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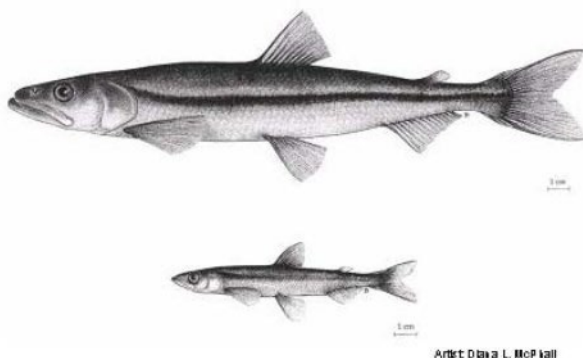
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Maritimes Region

ABUNDANCE ESTIMATES AND GENETIC ANALYSIS OF THE LARGE-BODIED POPULATION OF LAKE UTOPIA RAINBOW SMELT (*OSMERUS MORDAX*) IN MILL LAKE STREAM, 2023



Rainbow smelt (Osmerus Modax)

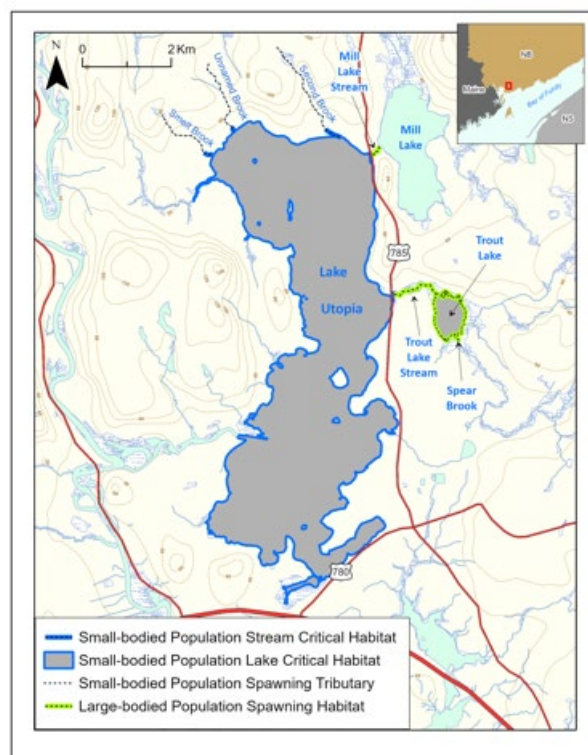


Figure 1. Areas of critical habitat identified for Lake Utopia Rainbow Smelt, Small-bodied Population (LURS-SbP) are shown outlined in blue. Portions of the spawning tributaries, shown in the green highlight, are where Lake Utopia Rainbow Smelt, Large-bodied Population (LURS-LbP) have been observed to spawn (Curry et al. 2004, DFO. 2011, Bradford et al. 2012).

CONTEXT

Lake Utopia is part of the Magaguadavic River watershed in southwestern New Brunswick. The native Rainbow Smelt (*Osmerus mordax*) inhabiting Lake Utopia consists of two sympatric morphologically, ecologically, and genetically differentiated populations: a small-bodied population (SbP) and a large-bodied population (LbP). The SbP has been protected under the *Species at Risk Act* (SARA) since 2003. The LbP was first designated as Threatened by the

Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in November 2008; the population was re-assessed as Endangered by COSEWIC in 2018 and listed as Threatened under the *Species at Risk Act* (SARA) in August 2019.

Former CSAS processes (DFO 2016b, 2018, 2024) have reviewed and updated the LbP abundance target, the minimum fork length for distinguishing the two populations, the maximum level of allowable harm that LbP can sustain without jeopardizing the population's survival or recovery, whether abundance estimates have met the abundance target, and indicators of whether the genetic objective is being met. An updated recovery abundance objective of "5,000 adults in Mill Lake Stream observed on nights of peak spawning" was recommended and considered feasible for this population (DFO 2018). To meet this objective, 5,000 fish would need to be quantitatively estimated on at least one night of sampling during the early spawning run in Mill Lake Stream. The threshold minimum fork length used to distinguish LbP from SbP was recommended to be reduced to 130 mm from previous thresholds of 143 (DFO 2018) and 170 (DFO 2011), based on genetic analysis (DFO 2024). The high variability of LbP spawner abundance estimates, declining minimum fork length criterion required to distinguish LbP smelt, a higher rate of gene flow from SbP into LbP, and the presence of hybrids, warrant further sampling, analyses, and review to track these trends and to determine if the LbP recovery abundance and genetic objectives are being met.

This Science Advisory Report is from the November 6–7, 2024, regional peer review on the Assessment of the Population Abundance for Lake Utopia Rainbow Smelt, Large-Bodied Population.

SUMMARY

- Lake Utopia Rainbow Smelt (LURS) comprise two sympatric populations, referred to as large-bodied (LbP) and small-bodied (SbP), that are differentiated by their morphology, choice of spawning streams and timing of spawning runs. The two populations are also genetically distinct, although there is evidence of gene flow between them.
- The updated recovery abundance objective for the LbP of a minimum of 5,000 fish in Mill Lake Stream observed on at least one night of spawning was met on both nights of mark-recapture sampling in 2023. Nightly estimates calculated using the adjusted Petersen method, were 25,014 and 29,093 for April 12 and 13, respectively.
- Nightly estimates scaled using the 170 mm, 142 mm and 130 mm fork length (FL) criteria to account for potential SbP and hybrid smelt present in Mill Lake Stream during nights of sampling range from 10,506 to 24,514 for April 12, 2023, and from 12,219 to 28,511 for April 13, 2023.
- Large and small-bodied smelt remain genetically and morphometrically distinguishable. Available genetic data do not support the use of one FL cutoff over another due to the range of sizes and genetic variability observed in fish spawning in Mill Lake Stream.
- While the LURS LbP has met the updated recovery abundance objective, the total number of spawners occupying Mill Lake Stream during annual spawning runs cannot be estimated given that sampling only covers a small portion of the spawning run and the proportion of the whole population spawning on any given night is unknown.
- Abundance estimates cannot be compared between years due to high annual variability in the estimates and associated error between sampling nights and the limited sampling

period. Similarly, the changes in the degree of hybridization over time cannot currently be assessed due to limited sampling and changes in the genetic marker panel post-2016.

- It is recommended that the updated recovery abundance target of 5,000 spawners be applied to nightly estimates of early (late March to mid-April) spawning fish in Mill Lake Stream until improved genetic and morphological metrics are developed to differentiate the LbP and SbP. Lack of certainty in the proportion of LbP smelt that make up the early spawning run of Mill Lake Stream can result in over-estimated abundance.

BACKGROUND

Lake Utopia Rainbow Smelt (LURS) represent a rare occurrence in Canada where divergent smelt populations co-exist in a lake system. There are morphological differences between the two LURS ecotypes, as well as differences in the choice of spawning streams and the timing of their spawning runs. Historical data suggest that the large-bodied population (LbP) spawns in Mill Lake Stream and the Trout Lake Stream-Spear Brook system between late-March and mid-April, whereas the small-bodied population (SbP) spawns from mid-April until mid-late May in Smelt Brook, Unnamed Brook, and Second (Scout) Brook (Figure 1). The body forms of the two populations are distinguishable by their relative eye and jaw to body size ratio, number of gill rakers, and body size at maturity (Bradbury et al. 2011). COSEWIC (2018) characterized the LbP as spawners at 136–227 mm fork length (FL) and SbP as spawners at 73–136 mm FL, whereas DFO had adopted a minimum fork length (FL) criterion of 170 mm for the LbP, based on a larger sample size and evaluation of phenotypic and genotypic diversity in the two populations (Bradbury et al. 2011, Bradford et al. 2012).

The two smelt populations in Lake Utopia are largely reproductively isolated and genetically distinct. However, the occurrence of hybrids indicates that gene flow occurs between the two populations (Bradbury et al. 2011). Analyses of spawning migrations in Mill Lake Stream in 2014, and in Second (Scout), Smelt, and Unnamed Brooks in 2015, found approximately 25% of the individuals sampled during the early (late March to mid-April) spawning migration in Mill Lake Stream (n=6/25) were SbP or hybrid individuals (Themelis 2018). LbP individuals showed more hybridization than the SbP, suggesting a higher level of gene flow from SbP to LbP when compared to 603 samples collected in 1990, 2002-2003, and 2010 (DFO 2018). A high proportion (>80%) of smelt sampled in Mill Lake Stream during the 2014 and 2017 assessments measured less than 170 mm FL, prompting the minimum FL criterion to be reduced to 143 mm (DFO 2018), then to 130 mm (DFO 2024). These findings raised concerns over the possible consequences of continued hybridization of the LURS populations, which could lead to introgressive meltdown and the potential for collapse of the two ecotypes into a single undifferentiated population (Themelis 2018).

The updated recovery abundance objective for the LbP is a minimum of 5,000 spawners in Mill Lake Stream during nights of peak spawning. To meet this objective, 5,000 fish would need to be quantitatively estimated on at least one night of sampling during the early spawning run in Mill Lake Stream. The value of 5,000 spawners is based on the concept of an effective population size (N_e) of 1,000 being necessary to maintain genetic diversity (Frankham et al. 2014). This value was scaled by a range of ratios (0.1-0.2) in effective population size/ minimum census population size (N_e/N_c) for salmonid populations, from which a target population size of 5,000 was derived by DFO (2018) to update the interim recovery target set at 2,000 for LbP smelt. Nightly spawner abundance has been estimated quantitatively for LbP smelt on a single night in April 2009, on 5 nights in April 2014, and on two nights in April 2017. Visual monitoring

of Mill Lake Stream, at least during daytime, was conducted every year from 2009 to 2024, with the exception of 2011.

This report provides abundance estimates for the LbP derived from mark-recapture events conducted over two nights during the 2023 Mill Lake Stream spawning run. It also includes genetic data for 83 of the sampled smelt, which were analyzed in comparison to previously collected samples from 2017/2018, to infer population structure and hybridization between the SbP and LbP morphs. No SbP spawning streams were sampled in 2023.

ASSESSMENT

Study Area

Mill Lake Stream flows into Lake Utopia through two concrete culverts under New Brunswick Route 785. The primary culvert measures 30 m × 4 m × 3.7 m, and is the main access from Lake Utopia into Mill Lake Stream. A secondary overflow culvert measuring 22.3 m X 1.5 m diameter is generally dry, except during times of high water flow. Both culverts were updated from their original smaller diameter, corrugated metal structures in 2021. Accessible spawning habitat in the stream is limited to a 30 m section between the culverts and a small (0.5 m) waterfall (45°12'21"N, 66°46'38"W) that acts as a natural barrier to upstream migration of LURS in most years (DFO 2011). The mean stream width is 4 m and less than 1 m depth, with water velocities reaching 1 m per second or more (MacDonald and Burbidge 2017, Caissie and Savoie 2017). Sampling was conducted in Mill Lake Stream, rather than Trout Lake Stream or Spear Brook, because Mill Lake Stream is identified as the principle spawning tributary for the LbP and the source of previous abundance estimates, whereas spawning activity has seldom been observed in the latter two streams (DFO 2018).

Sampling Methods and Data Analysis

Visual observations of Mill Lake Stream were conducted by members of the Passamaquoddy Recognition Group Inc. (PRGI) according to the methodology described in MacDonald and Burbidge (2017). In addition to daytime monitoring, nightly checks were conducted from March 28 to April 10, 2023 to inform the timing of mark-recapture efforts. The stream was checked every 2 to 3 nights, at 30-minute intervals between 11:30 pm – 2:00 am, from the main culvert to the waterfall, and in the outflow below the culvert. Sampling was undertaken when LURS were observed in abundance within the stream (i.e. visual observation of hundreds of fish). The first night when smelt were observed in abundance was April 10; sampling was undertaken on the nights of April 11 through 13, 2023.

On the first night of sampling, the number of smelt present in the stream was deemed too low to conduct on-the-night mark-recapture (several thousand smelt are required to draw samples of 500 for each marking and recapture event). Instead, 60 smelt were dip-netted along the stream banks between the culvert and waterfall and sampled for genetic analysis by clipping the upper portion of the caudal fin (UCF), after which all individuals were released into the stream. While no recapture efforts were undertaken on April 11, these fish were given unique marks so that they could be identified if caught in later sampling efforts.

Mark-recapture took place on April 12 and 13, 2023. On each night at about 11:00 pm, 500-600 smelt were dip-netted along the stream banks between the culvert and waterfall, marked and held in containers on shore. Smelt were marked by clipping the adipose fin (ADF) on April 12, and the lower portion of the caudal fin (LCF) on April 13. After all smelt were marked, they were released and allowed one hour to mix with other smelt present in the stream. A second sample

of 500-600 was then captured, the number of marked and unmarked smelt counted, and all smelt released. Unmarked smelt captured during the second (recapture) event on April 12 were marked before release. Fin clips from 40 of the smelt marked during the first capture event on April 12 were retained for genetic analysis. Samples were preserved in 95% ethanol.

As part of a pilot project to transition to the use of Passive Integrated Transponder (PIT) tagging in future LURS assessments, 99 smelt from the first capture event on April 12 were internally tagged with FDX PIT tags (8.5 by 2.12 millimeters, Biomark, Boise, Idaho, USA) in addition to receiving a fin clip. Tagged fish were otherwise treated the same as marked fish, and were held and released with the rest of the marked group. PIT tag retention and long term tagging effects were not assessed. All 99 PIT tagged individuals (140-220 mm), as well as the 100 smelt sampled for genetic analysis (119-232) and two very large (299 and 300 mm) smelt marked on April 12, were sexed and measured for fork length to the nearest millimeter (mm) to establish a length frequency and sex ratio of the spawning run.

Nightly abundance estimates were calculated with the R package *fishmethods* (Nelson 2023) using the adjusted Petersen method (Chapman estimator), and total abundance over the sampling period was estimated using the adjusted Schnabel method. The Marine Gene Probe Laboratory at Dalhousie University, Halifax, Nova Scotia, was contracted to conduct genetic analysis. Genetic data sampled in 2023 were analyzed using a panel of 63 microsatellite loci and a DNA sequencing-based approach as outlined in Bradbury et al. (2010). For detailed description of genetic analysis methods, see Appendix A.

Results were compared to SbP and LbP samples collected in 2017 and 2018 to infer population structure and hybridization between the two morphs. Samples collected in 2017 and 2018 were analyzed as a single group due to the low number of samples genotyped in 2017 (8 each for SbP and LbP). Nine samples collected from Mill Lake Stream on April 23, 2018 were provisionally assumed to be hybrid due to being collected after the peak of the LbP spawning run was presumed to be over. The final genetic dataset consisted of 297 viable Rainbow Smelt samples (Table 5; 2017 n=16, 2018 n=198, 2023 n=83).

Genetic assignment of individuals as either LbP, SbP, or hybrid is based on proportional Q-values. When tested for proportional assignment to two groups, individuals have two Q-values, each corresponding to either the SbP or LbP population. Fish whose genotype is primarily derived from a single population will have a Q-value approaching 1 for that population and 0 for the opposite population, while fish that are admixed (hybrids) will have intermediate Q-values. Historical genetic analyses indicate that presumed SbP individuals had Q-values greater than 0.9 for the SbP genotype, compared to 0.7 for the LbP genotype (Bradbury et al. 2011).

Available length data from previous sampling seasons (2014, 2017 and 2018) in Mill Lake Stream during the presumed LbP spawning run were compiled and compared with 2023 data to evaluate whether a noticeable change in the size of Mill Lake Stream spawners had occurred during this period. These data were obtained from published assessments (DFO 2016, DFO 2018, DFO 2024). A total of 378 lengths were recorded on April 2-10, 2014; 348 lengths were recorded on April 14-15, 2017; 95 lengths were recorded on April 15, 2018, and 201 lengths were recorded on April 11-12, 2023. Distributions were tested for normality using the Anderson-Darling (A-D) test implemented by the R package *nortests* (Gross and Ligges 2015) and compared to each other using the Kolmogorov-Smirnov (K-S) test implemented by the R package *dgof* (Arnold and Emerson 2011).

Length Frequency Distribution

The distributions of fork lengths for smelt sampled in Mill Lake Stream during the early spawning run (presumed LbP) were compared for 2014, 2017, 2018 and 2023 (Table 1). In 2014, sampled lengths followed a normal distribution (Figure 2; A-D test parameters $A=0.73$, $p=0.06$) with a mean FL of 155 mm (125-190 mm). Samples from 2017, 2018 and 2023 were not normally distributed (Figure 2; A-D test p -values < 0.05) and differed significantly from 2014 data (K-S test p -values < 0.00) but not from each-other (K-S test p -value range 0.06 to 0.49). The mean FL in 2017 and 2018 was 170 mm (103-277 mm and 124-262 mm range, respectively); the mean FL in 2023 was 168 mm (119-300 mm range; Table 1).

Table 1. Length frequency (FL, recorded in mm) of smelt sampled in Mill Lake Stream during the early spawning run (presumed LbP) in 2014, 2017, 2018, and 2023.

year	count	min	max	mean	sd	median	mode
2014	378	125	190	155	11.1	155	145
2017	348	103	277	170	25.9	170	204
2018	95	124	262	170	29.5	166	173
2023	201	119	300	168	24.4	165	160

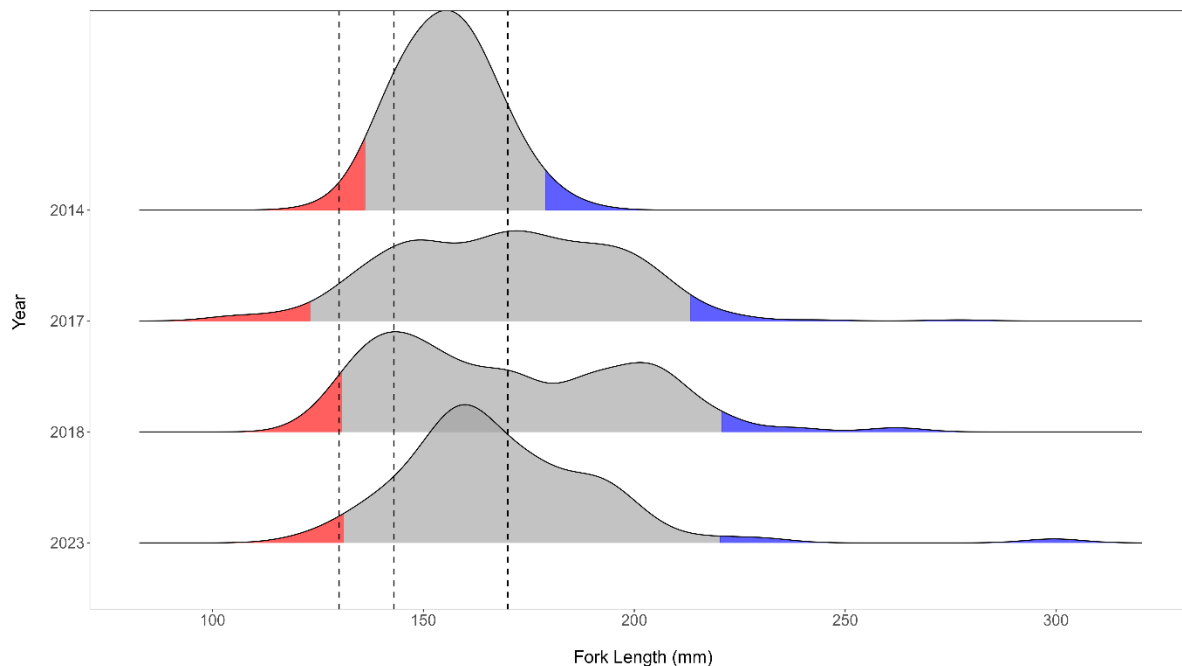


Figure 2. Distributions of fork length (mm) measurements of smelt sampled in Mill Lake Stream during the early spawning run (presumed LbP) in 2014 ($n=378$), 2017 ($n=348$), 2018 ($n=95$) and 2023 ($n=201$). Red and blue fill indicates the 2.5% and 97.5% tails, respectively. Dashed black lines indicate the 170 mm, 143 mm and 130 mm fork length criteria.

Population Estimate

A total of 2,134 smelt were sampled from Mill Lake Stream over three nights between April 11 and April 13, 2023. On April 11, 2023, it was judged that there were too few fish present to be

able to conduct robust on-the-night capture-mark-recapture. However, 60 fish were captured, measured, sexed, marked by fin clipping and the fin clips collected for genetic analysis.

Nightly marking efforts and recapture data for April 12th and 13th, 2023 are summarized in Table 2 and Table 3. The relatively constant rate of increase of recaptures with increasing marks (approximately 10 extra recaptures observed each time approximately 500 extra marks were added) suggests that the mark-recapture model assumption of closure may not be problematically violated. Of the 99 PIT tags deployed on April 12, three were recaptured that same night, and none recaptured on April 13. Because PIT tagged fish were also marked with a fin clip, they are counted within the recapture event in Table 3.

Using the Schnabel method (input data in Table 3), total abundance over the sampling period was estimated at 27,592 (95% confidence intervals 20,564 to 41,918). Nightly estimates calculated using the adjusted Petersen method (input data from Table 3), in which each night was treated as a separate mark-recapture event and only fish marked that night were counted in the nightly estimate, were 25,014 and 29,093 for April 12 and 13, respectively (Table 4). Compared with the previous LbP assessment (DFO 2018), the total number of fish sampled in 2023 was slightly lower than in 2017. However, both the Schnabel and Petersen population estimates were significantly higher in 2023 (Table 4).

In 2023, 98% (n=197/201) of the sampled Rainbow Smelt measured at or over 130 mm FL, 89% (n=179/201) measured at or over 143 mm FL, and 42% (n=85/201) measured at or over 170 mm FL. Adjusting nightly Petersen abundance estimates by these three length criteria produces estimates ranging from 10,506-24,514 smelt on April 12, and from 12,219-28,511 smelt on April 13.

Table 2. Summary of nightly marking efforts for Lake Utopia Rainbow Smelt large-bodied population in Mill Lake Stream. Smelt were marked by clipping the upper caudal fin (UCF) on April 11, the adipose fin (ADF) on April 12, and the lower caudal fin (LCF) on April 13. Recapture events began an hour after the marking event.

Study night	Date	Event	N Marked	Mark type	N PIT tagged
1	2023-04-11*	Marking	60	UCF	-
2	2023-04-12	Marking	536	ADF	99
2	2023-04-12	Recapture	547	ADF	-
3	2023-04-13	Marking	483	LCF	-
3	2023-04-13	Recapture	508	LCF	-

* No recapture efforts were undertaken on April 11, 2023; however, one fish was recaptured on April 14.

Table 3. Summary of mark-recapture data for Lake Utopia Rainbow Smelt large-bodied population in Mill Lake Stream during each study night, including total number of fish captured during each event, number of recaptures, number of newly marked fish, and total number of marked fish available for recapture from all previous periods (NA – not applicable; UCF-upper caudal fin clip; ADF - adipose fin clip; LCF - lower caudal fin clip).

Study night	Date	Event	Total catch	Recaptures (UCF, ADF, LCF)	New marked	Total marked
1	2023-04-11	Genetic sampling	60	NA	60	60
2	2023-04-12	Marking	536	0	536	596
2	2023-04-12	Recapture	558	11 (0, 11, NA)	547	1143
3	2023-04-13	Marking	506	23 (0, 23, 0)	483	1626

**Abundance Estimate and Genetic Analysis
of LbP LURS in Mill Lake Stream**

Maritimes Region

Study night	Date	Event	Total catch	Recaptures (UCF, ADF, LCF)	New marked	Total marked
3	2023-04-13	Recapture	540	32 (1, 23, 8)	NA	1626

Table 4. Comparison of sampling effort and abundance estimates between the 2017 and 2023 assessments of the Lake Utopia Rainbow Smelt large-bodied population in Mill Lake Stream. Values are unadjusted for minimum length criteria. (CI – confidence interval).

Date	April 13 and 14, 2017 (DFO 2018)	April 12 and 13, 2023
Nights sampled	2	2
Total fish sampled	2,276	2,074
Schnabel estimate (95% CI)	13,952 (11,111-18,743)	27,592 (20,565-41,918)
1st night Petersen (95% CI)	6,652 (5,200-9,200)	25,014 (15,741-49,848)
2nd night Petersen (95% CI)	12,843 (9,600-19,200)	29,093 (17,388-65,205)

Table 5. Estimates of nightly spawner abundance of Lake Utopia Rainbow Smelt in Mill Lake Stream on April 12 and April 13, 2023, based on the Petersen method adjusted with the 130, 143, and 170 mm fork length (FL) criteria.

Date	April 12, 2023	April 13, 2023
Unadjusted estimate	25,014	29,093
Adjusted for 130 mm FL	24,514	28,511
Adjusted for 143 mm FL	22,262	25,893
Adjusted for 170 mm FL	10,506	12,219

Genetic Analysis

A principal component analysis (PCA) of variation in allele frequencies showed two distinct clusters separated on the first axis of variance (PC1). Samples are coloured according to phenotypic association with the SbP and LbP based on the stream and timing that each sample was collected (Figure 3). Assumed hybrids from the 2018 late-April run in Mill Lake Stream (n=9) exhibited an intermediate centroid; however, many appeared to cluster with LbP or SbP. The same is true of several 2017–18 and 2023 individuals which were phenotypically LbP, but clustered with SbP; or vice versa. A number of individuals had intermediate PC1 values consistent with them being hybrids.

Figure 4 shows the smelt sampled in 2017, 2018 and 2023 ordered along the horizontal axis first by their spawning location and timing (assumed morph), then by increasing body length, while the vertical axis indicates the proportion of the genome attributable to SbP and LbP based on Q-values for each population (Q-value Small and Q-value Large). A Q-value Large of ≥ 0.53 was needed to capture 80% (n = 83) of assumed LbP morphs (defined as present in Mill Lake Stream during the LbP spawning migration in early April) in 2017-18 and ≥ 0.58 (n = 67) in 2023. A Q-value Small of ≥ 0.72 was needed to capture 80% (n = 82) of assumed SbP morphs in 2017-18.

Based on these Q-value cutoffs, the population identity of 2017–18 Mill Lake Stream samples (n=103) can be assigned as: 81% LbP (n=83), 6% SbP (n=6), and 14% hybrid (n=14; Table 6). The population identity of 2023 samples can be assigned as: 80% LbP (n=66), 2% SbP (n=2), and 18% hybrid (n=15; Table 6).

The range of Q-values varied significantly with fork length (Figure 5). At smaller body sizes (< 143 mm), Q-value Large ranged from 0.264 – 0.884 (mean = 0.616) for 2017-18 LbP, and from 0.168 – 0.849 (mean = 0.628) for 2023 LbP. Five 2017-18 LbP individuals (approximately 5%) had a Q-value Large >0.9 and mean FL of 195 mm (range: 160 – 238 mm). Five 2023 LbP individuals (about 6%) had a Q-value Large >0.9 with a mean FL of 176 mm (range: 160 – 230 mm). No genetic recaptures were detected between 2017, 2018, and 2023 sampling events.

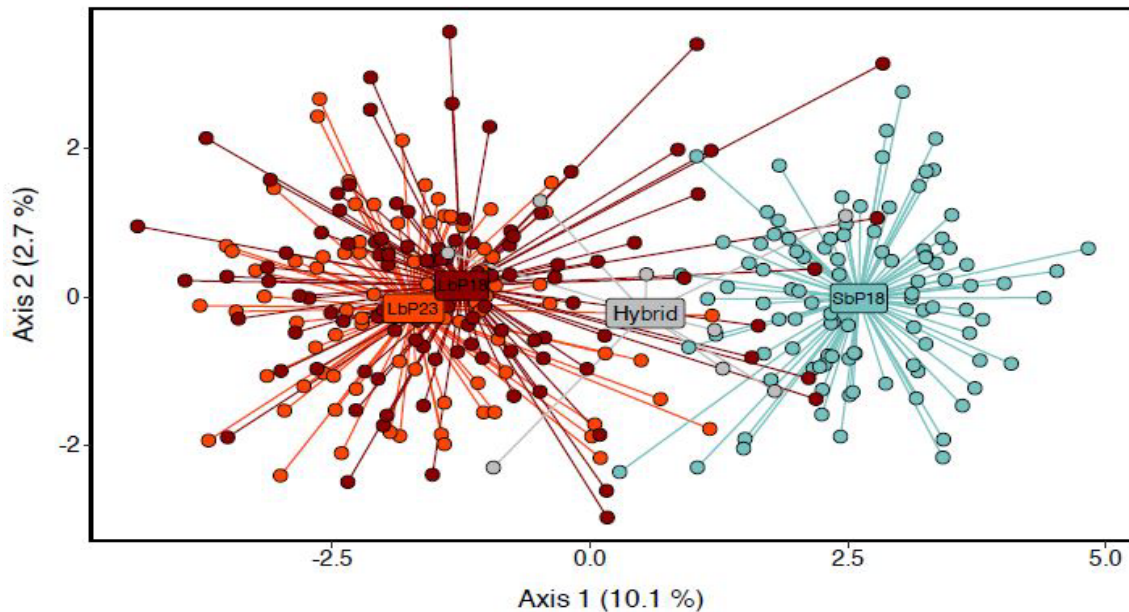


Figure 3. Principal component analysis of Lake Utopia Rainbow Smelt sampled during the small-bodied population (SbP) spawning run in 2017-18 (SbP18, $n = 102$), the large-bodied population (LbP) spawning run in 2018 (LbP18, $n = 103$) and 2023 (LbP23, $n = 83$), and assumed hybrids sampled post-LbP spawning run in 2018 (Hybrid18, $n = 9$). LbP are in red (2017-18) and orange (2023; left), SbP are in blue (right), and putative hybrids are in gray (middle). Figure provided by the Marine Gene Probe Laboratory, Dalhousie University¹ (unpublished report 2023).

¹ Dalhousie University. 2023. Lake Utopia Rainbow Smelt Genetic Analysis Report 2023. Unpublished Report.

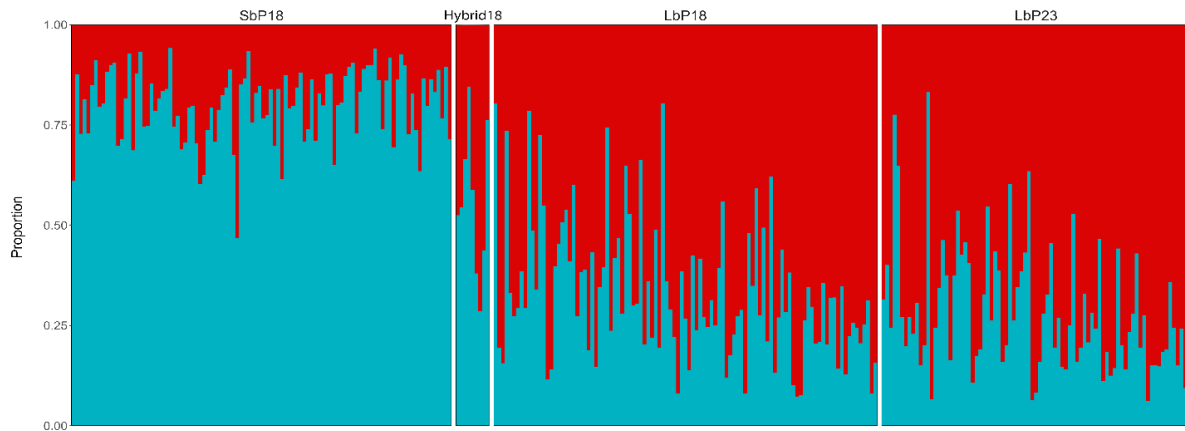


Figure 4. Proportion of genotype characteristic of large-bodied population (Q-value Large; red) or small-bodied population (Q-value Small; blue) of Lake Utopia Rainbow Smelt sampled during the presumed small-bodied population (SbP) spawning runs in 2017 and 2018 (SbP18, $n = 102$), the presumed large-bodied population (LbP) spawning run in 2018 (LbP18, $n = 103$) and 2023 (LbP23, $n = 83$), and the post-LbP spawning run in 2018 (Hybrid18, $n = 9$). Within each panel, smelt are ordered along the horizontal axis by fork length (SbP18 r axis by fork length (SbP18 range: 93–150 mm, Hybrid18 range: 122–144 mm, LbP18 range: 103–262 mm, LbP23 range: 119–232 mm).

Table 6. Genetic assignment of Lake Utopia Rainbow Smelt sampled in 2017, 2018, and 2023 from spawning streams (N -number; LbP – large-bodied population; SbP – small-bodied population).

Sampling site	N sampled	Assumed morph	N genetically-assigned as		
			LbP	SbP	hybrid
Mill Lake Stream 2017	8	LbP	3	2	3
Mill Lake Stream 2018	95	LbP	80	4	11
Mill Lake Stream 2018 (late run)	9	hybrid	3	2	4
Mill Lake Stream 2023	83	LbP	66	2	15
Second Brook 2017	8	SbP	0	7	1
Second Brook 2018	90	SbP	1	72	17
Smelt Brook 2018	4	SbP	0	3	1

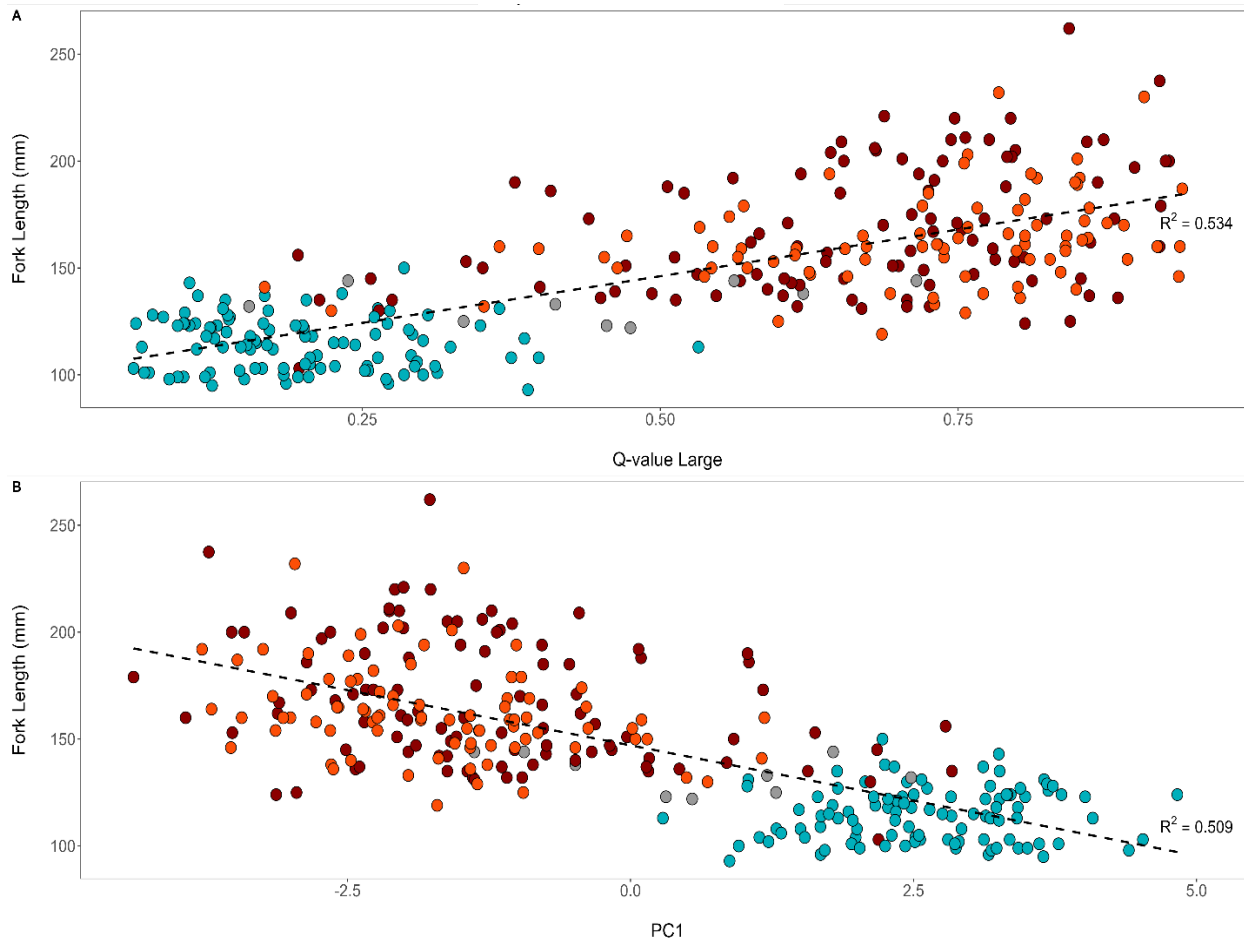


Figure 5. Relationship between fork length and Q-value Large (panel A) and fork length and PC1 (panel B) of Lake Utopia Rainbow Smelt sampled during the presumed small-bodied population (SbP) spawning run in 2017-18 (SbP18, $n = 102$, blue), the presumed large-bodied population (LbP) spawning run in 2017-18 (LbP18, $n = 103$, red) and 2023 (LbP23, $n = 83$, orange), and the post-LbP spawning run in 2018 (Hybrid18, $n = 9$, grey).

Sources of Uncertainty

While the LURS LbP has met the updated recovery abundance objective in 2023, the total number of adults occupying Mill Lake Stream during annual spawning runs cannot be estimated, given that sampling only covers a small portion of the spawning run. Therefore, the proportion of the whole population spawning on any given night is unknown. The relatively constant rate of increase of recaptures with increasing marks (approx. 10 extra recaptures each time approx. 500 extra marks were added) may indicate that the assumption of population closure for the Schnabel and Petersen capture-mark-recapture methods is not problematically violated, but the overall recapture rate is too low to say this with confidence. Abundance estimates cannot be compared between years due to high annual variability in the estimates and associated error between sampling nights and different sampling efforts. The timing of mark-recapture is based on visual observation, without a quantitative way of knowing whether the peak of the spawning run is being sampled in a given year.

There is a correlation between the genetic makeup of fish spawning in Mill Lake Stream and the timing of sampling. Most LURS population assessments to date have occurred in a short sampling window (2-3 nights). Based on visual observation, the earliest spawning run within Lake Utopia appears to occur in Mill Lake Stream (Curry et al. 2004). This run is comprised of smelt with a range of sizes and Q-values, which while broad, appear distinct from the distributions seen in the SbP that tends to occur later in Second, Smelt, and Unnamed brooks. Within Mill Lake Stream, mean body size has been observed to decline over the duration of the spawning period (Curry et al. 2004). In years when sampling of Mill Lake Stream was extended into late April and May, a second run of smelt with a smaller body size and a genome more characteristic of SbP (i.e. the 2014 spawning run) or hybrids (i.e. the 2018 spawning run) has been observed (DFO 2018). This presents a difficulty for assessing changes in hybridization between the two populations, as the detection of hybridized smelt in Mill Lake Stream may be dependent on the timing of sampling in relation to the early versus late spawning runs.

Given the overlap in length frequencies between the SbP and LbP, and available genetic data, minimum FL is not useful for indicating to which population an individual belongs. There is no evidence based on available length data that the size of LbP spawners has declined in recent years. The mean and range of lengths of LbP spawners is similar between 2017, 2018 and 2023, and generally comparable to the range of sizes reported by Curry et al. (2004) from the 1999 and 2000 runs. The relationship between body size and Q-value Large shows overlap between SbP and LbP spawners at lengths of 120-150 mm, which are also likely to include hybrids of the two morphs. Neither can the two populations be convincingly distinguished at a specific length using the relationship between body size and PC1. Therefore, there is no biological basis for discerning which of the previously applied Fork Length Criteria can be used to correct estimates of LbP spawners without over or under-estimating abundance. New morphometric data used in conjunction with genetic analysis should be used to confirm discreteness of the two populations. The feasibility of developing a new criterion, based on a combination of morphometric and genetic measurements, should be examined for future assessments.

The SbP morphs display less variability in Q-values, with approximately 80% of their genome (Q-value Small range 0.7-0.9) estimated to derive from the small-bodied form. In contrast, Q-values of LbP smelt have greater variability (Q-value Large range 0.5 to 0.9). The generally greater and more variable proportion of 'small' genotype present in smelt that are phenotypically 'large' provides evidence of gene flow from the small morph to the large morph. However, caution is recommended when interpreting results of genetic assessment in relation to levels of hybridization between the two populations. The number of genetic samples processed to date has been limited by cost and sample quality. Further uncertainty is added due to changes in the panel of genetic markers post-2016, which limits comparison of current data to past samples. Without a historical basis for comparison, and given the limited cross-section of the LbP spawning run sampled in any given year, evaluation of whether hybridization has increased in recent years is not possible at this time. Mean F_{st} was 0.045 but per locus values were highly variable (range: 0 - 0.30) suggesting observed genetic differentiation between large and small bodied morphs is driven primarily by a subset of the microsatellite loci examined (Figure A1; per locus F_{st} values are provided in Appendix A Table A1). Although these markers are microsatellites and are expected to be selectively neutral, they may be influenced by selection acting on adjacent genomic regions. Future comparison of the two populations should be conducted using a wider panel of microsatellites or whole-genome sequencing, alongside contemporaneous biological sampling (non-lethal fin clips) covering the spawning periods of both populations, before genetic data can effectively be used to inform LURS population size

and possible drivers promoting hybridization between the two morphs. Sampling should be both length and temporally stratified to ensure that changes in the character of the spawning run can be detected. Current length-at-age data are necessary to assess possible changes in life history that may be influencing the presence of small-bodied smelt in Mill Lake Stream. Genetic sampling can be further informed by monitoring of Mill Lake Stream to better resolve the start and duration of the spawning run(s) coupled with environmental data (e.g., water temperature, spring ice conditions).

Estimates of LbP abundance are currently assessed in relation to an interim target since information on total population abundance is lacking. Although an updated abundance target of 5,000 spawners was proposed following new scientific review of a met-analysis of N_e/N_c ratios and the average derived therefrom (DFO 2018), both of these targets were set based on the concept of an effective population size in salmonid populations (Frankham et al. 2014). Improved genetic sampling may enable future assessments to calculate estimates of effective population size (N_e) in LURS, which could be used to inform a species-specific recovery target. Genetic recaptures should be investigated and incorporated into future assessments where genetic data is available. Future recovery targets for Lake Utopia Rainbow Smelt should consider whole-lake population models to better reflect the inter-connected relationship between the two populations.

Mark-recapture using fin clipping has limitations because fin clips are not permanent, can be missed during sampling, and cannot be tied to individuals. The range of unique marks is limited by the number of fins available for clipping, thereby preventing numerous consecutive nights of mark-recapture in a single stream. When clips are collected for genetic analysis, the type of fin clipped can affect the quality of the sample. Refining marking methods would aid in calculating more accurate abundance estimates, particularly where the timeframe for conducting mark-recapture work is likely to remain limited to 2-3 nights a year for future assessments. The LURS responded well to preliminary PIT tagging trials, with no observed short-term behavioral changes or direct evidence of tagging mortality in the 99 individuals tagged as part of this study. PIT tagging is a well-established method for tracking fish survival and movement, with numerous studies successfully using the technology in Rainbow Smelt (e.g., Landsman and van den Heuvel 2017, Enterline et al. 2020). PIT tags reduce the chance of marks being missed during recapture, or taggers mistakenly confusing marking location between marking events. PIT tags can be retained long term (often for the animal's entire life) and are uniquely coded, allowing for each tag to be linked to individual metadata. This method opens the possibility for investigating more complex questions, such as year-to-year recapture, spawning periodicity, within-season repeat spawning, or movement between spawning streams. Coupled with genetic and length-at-age data, this practice may help inform future population abundance estimates. A more comprehensive assessment of tagging effect and rate of tag loss in LURS should be conducted so that it can be included in mark-recapture calculations.

CONCLUSIONS AND ADVICE

Abundance estimates for LURS LbP remain variable between sampling nights and between study years; however, the Peterson abundance estimate of 25,014 on April 12, 2023 and 29,093 on April 13, 2023 demonstrate that the population has met the updated recovery abundance objective proposed by DFO (2018) of 5,000 adults in Mill Lake Stream observed on nights of peak spawning. In application, 5,000 fish need to be quantitatively estimated on at least one night of sampling during the early spawning run in Mill Lake Stream in order to meet this target, as the exact timing of peak spawning is not known. Future updates to the LURS recovery strategy should reflect this discrepancy in wording.

Large-bodied and small-bodied populations remain genetically distinguishable. The results support conclusions based on historical samples that there is hybridization and introgression between the sympatric species pair. Introgression is bidirectional; however, gene flow is predominately from SbP to LbP as evidenced by occurrence of ecologically “large-bodied” individuals that are genetically intermediate. Current data are insufficient to assess the rate of change and degree of introgressive hybridization between the LURS sympatric species pair. Limited sampling and changes to the genetic marker panel make it difficult to confidently disentangle sampling biases from observed genetic change. Similarly, genetic data do not provide a clear length cutoff to distinguish LbP and SbP, and thus do not lend support for one FL criterion over another. Therefore, it is recommended that the timing and location of the spawning run should be used as the first indicators of population identity, and that the updated recovery abundance objective of 5,000 spawners be applied to nightly estimate of all early spawning fish in Mill Lake Stream until improved genetic and morphological metrics are developed to differentiate the LbP and SbP. Because lack of certainty in the proportion of LbP smelt that make up the early (March to mid-April) spawning run of Mill Lake Stream can result in over-estimated abundance, the three existing FL criteria can be used to provide a range of more or less conservative nightly estimates. While neither scaled estimate can be treated as being the most accurate, the range of estimates can be interpreted together to assess whether the target abundance is being met.

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APPENDIX A

Genetic Analysis Methodology

Fin clips (UCF: upper caudal or ADF: adipose) were collected from 100 Rainbow Smelt captured in Mill Lake Stream (MLS) on April 11-12, 2023. Tissue was preserved in 95% ethanol. Two samples were lost in transit to the Marine Gene Probe Lab. Genomic DNA was extracted from tissue using a silica-based procedure (Elphinstone et al. 2003) or, where the silica-based procedure failed to generate DNA of sufficient quantity and/or quality, a modified phenol-chloroform (PCI) procedure (Sambrook et al. 1989). DNA was visualized on a 1% agarose gel (0.5X TBE) to assess individual sample quality (i.e., presence of high molecular weight DNA). DNA of sufficient quantity and quality was extracted from 92 samples.

Samples were genotyped using a panel of 75 microsatellite loci and a DNA sequencing-based approach as outlined in Bradbury et al. (2018). The panel consisted of 70 loci developed for next-generation sequencing (NGS) and five legacy loci adapted for NGS. Loci were amplified in three multiplex polymerase chain reactions (PCRs), performed in 3.5 µL volumes using Q5 High-Fidelity 2X Mastermix (New England Biolabs (NEB) Ipswich, MA, USA, 1.75 µL), 0.35 µL Oligo Mix (1.0 µmol/L each oligonucleotide), 0.2 µL molecular-grade water, and 1.2 µL DNA normalized to 2ng/µL. PCRs were conducted on Eppendorf (Hamburg, Germany) Mastercycler ep 384 PCR machines using the following parameters: 98 °C for 4 min, followed by 25 cycles of 94 °C 30 s, 59 °C 1 min, 72 °C 60 s, with a final extension at 72 °C for 30 min. Multiplex PCR products were pooled (per sample) in equal ratios. Indexing sequences were then added to the multiplex PCR pools using an index PCR. Indexing PCRs were performed in 5 µL volumes with 0.26 U Phusion Taq DNA polymerase (NEB), 0.5 µL Thermopol 10x buffer (NEB), 0.5 µL of 0.2 nM each dNTPs, 0.5 µL each of 0.2 µM Index_1 and 0.2 µM Index_2 oligonucleotides (Illumina annealing adapter sequence, barcode, and sequencing primer), and 0.5 µL of multiplex-PCR product. Cycling parameters were: 95 °C for 2 min, followed by 20 cycles of 95 °C 20 s, 63 °C 60 s, 72 °C 60 s, with a final extension at 72 °C for 10 min. Indexed PCR products were pooled in equal volume amounts and 50 µL of pooled library was cleaned using Illumina Purification Beads (IPB, Illumina, San Diego, CA, USA) magnetic beads (2:1 bead: DNA library). Libraries were quantified using NEBNext Library Quantification Kit (NEB) for Illumina on a Roche Light Cycler (LC) 480 qPCR instrument using 7.5 µL volumes with 4.5 µL NEB Library Quant 2X Master Mix, 1 µL of molecular-grade water, and 2 µL of 100,000X diluted DNA library. Libraries were sequenced at 9 pM concentration using an Illumina MiSeq DNA sequencer with v2 chemistry (2 x 150 bp: Illumina, San Diego, CA), with a target depth of 1,000 reads per individual per locus. Individuals were demultiplexed with the MISEQ SEQUENCE ANALYSIS software. Genotypes were scored using MEGASAT (Zhan et al. 2017). One Rainbow Smelt sample from Lake Utopia that had previously been genotyped with the legacy microsatellite loci was used as a positive control to ensure comparability of genotypes obtained in this study with microsatellite genotypes previously obtained by the Marine Gene Probe Lab. A redundant sample was included as an additional positive control. A blank was included as a negative control.

Six loci failed to amplify, of which four were legacy loci. Seven samples failed to amplify (0 loci genotyped) and were excluded from downstream analysis; failure rate was higher for ADF than UCF samples. Eight genotypic matches were identified using ALLELEMATCH (Galpern et al. 2012). All putative matches had a score < 0.85. Genotypes of putative matches were manually inspected and determined to be unique.

The unique multilocus microsatellite genotypes obtained from 2023 samples ($n = 85$) were analyzed together with existing multilocus microsatellite genotypes from 214 Rainbow Smelt sampled from Lake Utopia in 2017 and 2018. This dataset, containing 70 microsatellite loci, was filtered for missingness: an additional six loci were excluded due to low variability using the *isPoly* function in the R package ADEGENT (Jombart, 2008) with default value for the parameter *thres* (0.01) and individuals with > 50% missing data (2 individuals) were removed from downstream analysis.

Principal component analysis (PCA), as implemented in the R package ADE4 (Dray & Drafour, 2007), was used to investigate the possibility of genetic structure among the samples. Bayesian clustering was performed using STRUCTURE v2.3.4 (Pritchard et al., 2000), as implemented through PARALLELSTRUCTURE (Besnier & Glover, 2013), to infer the number of distinct groups present. Ten independent Markov chain Monte Carlo (MCMC) runs using a burn-in of 100,000 and 500,000 iterations were performed for each value of K ranging from 2 to 4. The optimal number of clusters (K) was inferred using the DK selection method (Evanno et al. 2005) in STRUCTUREHARVESTER (Earl & Vonholdt 2012). Mean Q-values, admixture proportions of individuals, for each K value were plotted using modified R package STRATAG (Archer et al., 2017). In instances where hybridization between two groups occurs, intermediate Q-values may represent hybrid individuals. Associations between Q-values and body size were explored using linear regression. Tests for Hardy-Weinberg equilibrium (HWE) and F-statistics were calculated using the R packages PEGAS (Paradis, 2010) and HIERFSTAT (Goudet and Jombart 2022).

Table A1. Per locus fixation index (Fst) between Lake Utopia Rainbow Smelt large-bodied and small-bodied populations. Individuals from the small stream that were genotypically LbP, individuals from the large stream that were genotypically SbP, and admixed individuals are excluded from this analysis.

Locus	Fst
mpx1.33359	-0.0018
mpx1.51518	-0.0016
mpx1.63512	-0.0015
mpx2.43799	-0.0011
mpx2.8744	-0.0008
mpx1.49321	-0.0006
mpx1.64489	0.000
mpx2.19470	0.0001
mpx2.21975	0.0002
mpx3.27233	0.0002
mpx1.2368	0.0011
mpx1.43912	0.0012
mpx1.29750	0.0013
mpx1.44955	0.0014
mpx2.15256	0.0021

Locus	Fst
mpx1.44789	0.003
mpx2.48716	0.0034
mpx2.28203	0.0035
mpx3.38186	0.0044
mpx1.63377	0.0049
mpx2.65032	0.0049
mpx2.1813	0.0075
mpx2.6137	0.008
mpx1.29560	0.0086
mpx1.13341	0.0104
mpx1.30938	0.0133
mpx3.45969	0.0133
mpx1.8743	0.0156
mpx3.24318	0.0161
mpx3.47741	0.0163
mpx2.40959	0.0181
mpx1.64839	0.0182
mpx3.40758	0.0186
mpx2.14187	0.0292
mpx2.53750	0.0302
mpx1.27178	0.0304
mpx2.18581	0.0323
mpx3.Omo1	0.0329
mpx1.10206	0.0383
mpx2.24181	0.0418
mpx1.63471	0.046
mpx3.48088	0.0475
mpx2.4922	0.0477
mpx1.10280	0.0485
mpx3.49454	0.0537
mpx2.41276	0.0585

Locus	Fst
mpx3.8139	0.06
mpx2.60030	0.0605
mpx2.61749	0.0629
mpx3.22606	0.0663
mpx1.47001	0.0681
mpx2.16650	0.0727
mpx1.33692	0.077
mpx1.48656	0.0848
mpx2.31860	0.0906
mpx3.62317	0.1142
mpx2.37630	0.1194
mpx3.33791	0.1254
mpx2.22534	0.1457
mpx2.1534	0.168
mpx2.21158	0.2229
mpx2.45138	0.2647
mpx1.57397	0.3035

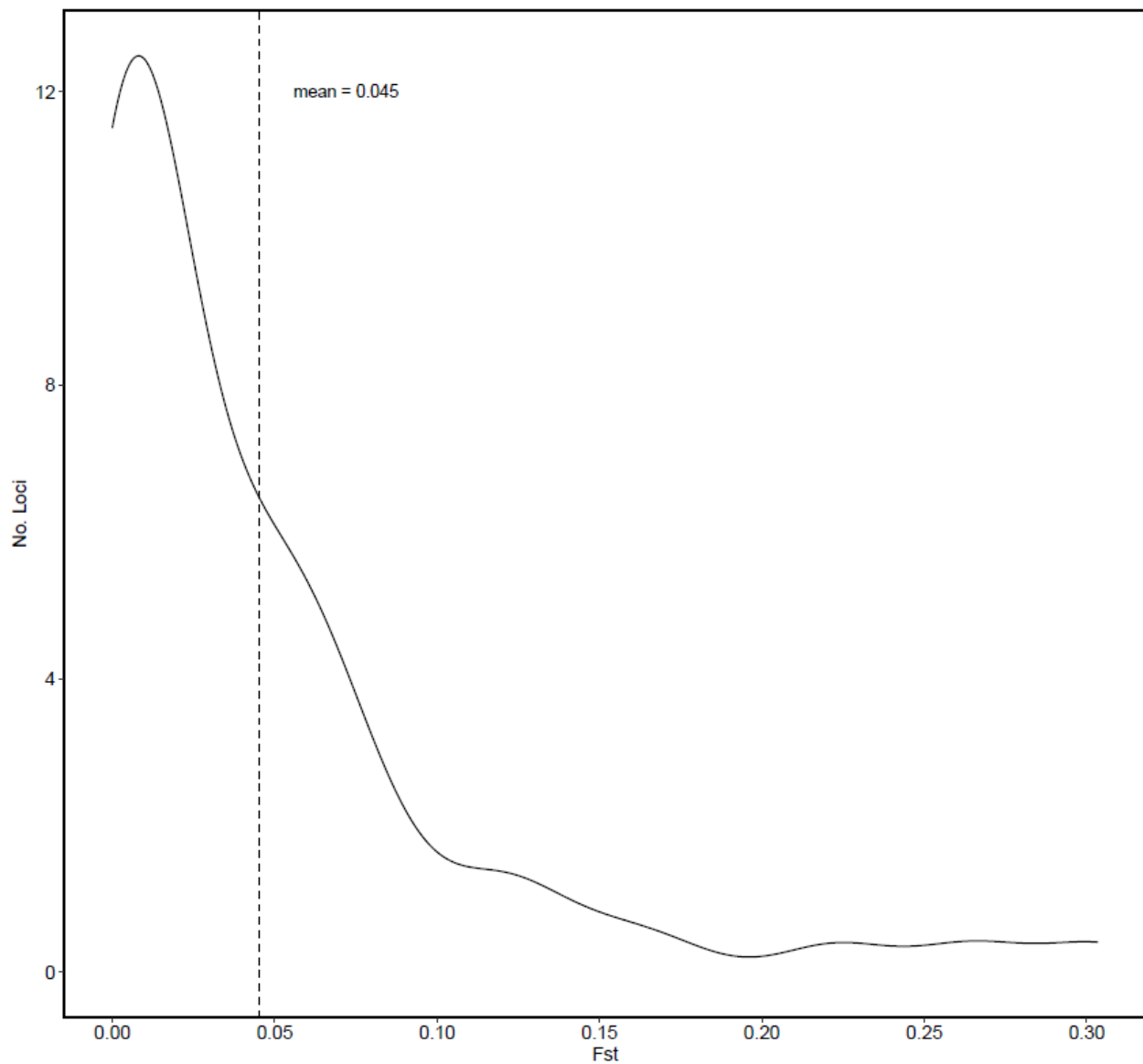


Figure A1. Per locus F_{st} between Lake Utopia Rainbow Smelt large-bodied and small-bodied populations. Individuals from the small stream that were genotypically LbP, individuals from the large stream that were genotypically SbP, and admixed individuals are excluded from this analysis.

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