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The rainbow smelt, *Osmerus mordax,* complex of Lake Utopia: threatened or misunderstood?

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Synopsis

We report on the spawning ecology, genetic characteristics, and predation threats to spawning groups of rainbow smelt, *Osmerus mordax*, in Lake Utopia, New Brunswick where a dwarf morpho-type has been listed as a threatened species. Two spawning groups in three inlet streams had been previously identified; we observed three groups using four inlet streams. The earliest group was the largest in body size (12–29 cm fork length (FL)), lowest in numbers (~1 000), and completed spawning approximately two weeks before the second group. The early spawners were previously identified as the normal morpho-type, but we now classify these as a giant morpho-type. The second group spawned in three different streams. They were intermediate in body size (10–15 cm FL) and numbers (~10 000). The dwarf group began spawning as the intermediate group completed spawning and within the same three streams. The dwarfs were numerous (~1 000 000), small in size (<12 cm), and with higher gill raker counts. Microsatellite analyses suggested that gene flow among groups occurred, but genetic divergence was high and genetic separation among populations of the same group among streams and within a stream occurred. Stable isotopes and stomach contents indicated the dwarf group were likely consumed by a variety of fishes, but they were not the sole food resource of any predator including a population of landlocked salmon. These are some of the complexities of smelt ecology, but there are clearly life history tactics that we do not yet understand.

Introduction

The rainbow smelt, *Osmerus mordax*, is one of the several species of north temperate, freshwater fishes that display a complex of morpho-types occurring in sympatry (e.g., Ryman et al. 1979, Skulason et al. 1996, Bernatchez 1997). Normal and dwarf morpho-types have been described for lake-dwelling rainbow smelt of North America (Kendall 1927, Nellbring 1989). Additionally, a larger body-sized, anadromous form exists (Frechet et al. 1983), although these populations have not been reported to occur in sympatry with other forms. Within anadromous populations, dwarf forms have been suggested (McKenzie 1964) and subpopulations separated by distinct spawning sites/times occur (Baby et al. 1991, Bernatchez & Martin 1996).

The lacustrine populations have provided strong evidence of distinct morpho-types existing in sympatry. Coexisting normal and dwarf forms were described in the early literature (see review by Lanteigne & McAllister 1983). Most recent studies describe morphological differences in body form

(Bridges & Delisle 1974, Copeman 1977, Copeman & McAllister 1978). Rupp & Redmond (1966) using experimental transplants concluded that body morphology was controlled by physical and biological characteristics of the environment more than genotypic differences between normal and dwarf forms. Copeman & McAllister (1978) re-examined the transplanted populations after several more generations and observed that the two forms were again appearing. A search for genetic differences found mixed levels of genetic separation between forms and concluded that smelt were monophyletic (Taylor & Bentzen 1993a,b).

Both morpho-types have been described in Lake Utopia of southwestern New Brunswick. First described in the early 1900s, a normal form was observed to be larger, spawning earlier, and using different tributary streams for spawning and incubation. The normal form begins to spawn as the lake becomes devoid of ice in spring (Lanteigne & McAllister 1983) with local fishers' accounts of spawning when the lake is still ice bound in some years. Spawning was reported in the two largest inlet tributaries located in the northeast of the lake. Dwarf smelt spawn in late April and May in two smaller streams located in the northwest.

The existing data suggest normal smelt range in body length from about 150 to 250 mm with 31–33 gill rakers. Dwarf smelt are 80–150 mm long with 33–37 gill rakers (Lanteigne & McAllister 1983, Taylor & Bentzen 1993a,b). An examination of mtDNA and mini-satellite DNA of normal and dwarf forms as well as allopatric, sympatric, and anadromous populations from across the region concluded the sympatric forms originated repeatedly within individual systems (Taylor & Bentzen 1993a,b). In Lake Utopia, the normal and dwarf forms were genetically more similar than differences between all dwarf and normal forms from across the region.

The few documented occurrences of the dwarf form combined with its genetic distinctness, apparent limited number of spawners, and apparent threats of predation and fishing pressure, were sufficient evidence for the Lake Utopia dwarf smelt to be classified as a 'threatened' species in Canada[1] (Taylor & Bentzen 1993b). However, there are ecological gaps in the knowledge base for the smelt of Lake Utopia and lacustrine populations in general. In Lake Utopia for example, there has been no complete description of the spawning ecology or habitats of the smelts. Collections in the lake have focused on four tributaries, yet there are at least two more inlet streams in the northern portion of the lake and a total of 17 inlet streams that represent potential spawning and incubation habitats. In addition, sampling of smelt has been of short duration (1 or 2 nights), small sample sizes (<50 smelt), and targeted the known spawners. This sampling may have biased results if the spawning period is longer or other streams support spawning and successful incubation. In addition, the lake is connected to the Magaguadavic River, which has a series of lakes where smelt exist and a fishway to the Bay of Fundy where anadromous smelt are common.

A dipnet fishery for smelt has occurred over many years in Lake Utopia and the northeast streams, Trout and Mill Streams, are the primary locations of fishing (i.e., the apparent normal smelt are targeted; Cronin, P. New Brunswick Department of Natural Resources and Energy, pers. comm.). A population of landlocked Atlantic salmon, *Salmo salar*, also inhabits the lake and supports an important recreational fishery. The population has received supplemental stocking of salmon 12 times since 1984 and presently is stocked at a rate of 3 400 salmon every other year (Collet et al. 1999). Landlocked salmon can target smelt as their principle prey in lakes and fisheries managers specifically use lakes with smelt populations for developing salmon fisheries, or they introduce smelt to enhance the forage base for salmon (Sayers et al. 1989, Kirn & Labar 1996). In Lake Utopia, there is no information on the diet of salmon or the trophic interactions of the fish community in general.

It is impossible to assess the threats accurately to the dwarf smelt of Lake Utopia without a more complete understanding of the spawning ecology and trophic status of the smelt complex. Moreover, such information may lead to resolution of questions concerning the ecological adaptability and evolutionary history of the species. In this context, our first objective was to describe the spawning ecology and genetic characteristics of the spawning groups of smelt in Lake Utopia. The second objective was to examine the trophic relationships within the fish community and assess the potential threats of predation for dwarf smelt in Lake Utopia. A third objective was to test the null hypothesis of no genetic differentiation among dwarf smelt spawning in different tributaries and assess their relationships with smelt of other forms in the lake.

[1] COSEWIC 2001. The Lake Utopia Dwarf Smelt. http://www.speciesatrisk.gc.ca.

Methods

Study area

Lake Utopia is located in southwestern New Brunswick, Canada (45°10′, 66°47′, Figure 1). It is a 1 400 ha oligotrophic lake averaging 11 m in depth. It is connected by its outflow to the Magaguadavic River that flows into the Bay of Fundy at St. George, New Brunswick. An unsurpassable waterfall exists at St. George where a fishway exists although there is no evidence that anadromous smelt can traverse the fishway (Carr, J. Atlantic Salmon Federation, St. Andrew's, N.B., pers. comm.). The river also drains Magaguadavic and Digideguash Lakes with known populations of smelt, but dwarf forms have not been reported.

In Lake Utopia, there are four inlet streams that have been identified as spawning areas for smelt (Figure 1). Mill (Lake) Stream and Trout (Lake) Stream are outflows from smaller lakes. A dam at Mill Lake prohibits upstream migration into the lake. Smelt can be captured moving upstream near the mouth of Trout Stream, which does not have apparent suitable habitat for smelt spawning and incubation. An inlet stream to Trout Lake may provide appropriate habitat (Spear Brook). Mill Stream averages 4 m wide and <1 m in depth.

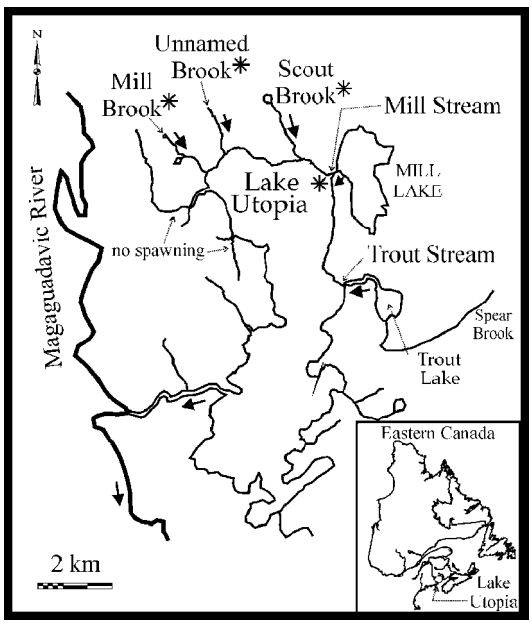

Figure 1. Lake Utopia and its tributaries. Spawning and incubation habitats are identified by an *.

Trout Stream averages 10 m wide with slow moving water and deeper pools. These streams were sampled in previous studies for what were described as 'normal' smelt in the system.

We surveyed a number of additional streams located around the north half of the lake (Figure 1). Two of these were previously described as Mill Brook (Lanteigne & McAllister 1983 – or Smelt Brook, Taylor & Bentzen 1993b) and Unnamed 'A' Brook (Taylor & Bentzen 1993b). A fourth stream, Scout (Second) Brook, with later spawning smelt was also discovered (Figure 1). All three of these inlet streams are small, ≤1 m wide with <500 m of accessible habitats. They drain upland, mostly forested areas that are undeveloped.

Field sampling

In 1998, sampling of spawning smelt began in mid-April and continued until mid-May. The objective was to identify streams used for spawning and examine basic morphometrics and spawning timing to match spawner groups to earlier studies. Sampling occurred weekly, but no smelt were encountered in Mill and Trout Streams and Scout Brook was not discovered until the completion of the spawning run.

In 1999, sampling began in late March while the lake was still ice covered. Some smelt were initially collected in Mill Stream, but none were encountered during the spring in Trout Stream. Bi-weekly sampling continued into mid-May. Approximately 30 females and 30 males roughly sorted into large and small smelt (four groups of 15 smelt) were collected by dip net and seine. Length (nearest 1 mm) and live wet weight (nearest 0.1 g) were recorded. After euthanization, individual fish were stored in 95% EtOH for later counts of lateral line scales (left side) and gill rakers (first gill arch, left side).

In the laboratory, otoliths were removed and stored dry. They were eventually mounted in epoxy, cut, ground, and aged (years) by three readers. Age was assigned only when there was a consensus among readers (n = 208 smelt in total). Tissue samples were taken and stored in 95% EtOH for later analyses of stable isotopes of carbon and nitrogen (epaxial muscle – Stable Isotopes in Nature Laboratory, Canadian Rivers Institute, University of New Brunswick) and microsatellite DNA (caudal fin – Laval University).

Estimates of spawner abundance were attempted for each stream and evening of sampling in 1999. A barrier net was placed across the stream mouth and seine net sweeps (1–3 over 1.5 h) of the stream mouth and

lake shoreline were made. Smelt were marked with fin clips (unique to each stream and evening) and returned immediately to the water. Schnabel estimates of abundances were calculated (Ricker 1975). These estimates represented the numbers of smelt entering the stream during the period of actual sampling (typically 1.5 h). From the 1998 and 1999 surveys, it was observed that spawners entered the streams from 21:30 to 04:30 h with an approximate normal distribution of numbers over time each evening, i.e., the peak was 00:00 to 01:30 h. From the distribution, evening and stream specific estimates for five time periods were summed to provide the total evening abundance for each stream. It is expected that this method underestimated the actual abundance. If too few smelt were encountered for a mark-recapture estimate, then numbers were estimated from actual counts of smelt observed in the stream. There were too many smelt in Unnamed Brook to enumerate with mark-recapture techniques on 3 May 1999. The estimate that evening was made by quickly isolating 1 m sections of the stream, counting all smelt within the section, and extrapolating across the entire length of accessible stream habitat.

In 2000, the earlier spawners detected in previous years in Mill Stream were targeted for tissue sampling for stable isotope and genetic analyses. No other analyses were undertaken.

The lake environment from March to May was generally similar among years based on lake levels: water levels were lowest in March in 1998; peak levels occurred in early April in each year; and levels declined during the remainder of smelt spawning except in 2000 when levels remained high into early May (J.D. Irving Limited, unpubl. data).

Comparisons of FL ($\log_{10}$ transformed) were made by an analysis of covariance (ANCOVA, $\alpha = 0.05$; SAS Institute Inc. 1990) with age as a covariate. Gill raker and scale counts ($\log_{10}$ transformed) were compared with an ANCOVA with FL as a covariate ($\alpha = 0.05$). Age was compared among sites by a one-way analysis of variance (ANOVA, $\alpha = 0.05$). A Tukey pairwise comparison of streams and dates followed analyses when appropriate ($\alpha = 0.05$). Analyses of stable isotopes of carbon and nitrogen were made graphically and with a cluster analysis of the Bray–Curtis similarity matrix (Primer version 6.0). Because of the potential of age-biased measures of morphological characteristics, examinations of age-3 smelt were also undertaken.

For isotope analyses, tissue samples were dried at 50°C for 48 h and ground into a fine powder with a mortar and pestle. Approximately 0.2 mg aliquots were packed into 3 mm × 5 mm tin cups. Samples were combusted to gas using a Thermoquest NC 2500 elemental analyzer, and gases were submitted via a continuous flow of helium to a Finnigan MAT Delta Plus isotope-ratio mass spectrometer. Results of $^{13}C:^{12}C$ and $^{15}N:^{14}N$ isotope ratios are reported as:

$$\delta X = [(R_{sample}/R_{standard}) - 1]*1000$$

where $X = {}^{15}N$ or ^{13}C and $R = {}^{15}N/{}^{14}N$ or $^{13}C/^{12}C$. Values are said to be enriched when values are more positive than a comparison sample and depleted when more negative. Replicates of commercially available isotope standards yielded results that were both accurate and precise (International Atomic Energy Agency (IAEA)). Ten percent of the samples were analysed in duplicate.

Genetic analysis

Total DNA was extracted from the muscle tissues using a standard proteinase K phenol–chloroform protocol (Sambrook et al. 1989). The extent of genetic differentiation and gene flow between ecotypes on each spawning ground was assessed using five microsatellite loci: four developed from rainbow smelt (Saint-Laurent & Bernatchez, unpubl. data) and one for the eulachon, *Thaleichthys pacificus* (McLean & Taylor 2001; Table 1; Appendix 1). Rainbow smelt specific loci were developed as detailed in Wirth et al. (1999). Microsatellite polymorphism was analysed by the fluorescent dye detection method. One of the primers of each locus was 5′-labelled with three different dye (colours); HEX (yellow) for *OSMO-Lav 12* and *OSMO-Lav 16*, TET (green) for *OSMO-Lav 45* and 6-FAM (blue) for *OSMO-Lav 16* and *TPA 26*. PCR was carried out in a 10 µl reaction volume containing 1 U of *Taq* polymerase, 1.0 µl of reaction buffer (10 mM Tris–HCl (pH 9.0), 1.5 mM $MgCl_2$, 0.1% Triton X-100, 50 mM KCl), 750 µmol of dNTPs, between 25 and 50 ng template DNA, and 0.03 pmol of each primer. Simplex and duplex (for *OSMO-Lav 12–OSMO-Lav 16*) PCR was used and performed in a Perkin-Elmer 9600 thermocycler (version 2.01) with the following profile: an initial denaturing step of 5 min at 95°C, followed by 30 cycles of 30 s at 94°C, 30 s at annealing temperature and 30 s at 72°C. Samples were heated to 95°C for 5 min and chilled on ice prior gel loading.

Electrophoresis procedures were conducted on a denaturing 5% polyacrylamide gel with an ABI 377 automated sequencer/gene scanner and analysis

Table 1. Allelic variability at five microsatellite loci from sympatric rainbow smelt ecotype of Lake Utopia. Number of samples successfully used for genetic analysis (N), number of alleles at each locus (A), mean number of alleles at six loci (Am) most common allele (Ac; in base pairs), frequencies of the most commun alleles (Fc), range of allele size (Ar), observed heterozygosity (Ho), gene diversity (He) at each locus and mean allelic diversity (Am) and heterozygosity (Hm) within each sample. d indicates significant heterozygote deficit following the sequential bonferoni correction ($\alpha = 0.005$, k = 30), whereas D indicates significant heterozygote deficit across sample (*OSMO-Lav45*) and across loci within sample (Unnamed Brook, dwarf) (global test, Fisher's method).

Sample		*OSMO-Lav16*	*OSMO-Lav157*	*OSMO-Lav12*	*OSMO-Lav45* (D)	*Tpa 26*	Am	Hm
Mill Stream	N	30	30	30	30	30		
giant (00)	A	3	6	10	11	9	8	
	Ac	80	225	168	233	217		
	Fc	0.9	0.6	0.36	0.34	0.5		
	Ar	80–84	221–239	152–180	195–289	215–231		
	He	0.10	0.56	0.78	0.81	0.65		0.58
	Ho	0.10	0.57	0.84	0.80	0.66		0.59
Mill Stream	N	12	12	12	11	12		
giant (99)	A	3	3	8	6	3	5	
	Ac	80	217	162	215	217		
	Fc	0.58	0.371	0.18	0.33	0.59		
	Ar	80–84	217–225	152–184	205–265	217–227		
	He	0.57	0.66	0.85	0.75	0.55		0.67
	Ho	0.59	0.85	0.81	0.50d	0.58		0.69
Mill Brook	N	30	30	30	30	30		
dwarf	A	6	6	16	18	6	10	
	Ac	80	221	170	215	217		
	Fc	0.6	0.41	0.12	0.18	0.56		
	Ar	74–88	217–231	150–184	189–277	213–221		
	He	0.56	0.66	0.91	0.91	0.61		0.73
	Ho	0.6	0.67	0.9	0.40d	0.62		0.74
Mill Brook	N	30	30	30	30	30		
intermediate	A	6	7	17	21	5	11	
	Ac	80	225	164	209	217		
	Fc	0.65	0.36	0.14	0.1	0.5		
	Ar	74–84	217–233	152–188	137–285	213–221		
	He	0.53	0.72	0.9	0.93	0.6		0.74
	He	0.55	0.72	0.92	0.53d	0.8		0.72
Scout Brook	N	30	30	30	30	30		
dwarf	A	8	11	17	18	6	12	
	Ac	80	227	176	211	217		
	Fc	0.45	0.48	0.16	0.18	0.75		
	Ar	72–88	215–239	156–190	173–285	209–225		
	He	0.65	0.72	0.9	0.91	0.4		0.71
	He	0.66	0.73	0.93	0.62d	0.4		0.73
Scout Brook	N	30	30	30	30	30		
intermediate	A	4	6	12	14	2	7	
	Ac	80	223	168	217	217		
	Fc	0.8	0.44	0.19	0.17	0.92		
	Ar	74–82	221–231	138–188	205–279	217–225		
	He	0.31	0.73	0.84	0.87	0.14		0.59
	He	0.30	0.75	0.88	0.69d	0.16		0.60

Table 1. (*Continued*)

Sample		*OSM O-Lav16*	*OSMO-Lav157*	*OSMO-Lav12*	*OSMO-Lav45* (D)	*Tpa 26*	Am	Hm
Unnamed	N	30	30	30	30	30		
Brook dwarf	A	6	5	17	20	6	10	
	Ac	80	225	164	219	217		
	Fc	0.62	0.36	0.2	0.14	0.78		
	Ar	74–86	217–229	148–190	183–305	213–221		
	He	0.56	0.73	0.9	0.92	0.37		0.70
	He	0.67	0.60d	0.89	0.53d	0.34d		0.61D
Unnamed	N	30	30	30	30	30		
Brook	A	6	6	15	14	4	9	
intermediate	Ac	72	227	180	235	217		
	Fc	0.55	0.46	0.13	0.17	0.56		
	Ar	70–92	211–231	148–188	191–285	215–227		
	He	0.63	0.68	0.91	0.89	0.53		0.73
	He	0.64	0.8	0.93	0.85	0.53		0.74

software. Loading product consisted of 0.2 µl internal sizing standard (TAMRA 350 bp red colour) and 2 µl deionized formamide that were combined to 0.8 µl (0.3 µl for *TPA 14–TPA 26*) of the PCR reaction. Gels were run for 2 h at 3 000 V. Allelic size was determined (using GENESCAN software version 2.1; ABI 1996a) by reference to the internal sizing standard and by comparison with the same standard sample of known allelic size that was run on each gel. The final scoring of allelic size and tabulation of data for each locus were conducted with the GENOTYPER software, version 2.0 (ABI 1996b).

Genetic polymorphism was quantified by the number of alleles per locus (A), observed heterozygosity (Ho), and unbiased gene diversity (He) with the GENETIX software, version 4.02 (Belkhir et al. 2000). Samples were tested for conformity with Hardy–Weinberg equilibrium under the alternative hypotheses of heterozygote excess or heterozygote deficiency with the score test (U-test) described in Rousset & Raymond (1995). Global tests across loci and across samples were also made with GENEPOP version 3.1d (Rousset & Raymond 1995).

The null hypothesis of no difference in allelic frequency distribution at each locus between all sample pairs was tested with the Markov chain method to obtain unbiased estimates of Fisher's exact test through 1 000 iterations (Guo & Thompson 1992) available in GENEPOP 3.1d. Probability values over all loci were obtained by the Fisher method (Sokal & Rohlf 1995). The extent of genetic differentiation between each population unit (defined as group of samples for which the above null hypothesis was not rejected) was estimated from the θ estimator (Weir & Cockerham 1984) of F_{st} with F-stat 1.2 (Goudet & Raymond 1996). Statistical significance levels in all the above tests were adjusted for multiple comparisons with a sequential Bonferroni adjustment (Rice 1989) to minimize Type 1 errors.

The hierarchical partitioning of genetic diversity among populations was quantified with an analysis of variance framework (AMOVA) using variance in allelic frequency (Weir & Cockerham 1984) in the program ARLEQUIN, version 1.1 (Schneider et al. 1997). By this procedure, we performed two hierarchical analyses of gene diversity in order to assess the component of genetic variance corresponding to: (i) variance among individuals within sample; (ii) among samples within groups (F_{SC}); and (iii) among groups (F_{CT}). In the first analysis, tributary of origin was considered the first grouping level, whereas the date of sampling was the grouping level in the second analysis.

We then assessed relationships among all populations analysed by chord distances (D_{CE}; Cavalli-Sforza & Edwards 1967), which assume pure genetic drift. Pairwise distances were computed with the GENEDIST program included in the PHYLIP computer package, version 3.57c (Felsenstein 1993). The matrix of pairwise distances obtained for D_{CE} was used to construct a population phenogram (Figure 2) with the neighbour-joining algorithm available in PHYLIP. Confidence levels on tree topology were estimated by the percentage of 2 000 bootstraps performed from re-sampling loci, and compiled with the CONSENSE program of PHYLIP.

Results

Spawning chronology

The most complete year of sampling took place in 1999. Spawning smelt were regularly encountered in four of six inlet streams in the northern portion of Lake Utopia (Figure 1). The earliest spawners were encountered in Mill Stream. They began spawning when stream temperatures were $\leqslant 6^{\circ}$C and the lake was ice covered (6°C) in the years they were observed (1999 and 2000). Their period of spawning ended by 2–5 April 1999 and may have lasted a total of 7–14 days, but numbers were sufficiently low that accurate estimates of abundance were not possible (i.e., no recaptures were made and estimates of removals by fishers was impossible).

After the completion of this early run, no smelt were observed in any stream until mid- to late-April, and no smelt were observed in Mill Stream for the remainder of the spawning period in any year. By 21 April 1999, spawning smelt were observed in Scout and Smelt Brooks (Table 2). Stream water temperatures were $\leqslant 6^{\circ}$C. Smelt continued to move into these streams until mid-May. Mill Brook was used from 26 April to 13 May at least. Stream temperatures were $\leqslant 9^{\circ}$C during the spawning period. There were regular occurrences of the mid- to late-spawning smelt moving between streams in one evening and between successive sampling dates. This occurred most often between Smelt Brook and Mill Brook.

In 1998, spawning smelt were captured in Mill Brook from 20 April until 5 May. Spawners were subsequently discovered in Unnamed and Scout Brooks on 5 May.

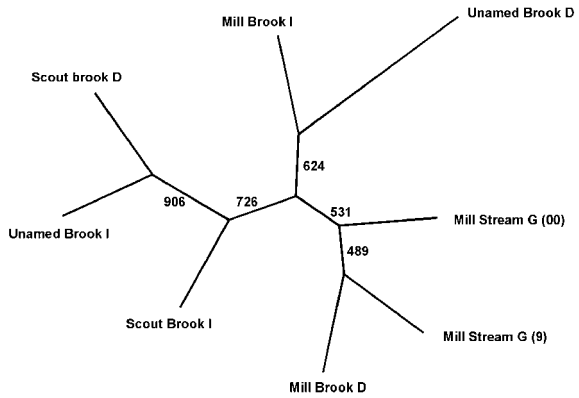

Figure 2. Population phenogram developed using the neighbour-joining algorithm (PHYLIP).

Body morphology

The early spawning, Mill Stream smelt were the largest in body size ranging from 12 to 26 cm FL in 1999 (Tukey test, $p < 0.05$; Figure 3 – smelt as large as 29.0 cm FL were captured in 2000). Body sizes declined on average over the duration of the spawning period, although there was substantial overlap among sample sites and dates. By early May, the average size of spawning, female smelt had declined from 17.6 ± 2.7 (Mill Stream; average ± 1SD) to 11.3 ± 1.2 cm. Mill Stream smelt had the fewest gill rakers ($F = 0.64$, $p < 0.0$; Figure 4). Gill raker counts suggested Mill Brook smelt spawning on 26 April were more similar to later spawners than concurrently spawning smelt in Unnamed and Scout Brooks. Mill Stream smelt (earliest spawning) were the oldest on average (3.1 ± 0.7 years), although the difference was not statistically

Table 2. Single evening estimates of abundance and 95% confidence limits of spawning smelt in four stream of Lake Utopia in 1999.

	Abundance			
Date	Mill Stream	Scout Brook	Unnamed Brook	Mill Brook
Apr. 2	<500[1]	0	0	0
Apr. 15	0	0	0	0
Apr. 21	0	43 000	64 000	
		39 000–48 000	52 000–82 000	0
Apr. 26	0	< 500[1]	100 000[2]	45 000
				31 000–61 000
May 3	0	<100[1]	169 000	17 000
			130 000–237 000	15 000–19 000
May 13	0	0	<100[1]	<100[1]

[1]Too few smelt to estimate.
[2]Density-based estimate.

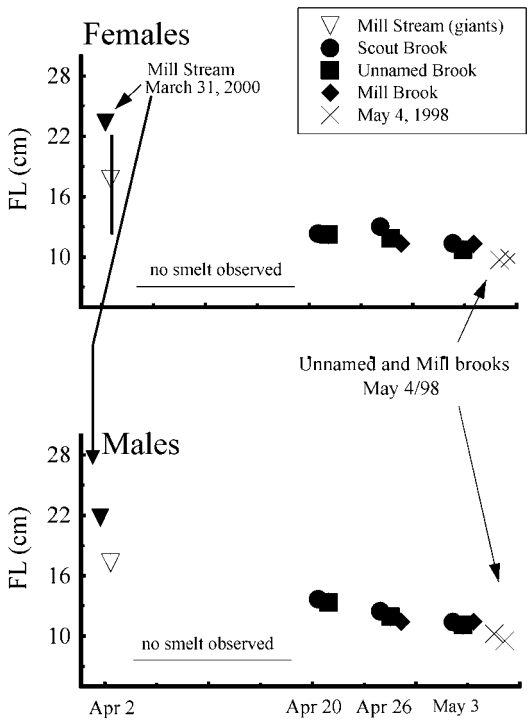

Figure 3. Size distribution of spawning smelt in Lake Utopia in 1998, 1999, and 2000.

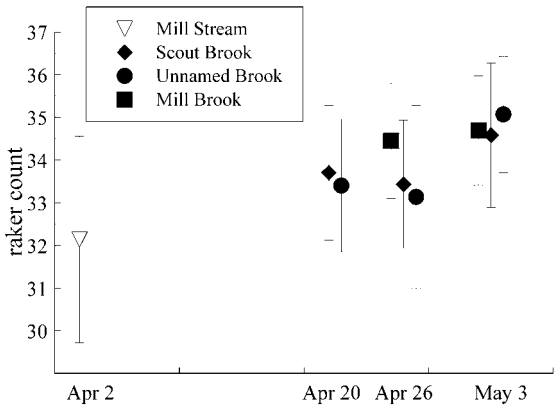

Figure 4. Gill raker counts for the smelt of Lake Utopia, 1999.

significant (overall average = 2.8 ± 0.6 years). Lateral line scale counts were consistent among sites and sampling dates (overall average = 64 ± 2). The overall trends remained the same for age-3 smelt only, i.e., early spawners were the largest and late spawners were smallest in size and gill raker counts increased from early to late spawners (Figure 5).

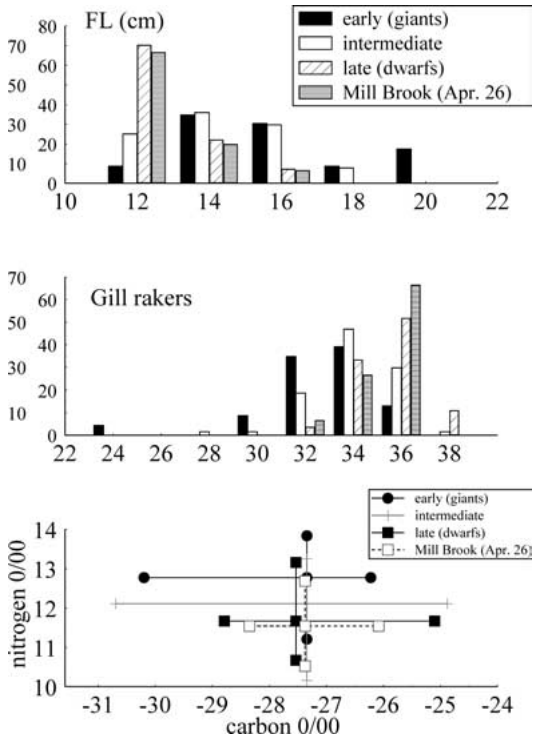

Figure 5. Size, gill raker counts, and stable isotopes of C and N of age-3 smelt of Lake Utopia, 1999.

Population abundance

The latest spawning group had the greatest abundance (Table 2). Single evening estimates were in the order of 100 000 suggesting a total population of 1–1.5 million spawners. The intermediate-spawning group had single evening estimates in the order of 50 000 suggesting a total population of 250 000–500 000. The early spawning Mill Stream group was substantially smaller and most probably numbered <5 000 spawners in total.

Trophic relationships

The fish community was relatively complex in Lake Utopia with 12 species encountered. Atlantic salmon (a landlocked population), brook trout, and smallmouth bass displayed enriched nitrogen and carbon signatures identifying them as potential predators of the late spawning, small smelt (Figure 6). Larger, predatory smelt were the only species with gut contents composed of smelt. The single gaspereau captured had a carbon signature of −18.5 0/00 and would be considered to have migrated to the lake from the marine environment.

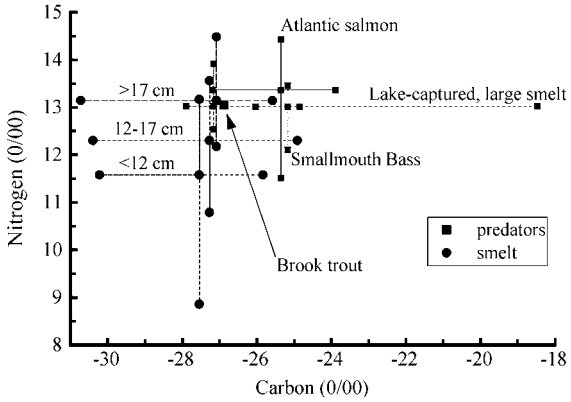

Figure 6. Trophic relationships among major predatory species (solid lines and squares) and spawning smelt of three size classes (dashed lines and solid circles) in Lake Utopia, 1999.

One large smelt captured offshore displayed a similar carbon signature (−18.4 0/00).

Genetic relationships

All microsatellite loci generally displayed high levels of polymorphism, with the total number of alleles per locus per sample varying between 3 and 21 (average = 10), and the mean gene diversity (He) per locus per sample varying between 0.10 and 0.93 (average = 0.68; Table 1). No significant departure from Hardy–Weinberg equilibrium was detected by multi-locus probability tests within each sample. However, a significant heterozygote deficiency was detected across sample for locus *Osmo-45*. This locus is the most variable, and had the widest allelic size range. It is therefore possible that this deficit was caused either by null alleles or miss-amplification of the largest alleles. Levels of intra-sample genetic diversity, expressed both in terms of number of alleles and gene diversity was generally very similar among samples, except for Mill Stream 'giant' and Scout Brook 'intermediate' smelts for which diversity was lower (Table 1). A t-test comparing mean number of alleles (A) and mean gene diversity (He) among each pair of parks for each locus revealed no significant differences in either mean values of number of alleles or in gene diversity.

All samples differed in their allelic composition at one or more loci (Figure 7). Consequently, homogeneity tests of allele frequency distributions revealed significant differences following Bonferroni sequential corrections in all pairwise sample comparisons (Table 3). This provided a first indication that each of the sample analysed represented a genetically distinct population. This was generally corroborated by θ estimates of population differentiation (Table 3). The average pairwise θ value was relatively high (average = 0.091), but varied substantially among comparisons (range = −0.010 and 0.233). The extent of differentiation between dwarf and intermediate smelt varied among tributaries. The lowest value was observed in Mill Brook. The extent of differentiation was intermediate in Scout Brook and highest in Unnamed Brook. Clearly, the giant smelt population from Mill Stream was the most genetically distinct from all others.

The AMOVAs did not reveal any significant pattern of hierarchical clustering (Table 4). The first AMOVA revealed that grouping by tributary of origin did not explain any significant component of total genetic variance. In contrast, the extent of genetic variance imputable to among populations within tributary was substantial and highly significant. Similarly, the component of genetic variance imputable to grouping by form (intermediate vs. dwarf) was null whereas genetic variance due to inter-population within form was important and highly significant (Table 3). Therefore, these results do not support either a more common origin of all smelt populations of a given form or more genetic exchange between populations within a given tributary relative to populations from other tributaries.

The topology of the population phenogram constructed from the pairwise D_{CE} matrix corroborated this pattern of population relationships; no apparent clustering either by form of tributary was observed (Figure 2).

Discussion

The evidence from the timing of spawning, body sizes, and gill raker counts, with some corroboration from trophic analyses has demonstrated that at least three identifiable groups compose the spawning population of the Lake Utopia smelt complex. Overall measures of genetic divergence among the groups were relatively high exhibiting greater differences than commonly reported among anadromous populations (e.g., Garant et al. 2000). In addition, genetic analyses indicated that two of these groups (dwarf and intermediate) each comprise several genetically distinct populations.

The first group is an early spawning, giant morphotype that used one inlet stream (Mill Stream) from a time when the lake was still ice covered in late March until the first week of April. This group appears to have been sampled in all earlier studies as 'normal' smelt.

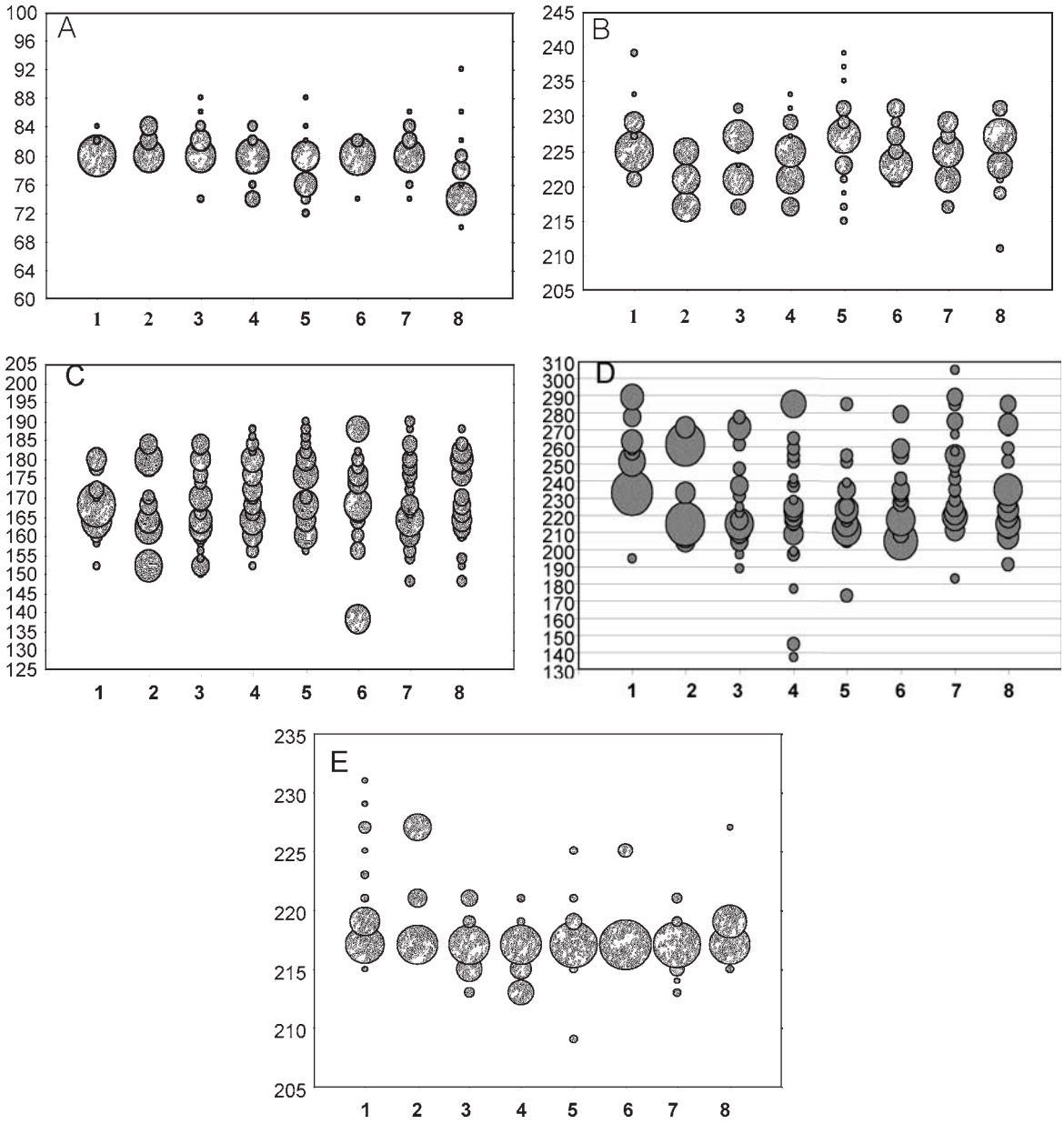

Figure 7. Allele frequency histogram of loci (A) *OSMO-Lav16*, (B) *OSMO-Lav157,* (C) *OSMO-Lav12*, (D) *OSMO-Lav45*, and (E) *Tpa-26.* Allele designation is in base pairs and size of circles is proportional to allele frequency within each sample. Populations: (1) Mill Stream giant (00); (2) Mill Stream giant (99); (3) Mill Brook dwarf; (4) Mill Brook intermediate; (5) Scout Brook dwarf; (6) Scout Brook intermediate; (7) Unnamed Brook dwarf; and (8) Unnamed Brook intermediate.

They were also previously sampled from a second stream, Trout Brook, but it produced very few smelt and not in all years of the present study. Interestingly, spawning in a tributary of Trout Stream's source lake was confirmed in 2002 (Currie, S. unpubl. data). An intermediate-sized group of smelt began spawning in three small, northwestern streams 1–5 km from the early spawning streams in mid-April. These smelt were most similar in body size to normal populations of smelt in other lakes (e.g., Lanteigne & McAllister 1983,

Table 3. Test of heterogeneity of allele frequency between samples (above main diagonal) over all loci with the Fisher's method and the level of significance adjusted after sequential Bonferroni correction (S = significant, $\alpha = 0.05$, k = 28). Below the main diagonal are the pairwise estimates of population differentiation (θ values, *statistically significant following sequential Bonferroni). G = giant, D = dwarf, and I = intermediate morph-types.

	1	2	3	4	5	6	7	8
1. Mill Stream G (1999)		S	S	S	S	S	S	S
2. Mill Stream G (2000)	0.070*		S	S	S	S	S	S
3. Mill Brook D	−0.010	0.159*		S	S	S	S	S
4. Mill Brook I	−0.010	0.089*	0.051*		S	S	S	S
5. Scout Brook D	0.058*	0.166*	0.057*	0.084*		S	S	S
6. Scout Brook I	0.061*	0.150*	0.100*	0.092*	0.079*		S	S
7. Unnamed Brook D	0.001	0.091*	0.052*	0.014*	0.070*	0.072*		S
8. Unnamed Brook I	0.118*	0.234*	0.120*	0.145*	0.078*	0.164*	0.155*	

Table 4. Hierarchical analyses of molecular variance (AMOVA) as shown by (A) grouping by tributary and (B) grouping by form. Mill Stream giant smelt samples from 1999 were not included in this analysis.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
(A) Grouping by tributary				
Among groups	2	20.940	0.00376	0.22^{ns}
Among populations within groups	4	36.701	0.14516	8.31*
Within populations	375	599.455	1.59855	91.48*
(B) Grouping by form				
Among groups	1	7.507	−0.02665	−1.50^{ns}
Among populations within groups	4	49.023	0.17825	10.01*
Within populations	352	573.372	1.62890	91.49*

ns = non-significant; *$p < 0.0001$.

Taylor & Bentzen 1993a). Two of the streams were previously identified as habitats for spawning 'dwarf' smelt (Mill Brook and Unnamed 'A' in Taylor & Bentzen 1993b) and the third stream, Scout (Second) Brook, was previously not known to support spawning. Within 7 days of the appearance of intermediate spawners, a third group of smaller smelt began spawning in the same streams on the same evenings. Their small size and increased number of gill rakers were previously used to characterize these late spawners as 'dwarf' smelt. The dwarf smelt were clearly distinct from the giant smelt in terms of size, gill raker counts, trophic status, and genetic characteristics.

It was previously reported that the giant smelt were abundant and dwarf smelt few in numbers (Taylor & Bentzen 1993b). Based on a more complete sampling of the spawning period and streams, we demonstrated that the actual abundances of spawning smelt were in the order of a few thousand for giants, hundreds of thousands for the intermediates, and over 1 000 000 for the dwarfs in 1999 with similar abundances observed in 2000. It was clear that the dwarf smelt abundance was limited by habitats. In both Mill and Unnamed Brooks, all suitable substrates, i.e., secure surfaces susceptible to egg attachment, were densely packed with eggs in some instances creating 5 cm deep mats of eggs covering the entire width of the streams for distances of 5 m. Smaller mats have also been observed in Scout Brook in 2001 and 2002 (Currie, S.L. unpubl. data). At the peak of their spawning, dwarf smelt completely clogged the streams after dark and significant numbers remained in the streams during the day such that they could be easily captured by hand in 1999 and 2000. There was no indication that they were spawning during the day, but there would be some reproductive advantages to being at a spawning area as opposed to attempting to enter the stream and access spawning areas once the observed mass of smelt entered at sunset. In 2001, we observed drifting smelt larvae at rates ranging from 1 to 44 larvae $cm^{-3}\ h^{-1}$ from these streams and therefore we know that embryos successfully develop to at least the larval interval (Curry, R.A. unpubl. data).

It would appear that one major limitation to dwarf smelt production in the lake is limited availability of spawning and incubation habitats. In addition, the over-saturation of available spawning habitats suggest the population size of small, dwarf smelt is near its maximum level.

The important role smelt can play as forage for landlocked Atlantic salmon populations is well known (e.g., Sayers et al. 1989). In Lake Utopia, there was evidence from stable isotope analysis that landlocked salmon consumed smaller bodied smelt. It was most probable that other predatory fishes also consumed dwarf smelt, including larger, predatory smelt which were the only species with smelt found in the stomach contents. How important the smaller smelt were to the landlocked salmon was not discernable, but smelt were clearly not the sole food of salmon and numerous other potential forage species displayed stable isotope signatures in the predicted region for prey of salmon, e.g., reduced carbon (1 0/00) and nitrogen (3.4 0/00) signatures (Vander Zanden et al. 1999). Salmon may target immature smelt which may have different isotope signatures than the mature smelt of the present study. Additional studies will be required to determine a more accurate description of the salmon and other predatory fishes' diets and the importance of the various life history stages of smelt as prey. Given the apparent abundance of the small, dwarf smelt, it does not appear that predatory species of the fish community are affecting the survival of the dwarf smelt.

Despite a significant increase in spatial and temporal sampling of the spawning smelt, there is evidence that more groups exist within the smelt complex of Lake Utopia. First, at the time of spawning for early, giant smelt, some smelt enter Trout Stream and these smelt spawn in a tributary to Trout Lake. This is some distance from Mill Stream where giant smelt spawn annually. Although straying among stream occurs for later spawners at least, the distance between Mill Stream and Trout Lake may represent a spatially isolating mechanism for an additional group of smelt. Second, one larger, lake-captured smelt displayed a stable isotope signature most similar to the one anadromous gaspereau captured. Both had signatures suggesting a marine origin, i.e., carbon $= -18$ 0/00 compared with the lake's freshwater clams at carbon $= -27$ 0/00 and marine-dwelling gaspereau can access Lake Utopia via the fishway on the Magaguadavic River (Carr, J. Atlantic Salmon Federation, pers. comm.). The possibility that anadromous smelt enter the lake and their contribution to reproduction and trophic dynamics requires further exploration. Third, shore or shoal spawning by smelt is not uncommon (Scott & Crossman 1973). We did not search for such spawning and incubation habitats; neither did we survey all the inlet streams representing potential habitats. Fourth, we observed intermediate spawners in Mill Brook at the onset of the second spawning on 20 April 1998 and some mix of intermediates and dwarf spawners a week after the second spawning began on 26 April 1999. An earlier study captured giant smelt, 19–27 cm in length from Mill Stream on 11 April 1922 (MacLeod 1922). Such observations suggest that the timing of spawning and patterns of stream use may vary annually. Indeed, in 2002 early spawning giants were not observed until 24 April, their abundance was $>5\,000$, and normal smelt began spawning 1 May (Currie, S.L. unpubl. data). We have demonstrated that the smelt of Lake Utopia present a more complex population structure, as well as behavioural and phenotypic variability than previously believed, but we may have only touched on the complexity that could exist.

The levels of genetic divergence among these three groups of smelt in Lake Utopia were higher than commonly observed among populations of anadromous salmon in geographically larger systems (e.g., Garant et al. 2000). In addition, there was clear evidence of genetic separation among populations of the same group among streams and within a stream over time (Scout, Unnamed, and Mill Brooks). This indicates that during the intermediate and late spawning, normal and dwarf smelt maintain some degree of assortive mating or suffer reduced hybrid survivability as embryos or a later life history stage. There is good evidence that all three groups mix in the lake because a distinct trophic separation was not apparent (although some giant smelt were top predators). Our examination of the spawning ecology begins to define the complexity of the smelt's intra- and inter-specific relationships in this lake and lacustrine environments in general, but there are clearly life history tactics that we do not yet understand.

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Appendix 1. Primer sequences for the five loci used in the study of Lake Utopia's rainbow smelt.

Name	Motif	Primer sequence (5′–3′)	Cloned allele (pb)	Ta (°C)
Osmo-Lav 12	$(GT)_{37}$	1: CTG TAA TAT TCC ACTGCT GC 2: CAA GTA GAC AGT AGO GAGA	164	55
Osmo-Lav 16	$(GT)_{15}$	1: GGA TCT TGG ATG AGA AC A T 2: GGC TCT TTC ATT ACA CAG G	92	55
Osmo-Lav 45	$(CT)_{33}$	1: CTG TTG ATA GAT TGG CAT C 2: CCC ATT CAA TTA GAC AGT G	201	55
Osmo-Lav 157	$(GT)_{33}$AAGAGA$(GT)_{7}$	1: CTT GCT TAT GTA AAG GTG GG 2: GAT CCA CCA GTT CTC ACA	246	55
Tpa-26		1 :AGG ACT GGC GTG GGA AAT 2: CTG CAC TGC TGT CTG GAG AA		57