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Information used in the Recovery Potential Assessment for the misty lake stickleback pair

Les renseignements servant à l'évaluation du potentiel de rétablissement de la paire d'épinoches du lac Misty

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ABSTRACT

Misty Lake drains into the Keogh River on northern Vancouver Island. The lake supports two forms of stickleback, a small coastal fish found in both fresh and salt waters: one form lives in the lake, the other in the inlet and outlet streams. It is one of only three clearly defined lake-stream pairs in Canada and has been studied for decades as a model for understanding the evolutionary process. The Misty Lake stickleback pair was designated Endangered by COSEWIC in 2006 because it is an endemic, highly divergent species pair restricted to a single lake-stream complex. It is presently being considered for listing under the Species At Risk Act (SARA). The B.C. Conservation Data Centre designates the species “S1” (Critically Imperiled and Red-listed). It was afforded some protection from creation of the Misty Lake Ecological Reserve in 1996.

There are insufficient data to describe the species’ critical habitat other than as “synonymous with its known distribution”, with the understanding that its penetration into inlet and outlet streams requires further study. Given the lack of knowledge of abundance or trends, it is not presently possible to establish population or distribution targets.

The main human-caused threats to the Misty Lake stickleback are introduction of alien species, runoff from the Highway 19 rest stop, and changes in water quality that affect light transmission, dissolved oxygen and productivity. All must be viewed not only in terms of their effect on population numbers, but also for their likelihood of causing hybridization between the lake and stream forms. Hybridization is the reason for the collapse of the Enos Lake stickleback pair on southern Vancouver Island. Because the main importance of the Misty Lake stickleback pair is its existence as a pair, the risk of their becoming a genetically homogeneous single population is as important as the risk of simply losing individuals. Critical habitat thus becomes not only that which is needed to maintain abundance of the two forms, but also that which is needed to keep them from interbreeding.

Options for minimizing human activities and threats to habitat include expanding the ecological reserve to include the full length of the inlet streams; moving the rest stop to another stretch of Highway 19; aggressive signage and other means of raising public awareness concerning invasive species; ensuring that timber harvesting does not alter dissolved organic carbon in the wetland where the inlet enters the lake; instituting a precautionary captive breeding program to preserve the gene pool; and sharing information and research.

RÉSUMÉ

Le lac Misty s'écoule dans la rivière Keogh au nord de l'île de Vancouver. Il héberge deux types d'épinoches, de petits poissons présents autant dans l'eau douce que dans l'eau salée de la zone côtière. Un type d'épinoches vit dans le lac Misty, tandis que l'autre type vit dans ses affluents et sa décharge. Il s'agit de l'un des trois types de paire d'épinoches bien définis au Canada, que l'on étudie depuis des décennies en vue de comprendre le processus d'évolution. En 2006, le Comité sur la situation des espèces en péril au Canada (COSEPAC) a désigné la paire d'épinoches du lac Misty comme « espèce en voie de disparition », car c'est une paire d'espèces endémique et très variable qu'on ne trouve que dans un seul complexe écologique (lac-ruisseau). À l'heure actuelle, on envisage de l'inscrire sur la liste des espèces en péril, en vertu de la *Loi sur les espèces en péril*. Le B.C. Conservation Data Centre attribue à l'épinoche du lac Misty la cote « S1 » (en grand péril et sur la liste rouge). En 1996, la mise sur pied de la Réserve écologique du lac Misty a permis d'assurer une certaine protection de cette espèce.

Nous ne disposons pas de données suffisantes pour décrire l'habitat essentiel de l'épinoche du lac Misty autrement que comme un habitat « correspondant à sa répartition connue », mais nous sommes conscients de la nécessité d'étudier davantage sa présence dans les affluents et la décharge du lac Misty. Puisque nous ne connaissons pas suffisamment l'abondance de l'épinoche du lac Misty ou les tendances en cette matière, nous ne pouvons présentement en établir la population ou les cibles de répartition.

L'introduction d'espèces aquatiques étrangères, l'eau de ruissellement provenant de l'aire de repos de l'autoroute 19 et la diminution de la qualité de l'eau qui nuit à la pénétration de la lumière, à la dissolution de l'oxygène et à la productivité dans le lac Misty constituent les menaces anthropiques principales à l'égard de l'épinoche du lac Misty. Il ne faut pas seulement prendre en considération leur effet sur le nombre d'individus, mais aussi la possibilité qu'il puisse en résulter une hybridation entre les deux types d'épinoches. L'hybridation est à l'origine de la disparition de la paire d'épinoches du lac Enos, qui se trouve au sud de l'île de Vancouver. Comme l'intérêt principal de la paire d'épinoches du lac Misty réside dans le fait qu'il y en a deux types, le risque que cette espèce devienne une seule population ayant un patrimoine génétique homogène est aussi important que le risque de seulement voir chuter la population. L'habitat essentiel devient alors non seulement nécessaire pour maintenir l'abondance des deux types d'épinoches, mais aussi pour empêcher un croisement.

Parmi les mesures que nous pouvons prendre pour réduire au minimum les activités néfastes des êtres humains et les menaces pesant sur l'habitat essentiel, mentionnons les suivantes : agrandir la réserve écologique pour que celle-ci comprenne tous les affluents du lac Misty; déplacer l'aire de repos dans une autre partie de l'autoroute 19; organiser une campagne agressive d'affichage et prendre d'autres moyens permettant de sensibiliser le public à la présence d'espèces aquatiques envahissantes; s'assurer que la récolte du bois ne modifie pas le carbone organique dissous dans la zone humide où les affluents se jettent dans le lac; mettre sur pied un programme préventif de sélection des espèces en captivité visant à préserver le patrimoine génétique des épinoches et le partage de l'information sur le sujet et les résultats des recherches.

INTRODUCTION

A Recovery Potential Assessment (RPA) provides technical advice to the Minister of Fisheries and Oceans concerning the scope of activities that might harm an aquatic species or its habitat without jeopardizing the survival or recovery of the species. This document contains information to support the RPA for the three-spined stickleback pair (Gasterosteus aculeatus) inhabiting Misty Lake and its associated inlet and outlet streams on northwest Vancouver Island.

Ideally, an RPA precedes listing of a species or population under SARA, and is used to help make the decision whether or not to list. If the species is already listed, the RPA contains information and technical advice on status, threats, critical habitat and abundance that can be used to develop recovery plans. Misty Lake stickleback belongs to the first category: it is designated Endangered by COSEWIC but is not listed under SARA.

The "allowable harm" described in an RPA anticipates Section 73 of SARA, under which the Minister may authorize activities that affect a listed aquatic species, any part of its critical habitat, or the residences of its individuals if all reasonable alternatives that would reduce the impact of the activity have been considered and the best solution adopted so that the activity will not jeopardize the survival or recovery of the species. The RPA attempts to answer the question: Can the species recover if human-induced mortality is greater than zero? Ideally, the RPA contains information the Minister must place on the SARA Public Registry to document the reasons for issuing a Section 73 permit.

This report generally adheres to the three-phase format based on the Moncton Protocol and summarized in DFO (2004), with some collapsing of categories (for example, where threats to habitat are human-caused). It begins with a description of the species and its status; next, threats to the species (including human activities) and their effects are listed; finally, various scenarios that would reduce the threats and potentially allow harm under Section 73 of SARA are presented.

As a risk assessment, an RPA reflects the data available. In a case like that of the Misty Lake stickleback, where there are limited data on the species' natural history and habitat use, an RPA can only provide the best advice with the information available, while noting specific information gaps that need to be filled. Where data from similar species are used to form an opinion on critical habitat and allowable harm, the RPA becomes a relative risk assessment. This is especially true for Misty Lake stickleback, to which we apply some principles developed for a different set of stickleback pairs, the limnetic-benthic pairs (Misty Lake stickleback are a lake-stream pair, which is different in some key respects). Maximum sustainable mortality and the importance of critical habitat are discussed together in this RPA because of the unique features of stickleback species pairs that make them susceptible not only to overall numerical decline but also to hybridization or "evolution in reverse."

This assessment was written for DFO by Brian Harvey under contract to the Science Branch of Fisheries and Oceans Canada. The author consulted with the following experts during its preparation: Chris Wood, Rick Singer, Steve Colwell, Gary Taccogna (DFO); Linda Philipp (B.C. Parks); Mac Willing (B.C. Ministry of Environment, Port Hardy); Mary Ann Jones (B.C. Ministry of Environment, Water Stewardship Division); Tracy Michalski (B.C. Ministry of Environment); Ross Peterson (Chair, Enos Lake

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PART I: CURRENT STATUS

BASIC BIOLOGY AND TAXONOMY

General stickleback characteristics

The three-spined stickleback *Gasterosteus aculeatus* Linnaeus is a small (average 51 mm total length), fish that is widely distributed in the Northern hemisphere, occupying a coastal band several hundred kilometres wide that includes both marine and fresh waters. In Canada it appears to be absent along the arctic coasts of the Northwest and Yukon territories but is common along the coast of the rest of the country. In British Columbia, threespine stickleback are found in most coastal lakes and streams, to a distance of several hundred kilometres inland; in the Fraser Valley, for example, it penetrates the Fraser system as far east as Kawkawa Lake, near Hope (Scott and Crossman 1973). Marine populations are generally confined to nearshore areas and always return to fresh water to reproduce.

The spines for which the species is named are all erectile and ahead of the dorsal fin; the third (most posterior) spine is much shorter than the other two. There are also paired pelvic spines and a single, shorter anal one. The other striking feature of the species is its lack of scales; instead, it bears a row of up to 30 protective bony plates (marine forms have more plates than freshwater). Generally drab colouration becomes brilliant during the reproductive period. The overall body shape is that of a voracious hunter: compressed and muscular, with large eyes, a protrusible jaw and many small teeth (Wooton 1976).

Stickleback pairs

Misty Lake supports two separate forms of stickleback, usually referred to as a “stickleback pair,” a term that often confuses people by suggesting two individual fish. The concept of stickleback pairs needs nevertheless to be understood before describing life histories, habitats and threats; it is crucial to the conservation of Misty Lake stickleback.

During the evolution of the species *Gasterosteus aculeatus* there have been many times when a marine population has colonized adjoining freshwater habitat, often after glacial retreat. What followed next was unusually rapid divergence in many phenotypic (visible) characteristics: morphology (appearance), behaviour, physiology and life history. Although the ‘new’ forms rarely persist long enough to become true new species, they offer unique insights into the process of species formation (speciation), and have been much studied by evolutionary biologists (McKinnon and Rundle 2002). Most (but not all) stickleback diversity is in fresh water.

There are six kinds of these morphologically and ecologically distinct pairs. Three kinds occur in coastal British Columbia: the anadromous-stream resident pair, the limnetic-benthic pair and the lake-stream pair. The first kind of pair is split between marine and fresh water; the second comprises one form that uses the open waters of a lake (limnetic), and a second form that lives near the bottom (benthic). In the third kind, the lake-stream pair, one form lives in a lake, while the other lives in its attached stream(s).

The second kind of stickleback pair, the limnetic-benthic, is presently the subject of much conservation attention in British Columbia. There are four such pairs, all SARA-listed: Hadley Lake, on Lasqueti Island; Paxton Lake and Vananda Creek, on Texada Island; and Enos Lake, on southern Vancouver Island. The Hadley lake pair is extinct. A Recovery Strategy for the remaining three pairs summarizes our knowledge of these species, threats, aspects of critical habitat and strategies for their recovery (National Recovery Team for Stickleback Species Pairs 2007).

While this recovery strategy summarizes the current wisdom on one kind of stickleback species pair and is a valuable starting point for conservation of the Misty Lake pair, its conclusions and strategies cannot be imported wholesale to Misty Lake because the latter has significant ecological differences from the benthic-limnetic pairs. Where justified, the present document will make conclusions based on evidence from the benthic-limnetic pairs, but there are limits beyond which this kind of relative risk assessment cannot go, and these will be noted.

The Misty Lake stickleback pair

Misty Lake (50°36’ 32” N, 127°15’ 46” W) is located 15 km upstream of the Pacific Ocean in the Keogh River system. Misty Lake stickleback is a parapatric lake-stream pair; one form lives in the lake, the other in the inlet and outlet streams. The term parapatric means that there is no physical barrier between the lake and stream forms. While lake fish may penetrate the stream for a short distance, stream forms are not found in the lake. It is one of only three clearly defined lake-stream pairs in Canada; the other two are in Mayer and Drizzle lakes on Graham Island, Queen Charlotte Islands (McPhail 1994). Lake-stream stickleback pairs do exist in several other coastal lakes in B.C., but research to date indicates the lake and stream forms in each are significantly less distinct, both morphologically and genetically, than those in Misty, Mayer and Drizzle lakes (Hendry and Taylor 2004).

The lake form of Misty Lake stickleback is larger than the stream form, slim and uniformly dark with numerous long gill rakers. The stream form is short, fuller-bodied and mottled, with relatively few short gill-rakers and more lateral plates than lake fish (Lavin and McPhail 1993). Hybrids do not appear to be common; they may be most likely to arise in the lengthy transition zone between the lake and the outlet stream (McPhail 2007, Professor Emeritus University of British Columbia, personal communications).

These morphological differences breed true, and have adaptive significance. The shallow body of the lake form is suited for sustained swimming, while the deeper body of the stream form is better suited to the kind of rapid swimming and maneuverability required in streams. The plentiful gill rakers of the lake form are well suited to the zooplankton prey items that predominate there, while fewer gill rakers are a good adaptation to the macro-invertebrates in streams (Hendry and Taylor 2004).

Life history

The natural history of the Misty Lake stickleback has received less scientific attention than its morphology, genetics and evolution, so some inferences need to be made from the much more complete accounts of other populations of the species (for example, Wooton 1976, from whom the description below is largely taken). Because sticklebacks were the subject of groundbreaking studies that laid the foundation for the science of animal behaviour (Tinbergen 1951), their reproduction has been very well studied—at least in the laboratory.

Outside the breeding season, sticklebacks tend to swim in loosely organized schools; in spring, however, they migrate into breeding grounds. Males separate from schools, build nests and receive up to seven gravid females at roughly hourly intervals, fertilizing the eggs and arranging them around the nest in adhesive masses. Elaborate courtship behaviour, aided by changes in colour, accompanies each fertilization. Spent females return to their schools, and the males tend the eggs until hatching (a period of around a week, depending on water temperature), defending the nests and surrounding territory until the hatched fry have dispersed. Males can build several nests during the breeding season, and females produce several clutches of eggs. Misty Lake stickleback breed from April to July (McPhail 1994). Maximum life span of most sticklebacks is around four years (Baker 1994). COSEWIC (2007) cites unpublished data that suggest Misty Lake females breed at ages one to five, with the inlet stream form breeding at the earlier end of the range. There are no data on the survival of Misty Lake sticklebacks after reproduction, although the general species pattern is for most post-spawned adults not to survive the winter.

Egg survival may be affected by water temperature, dissolved oxygen and siltation (Whoriskey and FitzGerald 1994); variations in these factors are presumably more easily dealt with by the free-swimming juveniles and adults.

Newly hatched larvae begin to feed on small zooplankton once the yolk is exhausted. The general adult stickleback feeding pattern is mainly carnivorous, with prey hunted by sight during daylight. The wide range of prey items includes small aquatic crustaceans (cladocerans, copepods), aquatic insects and their larvae, molluscs, fish eggs and plant material. The lake form of the Misty Lake stickleback, with its more plentiful gill rakers, is better suited to zooplankton, while the stream form, with fewer rakers, is better adapted to taking macro-invertebrates off the bottom (Hendry and Taylor 2004).

The diet of the threespined stickleback places it in competition with a variety of other fish species, including salmonids whose fry rear in lakes and streams. Stickleback eggs and larvae are eaten by other fish (including stickleback adults), while the adults are prey to piscivorous fish, birds and mammals; they may even be taken by large invertebrates. Reimchen (1994) reviews the influence of predator type on morphology of the stickleback in Drizzle Lake on the Queen Charlotte Islands, a population with similarities to that in Misty Lake. In Misty Lake, the main fish predators on stickleback are likely sculpin *Cottus asper* and several species of salmonid including cutthroat trout and coho salmon. Their locking spines and bony lateral plates provide deterrent and protection.

Taxonomy and evolutionary significance

Understanding the taxonomic relationships of a species or population allows us to comment on its uniqueness and make decisions about protection. As a model for understanding speciation, Misty Lake stickleback and other stickleback pairs have been studied for decades, using morphological analysis, breeding experiments, and increasingly sensitive biochemical analyses of their genetic makeup. The recovery strategy for the benthic-limnetic pairs describes these rapidly evolving stickleback as a “scientific treasure”, as significant to our understanding of evolution as the finches famously described by Charles Darwin on his visit to the Galapagos Islands. The study of the genetic basis of complex traits in stickleback is an active area in cancer research (Peichel 2007; personal communication), and the fish have received considerable media attention for their rarity and importance (Zimmer 2006).

COSEWIC (2007) discusses the evolutionary significance of each of the Misty Lake stickleback populations (lake, outlet, upper inlet, lower inlet) in the light of morphological, genetic and ecological evidence. For the purposes of this report, it is sufficient to note that current genetic data indicate that the Misty Lake stickleback pair is the result of post-glacial evolution independent of the other known stickleback pairs in British Columbia watersheds (Hendry and Taylor 2004) and should thus be considered unique and irreplaceable. The following discussion summarizes our present understanding of the Misty Lake population’s taxonomic position and its importance to science.

Morphological evidence

In the Misty Lake system, morphological differences between lake and stream forms are greatest for the upper and lower inlet, and least for the outlet. The upper inlet, which is farthest from the lake, is the most divergent environment (Hendry et al. 2002). Morphology of the lake-stream pairs in Misty, Drizzle and Mayer Lakes is similar, yet they occur on two widely separated islands. They may have diverged from a single ancestor, or represent three cases of independent parallel evolution; neither explanation has been ruled out (Lavin and McPhail 1994). Interestingly, in over 100 lake-stream systems examined in south-central Vancouver Island, only Misty Lake has such pairs, suggesting some unique factor common at least to Vancouver and the Queen Charlotte Islands.

Experimental evidence

Stickleback breed in the laboratory, so it is possible to test hypotheses of genetic uniqueness in what are called “common-garden” experiments where the offspring are raised in a neutral, controlled environment. Crosses between lake fish produce only lake fish; those between lower inlet fish produce only stream fish. Clearly the two forms represent separate gene pools (Lavin and McPhail 1993). More recent and comprehensive crosses (lake fish crossed with both lower inlet and outlet fish) and reciprocal transplants of lake and stream fish using enclosures confirmed that morphological differences have a genetic base, and that they are truly adaptive. The authors concluded that adaptive divergence was caused by natural selection (Hendry et al. 2002), a conclusion also reached by McKinnon and Rundle in their 2002 review of the evolutionary significance of stickleback pairs.

Genetic evidence

All the freshwater fish in the Strait of Georgia region are postglacial; that is, they have arisen within the last 11,000 years—an unusually short period in which marine populations quickly colonized the new freshwater environments, and divergence must have been rapid (McPhail 1994). The postglacial process by which there came to be two separate stickleback ecotypes (in the case of Misty Lake, the lake and stream forms) is still not clear; the two forms may have arisen through colonization by two separate marine lineages, or by a single lineage that later diverged into lake and stream forms (Hendry et al. 2002). At a broader evolutionary scale, mitochondrial DNA analysis of Misty Lake inlet, outlet and lake populations shows ties with both of the two known pre-glacial stickleback ancestral groups or 'clades;' the fact that one of these clades (the Trans North Pacific Clade) is relatively uncommon in British Columbia is a continuing stimulant to research on the species (Johnson and Taylor 2004).

Analysis of microsatellite DNA confirms the genetic distinction between lake and stream forms shown by the common-garden breeding experiments, with further refinement based on whether stream fish were sampled from the outlet, the lower inlet or the upper inlet. Gene flow is high between the lake and outlet, and may effectively limit range of the outlet population (Moore et al. 2007). The lake and outlet fish are more closely related to each other than to inlet fish in general, although the upper and lower inlet fish are also distinct from each other. The greatest genetic difference is between lake and upper inlet fish, which are also the most morphologically distinct (Hendry et al. 2002). Gene flow between lake and stream forms is greatest for the lake/outlet combination, although adult hybrids are rare (Hendry and Taylor 2004; McPhail 1994). The possible detrimental effect of increased hybridization is a key concern for conservation of stickleback pairs, and its relevance for Misty lake stickleback is discussed later in this RPA.

Listings and protection

The Misty Lake stickleback pair was designated *Endangered* by COSEWIC in 2006 because it is an endemic, highly divergent species pair restricted to a single lake-stream complex. It is presently being considered for listing under the Species At Risk Act (*SARA*). The B.C. Conservation Data Centre designates the species "S1" (Critically Imperiled and Red-listed).

The Misty Lake Ecological Reserve was formed in 1996 by the Government of British Columbia with two main purposes: to protect habitat for the lake form (then called the "giant black stickleback"), and to provide opportunities for biological research. An additional 13 ha were acquired from Western Forest Products Ltd. in 2001 to add to the original 55 ha. Camping and extractive activities are prohibited within the reserve. The present boundaries of the reserve are shown in Figure 2, from which it can be seen that less than 250m of the inlet and outlet streams are protected.

RANGE, CRITICAL HABITAT, RESIDENCE AND ABUNDANCE

Range

Misty Lake is a small (900 x 600 m; about 35 ha), shallow (mean depth 1.7 m), tannin-stained lake whose outlet drains into the Keogh River on northern Vancouver Island (Hendry et al. 2002). Figure 1 shows its connections, which are important to understand as reference points for the sites where lake and stream forms have been sampled for morphological and genetic study.

There is only one outlet, which drains the lake from the northwest. The inlet stream, however, is referred to as the “lower inlet” where it enters the lake, and as the “upper inlet” where it branches south about one kilometre upstream. A swampy area where the lower inlet enters the lake is an important transition zone where lake and stream stickleback are found together and is also likely an important source of the tannins that colour the lake (Willing 2007 pers. comm.). Roughly half of this wetland area is outside the current boundaries of the Misty Lake Ecological Reserve (Figure 2). The inlet stream flows through a culvert where it is crossed by Highway 19 about 400 metres from the swamp; formerly passable only at high water (Lavin and McPhail 1993). An improved culvert installed in 1998 now allows free fish passage (Broekhuizen 2007 pers. comm.).

The B.C. Parks Risk Assessment for Misty Lake Ecological Reserve characterizes the Misty Lake ecosystem as rare but not diverse, and little disturbed by human development (Philip 2007 personal communication). It notes that boundaries of the reserve do not conform well to natural landscape features or minimize edge effects; the protected area ranks low in encompassing the complete watershed.

While stickleback have often been sampled from the lake and connecting streams for purposes of evolutionary research, distribution of both forms has not been systematically studied. There are no conclusive data on how far the stickleback penetrate into the upper inlet; Moore and Hendry (2005) estimate occupation of around 2 km of the outlet stream; stickleback were very scarce at a sampling location 1.6 km upstream (Moore et al. 2007). The lake is 3.5 km from Rupert Inlet, a distance of only 11 km by water, so it is open to anadromous fish. The Misty Lake Ecological Reserve is intended also to protect habitat for prickly sculpin *Cottus asper* and a variety of salmonids; however, the only salmonids observed in the lake are cutthroat trout and coho salmon (Philip 2007 personal communication). The lake is likely too darkly stained and acidic to support rainbow and Dolly Varden trout (Don McPhail Professor Emeritus, University of British Columbia, personal communication).

Foraging habitat

Misty Lake stickleback inhabit both still and running freshwaters; the different foraging behaviours demanded by these two very different habitats are clearly evident in the morphological differences between lake and stream forms. The former are adapted to visual predation on zooplankton, homing in especially on concentrated patches. The latter likely feed both in the water column as well as on the bottom, which implies adaptation to the aquatic plant cover where invertebrate prey can be found (Hart and Gill 1994). Hendry et al. (2002) note that Misty Lake stream forms were commonly found in water 0.5 – 1.0 m deep, where current was slow.

Spawning and residence habitat

While reproduction has not been described for Misty Lake stickleback, the choice of breeding sites and nest materials (depth, substrate, cover and distance from shore) can all vary (Whoriskey and FitzGerald 1994). The lake form of Misty Lake stickleback likely moves into shallower water for nest construction and breeding. McPhail (1994) was unable to describe nesting sites of the stream form, and noted only a few were visible along the margin of the darkly stained lake.

Nest site and construction may affect female choice, hence reproductive success. Stream fish and lake fish are believed to restrict their breeding sites to their respective habitats, although small numbers of gravid females of both lake and stream forms have been collected in the inlet swamp transition area and breeding seasons appear to coincide (McPhail 1994); hybridization may also occur in the lower outlet, since gene flow between lake and stream forms is greatest for the lake/outlet combination.

It is important to realize that we know less about breeding habitat for Misty Lake than we do for the limnetic-benthic stickleback pairs, where both forms use the same geographic area (the shallow littoral zone) for building nests, differing only in fine detail like the amount of aquatic plant cover (Wood et al. 2004). This knowledge gap about where Misty Lake stickleback build their nests makes it hard to say how important this use of habitat is for maintaining reproductive isolation between the two forms, a key goal of conservation.

Designation of critical habitat

Threespined stickleback has been intensively studied for decades, and we know there is great variability in its natural history, depending on its general geographic location and its specific habitat. Unfortunately, there are few data that can tell us which strategies out of this extensive behavioural repertoire are used by Misty Lake stickleback, whose interest for science has been largely as a unique population (or collection of populations) that sheds important light on the evolutionary process.

A more detailed discussion on the unique role of habitat in maintaining genetic separation between stickleback species pairs is found below (Maximum Sustainable Mortality and the Importance of Critical Habitat); it describes certain habitat features that are uniquely important to sticklebacks. Without further research, however, designation of discrete areas is impossible. The precautionary and provisional designation of the species' critical habitat must therefore be "that synonymous with its known distribution", with the understanding that its penetration into inlet and outlet streams requires further study.

Abundance

While abundance has been estimated for the limnetic-benthic lake stickleback in Enos Lake, Paxton Lake and Vananda Creek, there have been no systematic censuses of lake or stream sticklebacks in Misty Lake, nor are any such studies planned or under way. Existing estimates of population size (COSEWIC 2007) are based on sample collection for other kinds of study and are unaccompanied by any idea of historical trends. Hendry et al. (2002) provide estimates of N_e (effective population size) for the outlet, lake, and upper and lower inlets calculated from the heterozygosity observed in genetic analyses. Ranges derived using two different methods for calculation are:

Lake:	8,288 - 13,451
Lower Inlet:	5,561 - 9,029
Upper Inlet:	3,624 - 5,018
Outlet:	5,134 - 14,616

Census population size can be expected to be 4-10 times N_e; even using the most conservative lower estimates still provides an estimated abundance of more than 10,000 adults for even the smallest (Upper Inlet) population. While these estimates are not based on systematic census sampling, they do provide some basis for evaluating the risk associated with differing levels of mortality.

POPULATION AND DISTRIBUTION TARGETS FOR RECOVERY

Given the lack of knowledge of abundance or trends, it is not presently possible to establish population or distribution targets. Even for the stickleback pairs in Enos Lake, Paxton Lake and Vananda Creek, for which there are more data on abundance than there are for Misty Lake, the Recovery Strategy simply targets a "self-sustaining population." Quite apart from the fact that there is no scientific evidence of any increase or decline in the numbers of Misty Lake stickleback, simply maintaining a stable population size is only one of two relevant concepts for recovery of stickleback pairs. A later section of this report (Maximum Sustainable Mortality and the Importance of Critical Habitat) describes the important second concept, namely prevention of hybridization between the members of the two pairs.

PART II: THREATS

SOURCES OF HUMAN-CAUSED MORTALITY AND HARM

A good starting point for a discussion of threats is the Purpose Statement for the Misty Lake Ecological Reserve (British Columbia Environmental Stewardship Division 2003). Management issues listed in the purpose statement include contamination from the highway rest stop; effects of logging on water quality and hydrology; introduced species and illegal recreational practices. A complementary perspective is provided by the B.C. Parks Risk Assessment for Misty Lake Ecological Reserve (Philipp 2007 pers. comm.). Road runoff and maintenance (both public and private roads), exotic species and forest harvest are noted as risk factors, along with unauthorized recreational use and collection of plants for extraction of botanical products.

The following section discusses these and other threats, presented here in declining order of importance.

Invasive species

The extinction of genetically unique populations of threespined stickleback from Hadley Lake, Lasqueti Island was the result of introducing brown bullhead *Ameiurus nebulosus*, to the lake, and illustrates the potential consequences of inserting an alien species into habitat that supports a stickleback pair (Hatfield 2001; Vamosi 2003). The mechanism was likely straightforward predation or interference with nest building (National Recovery Team for Stickleback Species Pairs 2007). Brown bullhead is a good example of an alien species whose value for recreational angling stimulates widespread deliberate introductions (B.C. Ministry of Environment 2006). It is presently confined to the southern third of Vancouver Island; McPhail (2007) describes the species as “a disaster” for small lakes in B.C. The stickleback pair in Enos lake, Vancouver Island, has been affected by introduction of crayfish (COSEWIC 2002). For Misty Lake stickleback, the likelihood of invasion is high and the consequences serious. The main uncertainty relates to the degree of protection already afforded by the ecological reserve, which prohibits fishing.

In benthic-limnetic lake pairs like these, the ecosystems are relatively simple, an “open field” for an alien species that may be able to have a dramatic effect by reducing the reproductive isolation between the two stickleback forms. How this occurs depends on how the invasive species alters habitat. In Enos lake, for example, the introduced crayfish may not only have eaten or disturbed the stickleback (as did the bullhead in Hadley Lake), they may also have removed benthic plants where benthic stickleback foraged (thus allowing less food for the benthic form and, and removing nest cover), or altered water clarity enough to disrupt mate selection. In such a case, the impact of an introduced species is by way of habitat change that leads to less reproductive isolation and more interbreeding (Rosenfeld and Hatfield 2006).

Generalizations should, however, be avoided. Enos and Hadley Lakes are less darkly stained than Misty Lake, where an introduced species would need to be able to thrive in tannin-rich waters. Not all can; for example, attempts to introduce rainbow trout into Misty Lake have failed (Don McPhail, Professor Emeritus, University of British Columbia, personal communication). Vamosi (2003) suggests that sympatric stickleback pairs may be especially vulnerable to introduced species, as they are normally found in lakes with few other fish species. Nevertheless, the vulnerability of Misty Lake stickleback, which live in a relatively simple ecological community easily accessed at the highway rest stop, is high. Speculation about the mechanism by which an introduced species might cause decline or extirpation (competition, habitat removal, promotion of hybridization) should not affect ranking the threat as the most severe facing Misty Lake stickleback. At present, the only invasive species so far recorded in Misty Lake is the common pet shop turtle *Trachemys scripta elegans* (Philip 2007 personal communication).

Runoff from Highway 19

Misty Lake is closely approached by Highway 19 as well as a number of logging roads; before re-siting of the highway in 1998, when the new culvert was installed, a logging truck actually ended up in the lake (Broekhuizen 2007 personal communication). The main point source of impact on the lake is the highway rest stop at the southwest corner of the lake, which is large enough to permit parking and truck turnaround and contains toilets. Construction of the rest station involved brush removal and addition of fill, some of which has now been cleaned up; however, it remains a source of chemical runoff as well as a convenient access point for introduction of exotic species (Philipp 2007 personal communication). The probability of runoff is high, as is uncertainty with regard to its consequences, which depend on amount, composition and location.

Forestry

Misty Lake lies within the Keogh-Cluxewe Resource Management Zone, currently designated an Enhanced Forestry Zone (defined as an "area managed to produce higher volumes and values of timber"). Misty Lake and its inlet and outlet streams are bordered by Crown and privately owned land; Western Forest Products Ltd. has harvesting rights in both. The inlet stream corridor has been heavily logged in the past, producing considerable accumulated debris; satellite photos show some remaining old growth to the north and west. The current Forest Stewardship Plan for Western Forest Products Ltd. identifies several areas as "subject to cutting permit", including three locations near the inlet stream (Mogensen 2007 personal communication). Runoff from logging roads can raise the level of sediment in rivers and lakes. Misty Lake stickleback may be particularly sensitive to changes in water turbidity if mate selection and zooplankton productivity are controlling factors in avoiding hybridization, as they are believed to be for benthic-limnetic pairs (Wood et al. 2004).

Heavily tannin-stained lakes like Misty Lake are high in dissolved organic carbon (DOC), which leaches out of marshy or boggy areas. Tannins affect visibility, and in Misty Lake may influence mate selection. Lake levels of DOC can be affected by logging, especially in lakes that are attached to wetlands: extensive research on small lakes in Ontario and Quebec has demonstrated a transient increase in lake DOC following logging adjacent to marshy areas (Quinby 2000), followed later by a longer-lasting decrease (Magnan and Bertolo 2007). A decrease in DOC could affect the makeup of the species assemblage in Misty Lake and erode barriers to hybridization (Rosenfeld 2007 personal communication).

Water use

Water extraction, which can raise temperatures and eliminate nesting habitat, appears to be much less of an issue for Misty Lake than it is for the limnetic-benthic pairs. Misty Lake is small and shallow, so any extraction of water could reduce littoral habitat believed important for nesting of the lake form. Protection of the lake in the ecological reserve prevents such extraction, but the majority of the inlet and outlet streams are outside the reserve boundaries. The only water licenses currently existing for the watershed are on the Cluxewe River (for use by OK Paving Ltd. in asphalt manufacture) and an Enterprise License held by B.C. Hydro on the Keogh River (Jones 2007 personal communication). Neither would appear to pose any threat to Misty Lake stickleback. The probability of harm is therefore low.

Scientific study

Misty Lake stickleback are periodically removed by pole seine or trap for collection of tissue samples or laboratory experimentation. Based on the arguments provided later in this report ("Maximum Sustainable Mortality"), removal of less than 5% of the total population can be considered allowable harm.

Climate change

Onset and duration of breeding in stickleback is strongly influenced by temperature, and more northerly populations tend to breed later in the spring (Whoriskey and FitzGerald 1994). Timing of reproduction can be expected to change in response to significant warming.

MAXIMUM SUSTAINABLE MORTALITY AND THE IMPORTANCE OF CRITICAL HABITAT

To add to the uncertainties surrounding abundance, another factor that confounds risk assessment is the lack of date on the productivity of Misty Lake stickleback. Importing knowledge from other stickleback populations requires some caution, because the natural history of sticklebacks is so variable. Productivity—which could ideally be used to judge the ability of a population to tolerate mortality—depends not only on the physical environment, but also many other variables including mate selection behaviour, lifespan, age at reproduction, adult size, clutch size, egg size and number of annual spawnings. All of these factors vary widely in B.C. sticklebacks (McPhail 2007).

However, there is no reason to believe that the Misty Lake stickleback populations have been declining or are now less productive than other freshwater sticklebacks, so there is no reason to think the natural mechanisms for compensation are compromised in this population. Sticklebacks are typically resilient and productive, with a minimum doubling time of <1.25y, compared with 1.4 - 4.4 years for sockeye salmon (Froese and Pauly 2008). The target rate for sustainable harvest of Fraser River populations of sockeye salmon is an annual removal of 67% of the mature population (age 4 individuals), which corresponds to a removal of 14% of the total population (all ages). This comparison suggests that an annual stickleback harvest rate of <10% (i.e., not exceeding 1,000 per population) for each of the four sub-populations of Misty Lake stickleback should not compromise their viability. Given the uncertainty in the population estimates and the high cost of error, the lower threshold for allowable harm should be set at 5%.

Stickleback pairs are the current result of an evolutionary process that has taken advantage of quite different habitats within a confined geographic area. They are thus very restricted in their distribution, and sensitive to changes in habitat or environmental factors. This sensitivity makes their conservation unusually challenging in that a population or species can be extirpated not only as a result of its falling below a minimum viable number, but also through the genetic mixing between the two members of a species pair that do not normally mate with each other because of their different size, mating colours and nest sites. This second process, which is very different from simple attrition in numbers, is called hybridization. It can be mediated by the same mechanisms that reduce overall numbers, or by different ones. Hybridization is notoriously the reason for the collapse of the Enos Lake stickleback pair on southern

Vancouver Island, and the mechanisms by which it came about are the subject of scientific study (National Recovery Team for Stickleback Species Pairs 2007).

The overwhelming importance of the Misty Lake stickleback pair is its existence as a pair. There is little "existence value" of a hybrid because unpaired lake populations of stickleback are common (Rosenfeld 2008 personal communication). The risk of their hybridizing and devolving into a genetically homogeneous single population as a result of human actions must be given at least as much weight as the risk of simply losing individuals; in the absence of any long term monitoring program, it should even get more. "Maximum sustainable mortality" is impossible to establish when the stickleback pair could cease to exist as two separate, genetically distinct populations, in which case there might still be enough individuals, but they wouldn't be lake or stream stickleback any more. Instead, it's helpful to think of the Misty Lake stickleback pair as two separate species balanced on a fine edge between staying separate and becoming a single species again – a process that has been called "evolution in reverse."

If hybridization is accepted as a mechanism by which Misty Lake stickleback could become extinct, we need to consider all those things that can cause the end of reproductive isolation. Different habitats (lake vs stream) are what caused reproductive isolation in the first place, so the challenge is to identify which habitat attributes are most important to preserve. Critical habitat thus becomes not only that which is needed to maintain abundance of the two forms, but also that which is needed to keep the two pairs from interbreeding. The threats already described must be viewed in this light. There is presently a high level of uncertainty because of our lack of knowledge about the way habitat changes could trigger hybridization.

In the discussion of critical habitat, no attempt was made to establish geographic boundaries. There are, however, attributes of critical habitat that can be identified, some of them quite specific for sticklebacks. Principles advanced for the limnetic-benthic lake pairs are relevant and provide a good starting point (Hatfield 2006). They must, however, be taken with the caveat that the life of a lake-stream pair is probably different from that of a limnetic-benthic pair. For example, lake and stream sticklebacks do not appear to share the same breeding habitat; apart from the transition zone between the inlet and the lake, they are probably less likely to encounter each other than are limnetic-benthic forms. Opportunities for hybridization may therefore be lower than for the limnetic-benthic pairs. Critical habitat criteria that affect both abundance and prevent hybridization in limnetic-benthic pairs include breeding areas and water quality.

Breeding areas

The shallow, littoral zone of the lake is a known breeding and rearing area; however, habitat used for breeding in streams is not well known. Aquatic plants are probably important for nest cover and production of macroinvertebrate food, and could be considered critical habitat. If the stream form uses shallow areas to breed, it may be affected by transient increases in filamentous algal growth that follow opening up of the stream canopy by logging (COSEWIC 2006).

Water quality

Maintenance of reproductive isolation probably depends to a great extent on mate recognition, which is affected by light transmission. Any changes in water quality that affect light transmission can disrupt vision and make it harder to choose mates; the severity of the effect depends of course on how closely the lake and stream pairs share breeding habitat. Light transmission is affected by suspended solids and the amount of dissolved organic carbon (already relatively high in tannin-rich Misty Lake).

Productivity is another key process affected by water quality, including turbidity and land practices that cause eutrophication. Any changes to productivity that alter the balance between zooplankton (eaten by the lake form) and macroinvertebrates (eaten by the stream form) could remove one of the main drivers of reproductive isolation (Schluter 1995).

PART III: SCENARIOS FOR MITIGATION AND ALTERNATIVES

MINIMIZING HUMAN ACTIVITIES AND THREATS TO HABITAT

Information sharing and research

The National Recovery Team for Stickleback Species Pairs has produced a comprehensive recovery strategy for the limnetic-benthic stickleback pairs, and Recovery Implementation Groups for Enos Lake stickleback and the species pairs on Texada Island are already active (Peterson 2007, personal communication). While the present report has taken pains to point out the differences between limnetic-benthic pairs and lake-stream pairs, there are enough similarities that recovery planning for Misty Lake stickleback can benefit enormously from the experience of those working on the lake pairs. While not technically minimizing risk to the Misty Lake pair, building of closer links between the two groups expands the repertoires for predicting and dealing with those risks.

The existence of the Misty lake Ecological Reserve is a powerful springboard for filling the many knowledge gaps. A local educational institution such as North Island College, for example, could promote conservation as well as its own visibility and growth through adopting Misty Lake as a research site.

Enlargement of the Ecological Reserve

The Misty Lake Ecological Reserve provides a degree of protection that is not currently available to the limnetic-benthic stickleback pairs in Enos Lake, Paxton Lake and Vananda Creek. For the latter species pairs, establishment of core Wildlife Habitat Areas could involve various boundaries around lakes, tributaries, wetlands and second order streams (Wood et al. 2004): the levels of protection suggested are generally already met or exceeded by the Misty Lake reserve. The exception is the parts of the inlet and outlet streams that are not included in the reserve; for these areas, which almost certainly contain habitat for the stream stickleback form, the only protection is through standard riparian management practices. Wood et al. (2004) argue that, given the global significance of stickleback pairs, standard practice is not sufficiently precautionary. Threats to Misty Lake stickleback habitat could thus be further reduced by expanding the ecological reserve to include the full length of the inlet streams.

Managing water levels

The improved culvert on the inlet stream approximately 1 km above the lake removes a barrier to stickleback that existed during low water periods (Lavin and McPhail 1993). Despite this improvement, the current low level of water extraction in the Misty Lake watershed should be maintained.

Rest stop runoff

Having a paved parking area on the margin of a small lake containing a globally important endangered species is an unnecessary risk. While the risk of highway runoff is not limited to the rest area (Highway 19 runs close to Misty Lake along its entire southern border), it could be greatly reduced by moving the rest stop to another stretch of Highway 19.

Invasive species

There are few easy access points to Misty Lake, so the accepted strategy of minimizing the risk of species introductions, namely aggressive signage and other means of raising public awareness, has some chance of working. Relocating the rest stop on Highway 19 would go a long way to reducing easy access.

As experience with Hadley Lake and Enos Lake stickleback has shown, once an alien species is introduced into a stickleback lake, the effects are rapid and severe. The virtual impossibility of actually preventing a determined person from inoculating a water body with an alien species means that precautionary captive breeding of Misty lake stickleback becomes an option for preserving the gene pool. This *ex situ* conservation strategy, understandably unpalatable to people who argue that existence of a captive population acts as a tacit license to abnegate responsibility, has nevertheless been included in recovery planning for Enos Lake stickleback (two *ex situ* captive populations exist).

Forestry

Western Forest Products' current plan for harvest of trees to the north and west of Misty Lake and along its inlet and outlet streams was not available at the time of writing this report. The likelihood of harm from forestry is greatest near the inlet stream, which contains the most genetically divergent forms. Sedimentation and reduction of stream canopy can be minimized or eliminated by application of the B.C. Riparian Area Regulations. However, these regulations do not apply on privately owned lands. There are presently three areas subject to cutting permit in the area of the upper and lower inlet. Two of these areas are mainly Crown land; the third, easternmost area is at least partly private (owned by Western Forest products (Tipper 2008, personal communication). An on-site survey would be required to refine these boundaries. The likelihood of harm from forestry in the inlet area depends on activities of the tenure holder and applicability of riparian area regulations. Severity is not possible to predict.

Particular attention should be paid to the wetland area where the inlet enters the lake, to avoid the known effects of logging on dissolved organic carbon (DOC). A further measure of protection would come from extending the ecological reserve along the length of the inlet and outlet streams.

ALTERNATIVES TO HUMAN ACTIVITIES AND THREATS TO HABITAT

Misty Lake is fortunate in that, with the creation of the Ecological Reserve, which involved cooperation of the forest license holder, most human activities and threats to habitat have effectively been removed. Those that remain can be minimized as suggested above.

RECOMMENDED PRODUCTIVITY AND MORTALITY VALUES

There is a large literature on the rationale for setting the minimum viable population (MVP) for wildlife species. Lack of data on the variables that go into calculating such values is common for many species besides stickleback, so there are rules of thumb that can give a general idea. An effective breeding number (N_e) of 1,000 and a population of 10,000 mature individuals are commonly cited, and these figures are close to the present indirect estimates of abundance. Hatfield (2006) reviews the principles of population viability analysis as they apply to limnetic-benthic stickleback pairs, making assumptions about vital rates (for example, fecundity and survival). The resulting mathematical models permit estimation of various impacts on habitat and strongly suggest a substantial portion of the lakes be defined as critical habitat. The effective breeding number arrived at (N_e = 1,000, representing a minimum viable population of 10,000) was not, however, adopted as a quantitative goal in the Recovery Strategy for the benthic-limnetic pairs.

Development of population viability models for Misty Lake stickleback would face similar challenges because of lack of data on abundance, spatial distribution, habitat availability and recruitment. Establishing a minimum viable population size for a listed species, especially one with no economic importance, is little more than an exercise if there is no long-term monitoring program.

FIGURES

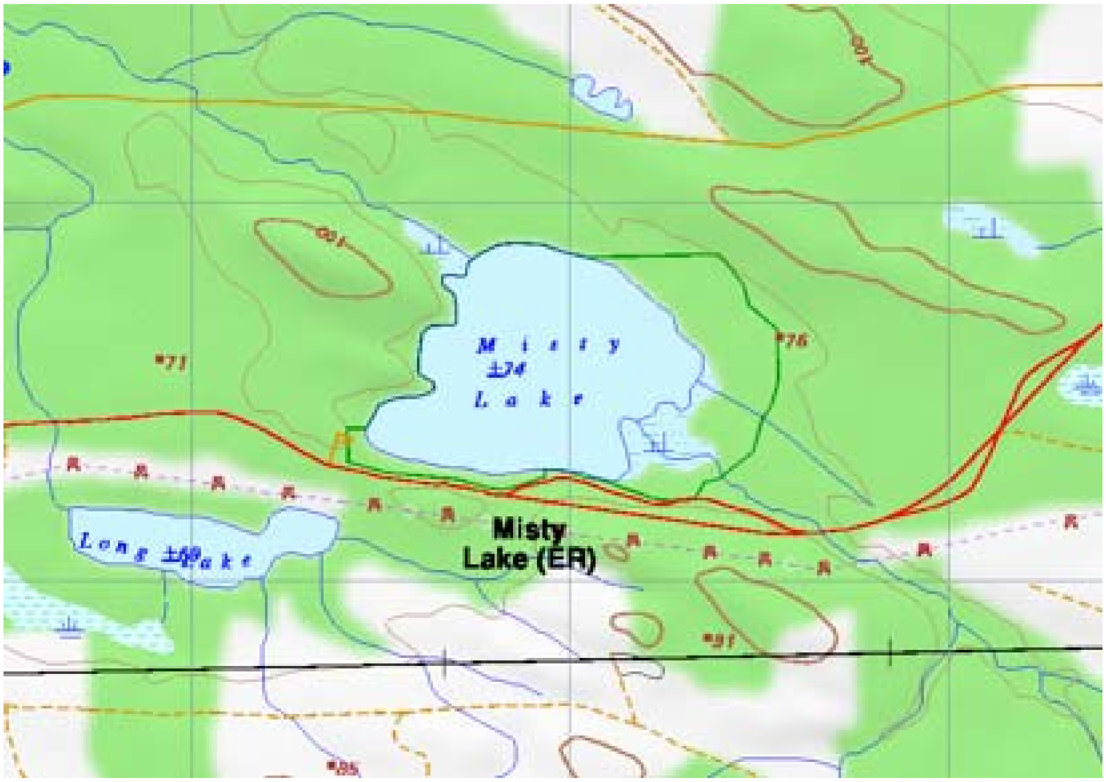

Figure 1. Misty Lake and its associated streams. The outlet exits to the northwest; the lower inlet enters at the southeast and branches south into the upper inlet. The red line is Highway 19 between Port McNeil and Port Alice.

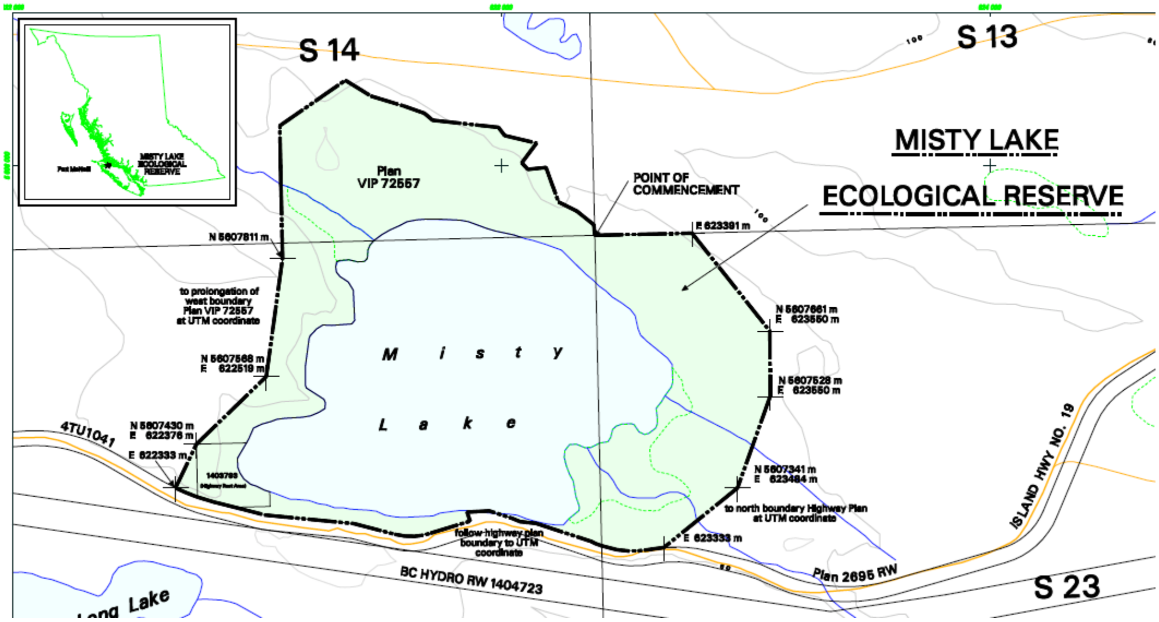

Figure 2. The Misty Lake Ecological Reserve. The highway rest stop is on the southwest corner of the lake.

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