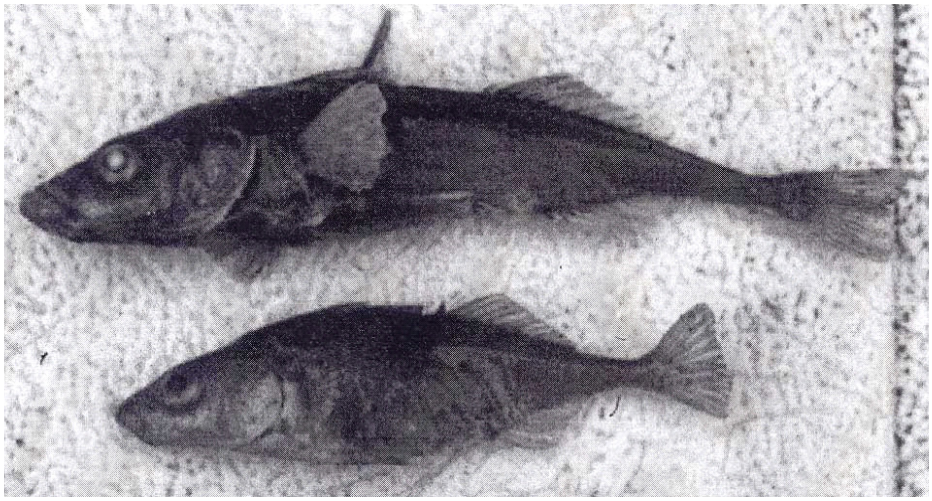
COSEWIC
Assessment and Status Report

on the

Misty Lake sticklebacks
Gasterosteus sp.

Misty Lake lentic stickleback
Misty Lake lotic stickleback

in Canada



Misty Lake lentic stickleback – ENDANGERED
Misty Lake lotic stickleback – ENDANGERED
2006

COSEWIC
COMMITTEE ON THE STATUS OF
ENDANGERED WILDLIFE
IN CANADA

COSEPAC
COMITÉ SUR LA SITUATION DES
ESPÈCES EN PÉRIL
AU CANADA

COSEWIC status reports are working documents used in assigning the status of wildlife species suspected of being at risk. This report may be cited as follows:

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Production note:

COSEWIC would like to acknowledge Juanita Ptolemy for writing the status report on the Misty Lake sticklebacks *Gasterosteus* sp. (Misty Lake lentic stickleback and Misty Lake lotic stickleback) in Canada, prepared under contract with Environment Canada, overseen and edited by Dr. Robert Campbell, Co-chair of the COSEWIC Freshwater Fishes Specialist Subcommittee

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Également disponible en français sous le titre Évaluation et Rapport de situation du COSEPAC sur les épinoches du lac Misty (*Gasterosteus* sp.) (Épinoche lentique et Épinoche lotique du lac Misty)

Cover illustration:
Misty Lake sticklebacks — the upper fish is typical of the lake form and the lower fish is an example of the inlet stream form (photo courtesy of Dr. Eric Taylor, UBC).

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COSEWIC
Assessment Summary

Assessment Summary – November 2006

Common name
Misty Lake Lentic Stickleback

Scientific name
Gasterosteus sp.

Status
Endangered

Reason for designation
This lake-dwelling fish is part of an endemic, highly divergent species pair restricted to a single stream-lake complex on Vancouver Island with an extremely small area of occurrence. This species pair could quickly become extinct due the introduction of non-native aquatic species or perturbations to the habitat. Proximity of this complex to a major highway and public access make an introduction likely. Logging activities in the watershed, as well as highway use and related maintenance, could impact habitat quality to some degree.

Occurrence
British Columbia

Status history
Designated Endangered in November 2006. Assessment based on a new status report.

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COSEWIC
Executive Summary

Misty Lake sticklebacks
Gasterosteus sp.

Misty Lake lentic stickleback
Misty Lake lotic stickleback

Species information

The Misty Lake sticklebacks, a highly divergent parapatric lake-stream pair of threespine stickleback, are found in a single small lake on Vancouver Island, British Columbia (BC). Similar parapatric lake-stream pairs have also been well documented in two other systems in BC, Mayer and Drizzle lakes on Graham Island (Queen Charlotte Islands). These pairs live in contact (parapatry) without a significant amount of overlap or interbreeding. Differences in body shape are believed to be adaptations to the different feeding strategies used by each member of the species pair. Genetic evidence suggests that each of the three highly divergent lake-stream pairs has evolved separately through parallel evolution. Of the lake-stream pairs examined to date, the Misty Lake inlet stream and lake populations appear to be the most genetically and ecologically differentiated from one another. The Misty Lake outlet stream population is intermediate in morphology between the inlet and lake populations and is considered part of the lake-stream complex.

Distribution

The lake-stream pair of threespine stickleback occurs only in Misty Lake and its inlet and outlet tributary system, on northeastern Vancouver Island, BC.

Habitat

Misty Lake, its outlet and inlet are darkly stained and small in size. Sticklebacks in Misty Lake feed in the limnetic zone and spawn in the littoral zone, while those in the outlet are found in slower, deeper water. The inlet stream form remains in either the inlet swamp or the inlet stream, where it is commonly found in deeper, low water velocity areas such as pools and sloughs. Most breeding activity occurs in the stream, with very little in the swamp. Distribution within the inlet system has not been mapped, but appears to be stable.

The lake is considered a natural ecosystem and appears to be stable. The inlet watershed has been logged, but impacts from forest harvesting activities are minor. Misty Lake and short sections of the lower portion of the inlet and the upper section of the outlet are contained within the Misty Lake Ecological Reserve, a part of the provincial protected areas system. However, the majority of the lake's watershed lies outside the reserve boundaries.

Biology

Although there is ongoing research into the evolutionary biology of the Misty Lake sticklebacks, no detailed studies have been completed on the specific biology of the these fish. In the absence of specific information, it is assumed that they are similar in many aspects to similar forms of the threespine stickleback.

Both forms begin reproductive activity in April and are finished in July (gravid females are common during May and June). There is some information to indicate that the inlet sticklebacks live shorter lives and produce a higher number of eggs at each spawning than lake or outlet fish. Generally, the male stickleback provides parental care. He protects and fans the nest and continues to care for the young fish until they are capable of moving into cover to feed. Eggs take about 7 – 10 days to hatch, depending on water temperature.

Mouth and gillraker morphology suggest that the lake population feeds on zooplankton in the open water of the lake and the stream populations feed on macroinvertebrates.

Studies have shown that the lake sticklebacks will move only in a downstream direction, while those in the inlet sticklebacks will move either upstream or downstream.

Population sizes and trends

Based on overall habitat stability, population trends are also likely stable, although in dry years decreasing water levels may lead to population declines.

Limiting factors and threats

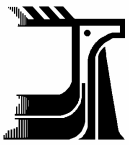
BC Parks has identified potential threats to the stickleback populations within the Misty Lake Ecological Reserve. These include: hydrocarbon and pesticide contamination from the adjacent highway and rest stop; water quality and hydrological changes from nearby logging; non-native species introductions (fish and plants); and non-conforming recreational uses of the lake (canoeing and illegal fishing). The introduction of non-native species has been detrimental to the stickleback pairs in Enos Lake (also on Vancouver Island) and Hadley Lake (on Lasqueti Island, near Vancouver Island).

Special significance of the species

The parapatric stickleback pair found in the Misty Lake system is one of three well-studied lake-stream pairs that display ecotype differences similar to the level seen in the benthic-limnetic stickleback pairs. These highly divergent parapatric pairs are invaluable to the study of evolutionary processes. Genetic data strongly suggest that each highly divergent lake-stream pair has evolved separately through parallel evolution as neither the Misty inlet stream form nor its lake form showed the same genetic ancestry as the pairs from the Drizzle or Mayer lake systems on Graham Island.

Existing protection

The lake is located within the boundaries of the Misty Lake Ecological Reserve, which was established for the protection of the larger lake stickleback. Most of the habitat for the stream form is located upstream of the reserve; this area and much of the Misty Lake watershed is not included in the reserve, making it difficult to ensure the long-term protection of habitat for the whole lake-stream complex.



COSEWIC HISTORY

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) was created in 1977 as a result of a recommendation at the Federal-Provincial Wildlife Conference held in 1976. It arose from the need for a single, official, scientifically sound, national listing of wildlife species at risk. In 1978, COSEWIC designated its first species and produced its first list of Canadian species at risk. Species designated at meetings of the full committee are added to the list. On June 5, 2003, the *Species at Risk Act* (SARA) was proclaimed. SARA establishes COSEWIC as an advisory body ensuring that species will continue to be assessed under a rigorous and independent scientific process.

COSEWIC MANDATE

The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) assesses the national status of wild species, subspecies, varieties, or other designatable units that are considered to be at risk in Canada. Designations are made on native species for the following taxonomic groups: mammals, birds, reptiles, amphibians, fishes, arthropods, molluscs, vascular plants, mosses, and lichens.

COSEWIC MEMBERSHIP

COSEWIC comprises members from each provincial and territorial government wildlife agency, four federal entities (Canadian Wildlife Service, Parks Canada Agency, Department of Fisheries and Oceans, and the Federal Biodiversity Information Partnership, chaired by the Canadian Museum of Nature), three non-government science members and the co-chairs of the species specialist subcommittees and the Aboriginal Traditional Knowledge subcommittee. The Committee meets to consider status reports on candidate species.

DEFINITIONS

Wildlife Species	A species, subspecies, variety, or geographically or genetically distinct population of animal, plant or other organism, other than a bacterium or virus, that is wild by nature and is either native to Canada or has extended its range into Canada without human intervention and has been present in Canada for at least 50 years.
Extinct (X)	A wildlife species that no longer exists.
Extirpated (XT)	A wildlife species no longer existing in the wild in Canada, but occurring elsewhere.
Endangered (E)	A wildlife species facing imminent extirpation or extinction.
Threatened (T)	A wildlife species likely to become endangered if limiting factors are not reversed.
Special Concern (SC)*	A wildlife species that may become a threatened or an endangered species because of a combination of biological characteristics and identified threats.
Not at Risk (NAR)**	A wildlife species that has been evaluated and found to be not at risk of extinction given the current circumstances.
Data Deficient (DD)***	A category that applies when the available information is insufficient (a) to resolve a species' eligibility for assessment or (b) to permit an assessment of the species' risk of extinction.

* Formerly described as "Vulnerable" from 1990 to 1999, or "Rare" prior to 1990.

** Formerly described as "Not In Any Category", or "No Designation Required."

*** Formerly described as "Indeterminate" from 1994 to 1999 or "ISIBD" (insufficient scientific information on which to base a designation) prior to 1994. Definition of the (DD) category revised in 2006.

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The Canadian Wildlife Service, Environment Canada, provides full administrative and financial support to the COSEWIC Secretariat.

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SPECIES INFORMATION

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Name and classification

Phylum:	Chordata
Class:	Actinopterygii (ray-finned fishes)
Order:	Gasterosteiformes
Family:	Gasterosteidae
Genus:	*Gasterosteus*
Lentic (Lake) form:	*Gasterosteus* sp.
Lotic (Stream) form:	*Gasterosteus* sp.
Common name	
English:	Misty Lake Lentic Stickleback, Lake Form
	Misty Lake Lotic Stickleback, Stream Form
French:	Épinoche lentique du lac Misty
	Épinoche lotique du lac Misty

The marine form of the threespine stickleback, sometimes called *trachurus* in the literature*,* migrates to freshwater to spawn (sea-run) while the freshwater type, referred to as *leiurus,* remains in fresh water for its entire life (Scott and Crossman 1973). The taxonomy of *Gasterosteus aculeatus* has been known to be problematic for some time (see e.g., Hagen and McPhail 1970; Scott and Crossman 1973). Classical approaches to systematics are based on morphometrics and assume that morphological traits rarely evolve in parallel. However, parallel evolution is widespread in freshwater sticklebacks and their taxonomy does not reflect the vast diversity that exists (e.g., Hagen and McPhail 1970; Hagen and Gilbertson 1972; Lavin and McPhail 1985; Reimchen *et al.* 1985; Schluter and McPhail 1992; Bell and Foster 1994; McPhail 1994). McPhail and Lindsey (1970), and Bell and Foster (1994) consider *G. aculeatus* to be a species complex.

Thompson *et al.* (1997) noted that the radiation of forms within *G. aculeatus* found in the eastern North Pacific is characterized partly by three sets of divergences involving sympatric or parapatric biological species (reviewed by McPhail 1994): parapatric sea-run and freshwater sticklebacks (e.g., Hagen 1967); sympatric lacustrine limnetic and benthic forms (Larson 1976; McPhail 1984, 1992); and parapatric lake dwelling (lentic) and stream dwelling (lotic) sticklebacks (Moodie 1972, Reimchen *et al.* 1985; Lavin and McPhail 1993). Highly divergent parapatric lake-stream pairs of sticklebacks are known to occur in three drainages: Mayer and Drizzle lakes, on northeast Graham Island (Queen Charlotte Islands), and Misty Lake on northeastern Vancouver Island (Fig. 1). These forms are endemic to British Columbia (BC). In particular, the magnitude of the ecological and genetic divergence seen in the Misty Lake parapatric lake-stream pair is similar to that found in benthic-limnetic species pairs (e.g., Paxton Lake and the Vananda Creek drainage on Texada Island, BC) (Taylor, pers. comm. 2005). Although several other lake-stream population pairs of sticklebacks have since been found in BC (Hendry and Taylor 2004), to date, none are known to combine the genetic, morphological and ecological differences observed in the Misty Lake pair (Taylor, pers. comm. 2005).

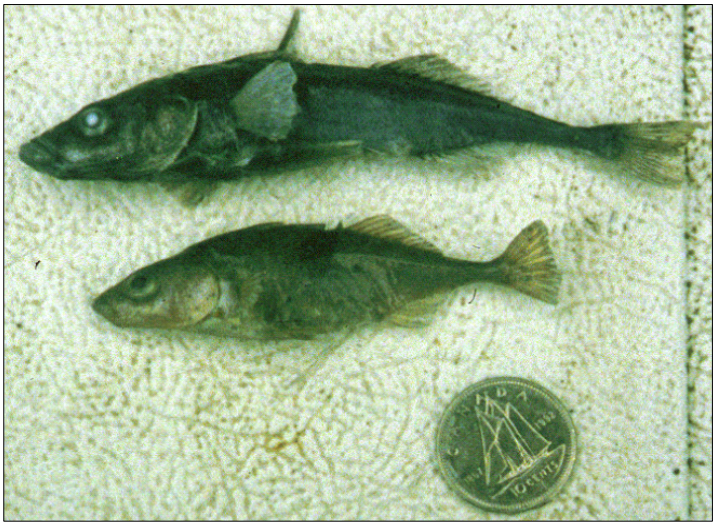

Figure 1. Misty Lake sticklebacks – the upper fish is typical of the lake form and the lower fish is an example of the inlet stream form (photo courtesy of Dr. Eric Taylor, UBC).

Hendry *et al*. (2002) conducted a detailed study of the sticklebacks from different sites in the Misty system. The authors concluded that morphological differences were greatest between the lake sticklebacks and the inlet sticklebacks (both in specimens collected in the wild and in crosses raised in common garden experiments conducted to determine if the differences seen in the wild had a genetic basis). These differences were thought to have evolved in response to their different foraging environments (Lavin and McPhail 1986; McPhail 1994). Genetic divergence (mitochondrial DNA and microsatellites) was also greatest between the sticklebacks from the lake and those in the inlet and least between those from the lake and fish from the outlet stream (Hendry *et al.* 2002). In their study on adaptive morphology, Moore and Hendry (2005) concluded that gene flow did not constrain adaptation in the inlet stream but strongly constrained adaptation in the outlet stream; unlike inlet fish (where a sharp shift in morphology occurred between lake and inlet) the outlet fish were characterized by a gradual increase in divergence from the lake fish as distance from the lake increased.

Morphological description

Generally, the threespine stickleback is a small fish (average about 51 mm in standard length); the body is compressed laterally and elongate, tapering to a slender, depressed caudal peduncle; there are three isolated, stout, serrated dorsal spines; the very short last spine, precedes, but is not attached to the soft rays; pelvics are thoracic with one strong spine and 1 soft ray; all fin spines can be locked in an erect position (Scott and Crossman 1973).

In the systems where highly divergent parapatric lake-stream pairs of sticklebacks have been observed the morphological divergence is almost identical (Lavin and

McPhail 1993). The inlet stream form is mottled brown and robust bodied while the lake form is melanistic, slender-bodied and has longer spines and more gill rakers than its corresponding parapatric stream form; the same ecotypes from different drainages are morphologically more similar than different ecotypes from the same drainage (Lavin and McPhail 1993).

In their study, Hendry *et al.* (2002) found that in the Misty Lake system, lake and outlet fish were usually longer than inlet fish (Table 1). Other data showed Misty Lake inlet sticklebacks usually had deeper bodies than either Misty Lake or Misty Lake outlet fish; lake fish had a higher number of gill rakers than stream fish; Misty Lake inlet fish had shorter pelvic spines than either Misty Lake or Misty Lake outlet fish; and Misty inlet fish had wider pelvic girdles than either lake or outlet fish. In morphology, lake and inlet fish were at the extremes while outlet fish were found to be intermediate, but more similar to lake fish (Moore and Hendry 2005).

Morphological differences between Misty Lake and inlet sticklebacks are inherited: crosses between stream forms produced only stream-form phenotypes; crosses between lake forms produced only lake-form phenotypes (Lavin and McPhail 1993; Hendry *et al.* 2002). This indicates that the two forms comprise separate gene pools, not a complex polymorphism (Lavin and McPhail 1993).

Table 1. Average morphological measurements of threespine stickleback from different collection sites in the Misty Lake system (adapted from Table 1, Hendry *et al*. 2002). Body depth, pelvic spine length and pelvic girdle width were standardized to a common body length of 55.4 mm. U indicates upper site (1.8 km upstream) and L the lower inlet site (0.9 km upstream). Homogenous subsets of collections based on Tukey tests are indicated with letter superscripts.

	U Inlet 1997	U Inlet 1999	L Inlet 1997	L Inlet 1998	Lake 1997	Lake 1998	Outlet 1998
N	7	30	21	30	30	30	29
Body length	$51.6^{b,c}$	49.1^{b}	36.2^{a}	$52.3^{b,c}$	62.8^{d}	61.6^{d}	$58.1^{c,d}$
Gill raker number	15.9^{a}	$16.7^{a,b}$	$16.8^{a,b}$	$16.1^{a,b}$	19.5^{d}	18.8^{d}	$16.8^{a,b}$
Body depth	14.5^{f}	13.6^{d}	$14.2^{e,f}$	$13.8^{d,e}$	12.4^{c}	11.6^{b}	12.7^{c}
Pelvic spine length	8.1^{a}	$8.7^{a,b}$	8.2^{a}	$9.0^{b,c}$	$9.3^{b,c}$	$9.2^{b,c}$	9.4^{c}
Pelvic girdle width	4.6^{e}	4.1^{d}	$4.5^{d,e}$	$4.3^{d,e}$	$3.1^{a,b}$	2.9^{a}	$3.2^{a,b,c}$

Genetic description

Different scales of evolutionary divergence occur in *Gasterosteus* of the North Pacific, an ancient divergence of mitochondrial DNA clades as well as a more recent, postglacial, divergence of ecotypes within the major clades (Thompson *et al.* 1997). Although it was initially only found around the Queen Charlotte Islands, Thompson *et al.* (1997) extended the known distribution of the Japanese (Argonaut Plain) clade to the Misty Lake drainage system and referred to it as the Trans-North Pacific clade (TNPC). Both the TNPC and the Eastern North Pacific lineage of the Euro-North American clade

(ENAC) are present in the Misty Lake system (Fig. 2). The TNPC is much less common in British Columbia than the ENAC, although Johnson and Taylor (2004) have found it to be more common and distributed more widely than previously thought. It is now known to occur in 12 of the 45 new locations surveyed in their study: four of nine Cook Inlet (Alaska) lake populations; three of four Quadra Island lake populations; one of 15 lake populations on northern Vancouver Island; one of four adjacent mainland populations; and four of 12 anadromous or marine populations. Johnson and Taylor (2004) reported a significant association between the presence of the TNPC and lake elevation; it was more common in lakes below 42 m than in lakes at higher elevation. This factor indicates that post-glacial lake accessibility was important in determining the distribution of the two clades in the eastern Pacific (Johnson and Taylor 2004).

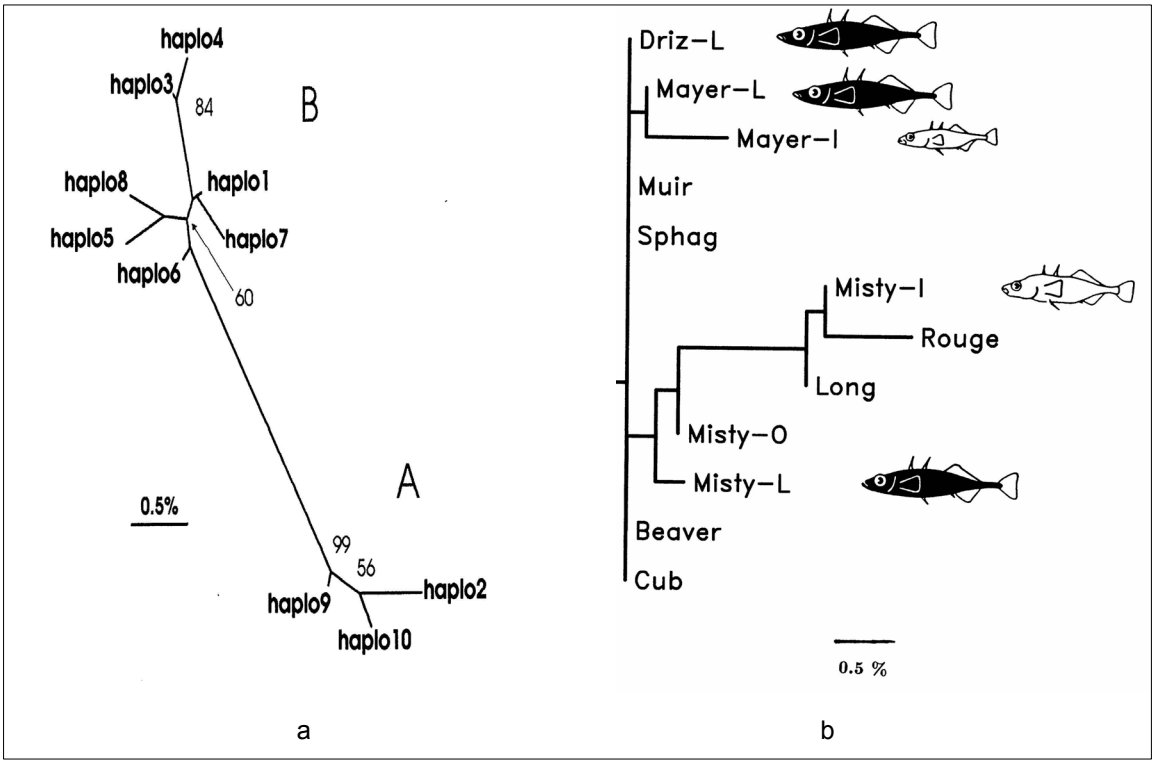

Figure 2. (a) Relationships among mtDNA haplotypes found in stickleback populations sampled from northern Vancouver Island and Graham Island, Queen Charlotte Islands, including Misty Lake inlet (predominantly haplo 2, plus haplo 3), and Misty Lake and outlet (predominantly haplo 3, plus 2 and 4). "A" and "B" refer to two major clades of mtDNA, the "Trans-North Pacific Clade (TNPC)" and the "Euro-North American Clade". Numbers on branch points represent % support from 1000 bootstrap replicates. (b) Neighbour-joining tree of estimated relationships among populations of lake and stream sticklebacks from Vancouver and Graham islands. Fish "cartoons" represent general shape and shading differences (both figures adapted from Thompson *et al*. 1997).

Hendry *et al.* (2002) were able to show that genetic divergence (mitochondrial DNA and microsatellites) was greatest between Misty Lake and the upper inlet (1.8 km upstream of the lake), intermediate between the lake and the lower inlet (0.9 km upstream) and least between the lake and the outlet stream (1.2 km downstream of the lake). The

TNPC was nearly fixed in upper inlet fish (95.5%), dominant in the lower inlet (67.5%), less common in outlet fish (20.5%) and rare in Misty Lake (4.4%); the TNPC was predominant in the inlet stream while the ENAC was dominant in lake and outlet stream fish (Thompson *et al.* 1997; Hendry *et al.* 2002). This juxtaposition of two populations each with a preponderance of a different clade appears to be unusual in these parapatric pairs.

Hendry *et al.* (2002) showed that within the Misty Lake system all pairs of collections (site- and year-specific) differed significantly in allelic frequencies at 5 nuclear DNA microsatellite loci ($P < 0.010$) and pairwise F_{ST} values were all significantly greater than zero ($P < 0.001$). Differentiation between years and within sites was small ($F_{ST} = 0.023 – 0.030$). Differentiation among sites ranged from small (lake vs. outlet, $F_{ST} = 0.005 – 0.046$) to moderate (lower inlet vs. lake, $F_{ST} = 0.129 – 0.157$) to large (upper inlet vs. lake, $F_{ST} = 0.289 – 0.345$), demonstrating concordance between nuclear and mitochondrial DNA differentiation. Misty Lake and outlet collections clustered together and were distinct from the lower inlet and upper inlet collections (98% bootstrap support). Lower inlet and upper inlet were also quite distinct from each other (99% bootstrap support), indicating the stream form may comprise more than one population (Fig. 3).

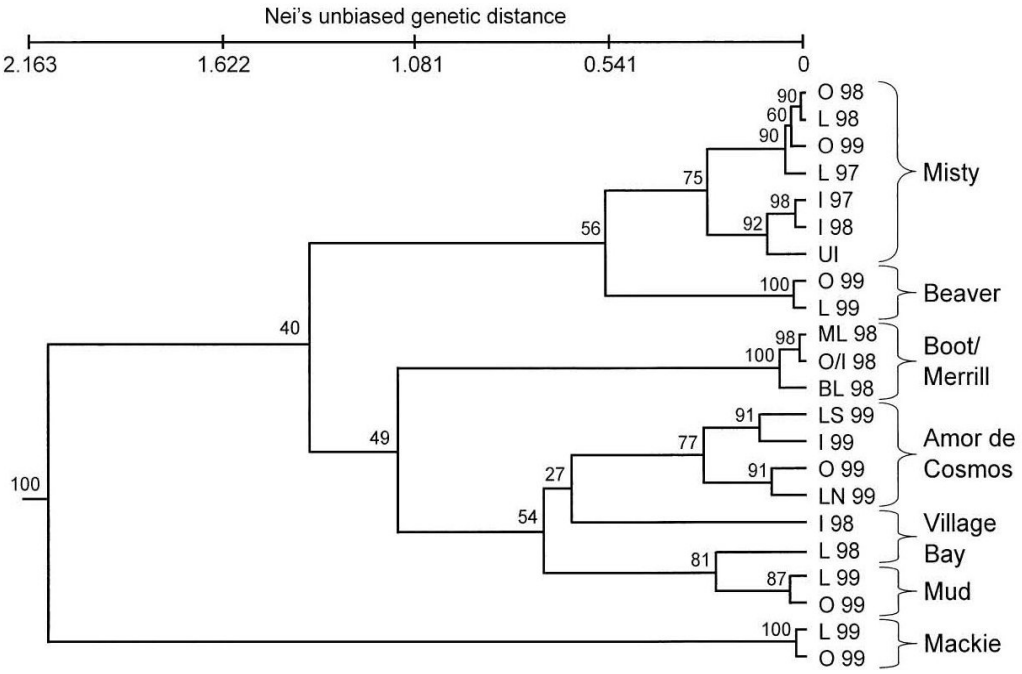

Figure 3. A dendrogram of Nei's (1978) unbiased genetic distance showing general relationships of Misty Lake stickleback from different habitats to each other and to other stream-lake pairs. Labels at the end of terminal branches refer to specific collections, with a location abbreviation followed by the collection year. O, outlet; I, inlet; and L, lake. Additional abbreviations appear in the Misty watershed (UI, upper inlet), the Boot/Merrill watershed (ML, Merrill Lake; BL, Boot Lake; O/I, the stream connecting these two lakes), and the Amor de Cosmos watershed (LS, the southern McCreight Lake collection, which was adjacent to the inlet; LN, the northern McCreight Lake collection, which was adjacent to the outlet). Bootstrap values are shown for all nodes and genetic distance is based on variation at five microsatellite loci. See Hendry and Taylor (2004) for details.

The estimated effective number of migrants between sites (total exchanged) was lowest between Misty Lake and upper inlet at 0.54 migrants per generation, slightly higher between Misty Lake and lower inlet at 1.59 migrants per generation, and much higher between Misty Lake and outlet at 18.23 migrants per generation (based on Wright's F_{ST} calculations, Hendry *et al.* 2002).

Designatable units

All of the stickleback populations in the Misty Lake watershed are important components of the evolutionary processes that are occurring in this system. The fact that these populations have diverged and co-exist as parapatric pairs is unusual and of conservation value. The morphological, genetic and ecological information indicate that the Misty Lake inlet stream and lake populations are divergent and reproductively isolated, behaving as two biological species. The two populations should be considered distinct designatable units much like benthic-limnetic stickleback species pairs. Currently, the outlet fish do not appear to meet the criteria for a separate designatable unit (DU) due to the reported similarities between the lake and outlet populations. Gene flow from the lake population to the outlet population is high enough to constrain adaptation in the outlet population, although some degree of ecological divergence is evident (Moore and Hendry 2005). For now, the outlet population could be considered a component of the lake DU. The outlet population contributes to the overall Misty Lake lake-stream complex and may have population dynamics separate from either the inlet stream or lake populations.

Thompson *et al.* (1997) argue that their molecular data strongly suggest the lake-stream pairs on Graham Island and northern Vancouver Island evolved separately through parallel evolution: neither the Misty Lake form nor its corresponding inlet stream form clustered with similar forms from the Drizzle or Mayer lakes' systems; the Misty Lake lake-stream pair did not share any mtDNA haplotypes with either of the pairs from Graham Island; and the haplotypes were phylogenetically independent from the Graham Island haplotypes. Microsatellite data (Hendry and Taylor 2004) support the conclusion that all of the pairs from different watersheds have a long history of evolutionary independence and represent independent events of post-glacial divergence.

Data from Thompson *et al.* (1997) also support an allopatric divergence for mtDNA clades seen in the Misty Lake pair, because mtDNA nucleotide divergence between the lake and inlet stream populations was estimated at 1.75% indicating that the predominant clades in the Misty Lake and Misty Lake inlet populations diverged about 875,000 years ago (assuming a whole molecule substitution rate of 2% per million years), whereas Misty Lake and its surrounding area were glaciated only 12,000 years ago. The two divergent mtDNA clades observed in the Misty Lake pair are believed to have originated from historical isolation in the two major glacial refugia proposed for the North Pacific, namely the Beringia and Cascadia (Thompson *et al*. 1997).

Lavin and McPhail (1993) noted that the lake form was predominantly found in the lake, but small numbers of lake fish were collected in the stream as far as 300 m

upstream. In contrast, none of the inlet stream fish were collected in the lake, but both forms were taken in the swamp, a transition zone, at the mouth of the inlet stream. The distribution of the two forms did overlap during breeding season. But the authors found that hybrids were rare in the wild; only one of the 274 fish collected from the Misty Lake system was found to be intermediate in morphology between the two forms, implying that hybridization is rare or hybrid survival is poor. The authors argue that this indicates that lake and inlet forms are distinct ecological and genetic entities and not an environmentally induced dimorphism within a single population.

DISTRIBUTION

Global range

The Misty Lake lake-stream stickleback species pair is only found in the Misty Lake drainage system on northern Vancouver Island, British Columbia, Canada (Fig. 4 and 5). Two other highly divergent lake-stream pairs are known to occur in Mayer Lake (Mayer River drainage) and in Drizzle Lake (Sangan River drainage) on Graham Island, the most northerly of the main Queen Charlotte Islands. The area in northern Vancouver Island where the Misty Lake pair is found is separated by about 400 km of ocean from Graham Island. The Graham Island pairs are morphologically similar but genetically distinct from the Misty Lake lake-stream pair (Thompson *et al.* 1997). Although several other lake-stream population pairs of sticklebacks have recently been documented in BC (Hendry and Taylor 2004), none are known to combine the genetic, morphological and ecological differences shown by these three highly divergent pairs, with the possible exception of the Village Bay pair found on Quadra Island near northern Vancouver Island (Hendry and Taylor 2004; Taylor, pers. comm. 2005). The two mtDNA clades are also known to occur in stickleback on Quadra Island; however, the significance of this for the lake-stream pairs found there is currently unknown as mtDNA has not been examined in the stream form (Taylor, pers. comm.). It is possible that a few additional highly divergent lake-stream pairs will be found in this area (Hendry and Taylor, unpubl. data).

Canadian range

This lake-stream pair of threespine stickleback is found only in Misty Lake (including its outlet) and its inlet stream, which are located approximately 11 km upstream from the ocean in the Keogh River system south of Port Hardy, Vancouver Island, British Columbia.

The extent of occurrence for the Misty Lake form is at maximum about 1.46 km^2, which includes the surface area of the lake (0.36 km^2) plus the watershed area of the outlet (1.1 km^2). The extent of occurrence for the inlet stream form is <9 km^2 (the area of the watershed for the inlet stream); the total length of stream in the inlet system is about 20 km, and the area of occupancy is between 0.008 to 0.04 km^2. Stickleback do not occupy the entire stream length; however the upper limit of their distribution is

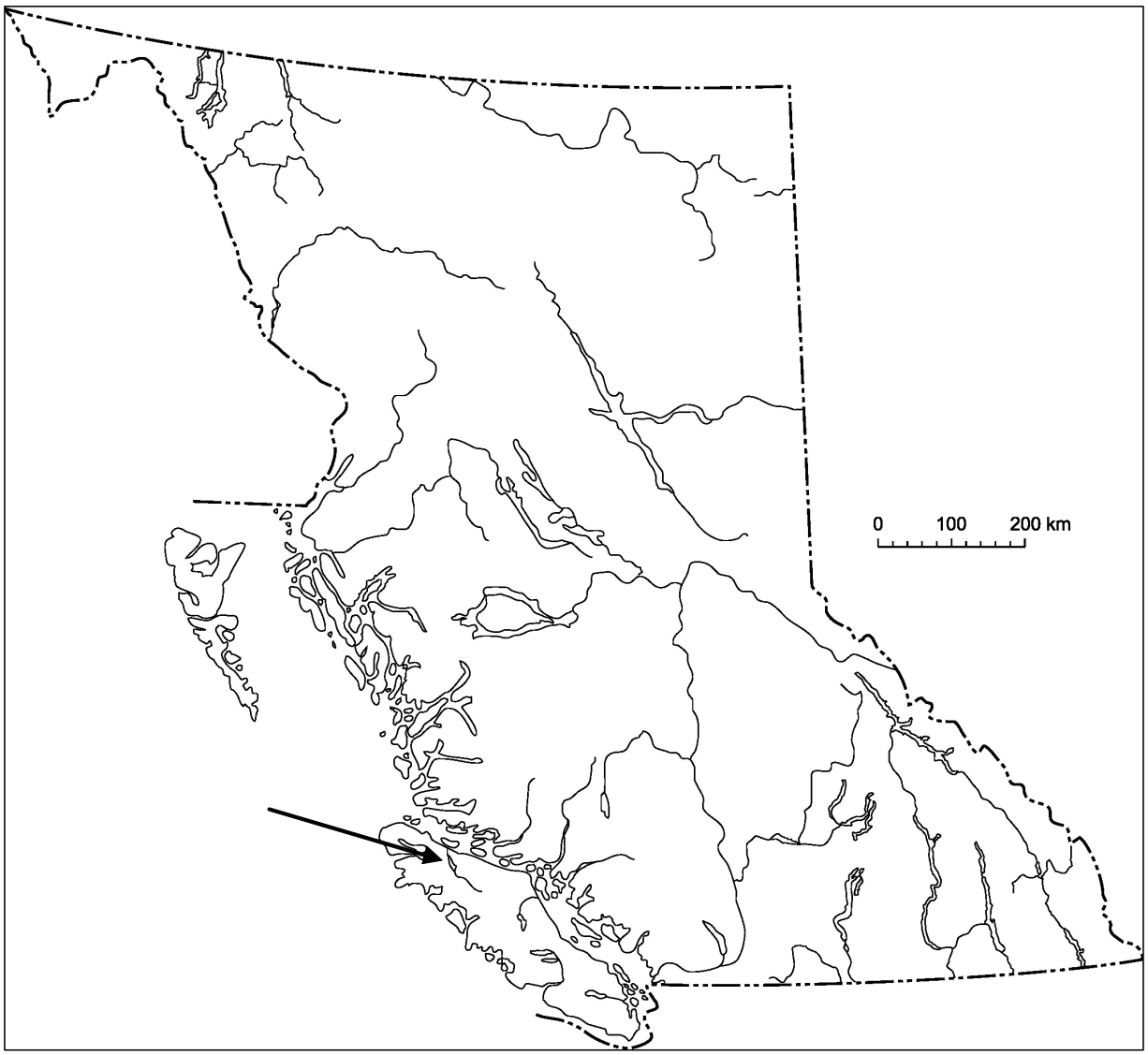

Figure 4. Map of British Columbia showing global and Canadian range of the Misty Lake sticklebacks on Vancouver Island. Arrow indicates location of Misty Lake drainage.

unknown. Known upper limits are shown in Fig. 6, (Hendry, pers. comm. 2005, 2006; Moore, pers. comm. 2005, 2006); however, no systematic survey of the inlet system has been completed for sticklebacks and they are likely to be found in areas upstream of those markers. The length of stream occupied by the outlet population is about 2.3 km (Moore and Hendry 2005), and area of occupancy is calculated at about 0.01 km^2. Area of occupancy is estimated using stream length, mean wetted width and an estimate of habitat use of 75% (Moore pers. comm. 2005, 2006) for stream populations.

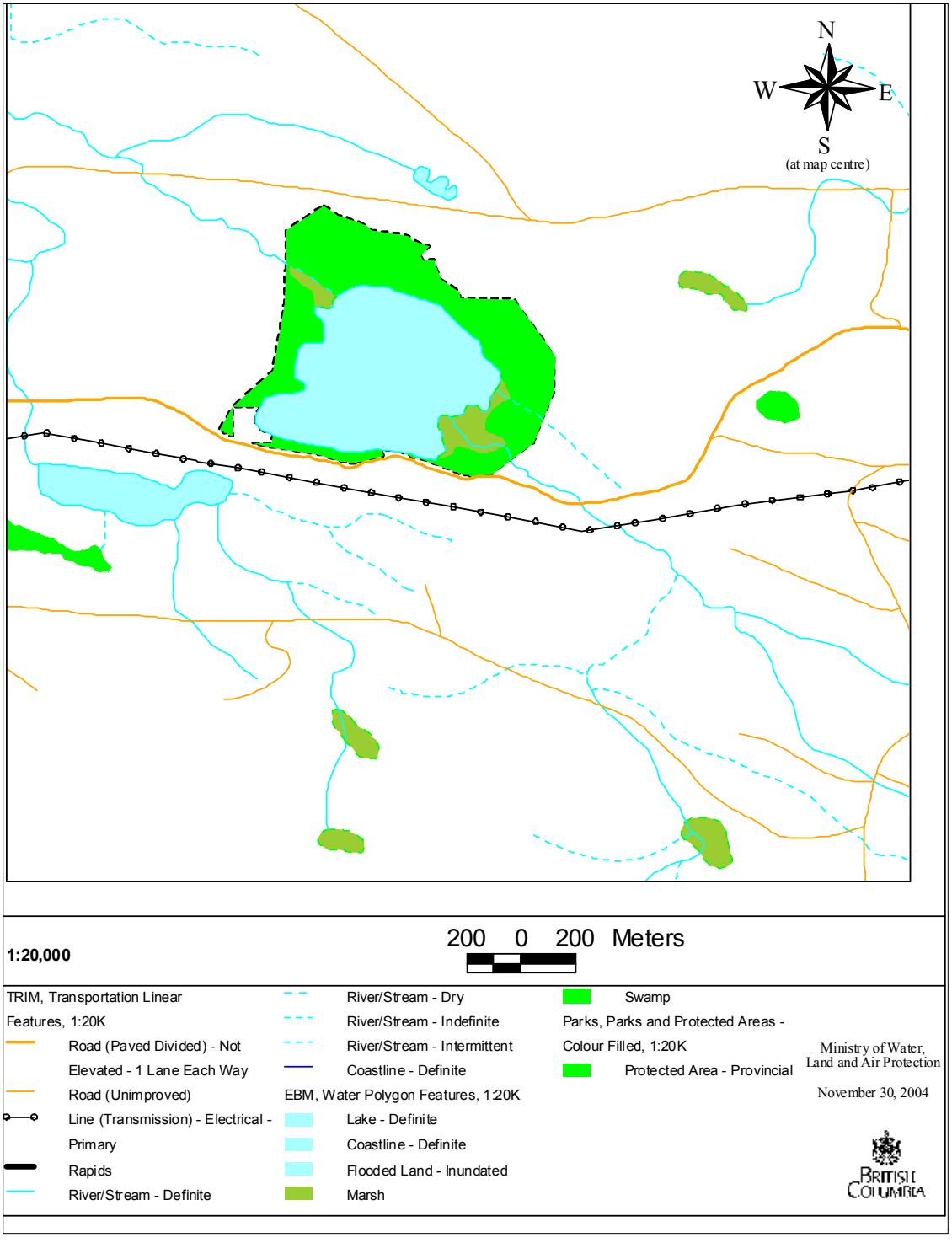

Figure 5. BC Parks map showing Misty Lake watershed, boundary of ecological reserve and highway adjacent to southern boundary of reserve.

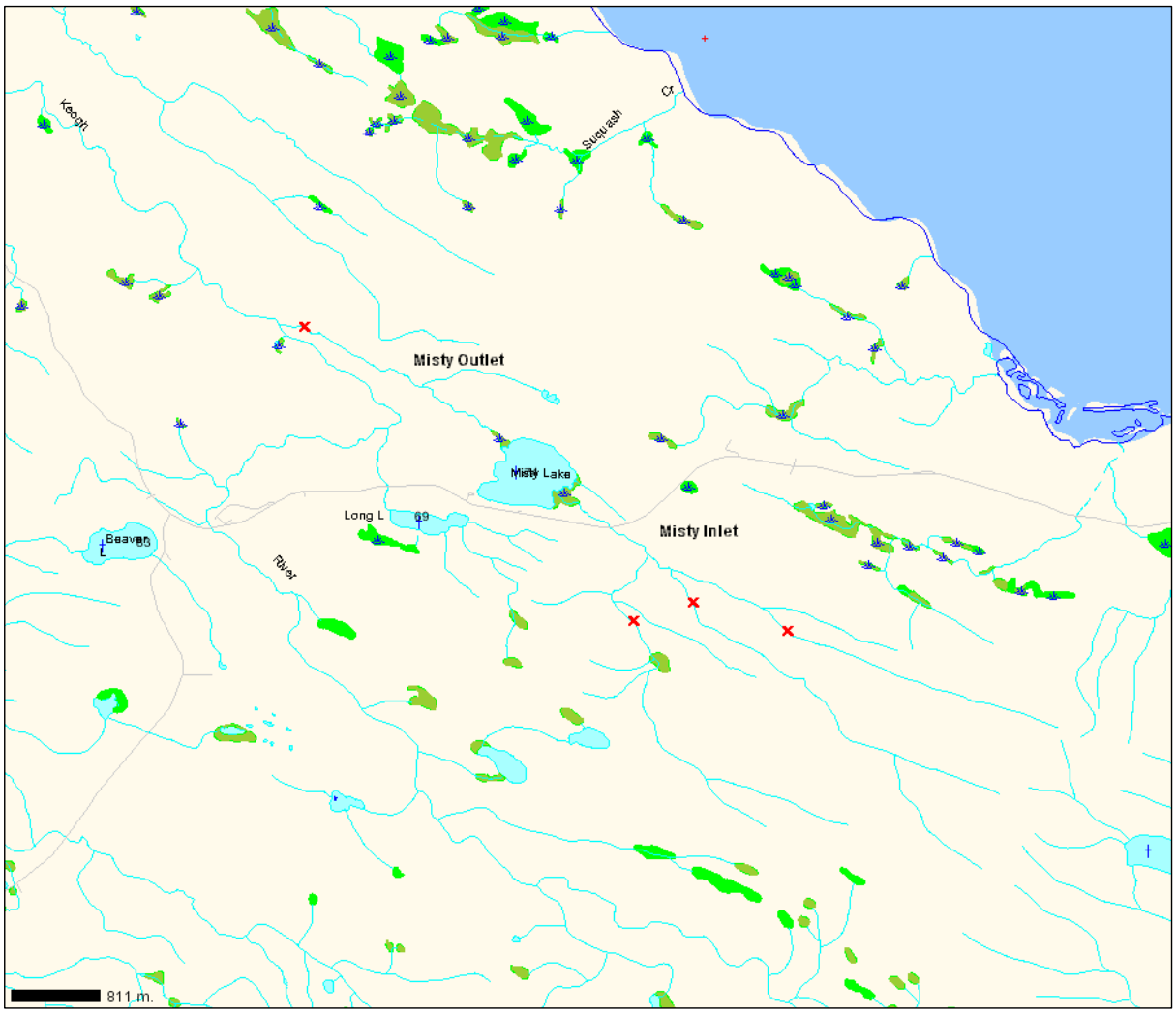

Figure 6. Known upper and lower limits of the Misty Lake lake-stream stickleback distribution (Moore, pers. comm.; Hendry, pers. comm. 2005, 2006).

HABITAT

Habitat requirements

Misty Lake is located at an elevation of around 74 m. It is considered a natural ecosystem and appears to be stable at this time. The lake is small (35.6 ha in surface area) and relatively shallow (mean depth = 1.7 m; maximum = 6.1 m). Loose detritus covers both the lakeshore and bottom; dense beds of *Potamogeton* and *Nuphar* cover the littoral zone during the summer. Misty Lake is oligotrophic and relatively low in productivity; this is typical for small lakes on Vancouver Island. The outlet stream is located at the northwestern end of the lake and filters through a swamp before it

develops and flows downstream to join the Keogh River. The water in the system is darkly stained with tannin lignins. Aside from *Gasterosteus*, native fish species in the watershed include Dolly Varden (*Salvelinus malma*), coastal cutthroat trout (*Oncorhynchus clarki*), coho salmon (*Oncorhynchus kisutch*), rainbow trout (*Oncorhynchus mykiss*) and prickly sculpin (*Cottus asper*).

The headwaters of the system are at approximately 260 m in elevation. The total watershed area is about 12.2 km^2. The inlet crosses Highway 19 (the Island Highway), through a culvert about 900 m above the lake. At high water the culvert is passable to fish but at low water the downstream end of the culvert sits about 0.3 m above the water surface. The stream flows through 500 m of swamp before reaching the southeast corner of the lake. Irvine and Johnston (1992) provide some physical characteristics for the mainstem inlet and outlet streams: the inlet has a mean wetted width of 2 m, length of 5.3 km, mean gradient equals 1.5%, and the water temperature ranges from 1 to 19ºC; the outlet has a mean wetted width of 3 m, a length of 2.3 km, mean gradient of 1.0%, and water temperature ranges from 2 to15ºC.

The lake form feeds in the limnetic zone. Nesting sites are likely concentrated in shallow areas characterized by sand substrate, a gentle gradient and underwater vegetation as described for the Mayer Lake stickleback (Moodie 1984). McPhail (1994) noted that most of the breeding activity seemed to occur in either the lake or the stream in the Misty Lake system, although some gravid females of both the lake and inlet forms were collected in the inlet swamp (a transition zone). Deeply stained water makes detection of nest location difficult in both the lake and the streams.

The inlet form remains in the inlet swamp or inlet stream where it is commonly found in low water velocity areas of about 0.5 – 1.0 m in depth (Hendry *et al.* 2002) such as pools and sloughs. Moore (pers. comm. 2005, 2006) and Hendry (pers. comm. 2005, 2006) have observed that the number of inlet sticklebacks decreases with an increase in distance upstream from the lake (similarly numbers decrease in the outlet with distance downstream from the lake). McPhail (1994) reported that no nests had been observed in the stream and could not describe nesting habitat. Stream residents in the Little Campbell River, in the BC lower mainland, have been found to utilize stream habitats with fine substrate (mud), no current and heavy vegetation for nesting (McPhail 1994). Although the sticklebacks are less likely to use fast riffle habitats, these areas are required for benthic invertebrate production. A complex of natural stream habitat would be required to maintain the population.

Habitat trends

Misty Lake and its inlet were described in Lavin and McPhail (1993); the habitat is not believed to have changed significantly since that time. The inlet stream is small (watershed area is less than 9 km^2). Much of the inlet watershed has been logged. Although forest harvesting impacts in this watershed have been relatively minor to date (R. Ptolemy, pers. comm. 2005), the recent loss of canopy cover has triggered an increase in algal growth resulting in some temporary loss of stickleback habitat (Moore,

pers. comm. 2005, 2006). Since algal production is likely related to an increase in water temperature and sunlight, regeneration of streamside vegetation should restore the stream to pre-logging conditions within a short period. Logging is planned for at least two blocks in the watershed in 2006, including blocks outside of the protected area around the lake and alongside the inlet stream (although some metres removed from the channel); harvesting activities could lead to changes in channel morphology, water quality, and/or indirectly affect benthic prey availability in the stream (T. Michalski, pers. comm. 2006).

Habitat protection/ownership

Misty Lake and short sections of the lower portion of the inlet (less than 50 m) and the upper section of the outlet (both mostly swampy segments) are contained within the Misty Lake Ecological Reserve (Fig. 5). The reserve was established under the *Protected Areas of British Columbia Act* in 1996 as a result of recommendations in the Vancouver Island Land Use Plan. Ecological reserves are areas in British Columbia selected to preserve representative and special natural ecosystems, plant and animal species (including distinct genotypes), features and phenomena. Consumptive uses such as hunting, fishing, camping and livestock grazing, or removal of materials, plants or animals are prohibited. The Misty Lake Ecological Reserve was primarily established to protect the lake form of *Gasterosteus*, which was red-listed by the BC Conservation Data Centre at the time the reserve was established. The secondary role of the reserve is to provide opportunities for biological research. The long-term effectiveness of the reserve may be compromised as the vast majority of the inlet tributary and the lake's watershed remain outside of the current boundaries of the reserve.

BIOLOGY

There is little information on the specific biology of either the lake or stream form of the Misty Lake sticklebacks. Until further studies are completed, it is assumed that much of their biology is similar to other *Gasterosteus* populations (for reviews of general biology see Wooton 1976; Bell and Foster 1994).

Life cycle and reproduction

Typically, the male stickleback provides parental care protecting and fanning the nest and continuing to defend and care for the young fish until they are capable of moving into cover to feed (Scott and Crossman 1973). The eggs take about 7 – 10 days to hatch, depending on water temperature. In general, threespine sticklebacks begin breeding in April and finish in September (Scott and Crossman 1973). McPhail (1994) described breeding timing for the Misty Lake stickleback pair; in both forms breeding began in April and ceased in July (gravid females were abundant during May and June).

Moodie (1972) described the life history of the lake form found in the Mayer Lake drainage. The lake form in Mayer Lake reproduces in its third summer. Males probably

complete about five nesting cycles during a single breeding season before dying; the number of clutches produced by the females is unknown, but it is probably less. Reimchen (1992) found that the lake form in Drizzle Lake is unusual as it lives well beyond the first breeding season. These fish can live up to eight years, about twice the age of other studied populations of threespine stickleback.

Baker (pers. comm. 2006) was able to provide some preliminary information on the life history of the Misty Lake pair, based on size frequency data from fish sampled in 2005. Inlet fish breed at an earlier age than either the lake or outlet fish. Lake form females breed at ages 1 to 5, with ages 2 to 4 being fairly common (only one age 5 fish was present in the sample). The outlet fish appeared to be similar to the lake fish. Inlet females displayed a different life history primarily breeding at ages 1 and 2; very few were older than 3 years of age (about 90% of the females examined were two years old or younger). Inlet sticklebacks produce a higher number of eggs per spawning than either lake or outlet fish; egg size does not differ among the three habitats, but the eggs are quite large relative to those from other stickleback populations.

Herbivory/predation

Threespine sticklebacks are highly predated upon both by fish (including cannibalistic nest predation by other sticklebacks) and piscivorous birds (Scott and Crossman 1973).

Physiology

There is no published information on the physiology of the sticklebacks in the Misty Lake watershed.

Dispersal/migration

Hendry *et al.* (2002) found that lake sticklebacks placed in a stream environment will move downstream but not upstream. Stream fish were more likely to remain at the release site, but when they did move they travelled in both directions (either upstream or downstream). This was supported by the work of Moore and Hendry (2005) on gene flow between the populations.

Interspecific interactions

The lake form coexists with coastal cutthroat (*Oncorhynchus clarkii*), rainbow trout (*O. mykiss*), coho salmon (*O. kisutch*), Dolly Varden (*Salvelinus malma*) and prickly sculpin (*Cottus asper*). The inlet stream form is known to coexist with coastal cutthroat, coho salmon and Dolly Varden.

Diet has not been studied, but based on differences in the morphology of the mouth and gillrakers, the lake form is adapted to feed on zooplankton in the open water

of the lake and the inlet stream form to forage on benthic macroinvertebrates (Lavin and McPhail 1986; McPhail 1994).

Adaptability

Although various forms of *Gasterosteus* appear to have evolved rapidly following the last glacial epoch, it is clear that these threespine stickleback forms rely on stability of the environment (more specifically, maintenance of the selective regime) to maintain adaptive differences. Thus, they are vulnerable to sudden changes in their environment, including species introductions and habitat change. This vulnerability has been demonstrated by the recent and rapid extinction of the Hadley Lake benthic-limnetic stickleback pair due to the introduction of brown catfish, *Ameiurus nebulosus*, (Hatfield 2001) and the collapse of the Enos Lake benthic-limnetic stickleback pair possibly as a result of habitat changes brought about by the introduction of signal crayfish, *Pacifastacus leniusculus* (COSEWIC 2002).

POPULATION SIZES AND TRENDS

Search effort

No systematic survey to determine distribution of stickleback has been completed in the inlet drainage system. It is likely that stickleback will be found in areas above the known limits shown in Fig. 6, but researchers (Hendry, pers. comm. 2005, 2006; Moore, pers. comm. 2005, 2006) have indicated that they do not occur in the highest reaches of the system.

Abundance

In parapatric lake-stream pairs, lake stickleback are much more numerous than stream stickleback (Hendry and Taylor 2004). To date, no population estimates have been completed for the lake-stream sticklebacks in the Misty Lake system.

Reimchen (1990) estimated an average population in Drizzle Lake (Queen Charlotte Islands, BC) of 75,000 adult sticklebacks based on mark-recapture methods. Drizzle Lake is 112 ha in size; by comparison Misty Lake is 35.6 ha. Moore (pers. comm. 2005, 2006) suggests that the adult population in Misty Lake inlet may be less than 2500 and is likely higher than 4000 in the outlet; these numbers, however, are preliminary estimates based on collection data and may underestimate population sizes.

Fluctuations and trends

It is possible that in drought cycles population declines may occur as a result of habitat loss due to the lowering of the water table; this may be particularly true for the stream form. It is also possible that temporary declines may occur as a result of increased algal growth in the streams following canopy removal due to forest harvesting

in the riparian zone (Moore, pers. comm.). However, habitat appears to have remained relatively stable over the longer term and population abundance has likely followed a similar overall trend. There is no other information available to determine trends or whether fluctuations in abundance have occurred in the system.

Rescue effect

Several hundred lakes along the British Columbia coast have been examined for sticklebacks. Although other population pairs have been found (Hendry and Taylor 2004), only three are currently known to contain highly differentiated lake-stream stickleback pairs. At present, extreme divergence of contiguous lake and stream pairs appears to be confined to the northern Vancouver Island area and Graham Island in the Queen Charlotte Islands (Moodie 1972; Reimchen *et* al. 1985; Lavin and McPhail 1993; McPhail 1994; Taylor, pers. comm. 2005). It seems likely that the adaptive divergence of these pairs requires an uncommon set of conditions and events (Taylor, pers. comm. 2005). If any of the Misty Lake forms are lost, no rescue effect is possible except perhaps between the lake and the outlet, where gene flow is apparent. Although the outlet stream fish are genetically similar to the Misty Lake population, they are not the same ecotype. Furthermore, the inlet stream form is genetically distinct from the lake and outlet populations (Thompson *et al.* 1997; Hendry *et al.* 2002). If it is possible for lake-stream forms to re-establish over time, it does not seem likely that each of the new forms would be defined by a preponderance of one of the two divergent ancestral clades as they are today.

LIMITING FACTORS AND THREATS

In general, continued reproductive isolation between divergent forms of stickleback requires the maintenance of the specific selective regime that drives the divergence. Changes to the selective regime can break down reproductive barriers and bring about hybridization (as seen in the benthic-limnetic pair found in Enos Lake, Vancouver Island). Populations can also be lost due to species introductions (as in Hadley Lake, Lasqueti Island, near Vancouver Island). Hybridization in lake-stream pairs may not be as great a concern as most of the inlet fish are believed to breed in the inlet, separate from the nesting sites of the lake fish (McPhail 1994).

The management issues identified in the Misty Lake Ecological Reserve Purpose Statement (BC Parks 2003) include: potential impacts of hydrocarbon and pesticide contamination from the adjacent highway and rest stop; water quality and hydrological changes due to nearby logging; non-native species introductions (fish and plants); and non-conforming recreational uses of the lake (canoeing and illegal fishing, which may increase the potential for detrimental impacts such as alterations to the aquatic community). Should crayfish be released to the lake, this could be detrimental to the lake population as reported for Enos Lake, which is also on Vancouver Island (COSEWIC 2002). Moodie (1984) identified increased beaver (*Castor canadensis*) activity as a threat to the giant black sticklebacks in Mayer Lake, Queen Charlotte

Islands, resulting in potential lake level changes. The author indicated that fluctuating water levels could influence the availability of nesting sites. In the case of the Misty Lake inlet stream population, beaver dams constructed in the inlet could temporarily impede fish movements. However, beaver are native to Vancouver Island and do not appear to be a serious threat in this area.

Moore (pers. comm. 2005, 2006) noted an increase in algal growth in the inlet subsequent to the opening of the stream canopy with forest harvesting. This thick filamentous algae occurs in shallow sites with good light penetration and may have an effect on habitat use. Any effect will likely be temporary, and the density of algae will decrease as the riparian vegetation returns. Cumulative logging impacts may be a concern, particularly to inlet habitat from erosion, sedimentation, altered flows and changes to the benthic community; however, there have been no apparent long-term impacts to date as a result of previous forest harvesting activity in the watershed. On small streams, green-up occurs quite quickly. Shrubs (like blackberry, salmonberry, red huckleberry, etc.) and alder grow in and provide shade relatively fast on the coast. Logging plans for 2006 indicated the block was to be some metres removed from the stream so that should leave some shade cover as well. Sticklebacks are mostly found in deeper water not in the shallow sites discussed, although they may use those areas at times (some feeding forays perhaps). It is unlikely that they rely on these areas too heavily as they would likely be exposed to heavier predation. In summary, this watershed has been exposed to logging activities previously with no apparent lasting impacts to the stickleback populations to date; however, future logging is still a concern depending on the extent and proximity of these activities relative to the stickleback habitat.

SPECIAL SIGNIFICANCE OF THE SPECIES

The parapatric stickleback pair found in the Misty Lake system is one of three highly divergent lake-stream stickleback pairs known to occur; this pair is invaluable to the study of evolutionary processes. The Misty Lake pair may be one of the best examples of parapatric divergence in nature (Taylor pers. comm. 2005), as it is likely that the Misty Lake system was colonized by a single dimorphic (for mtDNA clades) form that diverged in parapatry along a lake-stream ecotone through divergent selection (Hendry *et al*. 2002). Molecular data strongly suggest that the lake-stream pairs on Graham Island and northern Vancouver Island evolved separately through parallel evolution (Thompson *et al*.1997).

EXISTING PROTECTION OR OTHER STATUS DESIGNATIONS

The lake, and a small area around it, is located within the boundaries of the Misty Lake Ecological Reserve, established for the protection of the larger lake stickleback before it was recognized as part of a parapatric lake-stream pair. Less than 50 m of inlet stream habitat, above the swamp, is included within the boundaries of the

ecological reserve. The important habitat for the inlet form is located above the highway culvert outside of the reserve; none of the outlet is captured in the reserve. Thus, the ability of the ecological reserve to function even as it was originally intended to protect the lake population is limited and virtually useless in protecting the inlet population.

The British Columbia Conservation Data Centre ranks the Misty Lake sticklebacks as S1.

Paxton, Enos and Vananda sympatric benthic-limnetic pairs are currently listed as endangered under the federal *Species at Risk Act* (SARA) and the Hadley Lake benthic-limnetic pair is considered extirpated by COSEWIC.

TECHNICAL SUMMARY

***Gasterosteus* sp.**
Misty Lake Lentic stickleback — *Épinoche lentique du lac Misty*
Range of Occurrence in Canada: Endemic to Misty Lake, Vancouver Island, B.C.

Extent and Area Information	
• *Extent of occurrence (EO)(km²)* **Based on lake area and area of watershed for outlet for lake form**	0.36 km² lake plus 1.1 km^2 outlet = 1.46 km^2
• *Specify trend in EO*	Stable
• *Are there extreme fluctuations in EO?*	May decrease in dry years
• *Area of occupancy (AO) (km²)* Based on lake area plus length of outlet X mean wetted width X 75% habitat use for lake for	0.36 km² plus 0.01 km^2 outlet = 0.37 km^2
• *Specify trend in AO*	Stable
• *Are there extreme fluctuations in AO?*	Will decrease in very dry year
• *Number of known or inferred current locations*	1
• *Specify trend in #*	Stable
• *Are there extreme fluctuations in number of locations?*	None
• *Specify trend in area, extent or quality of habitat*	Stable, although additional logging is scheduled to begin in 2006 in the watershed
Population Information	
• *Generation time (average age of parents in the population)*	2-4 yr
• *Number of mature individuals*	Unknown
• *Total population trend:*	Both lake and outlet populations appear stable
• *% decline over the last/next 10 years or 3 generations*	
• *Are there extreme fluctuations in number of mature individuals?*	Unknown
• *Is the total population severely fragmented?*	No
• *Specify trend in number of populations*	Stable
• *Are there extreme fluctuations in number of populations?*	No
• List populations with number of mature individuals in each: Misty Lake - likely >10,000 (rough estimates based on Reimchen's (1990) estimate for Drizzle Lake); outlet stream – likely >4000 (Moore, pers. comm. 2006).	
Threats (actual or imminent threats to populations or habitats)	
Potential impacts of hydrocarbon and pesticide contamination from the adjacent highway and rest stop; habitat, water quality and hydrological changes from nearby logging or climate warming; non-native species introductions (including fish, invertebrates and plants); and non-conforming recreational uses of the lake.	
Rescue Effect (immigration from an outside source)	Not Applicable – Endemic Species
• *Status of outside population(s)?*	
• *Is immigration known or possible?*	
• *Would immigrants be adapted to survive in Canada?*	
• *Is there sufficient habitat for immigrants in Canada?*	
• *Is rescue from outside populations likely?*	
Quantitative Analysis	No Data
Current Status COSEWIC: Endangered (2006) B.C. Conservation Data Centre: S1	

Status and Reasons for Designation

Status: Endangered	**Alpha-numeric code**: A3e

Reasons for Designation:
This lake-dwelling fish is part of an endemic, highly divergent species pair restricted to a single stream-lake complex on Vancouver Island with an extremely small area of occurrence. This species pair could quickly become extinct due to the introduction of non-native aquatic species or perturbations in the habitat. Proximity of this complex to a major highway and public access makes an introduction likely. Logging activities in the watershed, as well as highway use and related maintenance, could impact habitat quality to some degree.

Applicability of Criteria

Criterion A: (Declining Total Population): Met endangered A3e. The possibility of the introduction of an exotic species is extremely high and such an event is projected to have the potential to drive the population to extinction within three generations.

Criterion B: (Small Distribution, and Decline or Fluctuation): Not Applicable, although the extent of occurrence and area of occupancy are less than 500 km^2, there is no evidence of decline or fluctuation.

Criterion C: (Small Total Population Size and Decline): Not Applicable, population size, although small, probably exceeds threshold value and there is no evidence of decline.

Criterion D: (Very Small Population or Restricted Distribution): Met threatened D2. An endemic with the total population in one location where it is restricted to an AO of less than 1 km^2.

Criterion E: (Quantitative Analysis): Not applicable, no data.

TECHNICAL SUMMARY

***Gasterosteus* sp.**
Misty Lake Lotic stickleback — *Épinoche lotique du lac Misty*
Range of Occurrence in Canada: Endemic to Misty Lake, Vancouver Island, B.C.

Extent and Area Information	
• *Extent of occurrence (EO)(km²)* **Based on area of watershed for inlet form**	<9 km^2 inlet stream
• *Specify trend in EO*	Stable
• *Are there extreme fluctuations in EO?*	May decrease in dry years
• *Area of occupancy (AO) (km²)* Maximum habitat available for stream form is estimated based on total length of stream and wetted widths (calculated from basin sizes and water yield (43L/s/km^2)); maximum available ~ 0.04 km^2; minimum from known distribution ~0.008 km^2.	0.008 to 0.04 km^2 for inlet stream form
• *Specify trend in AO*	Stable
• *Are there extreme fluctuations in AO?*	Will decrease in very dry years
• *Number of known or inferred current locations*	1
• *Specify trend in #*	Stable
• *Are there extreme fluctuations in number of locations?*	None
• *Specify trend in area, extent or quality of habitat*	Stable, although additional logging is scheduled to begin in 2006 in the watershed
Population Information	
• *Generation time (average age of parents in the population*	2 yr
• *Number of mature individuals*	Unknown
• *Total population trend:*	Both populations appear stable
• *% decline over the last/next 10 years or 3 generations.*	
• *Are there extreme fluctuations in number of mature individuals?*	Unknown
• *Is the total population severely fragmented?*	No
• *Specify trend in number of populations*	Stable
• *Are there extreme fluctuations in number of populations?*	No
• *List populations with number of mature individuals in each*: inlet stream – may be < 2500 (Moore pers. comm. 2006).	
Threats (actual or imminent threats to populations or habitats)	
Potential impacts of hydrocarbon and pesticide contamination from the adjacent highway and rest stop; habitat, water quality and hydrological changes from nearby logging or climate warming; non-native species introductions (including fish, invertebrates and plants); and non-conforming recreational uses of the lake.	
Rescue Effect (immigration from an outside source)	Not Applicable – Endemic Species
• *Status of outside population(s)?*	
• *Is immigration known or possible?*	Not possible
• *Would immigrants be adapted to survive in Canada?*	
• *Is there sufficient habitat for immigrants in Canada?*	
• *Is rescue from outside populations likely?*	
Quantitative Analysis	
Current Status COSEWIC: Endangered (2006) B.C. Conservation Data Centre: S1	

Status and Reasons for Designation

Status: Endangered	**Alpha-numeric code**: A3e

Reasons for Designation*:*
This stream-dwelling fish is part of an endemic, highly divergent species pair restricted to a single stream-lake complex on Vancouver Island with an extremely small area of occurrence. This species pair could quickly become extinct due to the introduction of non-native aquatic species or perturbations to the habitat. Proximity of this complex to a major highway and public access makes an introduction likely. Logging activities in the watershed, as well as highway use and related maintenance, could impact habitat quality to some degree.

Applicability of Criteria

Criterion A: (Declining Total Population): Met endangered A3e. The possibility of the introduction of an exotic species is extremely high and such an event is projected to have the potential to drive the population to extinction within three generations.

Criterion B: (Small Distribution, and Decline or Fluctuation): Not Applicable, although the extent of occurrence and area of occupancy are less than 500 km^2, there is no evidence of decline or fluctuation.

Criterion C: (Small Total Population Size and Decline): Not Applicable, although the population size may be approaching the threshold for endangered, there is no evidence of decline.

Criterion D: (Very Small Population or Restricted Distribution): Met threatened D2. An endemic with the total population in one location where it is restricted to an AO of less than 1 km^2.

Criterion E: (Quantitative Analysis): Not applicable, no data.

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COLLECTIONS EXAMINED

No collections have been examined for the preparation of this report.