the Meander Reach habitats downstream of Bonners Ferry, Idaho, and into Kootenay Lake, British Columbia (Figure 1). Across the recovery area, approximately 2 million age-0 juveniles (≥60 dph), 5 million feeding larvae (15-59 dph), 12 million pre-feeding larvae (≤14 dph), and more recent, ~30 million fertilized, incubating eggs have been strategically released/planted (Tables 3A and 3B). In the first phases of the program, age-0 juvenile releases were the focal life stage for release. Since 2015, when tank space for family group segregation significantly increased via Twin Rivers Hatchery, earlier life stages have been emphasized. Over the past decade, the program has successfully increased Burbot abundance from 50 remaining wild adults to ~50,000 hatchery-origin adults (Hardy et al. 2020), and since January 2019 the population has supported a fishery in Idaho. Also, since 2017, with the rebuilt in-river adult population providing an additional broodstock source for the program, the number of adults contributing to year-classes increased from 90 Moyie Lake adults to 300 adults combined (Moyie Lake and Kootenai River; Tables 2A and 2B).

Although hatchery Burbot have dispersed throughout the river and lake and have self-colonized several spawning tributaries, fish residing in the river and lake, respectively, have mixed less than expected (Stephenson et al. 2013; Hardy et al. 2015; Ross et al. 2018; Hardy et al. 2020). This led the co-managers to identify separate quantitative adult abundance objectives for the two portions of the recovery area, the Lower Kootenai River in Idaho and British Columbia, and Kootenay Lake, British Columbia (KTOI 2018b; KTOI 2019b). The river objective is >17,500 sexually mature adults and the lake objective is >20,000 sexually mature adults. The separate objectives necessitate segregating the two brood-source family groups and/or dedicating appropriate numbers of "family groups", adding several logistical considerations for staff and hatchery operations. Given the river objective has already been achieved, the two objectives are considered achievable and sustainable; however, changes in hatchery plans inherently carry some risk during initial implementation and require additional permitting and disease testing.

Other major lessons learned by the Burbot program address in-hatchery mortality and natural recruitment failure dynamics. Annual Burbot production is highly variable. Burbot are extremely fecund and typically spawn mid-winter under ice. Eggs are extremely small, possess little yolk-sac, and incubate at extremely cold temperatures (2°C) for 50-60 days. Incubation periods vary depending on temperature regime. It has been well established that 6°C is lethal to newly fertilized Burbot eggs; however, during late embryonic development, 6°C triggers egg chorion disintegration leading to hatch typically during late March to mid-April (Ashton et al. 2019 and 2021). Burbot free-embryos / newly hatched larvae have very little yolk sac; thus, during their first 7-10 dph, Burbot develop their mouth and gut in preparation for first feeding at average temperatures rising from 6-9°C. Larval first feeding begins 7-14 dph. Larvae will starve 48-72 hours after yolk-sac depletion if first feeding is not initiated and sustained. Spring ecological conditions, i.e., habitats with warming temperatures supporting fish metabolism/development and abundant zooplankton, must match in time and space. If temperature and food are not suitable, larval mortality is rapid and severe.

Burbot are pelagic planktivores until 60-90 dph; however, diet preference and prey selectivity are not well understood. In the hatchery, Burbot are successfully fed brackish Rotifers, smaller sized Artemia, and then larger sized Artemia. Therefore, at a minimum, larval Burbot require plankton-rich habitats with warming temperatures increasing from 6-12°C during April-May. Rearing Burbot intensively requires reliably providing these conditions, which are very taxing on hatchery infrastructure and staff. Even if the larval requirements are met, early life stage mortality is still significant and occurs rapidly over 24-48 hours, particularly during the numerous life stage transformations from free embryos to juveniles. For example, at any feed-type transition, 50% mortality is not uncommon. An increase in survival of one percentage point at each life stage transition can be the difference between meeting or not meeting the annual Burbot production targets.

At 60-90 dph, Burbot may become cannibals as they transition to a benthic feeding generalist. As size discrepancies emerge within any group of Burbot, the larger individuals tend toward cannibalizing their smaller cohorts. This is a natural strategy across the species. However, cannibalism does complicate hatchery rearing, necessitating additional attention and actions. Initially, cannibals were removed and segregated to improve overall year-class survival; now, cannibalism may be allowed as a more 'natural' strategy until juveniles are released. If cannibals are isolated/separated from their designated 'family-group' these cannibals are released at their designated family group release location(s) to maintain PBT program integrity. From 2015-2017, cannibals were removed from family groups and held separately. Cannibals were PIT-tagged to delineate them from their non-cannibal siblings, and to not confound PBT if cannibals survived disproportionately to the rest of their family group cohorts. Again, this protocol was changed to allow cannibalism, given the unique life strategy character is common in the largest, fastest growers of any Burbot cohort, resulting in significantly faster growth and life stage transitions. Cannibals are enumerated, and then reported under juvenile life stage releases.

Diversifying the general release strategy to include more early life stages (fertilized eggs, eyed eggs (embryos), and pre-feeding and post-feeding larvae) of Burbot has been a success. Releasing early life stages 1) reduces rearing densities and improves growth; 2) reduces live-feed needs during larviculture; 3) increases the probability of some contribution to a year-class in case of catastrophic hatchery events; and 4) provides a novel use of conservation aquaculture to simultaneously rebuild the population while supporting RM&E of Burbot recruitment dynamics related to habitat. From 2009-2018, the primary life stage released to restore the population was a 4-month or 6-month post-hatch juvenile. However, in 2011-2013, early life stages (larvae of variable ages) were released due to limited rearing space at UIARI. During 2013, as part of Burbot early life stage evaluations, KTOI's biomonitoring program *Project 199404900* expanded their lower-trophic level sampling to include April and May because this is when Burbot are expected to hatch and emerge to become obligate pelagic, planktivorous larvae needing warming temperatures and abundant zooplankton to support rapid growth and development.

In the meantime, the PBT genetic monitoring tool was completed, and its accuracy verified in 2015. Again, the premise of PBT is that if genetic samples are taken and analyzed for all broodstock, and the adult broodstock crosses are recorded, then the parents of any given group of Burbot reared and released together may be determined. Unique “family groups” are released into specific sites at specific times at various life-stages and sizes. Upon recapture of those progeny, a genetic sample is collected, resulting in everyone being assigned back to their parents or their respective “family group”. Thus, the release site and release time for any survivor is identified with high confidence. In subsequent years, if survivors of those habitats are recaptured and validated by PBT, one may correlate Burbot survival with the ecological conditions present at the time of release.

Encouraged by the PBT program progress, alarmed by extremely low zooplankton abundances in April and May biomonitoring samples, and having surpluses of larvae, the co-managers decided to release various larval stages ranging from 7-59 dph at different times and places. These releases shed light on temporal and spatial aspects of the altered ecosystem that are suspected causes of Kootenai Burbot recruitment failure. Through M&E efforts, the program has confirmed the survival of some of these early life releases via KTOI *Project 198806400* and IDFG *Project 198806500*. KTOI *Projects 199404900*, *200200200*, *200201100*, and *198806400* all contribute to extensive ecosystem biomonitoring, including phytoplankton and zooplankton abundance from April-October throughout the recovery area. Thus, the multiple programs have collaborated in such a way that temporal and spatial aspects of Burbot recruitment failure in the altered Kootenai River/Lake Ecosystem are now significantly better understood. This enhanced understanding is contributing to habitat restoration site-selection and design under KTOI *Projects 200200200* and *200201100*. In general, the continued discussion of this “major lesson learned” emphasizes insights about current ecosystem dysfunctions, which in turn negatively affect Burbot physiology, ecology, and ultimately recruitment.

During May 2015, with the proper hatchery implementation of PBT, feeding larvae were released to evaluate whether late spring ecosystem conditions support advanced larval and early juvenile stages. During mid-May, 650,000 45-50 dph larvae were released, with approximately 325,000 released near Bonners Ferry, Idaho (River Kilometer (RKM) 245) and another 325,000 released at the international border near Porthill, Idaho (RKM 170). Subsequent recaptures during the 2017-2019 IDFG winter hoop-net surveys confirmed survival from both release sites (Ross et al. 2018; Hardy et al. 2020). Recapture data indicates a low but quantifiable larval survival (approximately 0.03%) that is contributing more than expected to population rebuilding (Ross et al. 2018; Hardy et al. 2020). Detailed ecological conditions that supported these larvae were collected at the times of releases and suggested that the warmer than average spring may have enhanced metabolic and general physiological development even though abundance was low (KTOI unpublished data).

The next early life stage release occurred during mid-April 2017. The rationale for this release was to further investigate the window of potential recruitment bottlenecks by life-stage. Given that some advanced feeding larvae survived a mid-May release during 2015, the Burbot co-managers decided to release a large group of ~7.5 million newly hatched pre-feeding larvae into the mainstem Kootenai River during mid-April, matching the predicted time for natural Burbot larvae first-feeding. To date, recaptures from this release have been limited, which may suggest that April was too cold and/or zooplankton abundance was too low. Survival will be determined in collaboration with IDFG *Project 198806500*.

The post-feeding larval survival during 2015 is promising; however, the warmer spring that supported those fish was caused by a warmer winter. Winter and spring water temperatures in the Koocanusa Reservoir water mass behind Libby Dam create an "ecological Catch-22" in terms of Burbot spawning, egg incubation, and year-class recruitment downriver. During warm or even average winters, Koocanusa Reservoir stays too warm, i.e., warmer than pre-Libby Dam conditions. When Libby Dam discharges water temperatures exceeding the lethal limits of newly fertilized, and early incubating Burbot eggs, the result is mass egg mortality, and no cohort survives to take advantage of the subsequent warmer spring temperatures that promote larval development and zooplankton productivity to support first-feeding. On the flipside, a cold winter is better for Burbot egg fertilization and incubation, but then spring temperatures are suppressed by the cold Koocanusa Reservoir water mass behind Libby Dam. This results in suppressed post-hatch physiological development and suppressed ecosystem productivity resulting in extremely low zooplankton abundance at larval first feeding. Further, the extensive diking of the main-river channel and all the tributary channels has eliminated the connection to off-channel habitats that historically provided solar warming of shallow aquatic habitat, spurring productivity and supporting fish metabolism. Biomonitoring data also exhibited that these colder than historical April-May temperatures subdue ecosystem productivity in the main river-channel (KTOI unpublished data).

During 2016-2018, laboratory studies at the KTOI facility and UIARI explored this issue further. The main rationale for egg incubation studies was to evaluate the probability of egg survival during the variable winter scenarios created by Libby Dam operations in conjunction with recent climate variability (funded by IDFG 198806500 and KTOI 198806400). As discussed, post-Libby Dam average winter river temperatures are warmer than pre-dam natural conditions. The study validated that winter temperatures during February to mid-March of 2°C optimize egg survival; 4°C causes 50% mortality and deformity; and 6°C causes 100% mortality (Ashton et al. 2019). Surprisingly, warmer temperatures do not inhibit adult spawning. However, they do confirm the suspicion that warmer than natural winters likely kill Burbot eggs spawned in the main river, but tributary spawning may be successful given their colder temperatures. Thus, natural spawning success hinges on spawning locations selected by the restored population. Ashton et al. (2021) also showed that cold winter temperatures must then be followed by increasing spring water temperatures, from 6-12°C during April-May, in a timely progression from hatch through larval stages; otherwise, larvae will deform from suppressed metabolism, and/or starve from a lack of zooplankton due to suppressed ecosystem productivity (Ashton et al. 2021). These studies validated the spring ecological "Catch-22" hypothesis.

Advancing on the main-river channel releases of 2017, the 2018 and 2019 early-life evaluations switched focus to off-channel habitats based upon the premise that the more ecologically "productive" wetlands would provide warmer temperatures and higher zooplankton (prey) abundance during spring into early summer. Both pre-feeding (mid-April) and advanced feeding larvae (mid-May) were released at the same selected habitats:

1) Nimz Ranch (KTOI *Project 200201100*), a KTOI property with restored floodplain reconnection to the main river at RKM 221. At the Nimz Ranch "Big Pond", approximately 750,000 pre-feeding larvae from unique family groups were released mid-April, and another 750,000 feeding larvae from unique family groups were released mid-May before river levels rose to reconnect and maintain reconnection with the mainstem river from late-May to mid-June.

2) Ferry Island side-channel at RKM 205. At the Ferry Island back-channel, approximately 1,500,000 pre-feeding larvae from unique family groups were released mid-April, and another 1,500,000 feeding larvae from unique family groups were released mid-May before river levels rose to reconnect and maintain reconnection with the mainstem river from late-May to mid-June.

The degree to which Burbot survived post-release and out-migrated during the reconnection has exceeded expectations as those Burbot are now young adults recruiting to the winter hoopnet surveys in substantial numbers (IDFG and KTOI 2021, unpublished data). During August 2018, KTOI staff recaptured 138 Burbot that did not out-migrate (KTOI unpublished data reported by Hardy et al. 2020). Length, weight, and a fin clip for PBT analysis were collected for each recapture. IDFG PBT analyses confirmed that both pre-feeding and feeding larvae stocked in April and May survived (Hardy et al. 2020). When the collaborative efforts are combined, these data show that both pre-feeding and feeding larvae survived and thrived in a fast-warming, highly productive plankton bloom habitat (KTOI unpublished data) while also co-existing with a high abundance of non-native fish (KTOI unpublished data); and even more interesting, pre-feeding larvae survivors grew significantly larger than feeding larvae and their cohorts remaining in the hatchery until August (KTOI unpublished data; reported by Hardy et al. 2020). During 2019, KTOI replicated the Nimz Ranch habitat evaluation. Only 500,000 larvae split between pre-feeding and post-feeding larval stages were released; and the floodplain didn't reconnect to the main river. Even with smaller numbers and no wetland recharge, KTOI staff recaptured over 1,000 juveniles in August (KTOI unpublished data). These 2018 and 2019 results suggest that the off-channel habitat met habitat requirements for all larval and early juvenile stages; and that the earlier the Burbot are released in a habitat conducive to survival, a more rapid growth trajectory results and is maintained through life. This has also been corroborated from the recapture data from the above discussed 2015 early life releases (Ross et al. 2018; Hardy et al. 2020).

For the side-channel habitat aspect of these evaluations listed above, KTOI released ~1.5 million pre-feeding and ~1.5million feeding larvae into the large Ferry Island oxbow side-channel (KTOI unpublished data). Attempts to recapture juveniles the following fall were unsuccessful, and none have been recaptured during winter hoopnet surveys to date. The disappointing results are likely due to the high influence of the colder, unproductive main-river channel / side-channel dynamic that did not result in the same magnitude of warming and plankton blooms that the Nimz Ranch re-connected floodplain habitat produced. The lessons from this work have been shared to inform actions under KTOI *Projects 199404900, 200200200,* and *200201100*.

In conclusion, the development of a reliable PBT program has provided a valuable tool to evaluate recruitment bottlenecks leading to persistent Burbot recruitment failure. Extensive collaboration across agencies, and programs within agencies, has been instrumental to implementing such a novel approach to ecosystem-wide recruitment investigations. The results will help guide future habitat restoration that may boost Burbot natural recruitment. Now the ecological mismatches in time and space causing Burbot recruitment failure are considerably better understood. The preliminary results from multiple early life-stage releases suggest early life habitat suitability during February-May is highly variable due to variability of Libby Dam operations and almost no off-channel habitat availability due to extensive diking, which is also then affected by annual climate variability. During winter, Koocanusa Reservoir behind Libby Dam remains warmer than natural pre-dam river temperatures. Thus, river temperatures are frequently warmer than that needed for survival, egg fertilization, and subsequent embryo development during the relative long incubation period from mid-February to early April. If eggs do survive, then the month of April is also likely a leading cause of mortality and persistent recruitment failure. During this period, the main-channel and tributary main-channels remain too cold to support proper Burbot metabolism and development, and simultaneously subdue ecosystem productivity during the Burbot time of first feeding. Given the pelagic, planktivorous life strategy of Burbot, habitats devoid of timely plankton blooms in synchrony with their hatch and subsequent physiological development cause mortality at a scale that perpetuates recruitment failure.

The ecosystem-scale causation of this dilemma is Koocanusa Reservoir water storage and Libby Dam operations combined with the extensive diking – which precludes normative spring freshet flows and also creates non-normatively cool spring discharge (mainstem) temperatures due to isothermic, cold thermal mass stored in the reservoir – in conjunction with the widespread diking of almost all of the main channel and tributary channels disconnecting the floodplain and off-channel habitats. Given the results of the Nimz Ranch releases, the restoration of connections and functioning of transport processes to off-channel habitats is the most likely remedy to restore Burbot, and possibly other species, natural recruitment.

Spring flow management (timing and magnitude) needs to be re-evaluated annually to create enough suitable habitat to support Burbot early life stages. Spring flows are currently managed to minimize flood-risk and support Sturgeon spawning from late May through June; however, the timing of spring flows may be too late to support other species such as Burbot. Increased discharge from Libby Dam to support Sturgeon spawning typically begins in late May; whereas lower-trophic productivity increases in mid-March wherever temperatures begin to warm, mostly in disconnected off-channel habitats. Kootenai Burbot hatch around early April, and then immediately require a warming and zooplankton rich habitat. Withholding flows until late May, a strategy intended to better support Sturgeon recruitment, may not be as beneficial to Burbot and other species. For instance, if habitat projects create reconnections, these reconnects likely will not occur until

Sturgeon spawning flows are released in late May through June, missing 2-3 months of ecosystem warming and productivity needed by early life stages of Burbot and other species and reducing their benefits. The relationship between spring flow management and spring habitat conditions is a major factor driving recruitment dynamics of Burbot.

The KRNFCAP will continue to rebuild the river and lake Burbot populations in a manner that maintains genetic diversity, population structure, and abundance until natural recruitment is restored to a magnitude that is self-sustaining; and/or maintains adult abundance above minimum spawning adult targets to support cultural and recreational harvest. As of 2020, the long-term goal of restoring natural recruitment to self-sustaining levels that can also support fisheries has not been achieved (Ross et al. 2018; Hardy et al. 2020). However, on a positive note, a fishery is now open and sustained via hatchery releases; and M&E recapture data combined with PBT has confirmed a low level of natural recruitment from hatchery adults and a few wild remnant adults (Ross et al. 2018; Hardy et al. 2020 also included in Table 1 herein). Winter and spring temperature and flow alterations caused by hydro-operations; climate variability affecting hydro-operations; poor ecosystem productivity; and the magnitude of future in-channel improvements and off-channel habitat reconnections all combine to determine if natural recruitment of Burbot may be restored to self-sustaining levels. Last, and not least, Kootenai Burbot restoration will need to learn more about pollutants (e.g., toxic forms of Selenium (Se) and Mercury (Hg)) within the basin. Recent investigations are finding specific pollutant levels in fish tissues; and other ecological samples, that may exceed safe levels for fish development and reproduction. Human health related concerns associated with consumption may also need future attention.

1.0 INTRODUCTION

Overall, the Kootenai River Native Fish Conservation Aquaculture Program (KRNFCAP or program) has successfully met all interim biological objectives set forth in planning documents for hatchery fish (KVRI 2005; KTOI 2007; KTOI 2010; KTOI 2012a; KTOI 2012b; KTOI 2012c; KTOI 2012d; KTOI 2018a; KTOI 2018b; KTOI 2019a; KTOI 2019b; USFWS 2019). In doing so, the Kootenai Tribe of Idaho (KTOI) has recently re-evaluated and updated several long-standing biological and implementation objectives in collaboration with working-group members representing co-managing agencies through KTOI-hosted Annual Program Reviews (APRs) for both species. Burbot have been successfully reintroduced into the Lower Kootenai River and Kootenay Lake using genetically similar stock from Moyie Lake, British Columbia. In addition, the Burbot program now supports a popular fishery in Idaho. Although all interim goals have been achieved, the long-term goal of a self-sustaining population has not been met. The continued lack of significant natural recruitment necessitates continued conservation aquaculture for the foreseeable future. Given this need to continue conservation aquaculture of Burbot, this document has been developed to detail program progress, present updated goals, and describe the science used for program adaptive management.

KRNFCAP, *Project 198806400*, was initiated in 1989 as a response to the concerning lack of Kootenai River White Sturgeon recruitment. The program now includes both White Sturgeon and Burbot programs supported by two KTOI-owned and Bonneville Power Administration (BPA) supported conservation aquaculture facilities, Kootenai Tribal Sturgeon Hatchery constructed in 1989, *Project 198806400*, and Twin Rivers Tribal White Sturgeon and Burbot Hatchery constructed during 2013-14, *Project 200902400*, now operated under *Project 198806400*. This program, combined with Idaho Department of Fish and Game's (IDFG) *Project 198806500 Kootenai River Fishery Investigations* and Montana Fish Wildlife and Parks' (MFWP) *Project 200600800 Kootenai River White Sturgeon Investigations in Montana* provides an excellent example of how fish population restoration via conservation aquaculture may achieve objectives through collaborative adaptive management guided by research, monitoring, and evaluation (RM&E) results.

In addition, the above-mentioned projects collectively with other KTOI projects, *199404900 Kootenai River Ecosystem Restoration*, *200200200 Restore Natural Recruitment of Kootenai River White Sturgeon*, and *200201100 Kootenai River Operational Loss Assessment* demonstrate how habitat restoration in combination with conservation aquaculture may achieve ecosystem-scale goals. The future program will strive to achieve abundance goals while also providing experimental releases of early life stages to investigate the altered habitat dynamics that perpetuate recruitment failure of Kootenai Sturgeon and Burbot.

The purpose of this Hatchery Management Plan Supporting Lower Kootenai River Burbot is to provide a framework that summarizes program goals and objectives; describes the conservation aquaculture program components; introduces what is being monitored and evaluated; and details how all the working parts of the program are adaptively managed. This plan is intended to be another program iteration advancing the original *Kootenai River*

Burbot Conservation Strategy (KVRI 2005) developed through the Kootenai Valley Resource Initiative (KVRI) and agreed to and signed by numerous agencies and stakeholders. This plan uses and includes the best available science. Further, this plan also lists assumptions made when the aquaculture program transitioned to the facility at Twin Rivers Hatchery and compares these assumptions to observed results.

This plan is intended to be updated annually, revised according to Annual Program Reviews (Burbot APRs) decisions and agreements, and to guide the program for the next five years (2021-2025). Annual updates will include the most recent monitoring and evaluation (M&E) data, a description of any changes in the aquaculture program, and a discussion of decisions made during the annual adaptive management process. Following this Introduction, Section 2 provides an overview of the Lower Kootenai Burbot population status and recovery goals. Section 3 describes future program management and aquaculture program objectives; provides Status and Trends data for the program (hatchery and post-release); and summarizes the program's Key Assumptions, Decision Guidelines, and Biological Objectives. Section 4 describes the program's RM&E activities, focusing primarily on in-hatchery RM&E. Finally, Section 5 describes the Adaptive Management process, which includes an APR hosted by co-managing agencies.

Many people have dedicated their time and energy to this process. KTOI greatly appreciates the committed participation from federal agencies (BPA and NPCC, USACE, USFWS); state agencies (IDFG, MFWP); local governments (City of Bonners Ferry, Boundary County); Canadian provincial government (British Columbia Ministry of Forests [BC Ministry]); academia (University of Idaho); congressional delegation staff, Idaho Governor's Office of Species Conservation, KVRI board members and participants, private citizens, and conservation groups (American Wildlands, Idaho Conservation League, and The Nature Conservancy).

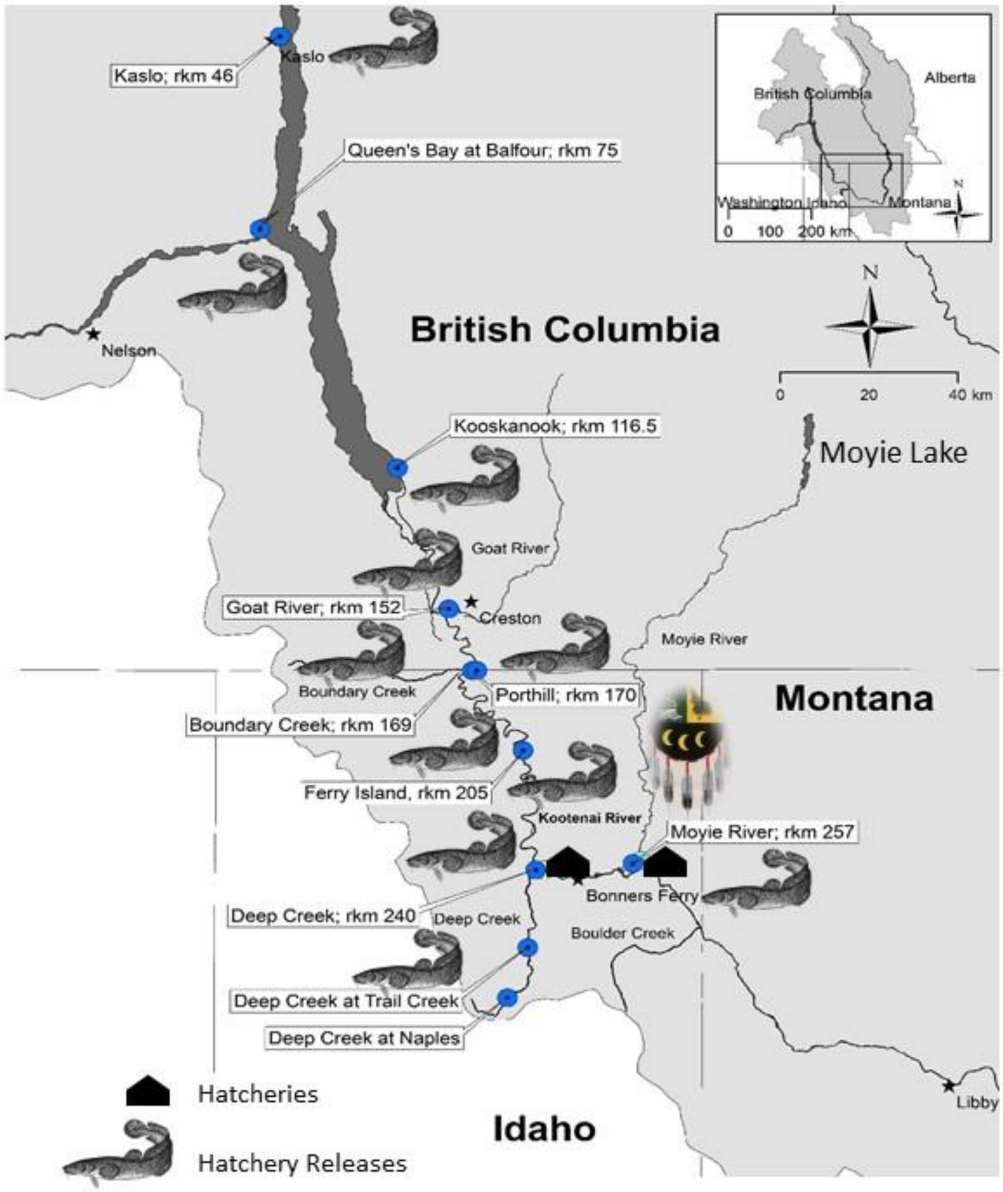

Figure 1. Project area with approximate release locations.

2.0 RECOVERY GOALS AND PLANNING OBJECTIVES

The Lower Kootenai/y River and Lake Burbot population historically was abundant, and had multiple components inhabiting Idaho, Montana, and British Columbia. These included: 1) fish that reared and spawned in the Kootenai River and tributaries (fluvial), 2) fish that reared and spawned in Kootenay Lake (lacustrine), and 3) fish that used both the river and lake to complete the life cycle (adfluvial). All three life history strategies were functionally extirpated by the 1990s. However, the restoration program made significant advances beginning in 2009, and the current population estimate is approximately 50,000 hatchery

Burbot in the recovery area (Hardy et al. 2020). As of January 2019, the population supports a sport fishery in Idaho.

The KRNFCAP will continue to rebuild the river and lake Burbot populations in a manner that 1) maintains genetic diversity, population structure, and abundance until natural recruitment is restored to a magnitude that is self-sustaining; and 2) maintains adult abundance above minimum adult spawning targets to support cultural and recreational harvest. As of 2020, the long-term goal of restoring natural recruitment to self-sustaining levels that can also support fisheries has still not been achieved (Ross et al. 2018; Hardy et al. 2020). However, on a positive note, a fishery is now open and sustained via hatchery releases; and M&E recapture data combined with PBT has confirmed a very low level of natural recruitment from hatchery adults and a few remnants wild adults (Ross et al. 2018; Hardy et al. 2020). Winter and spring temperature and flow alterations caused by hydro-operations; climate variability affecting hydro-operations; poor ecosystem productivity; magnitude of future in-channel improvements and off-channel habitat reconnections; and magnitude of bioaccumulating contaminants, all combine to determine if natural recruitment of Burbot may be restored to self-sustaining levels.

IDFG has been conducting Burbot population surveys since 1979 (Partridge 1983), before functional extirpation occurred. As habitat change appears to have been the main cause for the decline of Burbot and other species, KTOI, IDFG, MFWP, and BC Ministry have jointly assessed the health of the Kootenai system. In addition, multiple habitat restoration projects with the co-managers within the project area are expected to benefit Burbot restoration efforts. Previously, IDFG investigated the effects of an altered hydrograph and thermograph on Burbot behavior. Given recruitment failure persists even with increasing adult abundance, co-managers will continue to evaluate how hydro-operations and habitat availability affect recruitment dynamics. More recently, KTOI, IDFG, and BC Ministry have collaborated with the University of Idaho to further investigate the ecophysiology of Burbot early life stages (Ashton et al. 2019 and 2021).

The ultimate program goal is to restore a naturally reproducing Burbot population as close to the historical structure and abundance as possible that is in equilibrium with the carrying capacity of the present and future environment, and that can support tribal and non-tribal harvest.

This Plan follows several articles listed in Section 8.0 of the Conservation and Restoration Strategies of the KVRI BCS (KVRI 2005). The following KVRI BCS recommendations guide this Plan:

- *Implement an aggressive adaptive program of experimental recovery measures.*
- *Employ conservation aquaculture methods as a key near-term component for Burbot protection and restoration.*
- *Maintain a strong adaptive management and scientific monitoring and evaluation program to guide implementation of population conservation and recovery activities.*

2.1 PROGRAM HISTORY

The Kootenai Tribal Burbot project has been ongoing since the fall of 2001. Efforts began with broodstock, and gamete collections conducted by KTOI and BC Ministry. Adult fish and gametes were transported to the Kootenai Tribal Sturgeon Hatchery in Bonners Ferry where spawning, egg incubation, and larval rearing were successful; however, no juveniles were produced due to staff time and facility limitations. In 2003, efforts shifted, and collaboration expanded to the University of Idaho Aquaculture Research Institute (UIARI) in Moscow, Idaho, where initial work 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 (Jensen 2006; Jensen et al. 2008a; Jensen et al. 2008b; Jensen et al. 2008c; Jensen et al. 2011).

Studies defining specific pathogen susceptibility and carrier status of Burbot were also completed and provide a strong background for addressing Burbot health concerns (Polinski et al. 2010a, 2010b, and 2010c). In 2008, F1 progeny from the 2004 brood year successfully spawned, and F2 progeny were cultured to the juvenile life stage. During 2009-2013, research addressed semi-intensive rearing methods (Barron 2011a, Barron et al. 2013a), size grading methods to suppress cannibalism during culture of larval and juvenile Burbot (Barron et al. 2013b), the effects of temperature on larval and juvenile intensive culture performance (Barron et al. 2011b) and tagging (Ashton et al. 2014). To date, four graduate students have completed MS degrees with research focused on specific objectives related to Burbot conservation aquaculture development (Jensen 2006, Polinski 2009, Barron 2011a, and Ashton 2012).

During the above discussed UIARI role, Burbot were also reared at the UIARI and released to the Lower Kootenai to jump start the restoration from 2009-2014. Also, UIARI still contributes to Burbot releases as opportunities arise (Table 3A.) KTOI's Twin Rivers Hatchery was constructed during 2013-2014, and the first year-class of juvenile burbot was released in 2015. The Burbot conservation aquaculture program originally comprised four sequential implementation phases. These included: 1) aquaculture feasibility assessment, 2) post-release pilot study, 3) adaptive experimental evaluation, and 4) population rebuilding and management. As of 2020, the program has achieved all biological objectives outlined in previous planning documents except natural recruitment remains too low to maintain a self-sustaining population.

Moving forward, the goal is to maintain recent success, and further increase overall Burbot abundance in the river and lake habitats, with an emphasis on BC waters, while simultaneously supporting post-release RM&E of hatchery burbot and investigation of recruitment and habitat dynamics. To do so, during 2021-2025, the program will build upon the recent success of releasing all early life stages, fertilized eggs through 4- to 6-month-old juveniles, across the recovery area habitats using a PBT approach to support RM&E.

Although hatchery Burbot have dispersed throughout the river and lake and have self-colonized several spawning tributaries, fish residing in the river and lake, respectively, have mixed less than expected (Stephenson et al. 2013; Hardy et al. 2015; Ross et al. 2018; Hardy et al. 2020). This led the co-managers to identify separate quantitative adult abundance objectives for the two portions of the recovery area, the Lower Kootenai River in Idaho and British Columbia, and Kootenay Lake, British Columbia (KTOI 2018b; KTOI 2019b). The river objective is >17,500 sexually mature adults and the lake objective is >20,000 sexually mature adults. The separate objectives necessitate segregating numerous family groups, adding several logistical considerations for staff and hatchery operations. Given the river objective has already been achieved, the two objectives are considered achievable and sustainable. However, changes in hatchery operations, in this case to segregate family groups based on brood source, inherently carry some risk during initial implementation and require additional permitting and disease testing.

2.2 KTOI Goal

The Kootenai Tribe of Idaho's vision for Burbot restoration is:

> *To ensure the persistence and viability of a naturally-reproducing population as an essential element of an adequately functional ecosystem and a resource supporting traditional beneficial uses.*

Because the conservation aquaculture program is also designed to address Tribal trust responsibilities, the goal also incorporates traditional beneficial uses.

Another program goal is to transfer knowledge gleaned from KTOI and collaborating partners' Burbot recovery efforts to other Tribes, First Nations, and agencies. KTOI provides advice and technical expertise to the following Burbot recovery programs: Okanogan National Alliance (ONA), Upper Kootenai Basin Recovery, Upper Columbia Basin Recovery, and Colville Tribes' Lake Roosevelt Burbot Monitoring Program; and to other efforts across North America and Europe.

2.3 Population Objectives

Conservation and management of a naturally producing, self-sustaining Burbot population is the primary long-term goal of the program. This goal initially requires the combined implementation of conservation aquaculture and habitat restoration measures, both currently managed by KTOI and other collaborating agencies and entities. Twin Rivers Hatchery has been and will continue to be an integral program component contributing to a population that once again supports harvest and natural reproduction potential at levels consistent with conservation of the species in the Lower Kootenai/y River and Lake.

As of 2020, the co-managers, including KTOI, have identified the following population objectives for the Lower Kootenai River and Kootenay Lake:

Lower Kootenai River. Hatchery production sustains >17,500 sexually mature adults in Lower Kootenai River, Idaho and British Columbia combined (KTOI 2018b; KTOI 2019b) to provide adequate reproductive potential for consistent natural recruitment, and a harvestable surplus to support cultural and general recreational fisheries throughout the river (KVRI 2005; KTOI 2007; KTOI 2010; KTOI 2012a; KTOI 2012b; KTOI 2018b; KTOI 2019b). IDFG *Project 198806500* will monitor and evaluate post-release survival and model population structure and abundance.

Kootenay Lake. Hatchery production will achieve and sustain >20,000 sexually mature adults in Kootenay Lake by 2030 to provide adequate reproductive potential for consistent natural recruitment, and a harvestable surplus to support cultural and general recreational fisheries throughout the lake (KTOI 2018b; KTOI 2019b). BC Ministry will monitor and evaluate post-release survival in Kootenay Lake and will model population structure and abundance under sub-contracts funded by KTOI *Project 198806400* and IDFG *Project 198806500*.

2.4 Habitat Objectives

As suggested by the KVRI BCS, addressing habitat needs is critical to Burbot restoration. The following passage is found in Section 8 of the BCS:

> ***"2. Develop a broad-based habitat restoration program to address altered ecosystem problems that have contributed to the Burbot collapse****. Burbot declines are the result of an extended period of pervasive, large-scale changes in the Kootenai River and Kootenay Lake ecosystems. Declines, in some cases were exacerbated by past harvest (e.g., West Arm Burbot fishery). The changes extend from physical habitat and ecological function loss to primary and secondary system productivity, nutrient availability, and possible contaminant dynamics. Some factors such as harvest, levee construction, and hydro development are obviously implicated; population collapse resulted from the combined impacts of these multiple factors, rather than from the isolated effect of any single factor. The complex interactions of changes and their relative impacts on Burbot are difficult to partition. However, effective long-term persistence and viability of a sustainable, naturally producing Burbot population depends on significant conservation and restoration across the current ecosystem. Measures narrowly focused on increasing numbers of one species are likely to fail if by concentrating on the symptom, they overlook the underlying causes. Ecosystem-based approaches are given wide lip service but rarely translated into specific, scale-appropriate activities. In this Conservation Strategy an ecosystem-based approach includes a combination of mainstem habitat protection, tributary and mainstem habitat restoration, fish population protection and recovery measures, conservation aquaculture, fish community and primary productivity improvements, and pollution control. This Conservation Strategy also exists as part of a larger context of the Kootenai River Adaptive Management Program currently being developed."*

Diversifying the general release strategy to include more early life stages (fertilized eggs, eyed eggs (embryos), and pre-feeding and post-feeding larvae) of Burbot to investigate habitat dynamics relevant to Burbot reproduction and early life history has been a success. From 2009-2018, the primary life stage released to restore the population was a 4-month or

6-month post-hatch juvenile. However, additional early life stages (larvae of variable ages) were also released to evaluate numerous habitats suitable for larval survival. During 2013, as part of Burbot early life stage evaluations, KTOI's biomonitoring program *Project 199404900* expanded their lower-trophic level sampling to include April and May because this is when Burbot are expected to hatch and emerge to become obligate pelagic, planktivorous larvae needing warming temperatures and abundant zooplankton to support rapid growth and development.

As a result of these studies, the ecological mismatches in time and space causing Burbot recruitment failure are considerably better understood. The preliminary results from multiple early life-stage releases suggest early life habitat suitability during February-May is highly variable due to Libby Dam operations, and almost no off-channel habitat availability due to extensive diking. During winter, Koocanusa Reservoir behind Libby Dam typically remains warmer than natural pre-dam river temperatures. Thus, river temperatures are frequently warmer than that needed for survival, egg fertilization, and subsequent embryo development during the relative long incubation period from mid-February to early April. If eggs do survive, the month of April is also likely a leading cause of mortality and persistent recruitment failure. During this period, the main-channel and tributary main-channels remain too cold to support proper Burbot metabolism and development and simultaneously subdue ecosystem productivity during the Burbot time of first feeding. This is caused by the same isothermic water volume of Koocanusa Reservoir that was too warm in February for eggs remaining a constant temperature that then is subsequently too cold in April for young Burbot physiology and zooplankton physiology. Given the pelagic, planktivorous life strategy of Burbot, habitats devoid of timely plankton blooms in synchrony with their hatch and subsequent warming temperatures to support physiological development cause mortality at a scale that perpetuates recruitment failure.

Libby Dam management practices preclude normative spring freshet flows and create non-normatively cool spring discharge (mainstem) temperatures due to the isothermic, cold thermal mass stored in the reservoir, in conjunction with widespread diking of almost all of the main channel and tributary channels disconnecting the floodplain and off-channel habitats. Given the results of the Nimz Ranch releases, the restoration of connections and functioning of transport processes to off-channel habitats is the most likely remedy to restore Burbot, and possibly other species, natural recruitment.

Spring flow management (timing and magnitude) may need to be re-evaluated to create enough suitable habitat to support Burbot early life stages at levels that are self-supporting. Spring flows are currently managed to minimize flood-risk and support Sturgeon spawning from late May through June. However, the timing of spring flows may be too late to support other species such as Burbot. Increased discharge from Libby Dam to support Sturgeon spawning typically begins in late May, whereas lower-trophic productivity increases in mid-March wherever temperatures begin to warm, mostly in disconnected off-channel habitats. Kootenai Burbot hatch in early April and then immediately require a warming and zooplankton rich habitat. Withholding flows until late May, a strategy intended to better support Sturgeon recruitment, may be too late for Burbot recruitment. For instance, if habitat projects create reconnections, these reconnects likely will not occur until Sturgeon spawning flows are released in late May through June, thus missing 2-3 months of ecosystem warming and productivity needed by early life stages of Burbot and other species. The relationship between late winter and spring flow management and late winter and spring habitat conditions is a major factor driving recruitment dynamics of Burbot.

3.0 CONSERVATION AQUACULTURE PROGRAM

3.1 FUTURE PROGRAM OVERVIEW

Again, the original goal of the KRNFCAP was to reintroduce Burbot, a culturally significant resource to the Kootenai Tribe of Idaho, related First Nations, and the citizens of the Kootenai Basin, into the Lower Kootenai River and Kootenay Lake; and rebuild the population using genetically similar stock from within the Basin (KVRI 2005; KTOI 2007; KTOI 2010; KTOI 2012a; KTOI 2012b; KTOI 2018b; KTOI 2019b). Moyie Lake, British Columbia, was selected as the donor stock. KTOI's Twin Rivers Tribal Hatchery was constructed during 2013-2014, and the first year-class was reared and released in 2015. As noted previously herein, the KTOI's Burbot conservation aquaculture program originally comprised four sequential phases: 1) aquaculture feasibility assessment, 2) post-release pilot study, 3) adaptive experimental evaluation, and 4) population rebuilding and management. As of 2020, the program has achieved nearly all of the original biological objectives; however, natural recruitment remains too low to maintain a self-sustaining population.

The future program will maintain Burbot population abundance and age structure to 1) provide spawning potential that may ultimately create a self-sustaining population; and 2) support fisheries throughout the recovery area, which would include British Columbia opening a fishery. To achieve the goal of a naturally-recruiting, self-sustaining population, the program will continue to expand its releases of all life stages across habitats using PBT combined with monitoring abiotic and biotic habitat conditions (see Section 4.2 for more information on PBT). Results will continue to inform potential habitat restoration opportunities that may create a feedback loop that will reverse recruitment failure.

The KRNFCAP will continue to rebuild the river and lake Burbot populations in a manner that 1) maintains genetic diversity, population structure, and abundance until natural recruitment is restored to a magnitude that is self-sustaining and ecologically balanced with respect to carrying capacities; and 2) maintains adult abundance above minimum spawning adult targets to support cultural and recreational harvest. As of 2020, the long-term goal of restoring natural recruitment to self-sustaining levels that can also support fisheries has not been achieved (Ross et al. 2018; Hardy et al. 2020). However, on a positive note, a fishery is now open and sustained via hatchery releases; and M&E recapture data combined with PBT has confirmed a very low level of natural recruitment from hatchery adults and even a few wild remnant adults (Ross et al. 2018; Hardy et al. 2020, also included within Table 1 herein). Winter and spring temperature and flow alterations caused by hydro-operations; climate variability affecting hydro-operations; poor ecosystem productivity; and the magnitude of future in-channel improvements and off-channel habitat reconnections all combine to determine if natural recruitment of Burbot may be restored to self-sustaining levels.

3.2 PROGRAM GOALS AND OBJECTIVES

The KRNFCAP will continue to rebuild the Burbot populations in a manner that 1) maintains genetic diversity, population structure, and abundance until natural recruitment is restored to a magnitude that is self-sustaining; and/or 2) maintains adult abundance above minimum spawning adult targets to support cultural and recreational harvest (KVRI 2005; KTOI 2007; KTOI 2010; KTOI 2012a; KTOI 2012b; KTOI 2018b; KTOI 2019b). The KRNFCAP will adaptively manage hatchery production in collaboration with co-managers through Annual Program Reviews (APRs) to achieve the goals and objectives listed below.

Population Objectives

Kootenai River. Hatchery production sustains >17,500 sexually mature adults in Lower Kootenai River, Idaho and British Columbia combined (KTOI 2018b; KTOI 2019b) to provide adequate reproductive potential for consistent natural recruitment and a harvestable surplus to support cultural and general recreational fisheries throughout the river (KVRI 2005; KTOI 2007; KTOI 2010; KTOI 2012a; KTOI 2012b; KTOI 2018b; KTOI 2019b). IDFG *Project 198806500* will monitor and evaluate post-release survival and model population structure and abundance.

Kootenay Lake. Hatchery production will achieve and sustain >20,000 sexually mature adults in Kootenay Lake by 2030 to provide adequate reproductive potential for consistent natural recruitment and a harvestable surplus to support cultural and general recreational fisheries throughout the lake (KTOI 2018b; KTOI 2019b). BC Ministry will monitor and evaluate post-release survival in Kootenay Lake and model population structure and abundance under sub-contracts funded by KTOI *Project 198806400* and IDFG *Project 198806500*.

Broodstock and Spawning Objectives

Kootenai River. In collaboration with IDFG *Project 198806500*, KNFCAP will annually collect 200-300 hatchery-origin adults that have survived and matured in the Kootenai River. Adults will be transported to Twin Rivers Hatchery for in-tank volitional spawning. Sex ratios and spawning matrices will vary annually. The fertilized-egg collection target is >20 million annually. After Moyie Lake egg collection terminates (2021 final year under current agreement), annual egg collection from Kootenai River broodstock may increase as agreed upon by co-managers. Fertilized eggs collected from each tank of spawners will be a distinct “family group” (KTOI 2018b; KTOI 2019b). Additional, on-river manual spawning may also take place to supplement fertilized egg production targets and to investigate main river and tributary habitat. In the event natural recruits or remnant stocks are encountered, they will be carefully handled to ensure their genetic contributions are conserved.

Moyie Lake. Although use of the Moyie Lake Donor Population will terminate in 2021, the following details are included in the event the program has a need to return. The KRNFCAP, in collaboration with BC Ministry, will travel to Moyie Lake mid-February to collect and fertilize between 5-7 million eggs on the ice during Burbot spawning. All eggs will be transported to Twin Rivers Hatchery for incubation, and then reared up to 4-6 months (KTOI 2018b; KTOI 2019b). The eggs will be collected from 25 females and crossed with up to 125 males. The result will be 25 distinct "family groups" designated by female.

Rearing Objectives

Twin Rivers Hatchery rears all egg sources (see Broodstock and Spawning Objectives above) similarly throughout all life stages until release; however, all distinct family groups designated by females will be maintained. Some low abundance family groups will be combined, and all such combinations will be recorded. This protocol supports what has been a very successful PBT Program implemented by KTOI *Project 198806400* and analyzed by IDFG *Project 198806500*. The number of family groups each year class will vary and may be manipulated to accommodate RM&E and changes to the number of release locations. However, at a minimum, three distinct family groups from each broodstock source will be maintained for release in the Idaho and BC portions of the Kootenai River and Kootenay Lake. The actual number of family groups varies annually depending on family group abundances and general year-class strength (KTOI 2018b; KTOI 2019b). All family groups are tracked throughout hatchery production and are recorded for each year-class in the co-manager Burbot database.

Release Strategy Objectives

- Annually release ≥125,000 juveniles (≥60 dph) in the Kootenai River, Idaho and British Columbia (KTOI 2018b; KTOI 2019b).
- Annually release ≥100,000 juveniles (≥60 dph) in Kootenay Lake, British Columbia (KTOI 2018b; KTOI 2019b).
- Annually release a combination of 10-20 million fertilized eggs/embryos, pre-feeding larvae (7-14 dph), and/or feeding larvae (15-59 dph) into known spawning tributaries and/or key habitats (KTOI 2018b; KTOI 2019b).

Diversifying the general release strategy to include more early life stages (fertilized eggs, eyed eggs (embryos), and pre-feeding and post-feeding larvae <60 dph) of Burbot has been a success. Releasing early life stages 1) reduces rearing densities and improves growth; 2) reduces live-feed needs during larviculture; 3) increases the probability of some contribution to a year-class in case of catastrophic hatchery events; and 4) provides a novel use of conservation aquaculture to simultaneously rebuild the population while supporting RM&E of Burbot recruitment dynamics related to habitat. Releasing early life stages increases the probability of some contribution from each year-class to the population in case of catastrophic hatchery events. Release times and locations will be determined after year-class hatch is complete and assessed by KTOI hatchery staff. To date, and in the future, early life releases emphasize times and locations critical to Burbot recruitment. Based on survival we can characterize and quantify suitable and unsuitable habitat types.

Through the capabilities of PBT, the program has been and will continue to evaluate Burbot recruitment dynamics in relation to habitat restoration in general, specific habitat restoration sites, and hydro-operations. This novel use of conservation aquaculture will be a main component of the overall program. Early life stage releases serve the dual purpose of contributing to population rebuilding while simultaneously supporting RM&E of Burbot recruitment dynamics and habitat relationships and will continue to be an emphasis of program adaptive management. This strategy exemplifies the potential of conservation aquaculture in general, and the adaptation and innovation of the Kootenai Tribe's program specifically and may be used for other imperiled populations. This objective will be implemented collaboratively by KTOI *Projects 198806400, 199404900, 200200200, 200201100*, IDFG *Project 198806500*, and co-managers through APRs and related in-season working-group calls.

3.3 Status and Trends

Status and trends represent actual program outcomes and will be used to evaluate key assumptions (e.g., do the assumptions need to be modified?) and program performance (e.g., did we meet the biological objectives? Do the targets need to be modified?). This information is reported at the APR and incorporated into this plan. All information will be made available and shared with the public and other management entities. This section of the plan includes an overview of the most recent data on Burbot Population Status, Broodstock Collection and Spawning, Incubation and Rearing Outcomes, Release Strategies, and Post-release Survival.

This plan uses and includes the best available and relevant science, incorporating data from decades of research. Further, this plan also lists assumptions made when the aquaculture program transitioned to the facility at Twin Rivers Hatchery and compares these assumptions to observed results.

3.3.1 Population Status

IDFG tracks adult abundance and age structure of the restored Lower Kootenai/y River and Lake Burbot population. IDFG developed a stochastic, Leslie-Matrix population model for Burbot that indicated the 17,500 Kootenai River adult abundance target was met in 2019; this was based on an analysis of age-4+ Burbot (Figure 2; Hardy et al. 2020). The model indicated the population could withstand 10-15% annual fishing mortality without compromising attainment of population targets. This model assumes Burbot are not sexually mature until age-4, whereas more recent information indicates Burbot are mature at age-2 or 3 (KTOI unpublished data). The population estimate (as of 2019) for age-2+ adults is approximately 50,000 (Hardy et al. 2020).

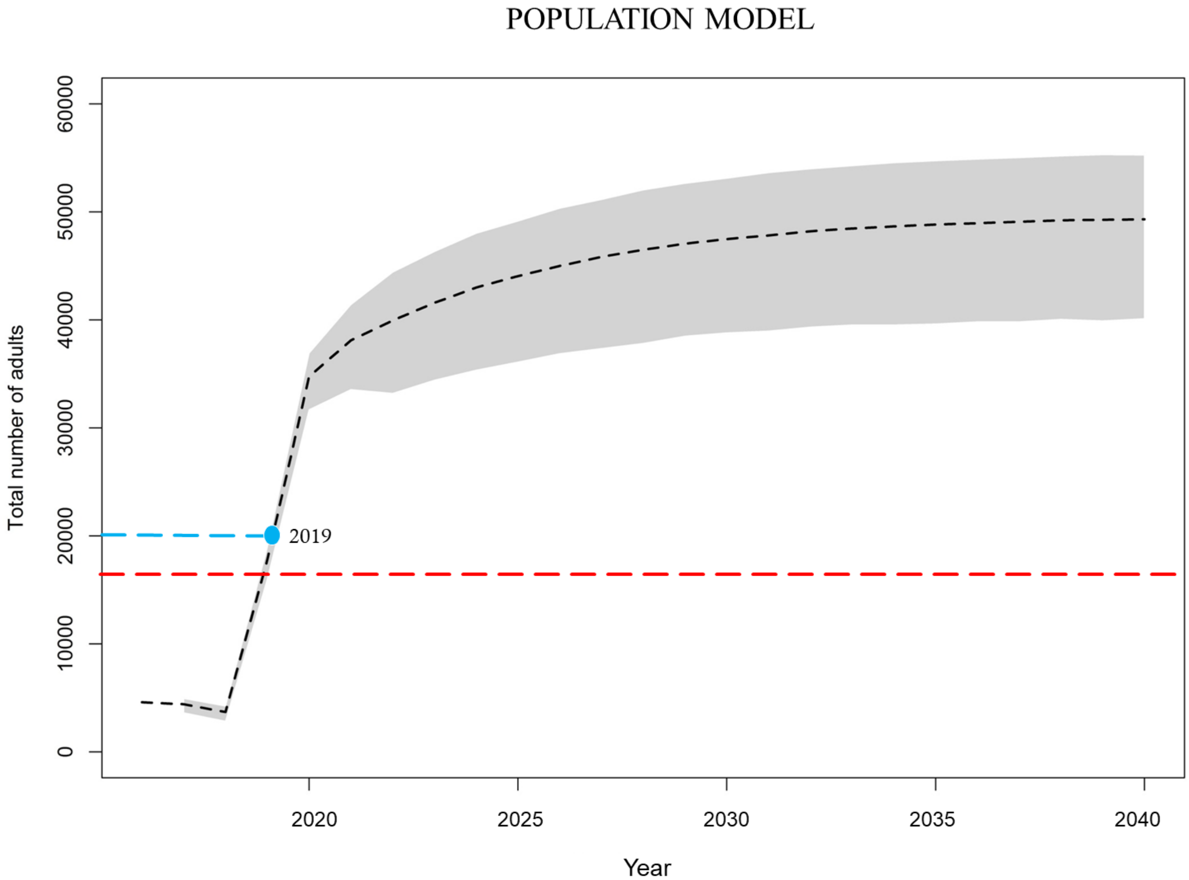

Figure 2. Results from the Burbot population model when annual fishing mortality is 15%. The red dashed line represents the restoration target of 17,500 age-4+ Burbot in the system. The blue point and dashed line represent the estimated abundance of age-4+ Burbot in the system as of January 2019.

Following extensive co-manager discussion and agreements, IDFG scoped and opened the Burbot fishery in the Kootenai River in January 2019 after nearly 30 years of being closed (Hardy et al. 2020). During the first year of the fishery, exploitation was well below the 10-15% threshold (i.e., between 0.2-7%). Public engagement and satisfaction with the fishery were high (Hardy et al. 2020).

The first releases of hatchery-reared burbot were in 2009. The catch per unit effort (CPU) of age 2+ Burbot reflects the increased population in river as fish recruited to the sampling gear (Figure 3). River conditions have also influenced sampling results. For 2017-2018 winter hoopnet surveys, 1,181 Burbot were captured at 18 sites downstream from Bonners Ferry; during 2018-2019 surveys, 612 Burbot were captured at the same sites, but the Kootenai River was frozen during the peak spawning period, precluding sampling for several weeks (Hardy et al. 2020).

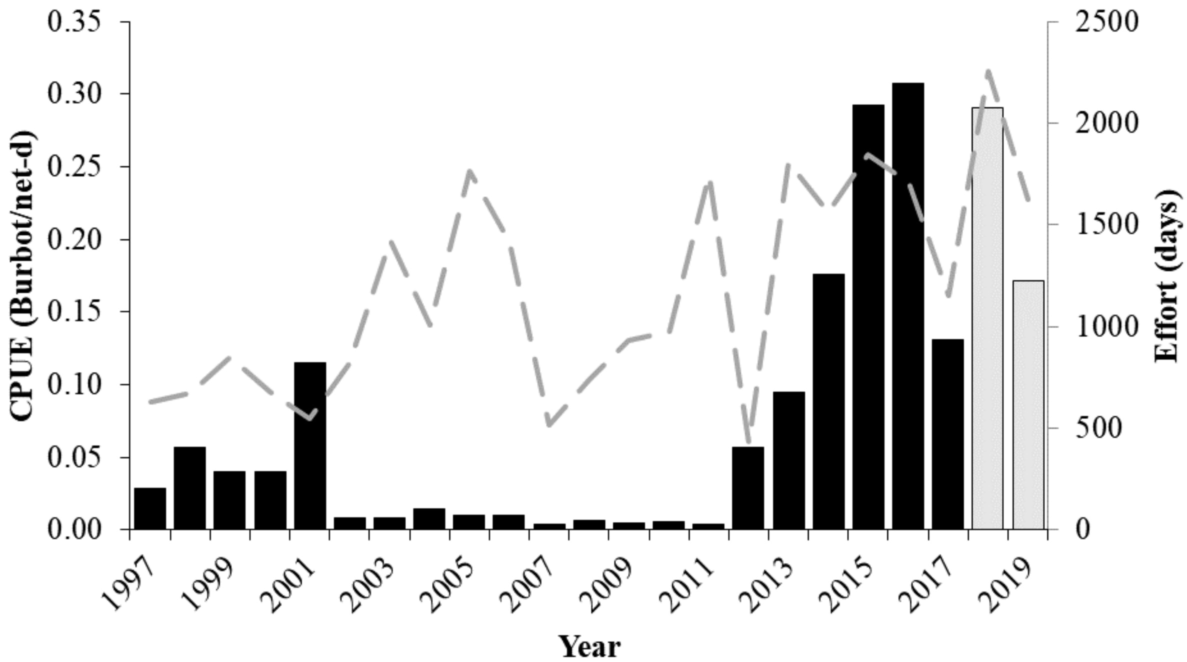

Figure 3. Catch-per-unit-of-effort of age 2+ Burbot (#Burbot/net-day) for hoopnet sampling from 1997-2019. Annual sampling started December 1 and ended March 31 in all years. Bars denote catch rates and dotted line denotes effort in days (Hardy et al. 2020).

Age-specific Survival

Annual age-specific survival was estimated for age 0 to age 5+ Burbot (Figure 4; Ross et al. 2018). Survival in the first-year post-release is approximately 10% and increases in subsequent years to approximately 80-90%. These estimates play a crucial role in assessing the status of the population and adjusting the production goals of KTOI's conservation aquaculture program to align with the population goals specified in the conservation strategy. Specifically, these estimates are the basis of the age-specific survival assumptions used to estimate the number of juvenile releases needed to produce the current goal of 37,500 adults in the Kootenai River and Kootenay Lake (see Section 3.6, Table 7B).

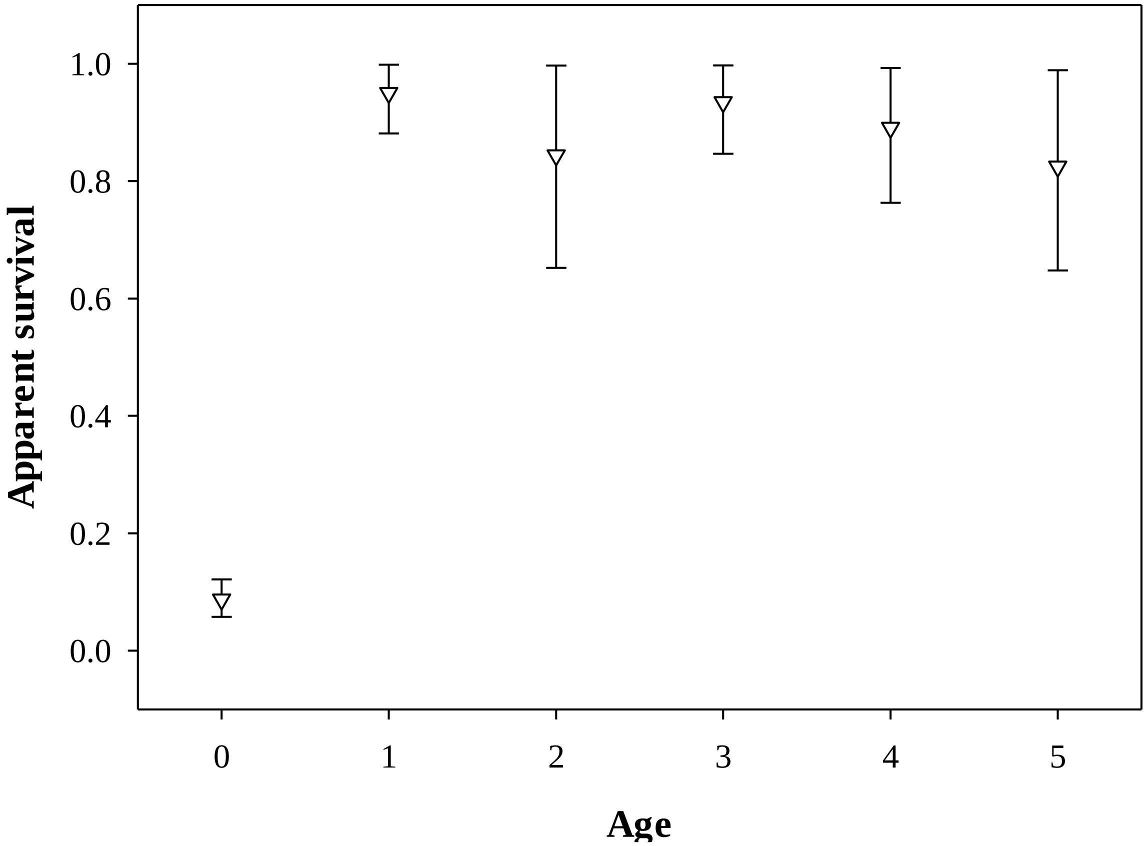

Figure 4. Age-specific annual apparent survival (95% credible interval) of Burbot in the Kootenai River system, 2009-2016. Estimates depicted are apparent survival and do not include any covariate effects (Ross et al. 2018).

Population Structure/Age Frequency

The Lower Kootenai/y River and Lake Burbot population age structure has shifted since the aquaculture program was initiated, increasing in age and size (Figure 5). Year-class strength will depend on 1) hatchery production, which is variable; 2) annual release strategies that may emphasize life stages for particular purposes; and 3) habitat conditions, which are also variable across space and time within and across years.

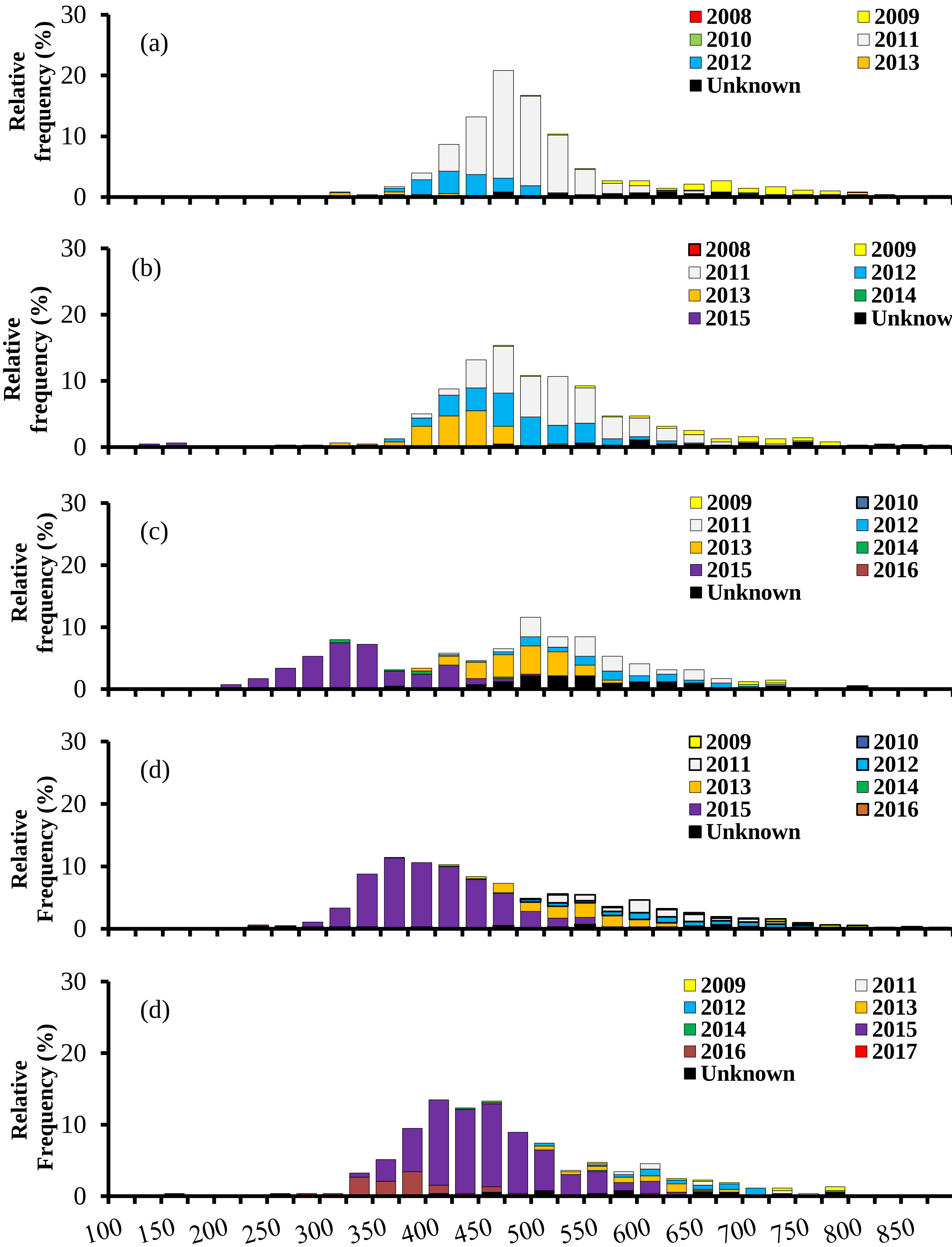

Figure 5. **Length frequency and year class assignments from PIT-tagged and PBT-assigned Burbot captured in hoopnets in the Kootenai River, December 1- March 31 during (a) 2015, (b) 2016, (c) 2017, (d) 2018, and (e) 2019 (Hardy et al. 2020).**

Natural Recruitment

Hardy et al. (2020) reported that winter 2017/18 was the first season when naturally recruited progeny from hatchery-origin adult spawning Burbot were captured in the Kootenai River; additional naturally recruited Burbot were captured in winter 2018/2019. Naturally recruited Burbot are identified as either natural origin from the remnant Kootenai River/Kootenay Lake population or hatchery origin from the Moyie Lake donor population. Burbot were identified as natural origin if they had no PIT tag and could not be assigned to the PBT baseline. Burbot were further identified as hatchery origin (but not hatchery reared) if assigned back to Moyie Lake donor genetics but could not be genetically assigned to any PBT hatchery broodstock. Additional genetic and biological criteria were used to confirm that Burbot were naturally produced.

In 2017/18, 15 fish were assumed to be natural recruits. Additional genetic evaluations suggested that 7 fish were of remnant Kootenai River genetic lineage, 2 were of remnant Kootenay Lake genetic lineage, 5 were of Moyie Lake genetic lineage, and one was unknown (Table 1). This was the first documented reproduction of hatchery-origin Burbot in the river since hatchery releases started in 2009. In winter 2018/2019, 17 Burbot were assumed to be natural recruits, including 4 of remnant Kootenai River genetic lineage, 11 of Moyie Lake genetic lineage, and 2 of unknown lineage. Natural recruitment is very low (<1% of total catch during hoopnet surveys; see Figure 3). We anticipate natural recruitment will increase in subsequent years as the number of mature Burbot increases and habitat restoration continues.

Table 1. Genetic profiles of naturally recruited Burbot captured during IDFG winter hoopnet surveys (Hardy et al. 2020).

Year	Kootenai River	Kootenay Lake	Moyie Lake	Unknown origin	Total
Winter 2017/2018	7	2	5	1	15
Winter 2018/2019	4	0	11	2	17

3.3.2 Broodstock Collection and Spawning

The KTOI Burbot Program initially used wild Moyie Lake broodstock as the donor population, collecting gametes and fertilizing eggs on location at Moyie Lake in mid-February 2009-2020. Beginning in 2017, the program developed a second broodstock spawning strategy that incorporates the now sexually mature hatchery-reared Burbot captured from the Kootenai River in Idaho during IDFG Project 198806500 winter hoop-net surveys. These adults volitionally spawn in-hatchery providing fertilized eggs at quantities supportive of program objectives detailed herein. The two broodstock sources provided a more reliable strategy to begin each rearing-cycle with sufficient fertilized eggs to 1) meet annual juvenile release targets, and 2) release early life stages to identify causes of persistent recruitment failure.

The program has rebuilt a healthy age-class structure by spawning approximately >2,000 adults and creating >1,000 unique families. Since 2017, with the rebuilt in-river adult population providing broodstock for the program, the number of adults contributing to year-classes has increased approximately 3-fold (e.g., ~90 Moyie Lake adults used up until 2017, now >250 Kootenai River adults (Tables 2A and 2B).

Twin Rivers Hatchery can still rear both egg sources similarly throughout all life stages until release if needed; however, Kootenai River Burbot are the primary source being used and distinct family groups are still being maintained. Some 'low-abundance family groups' may be combined, and all such combinations are recorded. This protocol supports what has been a very successful PBT Program implemented by KTOI *Project 198806400* and analyzed by IDFG *Project 198806500*. The actual number of family groups varies annually depending on family group abundances and general year-class strength (KTOI 2018b; KTOI 2019b).

Kootenai River. In collaboration with IDFG *Project 198806500*, KRNFCAP will annually collect up to 300 hatchery-origin adults that have survived and matured in the Kootenai River. Adults will be collected during IDFG hoopnet surveys in January-March. All broodstock receive a PIT tag and are fin-clipped upon capture. PIT tags are used to track individuals during transport to the hatchery, holding, spawning, and release. Adults will be transported to Twin Rivers Hatchery for in-tank volitional spawning. Additional, on-river manual spawning may also take place to supplement fertilized egg production targets and to investigate in-river embryo incubation bottlenecks. In the event natural recruits or remnant stocks are encountered they will be carefully handled to ensure their genetic contributions are conserved. Fin clips will be analyzed using PBT to determine parentage, which will designate family group, year class, and release location. This information will be used to create spawning matrices to prevent in-breeding of siblings. Females will be crossed with males from 1) different year classes, and/or 2) different release locations to reduce the probability of inbreeding siblings. Sex ratios and spawning matrices will vary annually. Fertilized-egg collections target >20 million annually. After Moyie Lake egg collection concluded (2021 final year under current agreements, with potential to revisit if deemed necessary by co-managers in the future), annual egg collection from Kootenai River broodstock may vary as agreed upon by co-managers. Fertilized eggs collected from each tank of spawners will be a distinct "family group" (KTOI 2018b; KTOI 2019b).

Moyie Lake. The KTOI Burbot program initially used wild-origin Moyie Lake broodstock as the donor population, collecting gametes and fertilizing eggs on location at Moyie Lake in mid-February 2009-2020. Although use of the Moyie Lake Donor Population will conclude in 2021, the following details are included in the event the program has a need to return. The KRNFCAP, in collaboration with BC Ministry, will travel to Moyie Lake mid-February to collect and fertilize between 5-7 million eggs on the ice during Burbot spawning. All eggs will be transported to Twin Rivers Hatchery for incubation, and then reared up to 6 months (KTOI 2018b; KTOI 2019b). The eggs may be collected from 25 females and then crossed with 125 males. The result will be 25 distinct "family groups" designated by female. The donor population of Moyie Lake, British Columbia was part of the program through 2021. The program has switched to 100% hatchery stock from the Kootenai River, and this will continue, unless co-managing agencies agree to alternative strategies within adaptive management framework.

Table 2. Summary of Burbot Program broodstock used and approximate numbers of eggs collected.

(A)

Year	Broodstock	Eggs
University of Idaho – Aquaculture Research Institute (UIARI)		
2003 – 2008	Research	Research
2009	45	7,000,000
2010	45	7,000,000
2011	45	7,000,000
2012	45	7,000,000
2013	45	7,000,000
2014	45	7,000,000
2015	Research	Research
2016	Research	Research
2017	Research	Research
2018	Research	Research
2019	Research	Research
2020	Research	Research
2021	Research	Research
2022	Research	Research
Total UIARI	***>270***	***>42,000,000***

(B)

Year	Broodstock	Eggs
KTOI – Twin Rivers Tribal White Sturgeon and Burbot Hatchery (TR)		
2015	90	7,000,000
2016	90	7,000,000
2017	234	12,000,000
2018	327	10,200,000
2019	302	13,270,000
2020	349	27,900,400
2021	243	28,548,000
2022	280	30,308,000
2023	264	16,734,407
Total KTOI Twin Rivers	***2,179***	***152,960,807***

3.3.3 Incubation and Rearing Outcomes

Twin Rivers Hatchery has three incubation systems consisting of 150 individual upwelling incubators. Incubation methods are described in detail in Jensen et al. 2008b and KTOI 2021 Burbot Aquaculture Manual (Chapter 11 – Burbot Incubation). Total egg incubation capacity is 150,000,000; however, only a fraction of total capacity is utilized. The primary purpose of maintaining this number of incubators and independent incubation systems is to allow for segregation supporting PBT implementation, and redundancy to safeguard against catastrophic loss due to mechanical failure. During incubation, individual spawns or parental crosses are held separately and may be moved and combined during the incubation phase after embryo pigmentation and a noticeable eyespot can be observed. Family groups are formed at the time of hatch based on co-manager agreements, available rearing space and fish health status; once this occurs, the ability to separate individual spawns is lost. The number of family groups that can currently be supported is ≤36. This is based on the number of rearing tanks, rearing systems at Twin Rivers Hatchery. Spawning and rearing plans are dictated by a comprehensive PBT program which requires that all adults selected for inclusion in the breeding program annually be genotyped. Specific combinations of adults, and then their progeny, are considered 'family groups' and are segregated while in the hatchery. It is not uncommon to have family groups unequal in size; however, this can be balanced during the incubation phase by combining individual spawns as mentioned above, and then during larval and juvenile phases as needed, and/or as agreed upon by the co-managers.

It should be noted that family size is inherently variable, whereas hatchery rearing tanks are standardized in size. This leads to variability in larval and juvenile densities on a per tank basis. This requires alternative feeding strategies (i.e., one tank may require additional hand-feedings or higher live-feed injection flows than another). During the different rearing phases, at each major biological, morphological, or physical rearing phase transition (e.g., swim bladder inflation, metamorphosis, feed type transitions, etc.), the mortality rate is assumed to be 50%. From fertilized egg to juvenile release, an overall 2% survival rate is needed to meet production/release targets. At the time of release, an entire family group is released at the same location to ensure post-release PBT analyses are accurate, and power maximized.

3.3.4 Release Strategies

All release events and respective details are recorded by KTOI and then provided to IDFG for incorporation into a co-manager Burbot database. The database also houses recapture data; broodstock data; genetic analysis data; and individual fish metadata.

The program has released Burbot into the Kootenai River and Kootenay Lake annually since 2009. UIARI successfully reared and released juveniles from 2009-2014 to jump start population abundance and structure and to provide sentinel fish to evaluate post-release survival and behavior. KTOI constructed a new conservation aquaculture facility during 2013-2014, Twin Rivers Tribal White Sturgeon and Burbot Hatchery. The first large-scale Burbot rearing at the new facility occurred during 2015, and in subsequent years, significantly larger annual year-classes have been released into Idaho and British Columbia. Much of the restoration strategy has focused on rearing 4-month to 6-month post-hatch juveniles for release. However, based upon RM&E results, the restoration strategy has diversified to releasing all major life stages for 0-6 months post-hatch; newly fertilized eggs; and advanced pre-hatch embryos.

Since 2009, the program has released millions of hatchery-origin Burbot; including: pre-feeding larvae (≤14 dph), post-feeding larvae (15-59 dph), and juvenile (≥60 dph) early life stages. The majority (~94%) of releases were produced at Twin Rivers Tribal White Sturgeon and Burbot Hatchery from 2015-present (Tables 3A and 3B). Post-release M&E of hatchery fish is primarily conducted by IDFG *Project 198806500*, BC Ministry subcontracted by IDFG *Project 198806500* and KTOI *Project 198806400*, and MFWP *Project 200600800* within their respective jurisdictions. KTOI conducts a significant part of early life stage release evaluations targeting off-channel habitats, for example Nimz Ranch Floodplain Reconnect. The program has successfully rebuilt an adult spawning-stock exceeding the original conservation strategy goal of 17,500 sexually mature adults. Hatchery Burbot have dispersed throughout the recovery area, which includes the Lower Kootenai River and Kootenay Lake (Stephenson et al. 2013; Hardy et al. 2015; Ross et al. 2018; Hardy et al. 2020). The restored population consists almost entirely of Burbot progeny from the Moyie Lake, BC, donor population. To date, hatchery reared Burbot have survived, grown, and matured, reversing extirpation, and are contributing a very low but quantifiable amount of natural recruitment (Ross et al. 2018; Hardy et al. 2020).

The program has now released multiple life stages throughout the Lower Kootenai River and Lake Recovery Area in Idaho and British Columbia, with the majority released into the Meander Reach habitats downstream of Bonners Ferry, Idaho, and Kootenay Lake, British Columbia (Figure 1). Of the millions of Burbot released across the recovery area, approximately 1.5 million age-0 juveniles (≥60 dph), >15 million feeding larvae (15-59 dph), and >10 million pre-feeding larvae (≤14 dph) have been strategically released (Table 3A, 3B.). Age-0 (≥60 dph) juvenile releases have been the focal life stage for release, while earlier life stages have been emphasized since 2015, when rearing space for family group segregation significantly increased via Twin Rivers Hatchery. The program has successfully increased Burbot abundance from 50 remaining wild adults to 50,000 hatchery-origin adults (age 2+) (Hardy et al. 2020), and the population now supports a fishery in Idaho.

At 60-90 dph, Burbot may become cannibals as they transition to a benthic feeding generalist. As size discrepancies emerge within any group of Burbot, the larger individuals tend toward cannibalizing their smaller cohorts. This is a natural strategy across the species. However, cannibalism does complicate hatchery rearing, necessitating additional attention and actions. Initially, cannibals were removed and segregated to improve overall year-class survival; now, cannibalism may be allowed as a natural strategy until cannibals are removed and released. If cannibals are isolated/separated, they are released at their designated “family group” locations. From 2015-2017, cannibals were removed from family groups and held separately. The cannibals were PIT-tagged to delineate them from their non-cannibal siblings, and to not confound PBT if cannibals survived disproportionately to the rest of their family group cohorts. Again, this protocol was changed to allow cannibalism, given the unique life strategy character is common in the largest, fastest growers of any Burbot cohort, resulting in significantly faster growth and life stage transitions. Cannibals are enumerated, and then reported under juvenile life stage releases.

Again, releasing early life stages 1) reduces rearing densities and improves growth, allowing for a larger size at release; 2) reduces live-feed needs during larviculture; 3) increases the probability of some contribution to a year-class in case of catastrophic hatchery events; and 4) provides a novel use of conservation aquaculture to simultaneously rebuild the population while supporting RM&E of Burbot recruitment dynamics related to habitat. From 2009-2018, the primary life stage released was a 4-month or 6-month post-hatch juvenile, which has been a successful strategy. However, from 2011-2013, early life stages were released out of necessity due to limited rearing space at UIARI. The co-managers suspected that some of these Burbot may have survived given the increasing catch during IDFG winter hoop-net surveys, but this could not be verified. During 2013, as part of Burbot early life stage evaluations, KTOI’s biomonitoring program *Project 199404900* expanded their lower-trophic level sampling to include April and May. This is the period when Burbot are expected to hatch and emerge, becoming obligate pelagic, planktivorous larvae needing warming temperatures and abundant zooplankton to support rapid growth and development.

Diversifying the general release strategy to include more early life stages (<60 dph) of Burbot has been a success. Releasing early life stages increases the probability of some contribution from a year-class to the population in case of catastrophic hatchery events. Release times and locations will be determined after year-class hatch is complete and assessed by KTOI hatchery staff. To date, and in the future, early life releases emphasize times and locations critical to Burbot recruitment. Based on survival we are able to characterize and quantify suitable and unsuitable habitat types. Through the capabilities of PBT, the program will continue to evaluate Burbot recruitment dynamics in relation to habitat restoration in general, specific habitat restoration sites, and hydro-operations. This novel use of conservation aquaculture will be a main component of the overall program. Releasing early life stages serves the dual purpose of contributing to population rebuilding while simultaneously supporting RM&E of Burbot recruitment dynamics and habitat relationships and will continue to be an emphasis of program adaptive management. This strategy exemplifies the adaptation and innovation of the Kootenai Tribe's Program and may be used for other imperiled populations. This objective will be implemented collaboratively by KTOI *Projects 198806400, 199404900, 200200200, 200201100*, IDFG *Project 198806500*, and co-managers through APRs and related in-season working-group calls.

As previously described in the Program Summary, the co-managers were encouraged by the progress of the PBT program and alarmed by extremely low zooplankton abundances in April and May biomonitoring samples. Given the surpluses of hatchery-reared larvae, the co-managers decided to release various larval stages ranging from 7-60 dph at different times and places. These releases shed light on temporal and spatial aspects of the altered ecosystem that are suspected causes of Kootenai Burbot recruitment failure. Through M&E, the program has confirmed the survival of some of these early life releases via KTOI *Project 198806400* and IDFG *Project 198806500*. KTOI *Projects 199404900*, *200200200*, and *200201100*, and *198806400* all contribute to extensive ecosystem biomonitoring, including phytoplankton and zooplankton abundance from April-October throughout the recovery area. Thus, the multiple programs have collaborated in such a way that temporal and spatial aspects of Burbot recruitment failure in the altered Kootenai River/Lake Ecosystem are now significantly better understood. This enhanced understanding is contributing to habitat restoration site-selection and design under KTOI *Project 200200200*. In general, the continued discussion of this "major lesson learned" emphasizes insights about current ecosystem dysfunctions, which in turn negatively affect Burbot physiology, ecology, and ultimately recruitment.

During May 2015, with the proper hatchery implementation of PBT, feeding larvae were released to evaluate whether late spring ecosystem conditions support advanced larval and early juvenile stages. During mid-May, 650,000 45-50 dph larvae were released, with approximately 325,000 released near Bonners Ferry, Idaho (RKM 245) and another 325,000 released at the international border near Porthill, Idaho (RKM 170). Subsequent recaptures during the 2017-2019 IDFG winter hoop-net surveys Project 198806500 have confirmed survival from both release sites (Ross et al. 2018; Hardy et al. 2020). Recapture data indicates a low but quantifiable larval survival (approximately 0.03%) that is contributing more than expected to population rebuilding (Ross et al. 2018; Hardy et al. 2020). Detailed ecological conditions that supported these larvae were collected at the times of releases and suggested that the warmer than average spring may have enhanced metabolic and general physiological development even though abundance was low (KTOI unpublished data). The next early life stage release occurred during mid-April 2017. The rationale for this release was to further investigate the window of potential recruitment bottlenecks by life stage. Given that some advanced feeding larvae survived a mid-May release during 2015, the Burbot co-managers decided to release a large group (7.5 million) of newly hatched pre-feeding larvae into the mainstem Kootenai River during mid-April, matching the predicted time for natural Burbot larvae hatch and first-feeding. Survival will be determined in collaboration with IDFG *Project 198806500*.

The post-feeding larval survival during 2015 is promising; however, the warmer spring that supported those fish was caused by a warmer winter. Winter and spring water temperatures in the Koocanusa Reservoir water mass behind Libby Dam create an "ecological Catch-22" in terms of Burbot recruitment downriver. During warm or even average winters, Koocanusa Reservoir stays too warm, i.e., warmer than pre-Libby Dam conditions. When Libby Dam discharges water exceeding the lethal limits of Burbot eggs, the result is mass egg mortality, and no cohort survives to take advantage of the subsequent warmer spring temperatures that promote larval development and zooplankton productivity to support first-feeding. On the flipside, a cold winter is better for Burbot egg incubation, but spring temperatures are suppressed by the cold Koocanusa Reservoir water mass behind Libby Dam. This results in suppressed post-hatch physiological development and suppressed ecosystem productivity resulting in extremely low zooplankton abundance at larval first feeding. Further, the extensive diking of the main-river channel and all the tributary channels has eliminated the connection to off-channel habitats that historically provided solar warming of shallow aquatic habitat, spurring productivity, and supporting fish metabolism. Biomonitoring data also exhibited that these colder than historical April-May temperatures subdue ecosystem productivity in the main river-channel (KTOI unpublished data).

During 2016-2018, laboratory studies at the KTOI facility and UIARI explored this issue further. The main rationale for egg incubation studies was to evaluate the probability of egg survival during the variable winter scenarios created by Libby Dam operations in conjunction with recent climate variability (funded by IDFG 198806500 and KTOI 198806400). As discussed, post-Libby Dam average winter river temperatures are warmer than pre-dam natural conditions. The study validated that winter temperatures during February to mid-March of 2°C optimize egg survival; 4°C causes 50% mortality and deformity; and 6°C causes 100% mortality (Ashton et al. 2019). Surprisingly, warmer temperatures do not inhibit adult spawning. However, they do confirm the suspicion that warmer than natural winters likely kill Burbot eggs spawned in the main river, but tributary spawning may be successful given their colder temperatures. Thus, natural spawning success hinges on spawning locations selected by the restored population. Ashton et al. (2021 In Progress) also showed that cold winter temperatures must then be followed by increasing spring water temperatures, from 6-12°C during April-May, in a timely progression from hatch through larval stages; otherwise, larvae will deform from suppressed metabolism, and/or starve from a lack of zooplankton due to suppressed ecosystem productivity (Ashton et al. 2021 In Progress). These studies validate the spring ecological "Catch-22" hypothesis.

Advancing on the main-river channel releases, the 2018 and 2019 early-life evaluations focused on off-channel habitats based upon the premise that the more ecologically "productive" wetlands would provide warmer temperatures and higher zooplankton (prey) abundance during spring through early summer. Both pre-feeding (mid-April) and advanced feeding larvae (mid-May) were released at the same selected habitats, 1) Nimz Ranch (KTOI

Project 200201100), a KTOI property with restored floodplain reconnection to the main river at RKM 221, and 2) the Ferry Island Side-channel at RKM 205. At Nimz Ranch, approximately 750,000 pre-feeding larvae from unique family groups were released mid-April, and another 750,000 feeding larvae from unique family groups were released mid-May before river levels rose to reconnect and maintain reconnection with the main-stem river from late-May to mid-June.

The degree to which Burbot out-migrated during the reconnection and drawdown remains unknown; however, at least one recapture in 2020 has verified some out-migration did occur. In August 2018, KTOI recaptured 138 Burbot that did not out-migrate, and an additional 400 winter-kill mortalities were collected during early April 2019 from this habitat (KTOI unpublished data). Length, weight, and a fin clip for PBT analysis were collected for each recapture. IDFG PBT analyses confirmed that both pre-feeding and feeding larvae stocked in April and May survived. These data showed that both pre-feeding and feeding larvae survived and thrived in co-existence with a high abundance of non-native fish; and even more interesting, pre-feeding larvae survivors grew significantly larger than feeding larvae and their cohorts remaining in the hatchery until August (KTOI unpublished data illustrated within Hardy et al. 2020). During 2019, KTOI replicated the Nimz Ranch habitat evaluation, releasing 500,000 larvae split between pre-feeding and post-feeding larval stages. The floodplain did not reconnect to the main river. Even with smaller numbers and no wetland recharge, KTOI staff recaptured over 1,000 juveniles in August (KTOI unpublished data). These 2018 and 2019 results suggest that the off-channel habitat met habitat requirements for all larval and early juvenile stages, and that releasing Burbot earlier in a habitat conducive to survival results in a more rapid growth trajectory that is maintained through life. This has also been corroborated from the recapture data from the 2015 early life releases discussed above (Ross et al. 2018; Hardy et al. 2020).

For the side-channel habitat aspect of these evaluations, KTOI released ~1.5 million pre-feeding and ~1.5 million feeding larvae into the large Ferry Island oxbow side-channel (KTOI unpublished data). Attempts to recapture juveniles the following fall were unsuccessful, and none have been recaptured during winter hoopnet surveys to date. Survivors may be large enough to be recaptured in IDFG 2020 winter hoop-net surveys, allowing for further evaluation of family group survival. The lessons from this work have been shared to inform actions under KTOI *Projects 199404900, 200200200, and 200201100*.

In conclusion, the development of a reliable PBT program has provided a valuable tool to evaluate recruitment bottlenecks leading to persistent Burbot recruitment failure. Extensive collaboration across agencies, and programs within agencies, has been instrumental to implementing such a novel approach to ecosystem-wide recruitment investigations. The results will help guide future habitat restoration that may boost Burbot natural recruitment.

Table 3. Summary of Burbot Program releases into the Kootenai River and Kootenay Lake by facility, year, and life stage since 2003.

(A) Burbot releases by life stage from the University of Idaho Aquaculture Research Institute.

Year	Releases			
	Fertilized Eggs	Pre-Feeding Larvae (≤14 dph)	Post-Feeding Larvae (15-59 dph)	Juveniles (≥60 dph)
University of Idaho – Aquaculture Research Institute (UIARI)				
2003 - 2008	Research	Research	Research	Research
2009	Research	Research	Research	209
2010	Research	Research	Research	2,013
2011	Research	Research	Research	70,416
2012	Research	Research	243,250	29,185
2013	Research	Research	450,000	11,555
2014	Research	Research	Research	4,024
2015	Research	Research	Research	~10,000*
2016	Research	Research	Research	~10,000*
2017	Research	Research	Research	~10,000*
2018	Research	Research	Research	~10,000*
2019	Research	Research	Research	~100,000*
2020	Research	Research	88,500	0
Total	**-**	**-**	***>781,750***	***>257,402***

* Juveniles reared from eggs and larvae used in ecophysiology studies at UIARI.

(B) Burbot releases by life stage from the Twin Rivers Hatchery.

Year	Releases			
	Fertilized/Eyed Eggs	Pre-Feeding Larvae (≤14 dph)	Post-Feeding Larvae (15-59 dph)	Juveniles (≥60 dph)
KTOI – Twin Rivers Tribal White Sturgeon and Burbot Hatchery				
2015	-	-	632,950	260,000
2016	-	-	-	138,000
2017	-	7,753,941	-	43,086
2018	-	2,600,000	2,300,000	96,711
2019	-	290,000	1,152,000	700,000
2020	27,900,400	0	0	0
2021	200,000	1,404,509	2,726,083	159,609
2022	716,000	7,473,702	9,241,706	0
2023	336,000/1,192,000	3,044,522	12,161,885	0
Total	***30,344,400***	***51,383,074***	***28,214,624***	***1,397,406***

(C) Total Burbot releases from Twin Rivers Hatchery by area and habitat.

Area	Habitat	Life Stage	Release Number
Idaho	River	Juvenile	1,164,689
		Post-feeding larvae	5,933,285
		Pre-feeding larvae	7,500,000
		Egg	0
	Off-Channel	Juvenile	0
		Post-feeding larvae	6,346,324
		Pre-feeding larvae	14,922,733
		Egg	4,000,000
	Tributary	Juvenile	50,000
		Post-feeding larvae	299,577
		Pre-feeding larvae	0
		Egg	26,444,000
	Juveniles released in Idaho at the USA/Canada Border		***450,000***
	Juveniles released in Idaho not at the border		***765,406***
	Total juveniles released in Idaho		***1,215,406***
BC	River	Juvenile	81,000
		Post-feeding larvae	0
		Pre-feeding larvae	0
		Egg	0
	Lake	Juvenile	81,000
		Post-feeding larvae	12,634,304
		Pre-feeding larvae	0
		Egg	0
	Tributary	Juvenile	20,000
		Post-feeding larvae	0
		Pre-feeding larvae	0
		Egg	500,000
	Off-Channel	Juveniles	0
		Post-feeding larvae	2,984,564
		Pre-feeding larvae	0
		Egg	0
	Total juveniles released in BC		***182,000***
Montana	Any	Any	0

3.3.5 Post-release Survival

Again, initial hatchery-reared Burbot releases were 6-month-old juveniles. Early results and analyses were promising as Burbot survived. Assumed survival post-release during age 0+ was lower than expected, only 10%. However, subsequent annual survival was higher than expected for ages 1+ and older, 80-90%. Age 0+ post-release survival has been consistent across release sites in the main river. The survival of Burbot released directly to Kootenay Lake remains unknown. However, a substantial number of Burbot from the Queens Bay release site have been captured by anglers in Idaho; and by IDFG hoopnets in Idaho (IDFG unpublished data). Also, post-release survival has been quantified for larval stage releases. Further, the Moyie River confluence release site has been proven to support higher survival than reflected in Figure 6.

Currently, post-release survival has been sufficient to rebuild substantial population abundance and structure across the recovery area. Changes to the diversified multiple life stages strategy; not segregating cannibals; and changing from 6-month-old to 4-month-old juvenile releases will likely result in changing survival estimates.

Updates to post-release survival across release sites/habitats and across ages will likely be completed during 2021-2022 by IDFG.

Post-release survival of age-0 (6-month-old juvenile) releases is depicted in Figure 6 (Ross et al. 2018). The highest survival rates were at Porthill (13.3%) and Deep Creek Confluence (12.5%) and the lowest at the Moyie River Confluence (1.5%). The average survival rate across all sites was 8.7%.

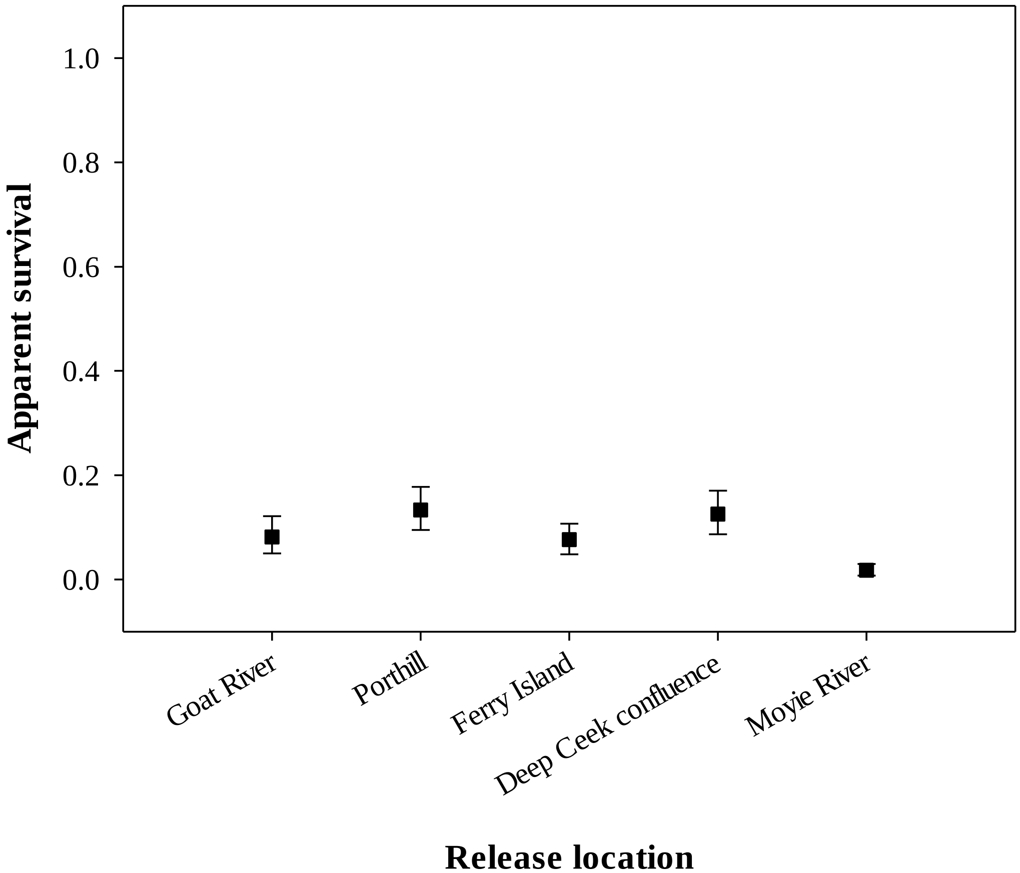

Figure 6. Annual apparent survival (95% credible interval) of Burbot released into the Kootenai River system as an age-0 (i.e., six-month-old juveniles), 2009-2016. Estimates depict survival to age-1 (Ross et al. 2018).

3.4 Key Assumptions

The key assumptions are a set of parameters that help the co-managers make in-season and long-term restoration decisions about the hatchery program (e.g., number of releases, life stages to be released, release locations, etc.). Generally, these assumptions are based on current culture techniques, recent M&E findings, and strategic goals. The parameters are grouped into four categories: 1) hatchery production, 2) natural production, 3) natural spawning, and 4) harvest parameters. Each parameter is defined in Table 4, and the assumed values are compared to recently observed values based on program M&E results.

Table 4. Key assumptions for hatchery production, natural production, natural spawning, and harvest.

Parameter	Definition	Assumed Value	Observed Value
Hatchery Production			
Age at sexual maturity	Age at which the majority of individuals are spawners	Age 4	Age 2
Broodstock/egg source(s)	The population from which broodstock and/or gametes are collected	Moyie Lake, Kootenai River	Moyie Lake, Kootenai River
Broodstock survival	Percentage of fish used for broodstock surviving handling procedures and released back to river/lake	100%	98%
Number of females: males	Ratio of females to males used for broodstock	1:3; females and males	1:1 to 1:5
Number of family groups	Distinct group of spawning adults' progeny	Up to 36 (Twin Rivers Hatchery)	Up to 36
Fecundity	Average number of eggs per female spawned	200,000 (200,000 eggs per kg body weight; mean weight per female = 2.0 kg)	200,000 (200,000 eggs per kg body weight; mean weight per female = 2.0 kg)
Number of fertilized eggs	Total number of eggs successfully fertilized as determined under microscope	20 million	5-27 million
Hatch	Percentage of fertilized eggs that successfully incubate and hatch	60%	90%
Pre-feeding (0-14 dph)	Percentage of fish that survive from hatch until feeding stage	20%	50%
Post-feeding (15-59 dph)	Percentage of fish that survive from live feeds to dry diets	25%	50%
Juvenile (≥60 dph)	Percentage of fish that survive from end of larval stage to 4-month juvenile	70%	50%
Natural Production			
Fertilized egg to Age-1 survival (in-river)	Survival from egg to Age-0 juvenile	0%	0.03% (estimate from KTOI early life stage releases, Hardy et al. 2020)
Natural Spawning			
Natural spawners	Total number of sexually mature adults	17,500	50,000 (total)
Age at sexual maturity	Age at first spawn	4 years old	2 years old

Parameter	Definition	Assumed Value	Observed Value
Spawning area	Any area where spawning aggregations are observed.	At least (3) congregations of mature adults.	≥ 3
Number of adults per spawning area	Number of sexually mature adults estimated to spawn in a common area	100% of adults estimated in a common area or tributary from mid-January to mid-March.	TBD (likely distributed as smaller groups among a particular river tributary and/or lake reach)
Harvest			
Fishing mortality	Percent of population that dies from fishing in a given year	<15%	0.2-7% (Hardy et al. 2020)

3.5 DECISION GUIDELINES

All available M&E data will be used to update status and trends information and key assumptions before each APR. Using this new information, cooperating agencies must decide whether to proceed with current Decision Guidelines or adjust to better address program goals. The cooperating agencies are collectively bound by the term "consensus, minus one" to determine biological objectives for the coming year. The biological objectives determine annual hatchery production, harvest, and any needed adjustments to M&E activities. Table 5 displays the initial program Decision Guidelines for each phase.

Table 5. Initial and current expected outcomes from KRNFCAP - Burbot Decision Guidelines for the phases of the program. Twin Rivers Hatchery started production in Phase 3 (shaded).

Metrics	Phase 1 2004-2008	Phase 2 2009-2013	Phase 3 2014-2018	Phase 4 2019-2020	Future 2021-2025
Donor Source	Moyie Lake	Moyie Lake	Moyie Lake	Moyie Lake	Kootenai River or other TBD by co-managing agencies
Percent Broodstock from Moyie Lake	100	100	50-100	0-100	0
Percent Broodstock from Kootenai River	0	0	0-50	0-100	100
Families/Family Groups	-	Up to 36 families	Up to 60 families	Up to 60 families	36 family groups
Fertilized Eggs Released	-	-	-	-	Up to 50 million
< 60 dph Larvae Released	-	0 – 350,000	500,000-7 million	0-10 million	0-10 million

Metrics	Phase 1 2004-2008	Phase 2 2009-2013	Phase 3 2014-2018	Phase 4 2019-2020	Future 2021-2025
≥60 dph Juveniles Released	-	5,000 - 20,000	20,000 - 100,000	≥125,000	≥225,000
Age-1 Released	-	100 - 500	0	0	0
Minimum Number Mature Adults	-	-	2,500	17,500 in Kootenai River	17,500 in Kootenai River and 20,000 in Kootenay Lake
Minimum Number of Spawning Areas	-	-	3	3	10
Natural Recruitment	-	Possible	Probable	Likely	Likely
Harvest					
Fishing Mortality	-	0	0	≤15%	TBD

3.6 Biological Objectives

Biological objectives for Burbot hatchery production will be determined by KTOI and co-managers at the APR. Currently, in-season management takes place once per quarter, typically via teleconference. Previously, an additional annual 2-day in-person meeting was standard. Most in-season management decisions involve updating the annual hatchery production plan and release strategy; but also includes reviewing and adapting RM&E activities. The annual plan will change as the program evolves depending on the status of Burbot restored to the system, and in the event of mechanical, biological, or public health emergencies.

An example of how biological objectives for Burbot production were initially estimated is shown in Table 6. Table 6(a) lists initial assumptions about in-hatchery production (number of eggs collected and life-stage survival rates) to produce the initial goal of 126,000 6-month-old juveniles for release into the Kootenai River. Table 6(b) shows how releasing 126,000 6-month-old juveniles was initially projected to meet the program's adult abundance objective for the Lower Kootenai River (17,500) based on the age-specific survival probabilities for each year-class. These in-hatchery and post-release survival assumptions were used to design and scale the Burbot program at Twin Rivers Hatchery and are updated annually at the APR. Tables 7(a) and 7(b) provide current assumptions about in-hatchery production and post-release survival to meet the program's release goals and adult abundance objective.

Table 6. Initial Master Plan (KTOI 2012a) assumptions about (A) in-hatchery production to produce the initial goal of 126,000 6-month-old juveniles for release into the Kootenai River and (B) post-release survival to produce the initial goal of 17,500 adults in the Kootenai River.

(A)

1 Family	Hatchery		
Life Stage	Numbers	Survival Probability	Stage
Eggs	100,000	0.60	Egg-Hatch
Hatched Larvae	60,000	0.20	Hatch-Larvae
Feeding Larvae	12,000	0.25	Larvae-Fry
Fry	3,000	0.70	Fry-6-mo juv
6-mo Juveniles	2,100		

60 Families	Hatchery		
Life Stage	Numbers	Survival Probability	Stage
Eggs	6,000,000	0.60	Egg-Hatch
Hatched Larvae	3,600,000	0.20	Hatch-Larvae
Feeding Larvae	720,000	0.25	Larvae-Fry
Fry	180,000	0.70	Fry-6-mo juv
6-mo Juveniles	126,000		

(B)

Year Class	Post-Release		
Life Stage	Numbers	Survival Probability*	Stage
6-mo Juveniles	126,000	0.275	6-mo juv-Age 1+
Age 1+	34,650	0.50	Age 1+ - Age 2+
Age 2+	17,325	0.63	Age 2+ - Age 3+
Age 3+	10,915	0.63	Age 3+ - Age 4+
Age 4+	6,876	0.63	Age 4+ - Age 5+
Age 5+	4,332	0.63	Age 5+ - Age 6+
Age 6+	2,729	0.63	Age 6+ - Age 7+
Age 7+	1,719	0.63	Age 7+ - Age 8+
Age 8+	1,083	0.63	Age 8+ - Age 9+
Age 9+	682	0.63	Age 9+ - Age 10+
Age 10+	430		
Total Adults 4-10+	17,851		

*Does not include fishing mortality (fishery began in 2019).

Table 7. Current assumptions about (A) in-hatchery production to produce the current goals for releases into the Kootenai River and Kootenay Lake (B) post-release survival to produce the current goal of 37,500 adults (17,500 in Idaho, 20,000 in BC) in the Kootenai River and Kootenay Lake

(A)

1 Family Group	Twin Rivers Hatchery		
Life Stage	**Numbers**	**Survival Probability**	**Stage**
Eggs (average no.)	500,000	0.90	Egg-Hatch
Embryos released	TBD		
Hatched Larvae	450,000	0.50	Hatch-Larvae
Pre-feeding Larvae released	TBD		
Feeding Larvae	225,000	0.50	Larvae-Fry
Feeding Larvae released	TBD		
Fry	112,500	0.50	Fry-4-mo juv
Fry/ cannibals released	TBD		
4-mo Juveniles	56,250 (if no other life stages released)		

36 Family Groups	Twin Rivers Hatchery		
Life Stage	**Numbers**	**Survival Probability**	**Stage**
Fertilized Eggs	18,000,000	0.90	Egg-Hatch
Embryos released	TBD		
Hatched Larvae	16,200,000	0.50	Hatch-Larvae
Pre-feeding Larvae released	TBD		
Feeding Larvae	8,100,000	0.50	Larvae-Fry
Feeding Larvae released	TBD		
Fry	4,050,000	0.50	Fry-4-mo juv
Fry/ cannibals released	TBD		
4-mo Juveniles released	2,025,000 (if no other life stages released)		

(B)

Year Class	Post-Release		
Life Stage	Numbers	Survival Probability*	Stage
4-mo Juveniles	225,000	0.10	4-mo juv-Age 1+
Age 1+	22,500	0.90	Age 1+ - Age 2+
Age 2+	20,250	0.90	Age 2+ - Age 3+
Age 3+	18,225	0.90	Age 3+ - Age 4+
Age 4+	15,491	0.85	Age 4+ - Age 5+
Age 5+	12,393	0.80	Age 5+ - Age 6+
Age 6+	9,914	0.80	Age 6+ - Age 7+
Age 7+	7,931	0.80	Age 7+ - Age 8+
Age 8+	6,345	0.80	Age 8+ - Age 9+
Age 9+	5,076	0.80	Age 9+ - Age 10+
Age 10+	4,060		
Total Adults 4-10+	79,435 (if all fish released as Juveniles)		

*Does not include fishing mortality (fishery began in 2019).

4.0 MONITORING AND EVALUATION

Conservation aquaculture for Burbot is a critical component of the recovery of this species within the Kootenai River subbasin of Idaho and British Columbia. All phases of the hatchery program incorporate best management practices as outlined in the hatchery operations manual initially developed by KTOI in 2016 and updated in 2021 (KTOI 2021). KTOI and UIARI continue to work together to refine and improve Burbot culture methods, with a focus on improving larval survival and transition to commercial feeds.

Monitoring and evaluation activities are prioritized based on three criteria:

1. **Impact on management decisions.** Metrics needed to implement the four-step In-Season Management Procedure (ISMP) and used in the Adaptive Management process. The ISMP steps include: 1) Update Status and Trends information, 2) Update Key Assumptions, 3) Review Decision Guidelines, and 4) Set Biological Objectives for the upcoming year.

2. **Degree of uncertainty.** Metrics with a high degree of variability due to random effects, or that vary in space and time.

3. **Feasibility of monitoring.** Metrics that can be monitored efficiently (i.e., with a reasonable investment of time and resources) and effectively (i.e., with sufficient accuracy and precision).

The purpose of this section is to:

- Identify the key metrics to be monitored.
- Identify the agency or agencies responsible for monitoring each metric.
- Explain how this information will be used to adaptively manage the Burbot restoration program.

4.1 Population Status

Monitoring and evaluation metrics used to assess the status of the Lower Kootenai/y River and Lake Burbot population are summarized in Table 8. These include metrics monitored in the field and in-hatchery by KTOI, IDFG, BC Ministry and MFWP. These metrics are used to evaluate the progress and success of the restoration program in meeting population goals stated in Section 3.1.

Table 8. Monitoring and evaluation metrics were used to assess the status of the Lower Kootenai/y River and Lake Burbot population.

Metric	Field or In-hatchery	Agency Responsible
Total adult abundance	Field	IDFG *Project 198806500*, BC Ministry (subcontracted under KTOI *Project 198806400* and IDFG *Project 198806500*) and MFWP *Project 200600800*
Population genetic structure	Field and In-hatchery	IDFG *Project 198806500*, KTOI *Project 198806400*, BC Ministry (subcontracted under KTOI *Project 198806400* and IDFG *Project 198806500*) and MFWP *Project 200600800*
Age composition of population and age-specific survival	Field	IDFG *Project 198806500* and BC Ministry (subcontracted under KTOI *Project 198806400* and IDFG *Project 198806500*)
Number of spawning locations and spawners per location	Field	IDFG *Project 198806500* and BC Ministry (subcontracted under KTOI *Project 198806400* and IDFG *Project 198806500*)
Sex ratio	Field and In-hatchery	IDFG *Project 198806500*, KTOI *Project 198806400*, and BC Ministry (subcontracted under KTOI *Project 198806400* and IDFG *Project 198806500*)
Fecundity and sperm motility	In-hatchery	KTOI *Project 198806400*
Egg fertilization rate	In-hatchery	KTOI *Project 198806400*
Egg hatch rate	In-hatchery	KTOI *Project 198806400*

Metric	Field or In-hatchery	Agency Responsible
Larval survival rate	Field and In-hatchery	KTOI *Project 198806400,* IDFG *Project 198806500* and BC Ministry (subcontracted under KTOI *Project 198806400* and IDFG *Project 198806500*)
Juvenile survival rate	Field and In-hatchery	KTOI *Project 198806400,* IDFG *Project 198806500* and BC Ministry (subcontracted under KTOI *Project 198806400* and IDFG *Project 198806500*)
Natural egg abundance	Field and In-hatchery	KTOI *Project 198806400,* IDFG *Project 198806500* and BC Ministry (subcontracted under KTOI *Project 198806400* and IDFG *Project 198806500*)
Natural larval abundance	Field	IDFG *Project 198806500* and BC Ministry (subcontracted under KTOI *Project 198806400* and IDFG *Project 198806500*), KTOI *Project 198806400*
Natural juvenile abundance	Field	IDFG *Project 198806500* and BC Ministry (subcontracted under KTOI *Project 198806400* and IDFG *Project 198806500*), KTOI *Project 198806400*
Water flows and temperatures	Field and In-hatchery	KTOI *Project 198806400;* IDFG *Project 198806500* and BC Ministry (subcontracted under KTOI *Project 198806400* and IDFG *Project 198806500*); and USACE
River productivity, plankton and algal communities, N:P ratio	Field	KTOI *Projects 198806400, 199404900 200200200, and 200201100;* IDFG *Project 198806500;* and BC Ministry (subcontracted under KTOI *Project 198806400* and *199404900);* and USACE

4.2 Genetics (Parental-Based Tagging)

A crucial component of M&E is Parental Based Tagging (PBT), which is used to monitor numerous post-release metrics and population genetic structure. PBT has been used by the Burbot program since 2015. It replaces the need for physical tagging (PIT tagging, VIE tags, etc.) of all hatchery-origin fish if done properly. The hatchery spawning plan emphasizes genetic management (KTOI 2018b; KTOI 2019b) as well as creating and maintaining "family groups" to fully utilize the power of PBT. PBT genetic techniques were developed by IDFG Eagle Genetics Lab via *Project 198806500* and have been integrated into the Kootenai Tribe's Conservation Aquaculture Program *Project 198806400*. The first and most important step is to sample fin clips from all broodstock spawned. Second, progeny from all crosses or spawning adult groups are segregated during hatchery rearing. These unique "families" form "family groups" when combined and are released at specific times and places. Recaptured progeny is fin-clipped, and DNA is extracted and genotyped to determine parentage and assign to original "family group", which provides information on hatchery versus wild origin, year-class, and release-site survival without applying traditional tags.

Another novel use of PBT has allowed for experimental release strategies of different life stages in the recovery area. By releasing specific "family groups" at key times and places and monitoring ecological conditions at those respective times and places, studies investigating habitat-related recruitment failure may be undertaken while simultaneously rebuilding the population. These experimental releases have provided valuable information about the spatial and temporal aspects of Burbot recruitment failure. Unique "family groups" are released into specific locations at specific times at various life-stages and sizes. Upon recapture of those progeny, a genetic sample is collected, resulting in each individual being correctly assigned back to their parents or their respective "family group" or not assigned to the broodstock catalog. Thus, the release site and release time for any hatchery-origin survivor is identified with high confidence. In subsequent years, if survivors of those habitats are recaptured and validated by PBT, one may correlate Burbot survival with the ecological conditions present at the time of release.

Metrics to be monitored

- Genetic lineage/parentage
- Post-release survival
- Gender
- Year-class
- Release location
- Post-release behavior (movement/ dispersal)
- Post-release growth
- Habitat suitability at release location

4.3 Broodstock Collection

Broodstock collection is a collaborative effort among multiple agencies (Table 2). The principal broodstock donor population for this program was from Moyie Lake, British Columbia, Canada. After approximately a decade of wild broodstock spawning and subsequent fertilized egg transport to the hatcheries (UIARI and KTOI hatcheries) for rearing to 4- to 6-month-old juveniles, it has been determined that the program has captured almost all the Moyie Lake burbot genetic lineage, and the progeny from the Moyie Lake donor population are now established in the Kootenai River and Kootenay Lake. The broodstock source for the program moving forward will come from the Kootenai/y River and its tributaries during winter hoop-netting efforts. This is a major change to broodstock collection efforts because adult fish are now transported to the KTOI hatchery where they are segregated and assigned to specific spawning groups based on age, size, and genetics. Broodstock that have been re-captured and have been used before, or those that are in poor condition, may not be used and are released back into the river. The annual objective for numbers of broodstock to be collected and transported to the KTOI hatchery is 300.

When broodstock are transported to the hatchery, they are treated for external pathogens during transport before entering the facility. After treatment, each new group of broodstock is isolated in a holding tank and observed for 24 hours before handling. The observation period is intended to identify fish that may be in poor condition and require additional treatment(s) or need to be released back into the river and not used for spawning. Briefly, each fish is measured (length and weight) and a portable ultrasound unit is used to determine gender. In addition, each fish that enters the hatchery is implanted with a PIT tag which is linked to a master database where additional information may be used for segregation and spawning matrix assignments. Select broodstock are first placed into 10 ft circular holding tanks and segregated by sex and size to prevent unplanned spawning and cannibalism. Typically, all PBT analyses for broodstock candidates are complete by mid-February and provided to KTOI and co-managers to assess broodstock status and assign adults within spawning matrices. Once spawning matrices are developed, broodstock are assigned to flow-through spawning tanks where they are allowed to spawn on their own volition, and the fertilized eggs are collected from the effluent pipe of each spawning tank. Adults are released back into the Kootenai River after spawning. For details, refer to the KTOI Burbot Fish Culture Manual (KTOI 2021).

Metrics to be monitored during brood collection and holding

- Broodstock survival in hatchery
- Fin clips (one fin clip in field and one fin clip in hatchery from each actual adult spawned for the year class)
- Number of broodstock, sex ratio, location collected or spawned
- Segregation (in tanks) by gender and size
- Tracking parents of each brood fish/spawning group to prevent inbreeding

4.4 Spawning

Spawning is the first critical in-hatchery phase following broodstock collection. The annual in-hatchery volitional fertilized-egg collection target is 20 million. Additionally, on-river manual spawning may also take place to supplement fertilized egg production targets and to investigate in-river embryo incubation bottlenecks. Based on multiple successive volitional spawning years at KTOI's Twin Rivers Hatchery, it has been observed that releases of larger year-classes of age-0 burbot could result in overrepresentation of a small number of family groups within a year-class of those; however, it is not known if this will affect future broodstock collection/spawning matrices now employed at the KTOI's Twin Rivers Hatchery or natural spawning/recruitment. Since the KTOI program incorporates strict hatchery family group segregation protocols and PBT, these over-represented year-class individuals can be identified and used with confidence that inbreeding will not occur. This finding plays a part in designing spawning matrices that eliminate sibling mating (i.e., inbreeding). For instance, if two broodstock are assigned to the same release location within the same year class, they are likely siblings and are not crossed or placed into the same volitional spawning tank. If fish are assigned to the same release location and year class, parentage needs to be evaluated before placement into spawning tank. Given multiple females and males are placed into spawning tanks, resulting in multiple spawning events, not all individuals from a "family group" are siblings. These three metrics determine crosses in the spawning matrix to minimize the chance of inbreeding. Furthermore, male: female sex ratios for broodstock captures transported to the hatchery each year have been nearly 1:1 (e.g., February 2023, 166 adults captured by February 10th: 84 were male and 82 were female). This was not expected, and each year there has been an apparent shortage of large males (>550mm). A highly skewed sex ratio dominated by males was expected based on experience from Moyie Lake activities. It remains unknown if these trends will continue.

Preferably, spawning matrices would include a balance of large (>550mm) and small (<550mm) burbot from each year-class; and include naturally recruited adults. Given the low numbers of adult burbot captured during winter hoopnet surveys that do not assign to a hatchery release, substantial contribution from natural recruits is unlikely for several years. Please refer to the KTOI Burbot Fish Culture Manual (KTOI 2021) for additional details on volitional spawning.

Metrics to be monitored during spawning (in hatchery)

- Number of fertilized eggs, eggs per female, spawning protocol
- Broodstock spawning groups (segregate all spawns until hatch)
- Tracking parentage of each spawning group to prevent inbreeding
- Year class
- Release location
- Parentage
- Condition factor
- Biomass/ density of broodstock in tanks

- Water temperature

4.5 INCUBATION

Egg incubation is the second critical phase in hatchery following successful spawn. Maintaining stable, cold-water temperatures (target 2°C) for developing embryos is crucial. This includes maintaining spawning tank water at 2°C and using pre-chilled equipment when handling eggs. In addition, the incubation system must be completely functional and ready to receive the eggs; we suggest setting up incubators and tanks at least a month before they will be needed. Water temperatures are monitored continuously (twice daily at minimum) at the water source; in the head tanks of rearing/incubation systems; in the incubators themselves; and in the incubator-hatch receiving tanks. The importance of temperature monitoring cannot be overemphasized because of the low temperatures required for burbot embryo development. For example, if 2°C is the target and 4°C is observed, the development rate doubles and could result in a 50% reduction in hatch success (Ashton et al. 2019). Thus, it is very important to track water temperature and tally temperature units or degree days during development. Simply tallying temperature units daily will help predict when hatch will occur. In addition, it is extremely important that each time an egg mass is moved, combined, or placed it be strictly documented and recorded for required family group tracking purposes.

When ova are hand-stripped from a female, the conversion factor KTOI uses to estimate *unfertilized ova* numbers based on the volume of a respective hand-stripped ova mass is 1,800 eggs/mL. When a volitional spawn is collected, it is transferred to a chilled graduated cylinder and allowed to settle for a minimum of 2 minutes. The settled egg mass volume is then recorded, and the eggs are transferred to a pre-chilled incubation vessel. Egg size and water hardening time may affect volumetric estimates of post-fertilized egg numbers, therefore it is important to document the time of fertilization. The conversion factor KTOI uses to estimate *fertilized, water-hardened egg* numbers based on the volume of a respective egg mass is 800 eggs/mL. Eggs are quantified volumetrically.

Example: 1,800 ova/mL X mL of ovum = number of eggs at onset of fertilization

800 eggs/mL X mL of eggs = number of eggs at onset of incubation

The next developmental observation is 10 days post-fertilization. This check is intended primarily to ensure embryos are developing properly (see KTOI 2021, Chapter 11 Incubation) and the egg mass is healthy. If a poor-quality egg mass is encountered at the 10-day check, it may be discarded or combined with another poor-quality egg mass. Entire incubators may be moved and combined to create family groups after body pigmentation dan eye spots are apparent. However, great care must be taken to ensure air temperatures during transfers do not warm the mass, water temperatures are ±1C between locations, and water flows are suitable between incubation systems.

Metrics monitored during incubation

- Egg mass volume (minimum 2 minute settling time; time varies based on mass)
- Percent fertilized eggs at 48-h (volumetric)
- Development at 10-d post fertilization (deformity may be estimated at this stage)
- Water temperature
- Fungal growth and control
- Percent hatch

4.6 LIVE FEED

Live feeds are required for intensive Burbot larviculture/production. Several commercially produced larval dry diets have been tried, but live diets have been observed to be better for production. Larval burbot are sight predators, obligate pelagic planktivores that prefer a moving prey item to trigger a feeding response, and do not recognize an inert food particle as a food item. Larval burbot may not have the proper gut microflora and fauna to digest a complex food particle when they consume a commercial diet. First feeding Burbot larvae require very small (~200 microns) prey items, and although the technology exists to create semi-stable food particles of sizes as small as 100 microns through a process known as Spheronization (Marumerization), other problems arise. Nutritional quality of commercial food particles may rapidly decrease after contact with water. Water soluble vitamins (e.g., Vitamin C) are leached out despite feed manufactured with water-stable binders (e.g., carboxymethyl cellulose or wheat gluten). If leached particles are eaten, a nutritional deficiency may occur that may lead to deformity or sub-optimal growth. Uneaten commercial feeds foul rearing tank water, become a basis for water mold and/or fungal manifestation and may cause bacterial gill disease if not removed. Although live prey may cause similar conditions if not distributed properly, the rate of consumption is much higher due to the innate feeding behavior of an altricial larval fish. Last, live feeds are preferred because they can be enriched with commercial products that boost their nutritional value directly. Zooplankton are obligate feeders and will eat whatever is presented to them as long as they can fit it in their mouth. Fatty acids (e.g., Arachidonic acid, Docosahexaenoic acid, HUFA and PUFAs), vitamins, minerals, and probiotics can all be fed to live prey to boost their nutritional value, which is subsequently fed to larval fish to deliver essential nutrients.

Please see KTOI Fish Culture Manual Chapter 13 (KTOI 2021) for details about the live feed system.

Metrics to be monitored

- Water quality and environmental conditions (Brackish water required)
- Rotifer density

- Artemia hatch
- Algae feed delivery system
- Live feed injection system calibration/function

4.7 REARING

The actual number of family groups varies annually depending on family group abundances and general year-class strength (KTOI 2018b; KTOI 2019b). Annual Burbot production is highly variable in general. Burbot are exceptionally fecund and typically spawn mid-winter under ice. Eggs are extremely small, possess little yolk-sac, and incubate at cold temperatures (2°C) for 50-60 days. Incubation periods vary depending on temperature regime and spawn timing. It has been well established that 6°C is lethal to fertilized Burbot eggs and developing embryos; however, during late embryonic development, 6°C triggers egg chorion disintegration leading to hatch typically during late March to mid-April (Ashton et al. 2019 and 2021). Burbot free embryos (newly hatched larvae) have very little yolk reserve. During the first 7-10 dph, embryos develop their mouth and gut in preparation for first feeding. Water temperatures are typically maintained near 9°C during this phase. Then, larval first feeding begins 7-14 dph. Larvae will starve 48-72 hours after yolk-sac depletion if live feeds are not presented at adequate densities (10 org./mL minimum). If temperature and food are not suitable, larval mortality is rapid and severe.

In the hatchery, Burbot are fed brackish Rotifers, SFB Artemia, and then larger sized GSL Artemia before dry diet transitions may begin. Thus, larval Burbot require plankton-rich environments with warming temperatures increasing from ≤12°C during April – May before dry diet transitions. Live feeds are injected into rearing tanks continuously to always provide fresh/live prey. Rearing Burbot intensively requires reliably providing these conditions. Even if the larval requirements are met, early life stage mortality is still significant and occurs rapidly over 24-48 hours, particularly during the numerous life stage transformations from free embryos to juveniles. For example, at any feed-type transition, 50% mortality is not uncommon. An increase in survival of one percentage point at each life stage transition can be the difference between meeting or not meeting annual Burbot production targets.

At 60-90 dph, Burbot may become cannibals as they transition to a benthic feeding generalist. As size discrepancies emerge within any group of Burbot, the larger individuals tend toward cannibalizing their smaller cohorts. This is a natural strategy across the species. However, cannibalism does complicate hatchery rearing, necessitating additional attention and actions. Initially, cannibals were removed and segregated to improve overall year-class survival; now, cannibalism is allowed as a natural strategy until cannibals are removed and released. Cannibals should be released at their designated "family group" locations. From 2015-2017, cannibals were removed from family groups and held separately. The cannibals were PIT-tagged to delineate from their non-cannibal siblings, and to not confound PBT if cannibals survived disproportionately to the rest of their family group cohorts. Again, this protocol was changed to allow cannibalism, given the unique life strategy character is common in the largest, fastest growers of any Burbot cohort, resulting in significantly faster growth and life stage transitions. Cannibals are enumerated, and then reported under juvenile life stage releases.

A priority during rearing has and will continue to be creating and maintaining "family groups" to fully utilize the power of PBT. Once again, it is extremely important that each combination of family groups be strictly documented and recorded for required family group tracking purposes.

Metrics monitored during rearing

- Water temperature, water flow, water mixing (to maintain temperature)
- Family group segregation and separation
- Survival / Deformities / Mortality
- Behavior (e.g., flight response, transition from pelagic to benthic swimming, etc.)
- Growth / Condition / Development Life Stage
- Biomass (per fish, per rearing tank)
- Feeding (feed amounts, feeding rates)
- Density
- Cannibalism

Variables estimated

- Percent larval survival / deformities (pre-feeding and post-feeding)
- Percent Young-of-Year (YOY) survival (transition to benthic early juvenile)
- Cannibal prevalence
- Number of 4-month-olds, average size (length and weight), and release location

4.8 RELEASE STRATEGIES

Evaluating life-stage specific release strategies is another major program objective for Burbot. Much of the restoration strategy has focused on rearing 4- to 6-month post-hatch juveniles for release. However, based upon positive RM&E results, the restoration strategy has been diversified to release all major life stages 0-6 months post-hatch. In 2018, with evidence that release of life stages <6 months was feasible, the in-hatchery rearing time was shortened to four months. In part, this decision was made to reduce risks associated with mechanical hatchery failures and reduce production costs, but also because in-river environmental conditions may be more suitable for survival (e.g., peak of prey abundance and warmer temperature for growth) in August compared to October.

Releasing early life stages 1) reduces rearing densities and improves growth; 2) reduces live-feed needs during larviculture; 3) increases the probability of some contribution to a year-class in case of catastrophic hatchery events; and 4) provides a novel use of conservation aquaculture to simultaneously rebuild the population while supporting RM&E of Burbot recruitment dynamics related to habitat. Diversifying the general release strategy to include more early life stages of Burbot has been a success. Releasing early life stages increases the probability of some contribution from a year-class to the population in case of catastrophic hatchery events.

Release times and locations will be determined by KTOI and co-managers after year-class is assessed by KTOI hatchery staff across life stages. Release sites for hatchery reared Burbot in the Kootenai River extend from the mouth of the Moyie River at RKM 260 downriver to / and in Kootenay Lake. Most lake releases have been at Queens Bay near the junction of the main lake and the West Arm. Lake releases will be expanded to several additional locations in the South, North, and West Arms. Release sites were selected based on current and historical distribution and the likelihood that these habitats will optimize survival. Other sites may be used if studies indicate they are necessary to meet objectives. Survival and growth will be compared to determine optimal mainstem, tributary, and off-channel release locations. All hatchery-origin Burbot will be identified as such by genetic parental based tagging because KTOI has implemented the concept across entire year classes. PBT may also be used on a smaller, more targeted level if logistics limit full year-class scale implementation.

Metrics to be monitored

- Number released by life stage and at each site
- Habitat conditions at release sites
- Apparent survival by release site
- Plankton/Zooplankton densities at release sites

Release Sites

Releases sites and strategies to distribute Burbot have changed across since 2009. Larval, juvenile, and subadult Burbot were originally stocked at four primary tributary locations and Kootenay Lake. Subsequently, tributaries were de-emphasized, and releases went into mainstem Kootenai River locations; from Moyie River (Idaho) confluence to Goat River (BC) confluence.

During 2009-2018, the standard practice was releasing all fish designated for a site en masse in small areas (within and area ≤1 river kilometer (RKM)) with river access. In 2019, KTOI, with co-manager agreement, changed that practice to distribute Burbot within "reaches". This practice had the effect of expanding the original "small" release sites to reaches spread out in areas of 5 to 20 RKMs in size/location. KTOI also adjusted protocols to release Burbot by boat into littoral areas, areas with abundant aquatic vegetation, woody debris, and rocky substrates. This provides Burbot with immediate cover and a higher probability of micro- and macro-invertebrate prey availability.

In the future, release sites will include more locations in Kootenay Lake, tributaries of Kootenai/y River/Lake, and habitat rehabilitation areas (Figure 1). General pathogen screening and permitting requirements for releases in Idaho and BC are detailed in Figure 7.

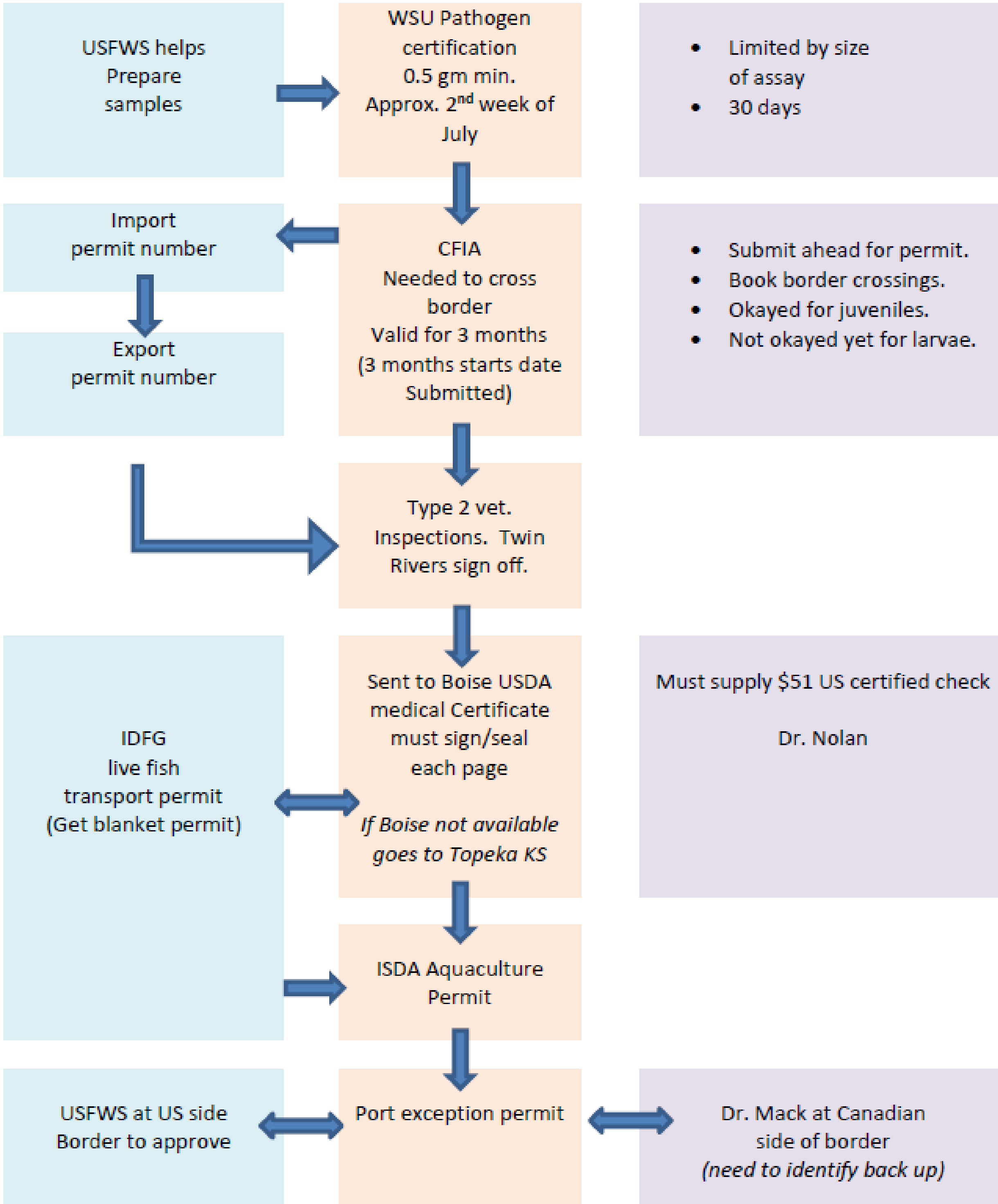

Figure 7. Permits required to release Burbot at sites in the U.S. and Canada.

2009-2018 Release Sites

- Site 1 KR - Mouth of Moyie River (RKM 260)
- Site 2 KR – Mouth of Deep Creek (RKM 240)
- Site 3 KR – Shorty's Island (RKM 230)
- Site 4 KR – Ferry Island (RKM 205)
- Site 5 KR – Mouth of Boundary Creek (RKM 170)
- Site 6 KR – Mouth of Goat River (RKM 130)
- Site 7 Boundary Creek, ID
- Site 8 Deep Creek, ID
- Site 9 Goat River, BC
- Site 10 Corn Creek, BC
- Site 11 Kootenay Lake – Queens Bay and Balfour, BC

2019 Release Sites (Kootenai River, Idaho)

- RKM 240-245
- RKM 229-240
- RKM 210-228
- RKM 200-210
- RKM 180-199
- RKM 170-175

2020 Release Sites (eyed eggs only; unique release strategy and locations due to Covid-19)

- Tributary Habitat Myrtle Creek (ID)
- Tributary Habitat Trout Creek (ID)
- Tributary Habitat Smith Creek (ID)
- Tributary Habitat Boundary Creek (ID)
- Tributary Habitat Moyie River (ID)
- Tributary Habitat Upper Deep Creek(ID)
- Tributary Habitat Dodge Creek (ID)
- Tributary Habitat Ball Creek (ID)
- Tributary Habitat Snow Creek (ID)
- Tributary Habitat Caribou Creek (ID)
- Off-Channel Boundary Creek WMA Canal

2021 Release Sites

- RKM 76-77 Kootenay Lake Chimney Bay
- RKM 76.5-77 Kootenay Lake Pilot Bay
- RKM 170-182.5 Porthill to Lucas Creek
- RKM 190-199.5 Parker Creek to Copleand
- RKM 204 – 225 Ferry Island to Flemming Creek

- RKM 229.5 – 244.5 Shortys Island to Ambush Rock
- Off-Channel Yaqan Nuiky Hunting Grounds (B.C.)
- Off-Channel Boundary Creek WMA (ID)
- Off-Channel Nimz Ranch (ID)
- Off-Channel TNC North and South Pond (ID)
- Tributary Habitat Ball Creek (ID)

2022 Release Sites (Variable life stages released)

- Boundary Creek Proper
- Flemming Creek Proper
- Dodge Creek Proper
- Upper Deep Creek Proper
- McArthur Lake
- McArthur Lake – Upper Deep Creek Mouth
- McArthur Lake – Dodge Creek Mouth
- Kootenai National Wildlife Refuge – Myrtle Pond
- Kootenai National Wildlife Refuge – Center Pond
- Nimz Ranch – Main Pond
- Nimz Ranch – North Quad
- Kootenay Lake – Eastside Lardeu Delta (RKM 17-20)
- Kootenay Lake – Westside Lardeau Delta (RKM 17-20)
- Kootenay Lake- Chimney Bay (RKM 74-76)
- Kootenay Lake – Westside Crawford Bay (RKM 76-82)
- Kootenay Lake – Northeastside Crawford Bay (RKM 76-82)
- Kootenay Lake – Southeastside Crawford Bay (RKM 76-82)
- Kootenay Lake – Redman Point (RKM 113)
- Kootenay Lake – Cultus Creek to Midge Creek (RKM 111-114)
- Kootenay Lake – Kooskanook (RKM 116.5)
- Kootenay Lake – Creston Delta (RKM 117-122)
- Yaqan Nuiky Hunting Grounds – Snake Pond
- Yaqan Nuiky Hunting Grounds – Frog Pond
- Yaqan Nuiky Hunting Grounds – Goat River Ox-bow.
- Kootenai River – Copeland to Porthill (RKM 200-170)
- Kootenai River – Flemming Creek to Ferry Island (RKM 205-225)
- Kootenai River – Elk Mountain Farms Alcoves (habitat restoration) (RKM 203,209.5)
- Kootenai River – Ambush to Shortys Island (RKM 244.5-230)
- Moyie River – Hatchery Effluent

Release sites listed above beginning in 2023 are all given consideration each year and selected based on water year, water temperatures, fish availability and permitting.

4.9 RESEARCH

- Ecophysiology across early life stages to investigate habitat/recruitment failure dynamics related to temperature.
- Large-scale early life stage releases to investigate habitat dynamics, ecosystem function, ecosystem productivity, identify suitable habitat characteristics based on post-release survival.
- Evaluation of contaminant concentrations in Kootenai River and Kootenay Lake Burbot.
- Investigate interactions between Burbot and nonnative fish species.
- Determine presence/absence of Burbot across multiple habitats using eDNA.
- Conduct laboratory studies to evaluate the effects of temperature on physiological development.
- Conduct laboratory studies to optimize feeding rates, rearing densities and reduce costs.
- Conduct investigations of alternative live feeds and large-scale zooplankton (native freshwater species) production.

5.0 ADAPTIVE MANAGEMENT

The Kootenai River Burbot Program undergoes annual reviews of critical variables and metrics as part of the ISMP and APR processes described below. These processes provide a solid adaptive management foundation for the Burbot aquaculture components of the program and the co-managers' RM&E programs. KTOI and co-managers may adjust release numbers and modify culture techniques in response to annually updated age-specific survival rates, ages at first maturity for males and females, and spawning frequency or periodicity values for Burbot in the Kootenai River. Thus, this suite of direct adaptive feedback loops will continue to serve the program well, as reflected in the program's relatively short but successful history.

5.1 IN-SEASON MANAGEMENT PROCEDURE AND GOALS

The goal of the ISMP is to provide a structured decision-making framework that guides hatchery operations, identifies M&E needs, and supports effective agency cooperation and communication consistent with the guidelines established each year. The Kootenai Tribe will implement the four-step ISMP (Figure 8) in cooperation with co-management agencies, research institutes, and stakeholders (as appropriate). The ISMP procedure is formalized in database(s) and a set of management tools, as well as through the APR to ensure consistency and accountability. The database will store and document data and assumptions, while management tools will use predictive models to arrive at outcomes from which decision guidelines and biological objectives may be derived. The tools document the basis for these targets and establish expectations for all performance indicators. They also help simplify the implementation process and document the rationale for recommended annual restoration actions. KTOI's biologist responsible for implementing in-season management will use these tools to prepare for the APR workshop, where analytical results will be presented and shared with all interested parties. The management tools used in the ISMP will be further refined over time through implementation of the ISMP and APR processes as new information is obtained and analyzed.

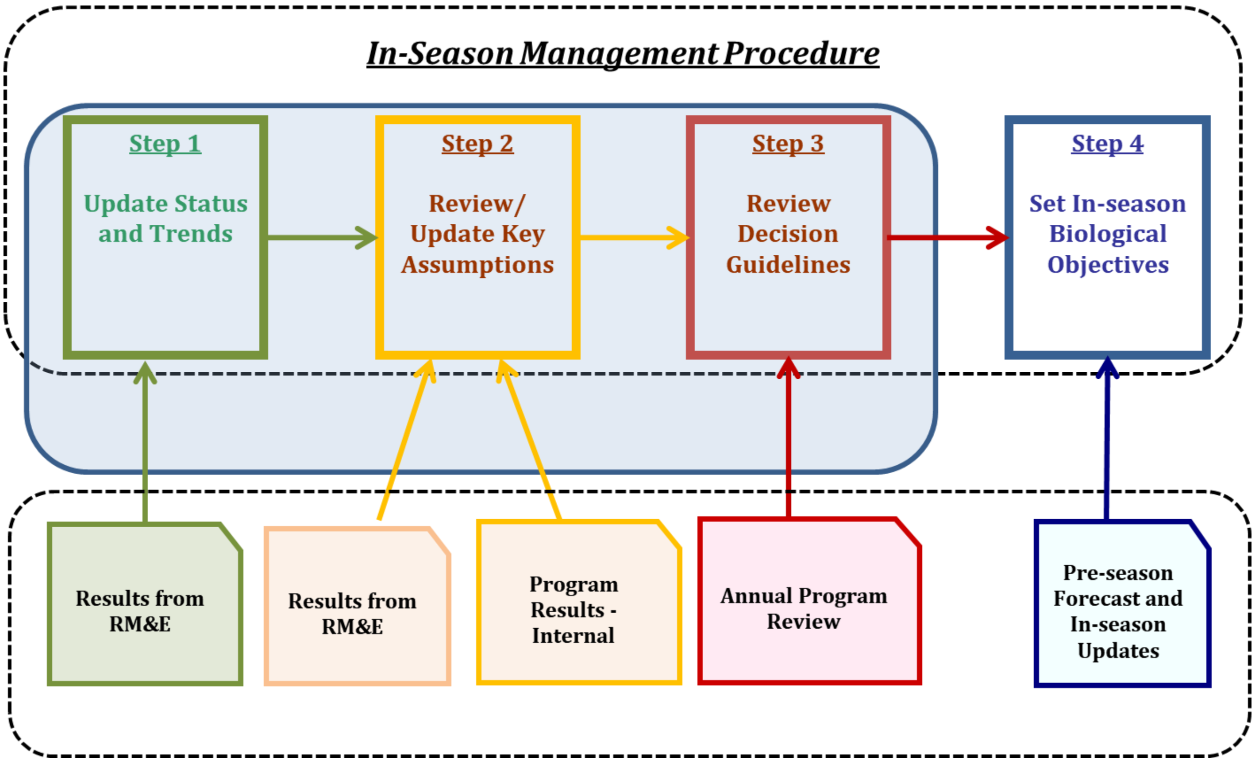

Figure 8. In-Season Management Procedure (ISMP) framework for the Kootenai River Burbot Aquaculture Program.

Due to inherent uncertainty and expected annual variability in abundance of natural-origin adult Burbot returns, the hatchery program is designed for flexible production and operations. This flexibility is reflected in the design and operation of the hatchery facilities and in the Decision Guidelines that will determine the annual hatchery production in balance with the natural population component.

5.1.1 Update Status and Trends Information

In this step, the most recent status and trends information will be evaluated for both the hatchery and natural components of the population. The initial predictive tool focused on production and survival of hatchery reared Burbot. Upon observation of natural recruitment, this tool will be modified to integrate the contribution of natural recruitment to the population. This step will occur at the APR workshop. KTOI monitors and evaluates in-hatchery metrics; and IDFG, BC Ministry, and MFWP monitor post-release metrics within their political jurisdictions in the Kootenai Basin. These data are consolidated, discussed, and reviewed at the APRs.

5.1.2 Update Key Assumptions

The annual ISMP will integrate newly acquired data and analyses to update a set of key assumptions (see Table 4). The key assumptions are crucial to setting biological objectives for the coming year.

5.1.3 Review Decision Guidelines

Once the key assumptions and population status have been updated, the Decision Guidelines (see Table 5) will be reviewed to determine if they need to be revised. This adaptive management step will occur at the APR workshop. Although not expected to change frequently, the Decision Guidelines may need to be periodically revised to account for 1) changes in conservation goals in the United States and Canada, 2) unequal goal achievement across the project area, 3) Libby Dam operations/habitat related issues, 4) new scientific discoveries, 5) other changes in management or environmental conditions in the subbasin or the region, and 6) annual harvest.

The purpose of the Decision Guidelines is to ensure that the hatchery program, co-managing agencies and fisheries align to restore and maintain a natural Burbot spawning stock. The goal of the Decision Guidelines is to establish a naturally reproducing population with a distribution similar to historical records. The Decision Guidelines within the ISMP are based on a set of key assumptions about our capability to accurately detect and respond to the annual abundance of hatchery and natural-origin fish. M&E Plans identify the information needed to update and apply these guidelines and help describe how data will be collected. KTOI expects to meet resource goals over time because of appropriate adaptive management actions through collaboration with co-managing agencies and local entities.

5.1.4 Set Biological Objectives for the Coming Year

Biological objectives are set based on co-manager agreements following the APR process. Adult-population abundance estimates, hatchery facility capacity, research needs and broodstock collection targets are all factors that play into setting biological objectives for future years. Release strategies based on specific biological life-stages are also considered to plan for the following year while also meeting target juvenile production numbers. The best available science is updated annually and presented at the APR. All updates are entered into modeling and analysis tools and the tool(s) then generate expected outcomes that are used to guide hatchery production methods and set biological objectives for the program annually.

5.2 ANNUAL PROGRAM REVIEW

Following the precedent of the KVRI BCS, APRs guide decisions about annual goals for broodstock management, gamete collection, aquaculture production, hatchery release strategies, harvest, and M&E activities. All cooperating agencies and stakeholders are urged to participate. The purpose of the APR is to implement the four-step ISMP described above with all stakeholders present to support information sharing, informed decision-making, and management to meet conservation objectives.

The agenda for the APR workshop will follow the steps outlined in the ISMP. The APR is a science-driven process that will result in an annual action plan that will be completed at the workshop. The APR participants will include appointed representatives from the cooperating agencies involved in this project. The workshop and completed action plan constitute the coordinated implementation component of the program.

The APR workshop will be conducted annually with additional coordination calls as needed. A facilitator selected by KTOI will guide the workshop to address four fundamental questions (Alison Squier, Ziji Creative Resources, facilitated 2013-present):

1. Given the information provided, what are the best estimates for the key assumptions (see ISMP Step 2)?

2. Do the Decision Guidelines need to be changed (see ISMP Step 3)?

3. What are the biological objectives for the coming year (see ISMP Step 4)?

4. How can the M&E plan be improved in the coming year?

The first part of the workshop will be devoted to presentations of results from M&E activities related to the key assumptions for the Tribal hatchery program (see ISMP Step 2). There will be sessions covering the following topics: 1) hatchery operations, 2) post-release survival and distribution, 3) habitat, 4) spawning and natural recruitment, and 5) harvest. Prior to the workshop, KTOI will coordinate with cooperating agencies to ensure the most up-to-date information for each of these subjects will be presented and discussed. The ISMP tool will also be populated with the most recent data and analytical results used to update status and trends (see ISMP Step 1).

In the second part of the workshop, the co-managers, which consists of policy and technical personnel, will review the implications and conclusions from part one that determine the Decision Guidelines (see ISMP Step 3). Participants will review and confirm conclusions and alternative modifications to the Decision Guidelines (see ISMP Step 3) and finalize a set of biological objectives for the upcoming year (see ISMP Step 4). The purpose of the Decision Guidelines is to ensure that the long-term goals established in the KVRI BCS, the Hatchery Master Plan, and this Plan are met over time. The product of the second part will be an updated action plan for the coming year.

The final step will be a review of parts 1 and 2 to ensure a consensus among the agencies and stakeholders has been reached. If consensus cannot be achieved with all participating agencies, the rule of “consensus minus one” is used for the final decision. Consensus minus one simply means that a single agency does not have the power to override the decisions of the majority. In addition, a ‘modified’ structured decision-making process is being developed. Each agency actively implementing the Recovery Plan will review how the workshop will guide their respective hatchery and/or field M&E activities/research during the upcoming year. After the workshop, the facilitator will provide a draft summary to all workshop participants incorporating findings, conclusions, and final decisions for review (Figure 9). Workshop participants will confirm (and if necessary, correct) the workshop summary and the facilitator will produce and distribute a final workshop record of agreements and required actions. The ISMP database, management tools, Decision Guidelines and other associated products will be retained along with the workshop summary for reference in subsequent APR workshops. Agency representatives will be responsible to relay activities, outcomes, and recommendations to/from their respective agencies to form agreements. The current Burbot Technical Co-managers will continue to communicate routinely throughout the year to coordinate logistics of program activities and reporting requirements.

Participants split into two breakout groups and identified the following initial ideas:

Group 1	***Group 2***

US
• Release guilds (PBT groups)
    o Eggs on boat → river (fertilized eggs; <24 hours)
    o Eggs → hatchery → river (eyed-eggs)
    o Pre-feeding larvae (<14 dph)
    o Post-feeding larvae (15-50 dph)
    o Juvenile (60-210 dph; ~ October)
• Family splitting
    o "All or none"
• Cannibals
    o All released at assigned family group location OR release in isolated water body (i.e., Bonner Lake)
    o Or isolated water body in MT?
    o Would need: permits, stocking plan, where, disease testing
Canada
• No family splitting
• If emergency release is necessary, release entire family at Porthill.
• If permitting issue is resolved, release underachievers and/or cannibals at predetermined release locations. Don't split families.
• Cannibals considered juvenile and stay in release location of future juvenile releases of same family
• Releases in both lake and river (focus on lake if low hatchery numbers).
	No splits of families outside of life stage "guild"
	    o If not possible determine best way to analyze/agree prior to release
	• Blanket permit with ability to stock in US within time window ~ would still want consultation with IDFG.
	• Canada releases will always need 2 CFIA permits 1) for larvae, 2) for juveniles. These will have time windows but must arrange border inspection and disease testing, so requires foresight and planning.
	    o If don't have permits and need to make room in hatchery because too many cannibals – ask Andy and Montana if okay with putting in any closed system. *Let's get answer to this now.*
	    o If need to get out underachievers that = entire family group.
	    o Keep cannibals and feed them underachievers. KTOI would have to approve this.
	• Cannibals: 1) release at assigned locations, 2) or grow to PIT tag size, 3) or release into closed system (not river or lake) i.e., small deep cool ponds.
	• Underachievers released at assigned locations. These are entire family groups (not cherry picked like cannibals)
	    o Not include these in M&E (if separate family groups)
	• Hatchery feeding techniques increase predictability with survival. If increased survival, we will want to think about egg take numbers and how to deal with too many fish and hatchery space.
	    o If survival so increased that need to get fish out door sooner.
	• BC will book Dr. Mack at border.
	    o 1 day – pre-feeding larvae mid-April
	    o 1 day – feeding larvae May
	    o 1-day cannibals July 1st
	    o 3 days – late August releases
	    o 1 day in beginning of August (just in case)
	• BC will get CFIA permitting done for August 1st and KTOI will get disease testing done too.

Figure 9. Example of Annual Program Review group exercise to review release strategies.

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