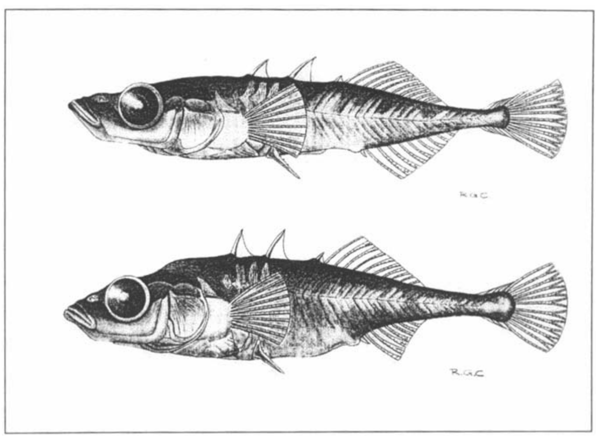
COSEWIC
Assessment and Status Report

on the

Enos Lake Benthic and Limnetic Threespine Stickleback Species Pair
Gasterosteus aculeatus

in Canada



ENDANGERED
2012

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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Previous report(s):

COSEWIC. 2002. COSEWIC assessment and update status report on the Enos Lake stickleback species pair *Gasterosteus* spp. in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vii + 27 pp.

McPhail, J.D. 1988. COSEWIC status report on the Enos Lake stickleback species pair *Gasterosteus* spp. in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 27 pp.

Production note:
COSEWIC would like to acknowledge Todd Hatfield for writing the status report on the Enos Lake Benthic and Limnetic Threespine Sticklebacks *Gasterosteus aculeatus* in Canada, prepared under contract with Environment Canada. This report was overseen and edited by Dr. Eric Taylor, Co-chair of the COSEWIC Freshwater Fishes Specialist Subcommittee.

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Enos Lake Benthic (lower image) and Limnetic (upper image) Threespine Stickleback Species Pair — drawn by R. Carveth.

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COSEWIC
Assessment Summary

Assessment Summary – May 2012

Common name
Enos Lake Benthic Threespine Stickleback

Scientific name
Gasterosteus aculeatus

Status
Endangered

Reason for designation
This small fish occurs in a single lake in south coastal British Columbia where it has now formed a hybrid swarm with a co-existing stickleback. Although it is possible that a small number of genetically pure fish still exist in the lake, the ongoing presence of an invasive crayfish, and associated habitat degradation, continue to place this species at a high risk of extinction.

Occurrence
British Columbia

Status history
Original designation (including both Benthic and Limnetic species) was Threatened in April 1988. Split into two species when re-examined in November 2002 and the Enos Lake Benthic Threespine Stickleback was designated Endangered. Status re-examined and confirmed in May 2012.

Assessment Summary – May 2012

Common name
Enos Lake Limnetic Threespine Stickleback

Scientific name
Gasterosteus aculeatus

Status
Endangered

Reason for designation
This small fish occurs in a single lake in south coastal British Columbia where it has now formed a hybrid swarm with a co-existing stickleback. Although it is possible that a small number of genetically pure fish still exist in the lake, the ongoing presence of an invasive crayfish, and associated habitat degradation, continue to place this species at a high risk of extinction.

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COSEWIC
Executive Summary

Enos Lake Benthic and Limnetic Threespine Stickleback Species Pair
Gasterosteus aculeatus

Species Information

The Enos Lake Benthic and Limnetic Threespine Sticklebacks were small-bodied (up to about 60 mm total length) freshwater fish species derived from the marine Threespine Stickleback. They were one of a handful of sympatric, reproductively isolated species pairs that occur in lakes in a restricted area of the Strait of Georgia, southwestern British Columbia. Limnetics primarily exploit plankton, and had traits that are considered adaptations to a zooplankton-consuming lifestyle. Benthics had traits that are considered to be advantageous in benthic feeding.

Detailed morphological, genetic and breeding analyses of the Enos Lake Benthic and Limnetic sticklebacks began in October 1977 and confirmed striking differences in general body shape. A laboratory breeding program demonstrated that differences between the limnetic and benthic sticklebacks had a genetic basis, yet there were no intrinsic barriers to hybridization, because hybrids were equally viable and fertile. The incidence of hybridization in the wild was inferred to be low because less than 1% of the total adult population had an intermediate morphology similar to lab-reared hybrids.

The Enos Lake Benthic and Limnetic sticklebacks satisfied all conditions of the biological species concept, and were therefore considered true species. Limnetics and benthics were easy to distinguish based on morphology, behaviour and ecology; differences in morphology and behaviour were genetically based; they showed strong assortative mating; and they constituted two genetically distinct populations.

Morphological and genetic evidence strongly indicates that Enos Lake sticklebacks now occur as a single hybrid swarm, and no longer satisfy the definition of distinct species.

Distribution

The Enos Lake Benthic and Limnetic sticklebacks were restricted to a single 17.6 ha lake, Enos Lake, on southeastern Vancouver Island, British Columbia.

Habitat

Habitat requirements for stickleback species pairs included features that limit size or viability of populations (e.g., lake productivity, juvenile rearing area, nesting habitat area), but also those features of the environment that prevent hybridization. These needs included moderate littoral and pelagic productivity, absence of exotic species, maintenance of natural light transmission levels, and maintenance of natural littoral macrophytes. The latter two were deemed especially important for maintaining mate recognition.

Enos Lake has been subjected to some human disturbance, including damming of the outlet stream, raising of the water level, and water extraction for irrigation and domestic use. Some of these disturbances initially occurred several decades ago and the species pair apparently continued to do well for a long period after. The biggest habitat change over the recent past is related to the introduction of American Signal Crayfish, which has considerably modified the littoral areas of the lake by removing almost all littoral vegetation. Prior to its introduction, the littoral area of Enos Lake had dense beds of littoral vegetation in the summer. Given the preference for different nesting microhabitats, this likely had a significant effect on the breakdown of mating barriers between the species and promoted the collapse of the species pair.

Biology

Limnetics were thought to mature on average as one-year-olds, and rarely live beyond a single breeding season. In the lab, reproductive females laid multiple batches of eggs in quick succession, and were thought to do the same in the wild. Nesting males would mate with several to many females, and may have nested more than once within a single breeding season.

Benthics delayed sexual maturation relative to limnetics. Although some individuals likely mated in their first year, many may have delayed mating until they were two-year-olds. They may have lived up to about five years, and mated in several breeding seasons. In the lab, reproductive females had fewer clutches within a breeding season than limnetics, and were thought to do the same in the wild. Nesting males would mate with several to many females, and may have nested more than once within a single breeding season.

Limnetics and benthics were similar to other Threespine Sticklebacks in their overall mode of reproduction. Males constructed nests, which they guarded and defended, until fry were about a week old. Eggs took up to a week to hatch, depending on temperature, and another three to five days before larvae were free-swimming. The nests and contents remained vulnerable to predators of different kinds, including other sticklebacks. Benthics built their nests under cover of macrophytes or other structure; limnetics tended to breed in open habitats.

Population Sizes and Trends

Population sizes in Enos Lake were estimated in 2001, based on multiple mark-recaptures, but these estimates were confounded by species identification problems due to substantial hybridization that had occurred by that time. When the sample was pooled, the population estimate was 26,630 ± 8,240, which gives an indication of the total number of sticklebacks in the lake that are one year of age and older.

Limiting Factors and Threats

Limits to Enos Lake stickleback abundance were poorly understood and there were few relevant data. Sticklebacks in the lake were abundant; the primary factor determining their original conservation status was extreme endemism and threats from introduced species, not decline in abundance or distribution.

Threats to Enos Lake Benthic and Limnetic sticklebacks have been described in the National Recovery Strategy. The primary threats to persistence of stickleback species pairs is introduction of exotic species.

Special Significance of the Species

The significance of Enos Lake Benthic and Limnetic sticklebacks was primarily aesthetic and scientific. Stickleback species pairs are widely regarded as a scientific treasure and have become a textbook case of recent adaptive radiation. There was no direct commercial value of Enos Lake Benthic and Limnetic sticklebacks.

Existing Protection

Enos Lake Benthic and Limnetic sticklebacks are listed as Endangered under Schedule 1 of the federal *Species at Risk Act*. They are designated Endangered by COSEWIC, based on a 2002 and 2012 update status report. They are currently designated Endangered by the BC Conservation Data Centre. There are no habitat protection provisions specifically for Enos Lake Benthic and Limnetic sticklebacks.

TECHNICAL SUMMARY 1

Gasterosteus aculeatus

Enos Lake Benthic Threespine Stickleback — Épinoche à trois épines benthique du lac Enos

Endemic to Enos Lake, British Columbia

Demographic Information

Generation time	2 yr
Is there a continuing decline in number of mature individuals? *There are no Enos benthics in the wild or in captivity*	Not Applicable
Estimated percent of continuing decline in total number of mature individuals within 5 years	Not Applicable
Percent decline in total number of mature individuals over the last 10 years.	>70%
Percent decline in total number of mature individuals over the next 10 years.	Unknown, but probably >70%
Percent decline in total number of mature individuals over any 10 year period, over a time period including both the past and the future. *Although sampling indicates the existence of a hybrid swarm, some benthic individuals may exist in the total population*	>70%
Are the causes of the decline clearly reversible and understood and ceased? *The decline is thought to have been driven by the introduction of an exotic crayfish, but the exact mechanism remains uncertain and does not appear to be reversible.*	No
Are there extreme fluctuations in number of mature individuals?	No

Extent and Occupancy Information

Estimated extent of occurrence *(This is an extreme endemic, restricted to a single small lake. McPhail (1984) lists the area of Enos Lake at 17.6 ha. = 0.20 km²). If all fish are indeed hybrids, EO will be 0*	8 km²
Index of area of occupancy (IAO) 2 X 2 km grid *If all fish are indeed hybrids, IAO will be 0*	8 km^2
Is the total population severely fragmented?	No
Number of “locations”	1
Is there continuing decline in extent of occurrence? *There are probably no Enos benthics in the wild and there are none in captivity*	Yes
Is there continuing decline in index of area of occupancy?	Yes
Is there continuing decline in number of populations?	No
Is there continuing decline in number of locations?	No
Is there continuing decline in area, extent or quality of habitat? *Habitat has been substantially altered by introduced crayfish and is ongoing*	Yes
Are there extreme fluctuations in number of populations?	No
Are there extreme fluctuations in number of locations?	No
Are there extreme fluctuations in extent of occurrence?	No
Are there extreme fluctuations in index of area of occupancy?	No

Number of Mature Individuals (in each population)

Population	N Mature Individuals
Total	Unknown, but thought to be 0

Quantitative Analysis

Repeated genetic analyses strongly suggest that there are no "pure" Enos benthics in the wild or in captivity	

Threats (actual or imminent, to populations or habitats)

Immediate
- Ecological effects of exotic species
- Water diversion and drawdown
- Habitat loss and degradation from land use practices

Potential
- Excessive harvest for research purposes

Rescue Effect (immigration from outside Canada)

Status of outside population(s)? Not Applicable (endemic to Enos Lake)	
Is immigration known or possible?	No
Would immigrants be adapted to survive in Canada?	Not Applicable
Is there sufficient habitat for immigrants in Canada?	Not Applicable
Is rescue from outside populations likely?	Not Applicable

Current Status

COSEWIC: Endangered 2012

Status and Reasons for Designation

Status: **Endangered**	**Alpha-numeric code:** A2ace; B1ab(iii,v)+2ab(iii,v); C2a(ii)
Reasons for designation: This small fish occurs in a single lake in south coastal British Columbia where it has now formed a hybrid swarm with a co-existing stickleback. Although it is possible that a small number of genetically pure fish still exist in the lake, the ongoing presence of an invasive crayfish, and associated habitat degradation, continue to place this species at a high risk of extinction.	

Applicability of Criteria

Criterion A:
Meets Endangered for A2, sub-criteria a, c, and e as declines in abundance inferred to be > 70% from genetic and morphological samples from the lake as a consequence of the combined effects of an invasive species and its detrimental effect on habitat quality.

Criterion B:
Meets Endangered for B1 and B2, sub-criterion b(iii, v) as EO, IAO, and number of locations (8km², 8km², 1, respectively) are all well below thresholds, and a continuing decline in both quality of habitat and number of mature individuals is inferred from the continuing presence of an exotic crayfish.

Criterion C:
Meets Endangered for C2a(ii) as a continuing decline is inferred and a single population contains > 95% of any remaining mature individuals.

Criterion D:
Meets Threatened D2 as it occurs in one location.

Criterion E:
Not applicable, as necessary data unavailable.

TECHNICAL SUMMARY 2

Gasterosteus aculeatus
Enos Lake Limnetic Threespine Stickleback — Épinoche à trois épines limnétique du lac Enos
Endemic to Enos Lake, British Columbia

Demographic Information

Generation time	1 yr
Is there a continuing decline in number of mature individuals? *There are no Enos limnetics in the wild. There is a morphologically divergent, captive breeding population in a golf course pond in Murdo Fraser Park, North Vancouver*	Probably
Estimated percent of continuing decline in total number of mature individuals within 5 years	>70%
Percent decline in total number of mature individuals over the last 10 years.	>70%
Percent decline in total number of mature individuals over the next 10 years.	Unknown, but probably > 70%
Percent decline in total number of mature individuals over any 10 year period, over a time period including both the past and the future. *Although sampling indicates the existence of a hybrid swarm, some limnetic individuals may exist in the total population*	>70%
Are the causes of the decline clearly reversible and understood and ceased? *The decline is thought to have been driven by the introduction of an exotic crayfish, but the exact mechanism remains uncertain and does not appear to be reversible*.	No
Are there extreme fluctuations in number of mature individuals?	No

Extent and Occupancy Information

Estimated extent of occurrence *(This is an extreme endemic, restricted to a single small lake. McPhail (1984) lists the area of Enos Lake at 17.6 ha = 0.20 km²).* *If all fish are indeed hybrids, EO will be 0*	8 km²
Index of area of occupancy (IAO) 2 X 2 km grid *If all fish are indeed hybrids, IAO will be 0*	8 km^2
Is the total population severely fragmented?	No
Number of "locations"	1
Is there continuing decline in extent of occurrence? *There are no Enos limnetics in the wild. There is a morphologically divergent, captive breeding population in a golf course pond in Murdo Fraser Park, North Vancouver*.	Yes
Is there continuing decline in index of area of occupancy? *There are probably no Enos limnetics in the wild*.	Yes
Is there continuing decline in number of populations?	No
Is there continuing decline in number of locations?	No
Is there continuing decline in area, extent or quality of habitat? *Habitat has been substantially altered by introduced crayfish and is ongoing*	Yes
Are there extreme fluctuations in number of populations?	No
Are there extreme fluctuations in number of locations?	No
Are there extreme fluctuations in extent of occurrence?	No
Are there extreme fluctuations in index of area of occupancy?	No

Number of Mature Individuals (in each population)

Population	N Mature Individuals
Total	Unknown, but probably 0

Quantitative Analysis

Repeated genetic analyses strongly suggest that there are no "pure" Enos limnetics in the wild	Not conducted

Threats (actual or imminent, to populations or habitats)

Immediate

- Ecological effects of exotic species
- Water diversion and drawdown
- Habitat loss and degradation from land use practices

Potential

- Excessive harvest for research purposes

Rescue Effect (immigration from outside Canada)

Status of outside population(s)? Not Applicable (endemic to Enos Lake)	
Is immigration known or possible?	No
Would immigrants be adapted to survive in Canada?	Not Applicable
Is there sufficient habitat for immigrants in Canada?	Not Applicable
Is rescue from outside populations likely?	Not Applicable

Current Status

COSEWIC: Endangered 2012

Recommended Status and Reasons for Designation

Recommended Status: Endangered	**Alpha-numeric code:** A2ace; B1ab(iii,v)+2ab(iii,v); C2a(ii)

Reasons for designation: This small fish occurs in a single lake in south coastal British Columbia where it has now formed a hybrid swarm with a co-existing stickleback. Although it is possible that a small number of genetically pure fish still exist in the lake, the ongoing presence of an invasive crayfish, and associated habitat degradation, continue to place this species at a high risk of extinction.

Applicability of Criteria

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Meets Endangered for A2, sub-criteria a, c, and e as declines in abundance inferred to be > 70% from genetic and morphological samples from the lake as a consequence of the combined effects of an invasive species and its detrimental effect on habitat quality.

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Criterion C:
Meets Endangered for C2a(ii) as a continuing decline is inferred and a single population contains > 95% of any remaining mature individuals.

Criterion D:
Meets Threatened D2 as it occurs in one location.

Criterion E:
Not applicable, as necessary data unavailable.

PREFACE

Enos Lake Benthic and Limnetic sticklebacks (*Gasterosteus aculeatus*) were sympatric, reproductively isolated, but undescribed, species that were restricted to a single lake (Enos Lake) on Vancouver Island, British Columbia. The two species were considered as a species pair and both species were designated Threatened in April 1988. They were assessed as two species when re-examined and were designated Endangered in November 2002 and April 2012. Status was determined based primarily on an extremely restricted distribution and ongoing threats from introduction of exotic species and habitat loss and/or degradation from human disturbance. The species were listed as Endangered under *SARA* in 2005.

A recovery strategy, co-led by Fisheries and Oceans Canada – Pacific Region and the British Columbia Ministry of the Environment, was completed in 2007. The recovery strategy lists a series of threats, the greatest of which is introduction of exotic species. In the late 1990s non-native American Signal Crayfish (*Pacifastacus leniusculus*) became well-established in the lake. Formerly lush littoral macrophytes disappeared from the lake. At about the same time, hybridization levels between Enos Lake limnetics and benthics increased. The species pair has now collapsed into a hybrid swarm, and there appears to be little likelihood of recovery. Another species pair in Hadley Lake on Lasqueti Island, southwestern British Columbia, went extinct following introduction of Brown Bullhead. Given the development of the hybrid swarm between Enos Lake Benthic and Limnetic Threespine Sticklebacks and the small possibility that some pure individuals of either species may still exist in the lake, the following status report describes and assesses the original two designatable units (DUs). Because of the unique nature of this situation (two DUs have collapsed into a hybrid swarm), the report presents both historical context (two reproductively isolated DUs) and the current context (a single hybrid swarm) for most sections of the report.

The sticklebacks of Enos Lake continue to be studied by researchers interested in ecology, evolution and genetics, particularly in terms of the apparent fragility of ecologically based mating barriers in recently derived species.

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
(2012)

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.

Environment Canada / Environnement Canada
Canadian Wildlife Service / Service canadien de la faune

Canada

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

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SPECIES INFORMATION

Name and Classification

Phylum:	Chordata	
Class:	Actinopterygii (ray-finned fishes)	
Order:	Gasterosteiformes	
Family:	Gasterosteidae	
Genus:	Gasterosteus	
Limnetic Species:	*Gasterosteus aculeatus*	
Benthic Species:	*Gasterosteus aculeatus*	
Common Name	English:	Enos Lake Limnetic Threespine Stickleback Enos Lake Benthic Threespine Stickleback
	French:	Épinoche à trois épines limnétique du lac Enos Épinoche à trios épines benthique du lac Enos

Morphological Description

Enos Lake Benthic and Limnetic sticklebacks were freshwater fish species derived from the marine Threespine Stickleback (*Gasterosteus aculeatus*). Understanding the uniqueness of these species requires understanding the broader postglacial radiation of sticklebacks in British Columbia.

The Threespine Stickleback is a small (average length 51 mm) fish that is common in coastal marine and freshwater habitats throughout the Northern Hemisphere (Scott and Crossman 1973; McPhail 2007). The marine form is ancestral to most freshwater forms, and is usually anadromous, meaning it returns to freshwater to reproduce (Schluter and McPhail 1992,1993; McKinnon and Rundle 2002). The marine form has given rise to isolated freshwater forms in numerous postglacial lakes and streams (McPhail 1994, 2007).

The Threespine Stickleback has a laterally compressed body with delicate pectoral and caudal fins. Individuals in most populations are well-armoured with calcified lateral plates, and pelvic and dorsal spines that can be rigidly locked in an erect position (Scott and Crossman 1973; Wooton 1976; Reimchen 1994). Freshwater populations are variable in extent of armour but usually have less than the marine form (Reimchen 1994). Body colour varies from silver to mottled green and brown. Sexually mature males develop bright red throats during the breeding season, although in a few freshwater populations males turn completely black instead (McPhail 1969; Ridgway and McPhail 1987; Reimchen 1989).

Marine sticklebacks are phenotypically similar throughout their range, whereas freshwater sticklebacks are ecologically, behaviourally and morphologically diverse (McPhail 1994). Three sets of genetically and morphologically divergent “pairs” are known from coastal British Columbia (McPhail 1994): parapatric anadromous and stream-resident pairs (i.e., spatial distribution is contiguous and only overlapping in a relatively small area of contact), sympatric limnetic and benthic pairs (i.e., spatial distribution is entirely or mostly overlapping), and parapatric lake and stream pairs. In each case these are referred to as “species pairs” because there are strong ecological interactions between each member of a pair. Detailed descriptions of solitary freshwater populations, and of each kind of species pair, are provided in McPhail (1994).

Enos Lake Benthic and Limnetic sticklebacks (Figure 1) were one of five known sympatric, reproductively isolated species pairs that occur in lakes on islands in a restricted area of the Strait of Georgia, BC (McPhail 1984,1992; Schluter and McPhail 1992; McPhail 1993,1994; Gow *et al.* 2008). In each case, limnetics primarily exploit plankton, and have morphological traits such as a fusiform body, narrow mouth and many, long gill rakers, which are traits considered adaptations to a zooplankton-consuming lifestyle (Schluter and McPhail 1992,1993). Benthics mainly eat benthic invertebrates in the littoral zone, and have a robust body form, wide gape and few, short gill rakers, traits considered to be advantageous in benthic feeding (Schluter and McPhail 1992,1993). The pattern of morphological and ecological divergence is similar in each of the lakes (Schluter and McPhail 1992; Gow *et al.* 2008), such that limnetics from all species pair lakes look relatively similar to each other, as do all benthics, but there are also some minor morphological differences (McPhail 1994). The available genetic and biogeographic evidence suggests that species pairs of Threespine Sticklebacks have arisen independently in each lake through a process of double invasion by marine sticklebacks, divergence in allopatry and subsequent ecological speciation (McPhail 1993; Hatfield and Schluter 1999; Taylor and McPhail 2000). The following discussion reviews results of studies before and after the start of increasing hybridization of Enos Lake Benthic and Limnetic sticklebacks. A similar separation is adhered to in two subsequent sections on the main biology of the species pair.

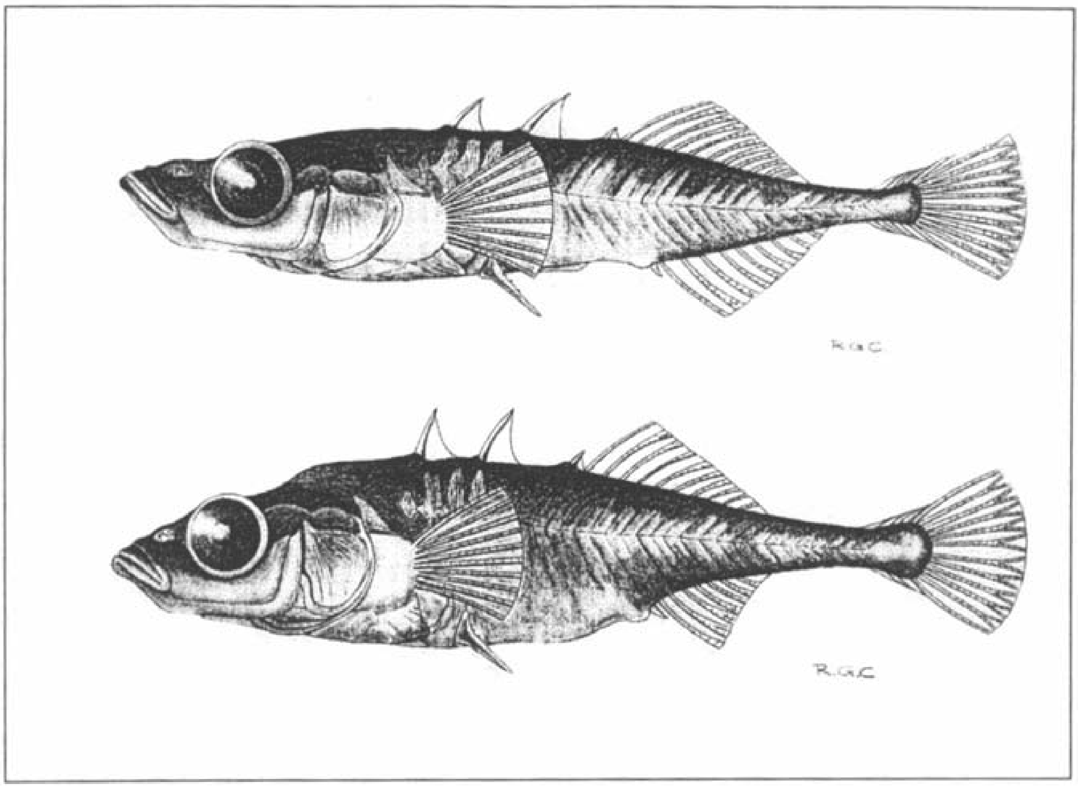

Figure 1. Enos Lake Limnetic (top) and Benthic (bottom) sticklebacks. (Drawing by R. Carveth)

Historical context.

Enos Lake Benthic and Limnetic sticklebacks were distinguished by several morphological and meristic features. McPhail (1984) undertook detailed morphological, genetic and breeding analyses of Enos Lake sticklebacks. Based on collections from the wild taken in October 1977, and subsequent laboratory-reared offspring, the most notable meristic difference between the two forms was in gill raker counts: a mean of 18.5 in benthics vs. 25.9 in limnetics, with almost no overlap in distribution (McPhail 1984). Analysis of morphometric traits like body depth, head length, eye diameter and gill raker length confirmed striking differences in general body shape (McPhail 1984). These patterns were confirmed with additional analysis of fish captured in 1988 (Schluter and McPhail 1992). McPhail's laboratory breeding program also demonstrated that these morphological and meristic differences had a genetic basis, yet there were no intrinsic barriers to hybridization, because hybrids did not show reduced viability or fertility (McPhail 1984). The incidence of hybridization in the wild was nevertheless inferred to be low because less than 1% of the total adult population had an intermediate morphology consistent with lab-reared hybrids (McPhail 1984).

Enos Lake Benthic and Limnetic sticklebacks satisfied all conditions of the biological species concept (Mayr 1942), and most other species concepts, and were therefore considered true biological species. Enos Lake Benthic and Limnetic sticklebacks were easy to distinguish based on morphology, behaviour and ecology (Bentzen and McPhail 1984; Bentzen *et al.* 1984; McPhail 1984; Schluter and McPhail 1992); differences in morphology and behaviour were genetically based (McPhail 1984); they showed strong assortative mating (Ridgway and McPhail 1984); and they showed strong biochemical and molecular genetic differences (McPhail 1984; Taylor and McPhail 1999; Taylor and McPhail 2000). Similar evidence exists for other limnetic-benthic pairs (McPhail 1994).

Current context.

Enos Lake Benthic and Limnetic sticklebacks were intensively studied by zoologists, primarily at the University of British Columbia (UBC), from the 1980s onward. In July 1999, Kraak *et al.* (2001) collected and analyzed 50 sticklebacks from Enos Lake. Of the 50 fish, 19 seemed to be limnetics, 23 seemed to be benthics, and 8 could not be easily classified. Morphometric analysis indicated that about 12% of the fish in their sample were likely to be hybrids, a considerably higher proportion than in previous samples. It appeared a collapse of the pair was under way by 1999 at the latest (Kraak *et al.* 2001).

Following the initial investigation by Kraak *et al.* (2001), Taylor *et al.* (2006) conducted detailed morphological and genetic analyses on samples spanning 1977 to 2002 to assess whether the Enos Lake pair was collapsing into a hybrid swarm. Their morphological (and genetic - see below) analysis showed a clear breakdown between benthics and limnetics: the two morphological clusters evident in 1977 and 1988 were replaced in the 1997 sample with a single highly variable cluster. Samples from 2000 and 2002 confirmed this pattern. Samples taken in 2005 again confirmed the absence of distinct morphological clusters (Behm *et al.* 2010).

The morphological and genetic evidence strongly suggests that Enos Lake Benthic and Limnetic sticklebacks no longer occur in the wild as distinct species and the sticklebacks in the lake probably constitute a hybrid swarm with no assortative mating. Given, however, that the studies described above were not designed from a sampling perspective to assess the temporal and spatial extent of hybridization (i.e., the studies were designed for more general aspects of species pair biology), it is possible that some pure benthics and limnetics may still exist in the lake. Only more exhaustive and targeted genetic and morphological surveys can eliminate this possibility beyond a reasonable doubt.

Spatial Population Structure and Variability

<u>Historical context</u>

A key research question has been whether sympatric stickleback species pairs are the result of a single speciation event or multiple, independent events. The question has been addressed most directly through the examination of microsatellite DNA variation in benthic and limnetic populations from different species pair lakes (Taylor and McPhail 2000). Phylogenetic and genetic distance analyses support the hypothesis that pairs of sympatric species have evolved multiple times. In other words, despite similar appearance among lakes, molecular phylogenies strongly indicate that the pairs are independently derived. Thus, a stickleback species pair from one watershed is genetically and evolutionarily distinct from pairs in other watersheds. Consequently, the different forms within lakes are considered distinct species as are the same morphological forms between lakes (see COSEWIC 2010a,b). Another focus of research has been the intrinsic and extrinsic barriers to gene flow between limnetics and benthics within lakes. Enos Lake Limnetic and Benthic sticklebacks were each assumed to be single panmictic populations, as there was no reason *a priori* to think there is population structuring within each species. There were no intrinsic barriers to gene flow between the species: F_1 hybrids between limnetics and benthics are fully fertile and fitness in the laboratory is equivalent to the parental species (McPhail 1984). In contrast, limnetics and benthics showed strong assortative mating in the lab and in the wild (Ridgway and McPhail 1984; Nagel and Schluter 1998; Boughman 2001), and several studies indicated that hybrids are selected against in the wild due to ecologically mediated selection against intermediate trophic morphology (Schluter and McPhail 1993; Schluter 1994,1995; Hatfield and Schluter 1999; Gow *et al.* 2007).

Hybridization between Enos Lake Benthic and Limnetic sticklebacks occurred naturally in the wild at a low rate. Based on examination of morphology McPhail (1984) and Schluter and McPhail (1992) estimated that about 1% of adults in Enos Lake were hybrids. Despite some hybridization, Enos Lake Benthics showed fixed allele differences from Enos Lake Limnetics at the allozyme locus *Mdh*-3, indicating the maintenance of separate gene pools (McPhail 1984).

<u>Current context</u>

After Kraak *et al.*'s (2001) investigation, Taylor *et al.* (2006) conducted genetic analysis on several samples collected from 1994 on, using five microsatellite loci that had previously provided excellent discrimination between benthics and limnetics. The 1994 samples indicated two genetically distinct populations in Enos Lake, but samples collected in 1997, 2000, and 2002 showed only a single genetic population was evident. In addition, genetic analyses of samples collected in 1997, 2000, and 2002 showed strong signals of being hybrids because they were genetically intermediate to parental genotypes (Taylor *et al.* 2006). These results corroborated the morphological results (Kraak *et al.* 2001; Taylor *et al.* 2006). Further, in 2006-2007, 508 male sticklebacks were examined for male breeding colour, which historically was distinct between benthics (black throat colour) and limnetics (red throat colour, Ridgway and McPhail 1984). A total of 17 of the "reddest males", 17 of the "blackest males" and 13 intermediate-coloured males were selected for subsequent DNA analysis. These 47 fish were then examined at four diagnostic microsatellite DNA loci (Gow *et al.* 2007) to determine if: (i) genetically "pure" limnetic and benthic males could be detected, and (ii) any benthic and limnetic males resolved genetically matched their classification as benthics or limnetics based on male throat colour. This analysis showed a complete lack of genetic structure (i.e., there was an absence of two genetic clusters that matched historical patterns of benthic and limnetic clusters – see Taylor *et al.* 2006) and that a morphological feature historically diagnostic for difference between benthic and limnetic males (throat colour) was not associated in any way with genetic markers historically diagnostic for benthic and limnetics (C. Peichel, pers. comm., 2010). Consequently, this lack of genetic structure in the population is consistent with a panmictic (i.e., no assortative mating) hybrid swarm. Like the morphological evidence, the repeated genetic analyses strongly suggest that Enos Lake Benthic and Limnetic sticklebacks have become a hybrid swarm, and no longer satisfy the definition of distinct sympatric species (see Taylor *et al.* 2006). In total, however, perhaps 1,000 fish have been sampled and examined genetically and morphologically over the last 10 years. While these data provide strong evidence of a hybrid swarm in the lake, the estimated total population size of sticklebacks is on the order of at least 20,000 mature individuals (see **Abundance** section**).** Consequently, without further massive sampling and analytical efforts, it is impossible at present to disprove the idea that some "genetically pure" benthics and limnetics may remain within Enos Lake.

Designatable Units

Historical context

The Benthic and Limnetic sticklebacks from Enos Lake warranted status as separate designatable units (DUs) within *Gasterosteus aculeatus* because they satisfied the "discrete" and "significance" criteria of COSEWIC (COSEWIC 2011). First, both were genetically distinct from other sticklebacks as evidenced by an assemblage of allozyme, microsatellite, and morphological data (e.g., McPhail 1984; Taylor and McPhail 2000). In addition, the Enos Lake pair was only one of three existing cases (occurring in three different watersheds on two different islands) of sympatric pairs in *Gasterosteus* despite the sampling of hundreds of coastal lakes (McPhail 1994). In addition, all three sets of pairs evolved independently from one another (Taylor and McPhail 2000). The existence of a sympatric pair in Enos Lake was, therefore, the result of a unique evolutionary divergence. This unique divergence meets the significance criterion in that it supports the view that Benthic and Limnetic sticklebacks in Enos Lake existed within a unique ecological and evolutionary setting: divergent populations in sympatry with the associated adaptations (feeding and reproductive) that are crucial to their persistence in sympatry. Also, given that Benthic and Limnetic sticklebacks in Enos Lake acted as distinct biological species (they were genetically, ecologically, morphologically, and behaviourally distinct in sympatry), they merited recognition as two DUs independent from *G. aculeatus* as a whole.

It is also appropriate and important that the status of both members of the pair be assessed in the same report for three reasons. First, the significance of the Enos Lake stickleback pair rests on their distinctions and persistence in sympatry; neither form considered in isolation from the other is particularly unique within *Gasterosteus aculeatus*. Second, interactions between them have contributed to their evolution and persistence (and now, via hybridization and introgression, their demise). Third, the Limnetic and Benthic sticklebacks shared common threats to their habitats, especially breeding habitats, and disturbance to such habitats could lead to increased hybridization between Limnetic and Benthic sticklebacks as has been documented for other sympatric pairs of *Gasterosteus* (Hatfield 2001).

Current context

Given the evidence of the collapse of the Enos Lake Benthic and Limnetic sticklebacks, the arguments for their status as distinct biological species, by definition, no longer apply. As such, the current population of sticklebacks in Enos Lake does not appear to qualify as a designatable unit within the Threespine Stickleback; all indications are that the two designatable units that did exist are now probably extinct.

Special Significance

The significance of Enos Lake Benthic and Limnetic sticklebacks was primarily aesthetic and scientific. Stickleback species pairs are widely regarded as a scientific treasure and have become a textbook case of recent adaptive radiation. They are as valuable to science as cichlid fishes in the Great Lakes of Africa, and Darwin's finches in the Galapagos Islands. Their value to science is in large part because they are among the youngest species on earth: scientists believe the species pairs have arisen since the end of the last glaciation, a mere 13,000 years ago (Schluter and McPhail 1992; McPhail 1994). They are part of a remarkable research system that is being used to understand the biological and physical processes that give rise to the biodiversity we see around us (e.g., see perspective by Gibson 2005). Newspapers, magazines and scientific journals have published the story of the discovery of these species, and regularly report on the results of ongoing scientific studies.

There was no direct commercial value of Enos Lake Benthic and Limnetic sticklebacks. The species were part of Canada's native fauna, with its own intrinsic value including its contribution to biodiversity, ecology, education and science.

DISTRIBUTION

Global Range

The Enos Lake Benthic and Limnetic sticklebacks occurred only in Enos Lake, on southeastern Vancouver Island, British Columbia (Figure 2). The Enos Lake sticklebacks have collapsed into a hybrid swarm with few or no limnetics or benthics (Kraak *et al.* 2001; Taylor *et al.* 2006) and, therefore, neither benthics nor limnetics exist as distinct biological species. A self-sustaining population of limnetics was established in a golf course pond in Murdo Frazer Park in North Vancouver, British Columbia. The population was established with 445 lab-raised Enos Lake Limnetic sticklebacks released 30 September 1988, and 150 wild limnetics introduced on 6 May 1989. The Murdo Fraser limnetic population, however, is established outside the native range of Enos Lake Limnetic Threespine Sticklebacks. They were not assayed genetically before introduction (nor since) and they are now apparently quite distinct morphologically from “typical” Enos Lake Limnetic Threespine Sticklebacks and tend to resemble the benthic phenotype (D. Schluter, University of British Columbia, pers. comm. 2011). Furthermore, the basis for designatable unit status both of benthic and limnetic sticklebacks rests crucially on their sympatric occurrence with each other. Consequently, introductions of populations into allopatric situations such as Murdo Fraser Pond are not considered in this status assessment. A separate population of benthics has never been established.

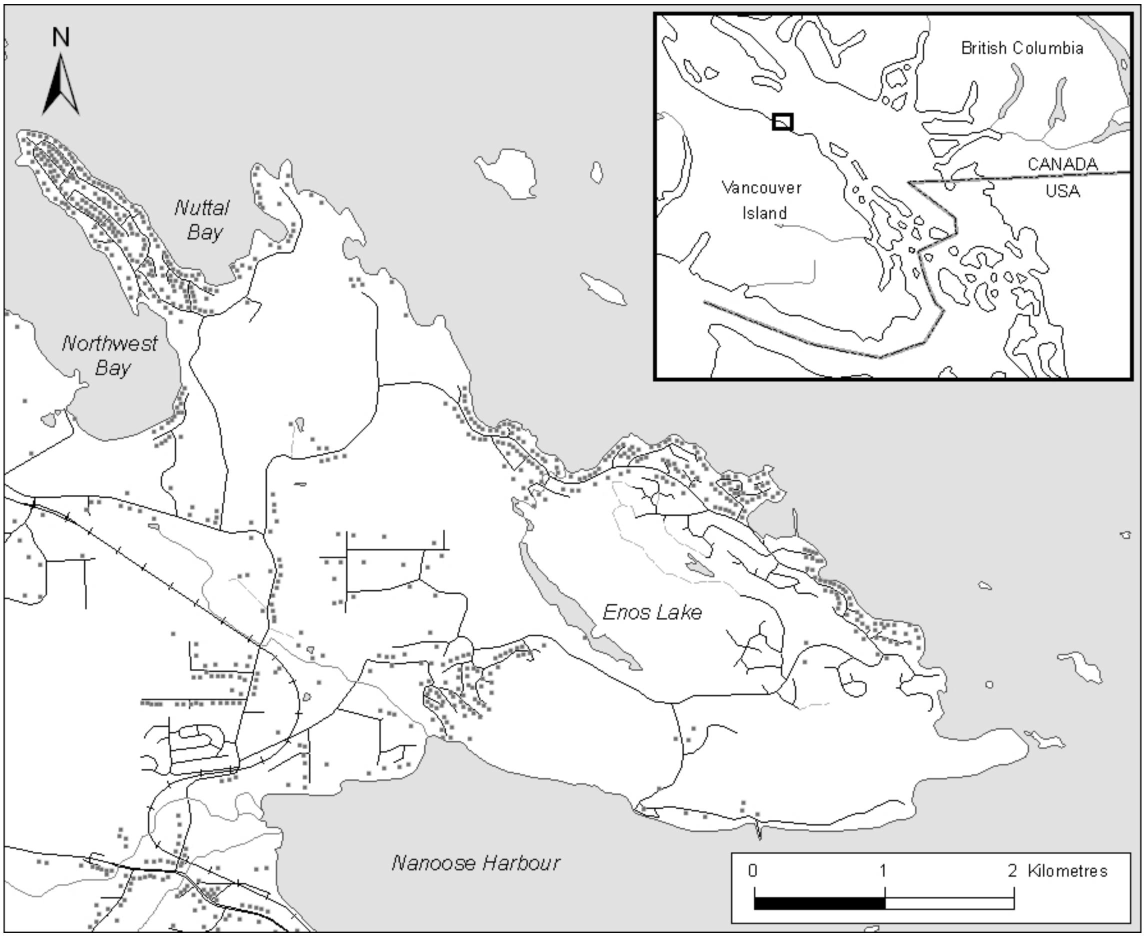

Figure 2. Distribution of Enos Lake Benthic and Limnetic sticklebacks in Canada. (Prepared by Jenny Wu, Environment Canada).

Independently evolved benthic-limnetic species pairs are still found in three lakes in the Vananda Creek watershed and in Paxton Lake on Texada Island, BC, and Little Quarry Lake on Nelson Island, BC. Another pair, in Hadley Lake on Lasqueti Island, is extinct (Hatfield 2001).

Canadian Range

The Canadian range of Enos Lake Benthic and Limnetic sticklebacks was a single 17.6 ha lake, Enos Lake, on southeastern Vancouver Island, British Columbia (Figure 2). The Canadian and global range were identical. An index of area of occupancy (IAO) calculated using a 2 km × 2 km (1 km x 1 km) overlaid grid (COSEWIC 2009) was estimated to be 8 km^2 (2 km^2). The extent of occurrence (EO) was 8 km^2, based on the convention that the EO cannot be less than the IAO.

HABITAT

Habitat Requirements

Solitary stickleback populations (i.e., those populations for which a single form inhabits a lake) are widely distributed and generally tolerant of significant changes in habitat or water quality. In contrast, stickleback species pairs are highly restricted in their distribution and sensitive to changes in habitat or other environmental factors. As evolutionarily young species that are not yet intrinsically reproductively isolated (i.e., they can produce viable hybrids), environmental changes can disrupt barriers to hybridization and lead to collapse of co-existing species into a hybrid swarm. Therefore, habitat requirements for stickleback species pairs include those same features that limit size or viability of solitary populations (e.g., lake productivity, juvenile rearing area, nesting habitat area), but also those features of the environment that prevent hybridization. In other words, habitat needs for species pairs include features whose alteration or loss will lead to reduction in abundance to an unviable population level, or breakdown of reproductive barriers sufficient to cause collapse into a hybrid swarm. These needs include moderate littoral and pelagic productivity, absence of invasive species, maintenance of natural light transmission levels, and maintenance of natural littoral macrophytes. The latter two are deemed especially important for maintaining mate recognition and are discussed in greater detail in Hatfield (2009). Our understanding of habitat requirements comes from studies in several species pair lakes.

Enos Lake Benthic and Limnetic sticklebacks had different habitat requirements (Bentzen and McPhail 1984; Bentzen *et al.* 1984; McPhail 1984; Ridgway and McPhail 1987; McPhail 1993,1994). These requirements varied throughout the year, and are described here for each major life stage. In general, limnetic and benthic sticklebacks spawned in littoral areas in the spring, reared in pelagic and littoral areas in spring and summer, and overwintered in deep water habitats during the fall and winter.

<u>Spawning Habitat</u>

Limnetic and benthic sticklebacks spawned in the shallow littoral area of lakes (Bentzen *et al.* 1984; McPhail 1984,1993). Benthic males built their nests (small cave-like structures with a single entrance and exit made on the lake bottom and constructed from small sticks and other organic debris) under cover of macrophytes or other structure, whereas limnetic males built their nests in open habitats (Ridgway and McPhail 1987; McPhail 1994).

Juvenile Rearing Habitat

Immediately after leaving the protection of paternal care, both limnetic and benthic fry used the littoral zone, where there is abundant food and cover from predators. The extent of habitat partitioning by benthic and limnetic fry is not understood well, but in nearby Paxton Lake Limnetic juveniles are common along steep, rocky, unvegetated littoral shoreline compared to benthic juveniles, which shelter around macrophytes (J. Gow, UBC Zoology Department, pers. comm., 2008). Eventually, limnetics moved offshore to feed in pelagic areas (Bentzen *et al.* 1984; Schluter 1995).

Adult Rearing Habitat

Adult limnetics (with the exception of nesting males) fed on zooplankton in the pelagic zone of the lake, whereas adult benthics fed on benthic invertebrates in the littoral zone (Bentzen and McPhail 1984; Bentzen *et al.* 1984; Schluter 1995). Productive littoral and pelagic habitats are required for the persistence of benthic-limnetic pairs. Because lakes with stickleback species pairs are approximately the same size, the proportion of pelagic to littoral productivity is also thought to be important.

Overwintering habitat

By late summer, individuals moved to deeper habitats where they overwintered. Little is known about habitat requirements of limnetics and benthics during this stage, except that trapping and seining consistently indicated use of deeper water by early fall (Bentzen *et al.* 1984).

Habitat Trends

Enos Lake has been subjected to some human disturbance, including damming of the outlet stream, raising of the water level, and water extraction for irrigation and domestic use. Some of these disturbances occurred several decades ago and the species pair continued to apparently do well for a long period after. Trends in habitat quantity and quality can be assessed only qualitatively, since there has been no long-term monitoring of habitat in Enos Lake.

The lake outlet was dammed many years ago, and presumably relates to a water licence issued in 1955. McPhail (1984) noted that this raised water levels by about 1 m, and that the lake was used as a domestic water supply for the Nanoose Bay area. Data are not available that describe how much water is actually used at present.

The lake’s shoreline remains mostly undisturbed, but sediment-laden runoff from nearby residential development has caused concern in the recent past.

The biggest habitat change is related to the introduction (by unknown means) of American Signal Crayfish, *Pacifastacus leniusculus*, which has considerably modified the littoral areas of the lake by removing almost all littoral vegetation. Prior to its entry to the lake sometime in the 1990s, McPhail (1989) described the littoral area of Enos Lake as having dense beds of *Potamogeton* and *Utricularia* in the summer. Given the preference for different nesting microhabitats—limnetic males preferred open areas and benthic males preferred to nest on substrate under vegetation (Ridgway and McPhail 1987)—this likely had a significant effect on the breakdown of assortative mating and the collapse of the species pair.

Habitat Protection/Ownership

Lands surrounding Enos Lake were originally within a military reserve without public access, but are now privately owned (COSEWIC 2002). There are no habitat protection provisions specifically for the habitat of Enos Lake Benthic and Limnetic Threespine Sticklebacks.

BIOLOGY

Life Cycle and Reproduction

Enos Lake Benthic and Limnetic sticklebacks had similar life histories (McPhail 1993,1994). Life history information for both species comes from observations of wild and laboratory-reared populations, but the data are mostly anecdotal. Limnetics are thought to have matured on average as one-year-olds, and rarely lived beyond a single breeding season. In the lab, reproductive females had multiple clutches in quick succession, and are thought to have done the same in the wild. Nesting males would mate with several to many females, and may have nested more than once within a single breeding season.

Benthics delayed sexual maturation relative to limnetics. Although some individuals likely mated in their first year, many may have delayed mating until they were two-year-olds. They may have lived up to about five years, and mated in several breeding seasons. In the lab, reproductive females had fewer clutches within a breeding season than limnetics, and are thought to have done the same in the wild. Nesting males would mate with several to many females, and may have nested more than once within a single breeding season.

The sex ratio of both limnetics and benthics was approximately 1:1. Enos Lake Benthic and Limnetic sticklebacks were similar to other Threespine Sticklebacks in their overall mode of reproduction (McPhail 1994). Males constructed nests, which they guarded and defended, until fry were about a week old. Eggs took up to a week to hatch, depending on temperature, and another three to five days before larvae were free-swimming (McPhail 2007). The nests and contents remained vulnerable to predators of different kinds, including other sticklebacks (Foster 1994). Benthics built their nests under cover of macrophytes or other structure; limnetics tended to spawn in open habitats (Ridgway and McPhail 1987; McPhail 1994; Hatfield and Schluter 1996).

In the wild, benthics reproduced earlier in the year than limnetics, but there was considerable overlap in their spawning times (Table 1). There was strong assortative mating (Ridgway and McPhail 1984; Nagel and Schluter 1998; Boughman 2001), and hybridization occurred naturally in the wild at a low rate. Male benthics from Enos Lake developed black nuptial colouration and male limnetics developed red colouration (Ridgway and McPhail 1984).

Table 1. Life history timing for benthic-limnetic stickleback species pairs (general time frame for all lakes with species pairs).

		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Speices	**Life Stage**	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
Limnetic	Spawning				x x x	x x x x	x						
	Incubation				x x	x x x x	x x						
	Juvenile rearing				x	x x x x	x x x x	x x x x	x x x x	x x			
	Adult rearing		x x	x x x x	x x x x	x x x x	x x x x	x x x x	x x x x	x x			
	Overwintering	x x x x	x x							x x	x x x x	x x x x	x x x x
Benthic	Spawning			x x	x x x x	x x							
	Incubation			x	x x x x	x x x							
	Juvenile rearing				x x x x	x x x x	x x x x	x x x x	x x x x	x x			
	Adult rearing		x x	x x x x	x x x x	x x x x	x x x x	x x x x	x x x x	x x			
	Overwintering	x x x x	x x							x x	x x x x	x x x x	x x x x
		1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4	1 2 3 4
		Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**

Immediately after leaving the nest, both limnetic and benthic fry used inshore areas, where there is abundant food and cover from predators. Eventually limnetics moved offshore to feed in pelagic areas (Bentzen *et al.* 1984; Schluter 1995). The timing of this movement was likely dictated by a combination of relative growth rates and predation risk in littoral and pelagic habitats (Schluter 2003). Benthics remained in littoral areas throughout their life.

Adult limnetics (with the exception of nesting males) fed in the pelagic zone of the lake, whereas adult benthics fed in the littoral zone (Schluter 1995). By late summer, individuals began moving to deeper water habitats where they overwintered (Bentzen *et al.* 1984).

Physiology

Physiological requirements and tolerances have not been described for Enos Lake Benthic and Limnetic sticklebacks. As a group, Threespine Sticklebacks occur in a wide array of environments and they are known to have broad tolerances of many water quality characteristics (e.g., turbidity, water velocity, temperature, depth, pH, alkalinity, calcium and total hardness, salinity, conductivity, etc.). Enos Lake itself is moderately stained, productive, and presumably minimally affected by toxic inputs. Concerns have been expressed (e.g., National Recovery Team for Stickleback Species Pairs 2007; Wood 2007; Ormond *et al.* 2011) that deviations from these natural conditions pose a threat to persistence of the species pairs, but the concern is usually expressed in connection with reproductive isolation between limnetics and benthics (e.g., mate recognition), rather than physiological tolerance *per se*.

Dispersal/Migration

Enos Lake Benthic and Limnetic sticklebacks did not migrate beyond the limits of Enos Lake. A few individuals likely became entrained in the outlet stream, but these would be lost to the population and were likely of little consequence to general population dynamics. Within Enos Lake, there were short-distance, seasonal movements associated with spawning, rearing and overwintering (Bentzen *et al.* 1984).

Interspecific Interactions

As a group, sympatric stickleback species pairs evolved and persisted in the presence of only one other fish species, Coastal Cutthroat Trout (*Oncorhynchus clarkii clarkii*; Vamosi 2003). Maintaining a simple ecological community appears to be necessary for persistence of the sympatric pairs, as underscored by the rapid extinction of the Hadley Lake species pair following introduction of Brown Bullhead (*Ameiurus nebulosus*; Hatfield 2001) and the collapse of the Enos Lake pair following the appearance of American Signal Crayfish (Taylor *et al.* 2006; Rosenfeld *et al.* 2008; Ormond *et al.* 2011).

Recent and current predation of sticklebacks in Enos Lake is likely less than it was historically, because piscivorous Coastal Cutthroat Trout are rare after damming of the outlet stream. The few cutthroat now present in the lake are thought to have been clandestinely stocked by locals (McPhail 1984). The lake is inhabited by numerous invertebrates that feed on young sticklebacks, and is regularly visited by piscivorous birds (e.g., Great Blue Heron (*Ardea herodias*), Belted Kingfisher (*Megaceryle alcyon*) and Common Loon (*Gavia immer*)). Their presence, however, was not considered a threat to the stickleback species pair.

The greatest interspecific competitors for limnetics were likely benthics, and vice versa. Several studies have demonstrated character displacement between limnetics and benthics, and competition between the two species (Schluter and McPhail 1993; Schluter 1994,1995).

Adaptability

The extent to which limnetics and benthics can be considered "adaptable" is debatable. On the one hand, both limnetics and benthics had a high intrinsic rate of population growth and could therefore recover quickly from small to moderate population reductions (Hatfield 2009). They are fairly hardy fish, as evidenced by the relative ease of rearing them in large numbers in the laboratory or in experimental ponds. On the other hand, it has proven difficult to rear both species together in captivity. For example, in experimental ponds at UBC, limnetics and benthics hybridized at very high levels, which ultimately led to collapse into a hybrid swarm (D. Schluter, UBC Zoology Department, pers. comm. 2010). The extirpation of the Hadley Lake pair and the collapse via hybridization of the Enos Lake pair, highlight the sensitivity of the pairs to certain types of environmental perturbation. Whereas population modelling indicates the sticklebacks are resilient to generalized environmental perturbations, other observations indicate that continued reproductive isolation is contingent on specific environmental factors, which to date have been only qualitatively assessed. In this context, the species pairs are not adaptable, and are not particularly resilient to environmental disturbance.

POPULATION SIZES AND TRENDS

Search Effort

Threespine Sticklebacks are common in coastal marine and freshwater throughout the Northern Hemisphere. Physically isolated populations exist in numerous low-elevation lakes, but only five sympatric stickleback species pairs have been discovered, all within a highly confined geographic area in southwestern British Columbia. Biologists have surveyed hundreds of lakes along the BC, Washington and Alaska coasts and found stickleback species pairs in only this small area of BC. A species pair was recently discovered within this area, in Little Quarry Lake on Nelson Island (Gow *et al.* 2008), but it seems unlikely that many more unidentified pairs exist. To date, genetic data indicate that each pair was independently derived from a marine ancestor (i.e., the pair in Enos Lake was distinct from all other sympatric species pairs, Taylor and McPhail 1999; Taylor and McPhail 2000). The Enos Lake Benthic and Limnetic sticklebacks were unique BC endemics.

Abundance

Good, empirically derived population estimates do not exist for most of the stickleback species pairs. McPhail (1989) suggested that population sizes were on the order of 100,000 for each of the species in Enos Lake, prior to their collapse, but this was not a direct estimate based on captures, and more recent data suggest this may be generous, at least for individuals one year and older.

Matthews *et al.* (2001) estimated population sizes in Enos Lake, based on multiple mark-recaptures and a Bayesian estimation technique (Gazey and Staley 1986), but these estimates were confounded by species identification problems due to substantial hybridization between limnetics and benthics that had occurred by that time (i.e., the inability of the study team to distinguish between limnetics, benthics and hybrids). When the sample is pooled, the population estimate is 26,630 ± 8,240, which gives an indication of the total number of sticklebacks in the lake one year of age and older.

Nomura (2005) completed abundance estimates of the Paxton species pair using mark-recapture methods, and the modified Peterson estimator. This study was not hampered by identification issues. Abundances have been extrapolated to other species pairs based on lake perimeter for benthics, and lake area for limnetics (Hatfield 2009). Using this method, the population estimate for Enos Lake Limnetic sticklebacks was 85,050 (95% CI 50,208 – 147,962) and for Enos Lake Benthic sticklebacks was 20,761 (95% CI 3,124 – 27,721), or 105,811 for both species combined.

Fluctuations and Trends

There has been no systematic monitoring of abundance in Enos Lake, so population trends are unknown. Enos Lake Benthic and Limnetic sticklebacks, however, have been studied by zoologists at UBC for the last three decades or more (e.g., Bentzen and McPhail 1984; Bentzen *et al.* 1984; McPhail 1984; Ridgway and McPhail 1984; Schluter and McPhail 1992; McPhail 1994; Taylor and McPhail 1999, 2000). Throughout this time sticklebacks remained fairly easy to trap in large numbers in Gee traps. Since at least 1994, however, the distinctions between benthics and limnetics as assayed using genetic and morphological techniques have declined dramatically (see above sections on **Morphological description** and **Spatial population structure and variability**) such that genetically pure forms of both species are thought to no longer exist and have been replaced by a hybrid swarm.

Rescue Effect

The global range of Enos Lake Benthic and Limnetic sticklebacks was a single lake entirely within Canada, so the concept of rescue effect does not apply to these species.

LIMITING FACTORS AND THREATS

Limits to Benthic and Limnetic Enos Lake stickleback abundance are poorly understood. It is not known whether abundance was limited by food production, cover, predation, spawning habitat or other factors. It is likely that the main limiting factor was food supply—the capability of the lake to produce plankton and benthos—but there are no data to support this view. In any case, sticklebacks in the lake were abundant, and not in apparent decline; the primary factor determining their original conservation status (COSEWIC 2002) was extreme endemism, not small and/or declining abundance or declining distribution.

Threats to Enos Lake Benthic and Limnetic sticklebacks have been described in the National Recovery Strategy (National Recovery Team for Stickleback Species Pairs 2007). As noted in the Recovery Strategy, the discussion of threats is based primarily on professional opinion, not on quantitative risk assessment. This is because there is an absence of information on the effects of different threats on population vital rates (e.g., hybridization, growth, survival, reproductive success). The threats analysis is nevertheless deemed to be robust.

Invasive Species

The primary threat to persistence of stickleback species pairs as a group is spread of exotic species. (The term "invasive species" in this context refers to any species that becomes established in a location where it does not occur naturally and where it causes some actual or inferred harm to native species). The species pairs seem to depend critically on the maintenance of several ecological factors, including a simple fish community. Species pairs occur in lakes that naturally have only stickleback, or stickleback and Coastal Cutthroat Trout (Vamosi 2003; Gow *et al.* 2008; Ormond *et al.* 2011).

The Hadley Lake species pair quickly became extinct following the introduction of Brown Bullhead, which is thought to have preyed on or interfered with nesting stickleback, ultimately leading to complete recruitment failure (Hatfield 2001). Brown Bullhead were introduced to Hadley Lake in the early 1990s and all stickleback were absent by 1995 (Hatfield 2001). This highlights the vulnerability of the stickleback species pairs and the speed with which a pair can be affected by an introduced species. The Enos Lake species pair collapsed due to hybridization (Kraak *et al.* 2001; Taylor *et al.* 2006), and the appearance of the American Signal Crayfish in the 1990s is implicated. The mechanism by which the crayfish affected sticklebacks appears to be through littoral habitat disturbance and alteration (Rosenfeld *et al.* 2008), although differential impacts on limnetic breeding success are also a plausible mechanism (Velema 2010).

Additional introductions are possible, as evidenced by a number of other invasive species that are in nearby lakes and spreading throughout the region. For example, Largemouth and Smallmouth basses (*Micropterus salmoides* and *M. dolomieu*), Pumpkinseed Sunfish (*Lepomis gibbosus*), and Yellow Perch (*Perca flavescens*) are spread by anglers and other members of the public. Bradford *et al.* (2008a,b) conducted qualitative risk assessments and concluded that for most regions of BC the probability of becoming established after release is high or very high, and the likely magnitude of ecological impact in small water bodies is very high. Other threats include the spread of amphibians like the Bullfrog (*Rana catesbeiana*) and invasive aquatic vegetation such as Eurasian Milfoil (*Myriophyllum spicatum*) and Purple Loosestrife (*Lythrum salicaria*).

Water Use

Existing water licences allow both storage and diversion of water from Enos Lake that has raised lake levels due to a dam at the outlet and increased annual drawdowns. A water licence for 140 acre feet of storage dates to 1955, and presumably relates to the original damming of the lake outlet. A licence for 140 acre feet of diversion dates to 1984, and represents about 20% of lake volume. More recent water licences date to 1992 for 201 acre feet of storage and 150 acre feet of diversion for “watering.” Actual water use is not known, although seasonal reductions of about 1 m over a period of 4-6 weeks in late summer were common (J. Rosenfeld, BC Ministry of Environment, pers. comm., 2011). Because there is no defined inlet channel to the lake, inflows are likely fairly small and although they may offset diversions on an annual basis, the diversions probably exacerbate water level fluctuations. Large fluctuations have impacts on littoral productivity and pelagic volume and would be expected to have a direct effect on sticklebacks, limiting both spawning and feeding habitats. Limiting available spawning habitat from water level reductions could influence hybridization dynamics between the two species by limiting opportunities for reproductive habitat segregation.

Land Use

Lands surrounding Enos Lake were originally within a military reserve without public access, but are now privately owned (COSEWIC 2002). Historically, the land was likely logged, but in general land use appears to have been a fairly minor concern. The lands are now privately owned and are being developed as residential area, and there have been concerns about introduction of suspended sediments (i.e., increased turbidity), water extraction from the lake for irrigating the associated golf course, and loss of riparian vegetation.

Collections for Research

Stickleback species pairs have been the focus of intense scientific study since the 1980s and there is an increasing demand for wild stock for use in laboratory-based studies and for permits to conduct *in situ* scientific study. Collecting activities have the potential to be a significant source of mortality on adult fish, and constitute a threat to the species pairs that should be carefully managed. The Recovery Team for Non-Game Freshwater Fish Species in BC (2008) produced guidelines for the collection of stickleback species pairs that recommend limiting the number of individuals removed from each lake, limiting collecting activities to only half the lake, thorough cleaning of all sampling gear, and prohibitions on the use of hybrids or exotic species in any *in situ* studies. With the collapse of the Enos Lake Benthic and Limnetic sticklebacks there is now little concern related to collecting activities in this lake.

Other

Impacts may occur from other activities, including bait release from anglers, pollution from recreational boating, introduction of disease, and effects of climate change and pollution. These threats are thought to present a lower risk than the threats noted above.

EXISTING PROTECTION OR OTHER STATUS DESIGNATIONS

Enos Lake Benthic and Limnetic sticklebacks are listed as Endangered under Schedule 1 of the *Species at Risk Act*. Under SARA, it is illegal to “kill, harm, harass, capture or take an individual of a wildlife species that is listed as an extirpated species, an endangered species or a threatened species” [s 32. (1)]. Also under SARA, no person may “damage or destroy the residence of one or more individuals of a wildlife species that is listed as an endangered species or a threatened species...” [s 33]. And, “no person shall destroy any part of the critical habitat of any listed endangered species or of any listed threatened species—or of any listed extirpated species if a recovery strategy has recommended the reintroduction of the species into the wild in Canada—if … the listed species is an aquatic species…”[s 58. (1)].

Enos Lake Benthic and Limnetic sticklebacks are designated Endangered by COSEWIC, based on a 2002 and 2012 update status report (COSEWIC 2002). They are currently designated Endangered by the BC Conservation Data Centre (B.C. Conservation Data Centre 2012).

At this time there are no habitat protection provisions specifically for Enos Lake Benthic and Limnetic sticklebacks. The Recovery Team for Stickleback Species Pairs, however, identified critical habitat for the species, and a report (Hatfield 2009) has been reviewed and accepted by DFO's Pacific Scientific Advice Review Committee. The report recommends critical habitat identification of the entire wetted area of Enos Lake, plus a riparian buffer.

All lands adjacent to Enos Lake are privately owned, so the *BC Forest and Range Practices Act*, which has provisions to protect fish habitat from forestry activities, does not apply.

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Todd Hatfield is a consulting biologist, based in Victoria, British Columbia. In 1995, he completed a Ph.D. at UBC, focusing on the evolutionary ecology of Paxton Lake Limnetic and Benthic Threespine Sticklebacks. His consulting work focuses on applying scientific methods and decision-making techniques to the resolution of natural resource management issues and environmental conflicts. He works extensively on species at risk and water management issues. He has coordinated the Recovery Team for Non-game Freshwater Fish Species in BC, since 2003, and has been a member of the COSEWIC Freshwater Fishes Specialist Subcommittee, since 2010.

COLLECTIONS EXAMINED

No museum collections were examined for this report.

Appendix: Opportunity for recovery of the Enos Lake Benthic and Limnetic Threespine Stickleback

Theoretically, the recovery of the Enos Lake stickleback species pair might be accomplished in either of two ways. The first is to restore habitat conditions in Enos Lake to their native state in the hopes that this would encourage the hybrid swarm to reconstitute the original limnetic and benthic phenotypes and genotypes. The second is to restore habitat conditions in the lake and reintroduce pure limnetics and benthics from a captive breeding program.

Both options require restoring Enos Lake to conditions as they were prior to establishment by the American Signal Crayfish. This would mean complete removal of crayfish or maintenance at a very low abundance in perpetuity, which would in turn allow macrophytes to re-establish in the littoral zone. There is good evidence, albeit somewhat circumstantial, that macrophytes play a critical role in premating isolation through selection of nesting sites (Ridgway and McPhail 1987; McPhail 1994), so this seems a reasonable pre-condition for recovery. Complete removal of crayfish from Enos Lake seems unlikely given current technologies. Introduced crayfish have caused numerous ecological impacts in Europe (Lodge *et al.* 2000; Gherardi 2007) and there are no published accounts of effective long-term eradication methods. It may be possible to harvest crayfish in sufficient quantities to substantially suppress total abundance (Hein *et al.* 2007), but it would require continual harvest using trapping techniques that would not harm sticklebacks, and the degree of crayfish depletion required to re-establish historical limnological (e.g., macrophyte) conditions is unknown. Gee traps collect crayfish in great numbers, but they also trap sticklebacks with high efficiency. The crayfish source population and original vector of introduction is unknown, but reintroduction could occur if they were eradicated. Assuming crayfish could be eliminated it would likely take at least several more years for macrophytes to re-establish naturally. Transplanting from nearby sources may speed up the colonization, but presumably it would still take at least a few years to develop a natural-like macrophyte community. There are a number of real challenges to controlling crayfish and restoring a vegetated littoral zone, including a commitment to actively manage crayfish populations for the foreseeable future.

Following restoration of Enos Lake, one option is to hope that this environment will select for the original phenotypes and reconstitute the Enos Lake Benthic and Limnetic sticklebacks from the hybrid swarm. In some respects this is a reasonable hypothesis, because all limnetic and benthic genes presumably still exist in the hybrid swarm, though recombined through repeated hybridization. Yet, in fact, this is little different than expecting sympatric speciation in numerous other lakes with solitary forms. It would require strong selection for the benthic and limnetic phenotypes and the simultaneous development of strong assortative mating. There is certainly plenty of evidence of natural selection against intermediate phenotypes, particularly when the limnetic and benthic phenotypes are present in high frequency (Schluter and McPhail 1992,1993; Schluter 1994,1995; Hatfield and Schluter 1999; Gow *et al.* 2007). In addition, the current environment in Enos Lake, however, does not appear to select against intermediate phenotypes (Behm *et al.* 2010), but some selection against intermediates occurs in solitary populations (Robinson 2000), suggesting that disruptive selection may recur if the lake is restored. It is also true, however, that hybrids are reproductively and ecologically viable and capable of surviving in the wild (McPhail 1984; Schluter 1995; Hatfield and Schluter 1996,1999; Gow *et al.* 2006; Gow *et al.* 2007), indicating that selection against intermediates is far from 100% (see also Taylor *et al.* 2011). Even if selection were to strongly favour the limnetic and benthic phenotypes there is little expectation that assortative mating will recur if genes controlling mate choice are now distributed randomly in the current hybrid swarm (see discussion above concerning male throat colour under Spatial Population Structure and Variability).

Most sympatric speciation models require considerable shifts in phenology or mating habitats (Via 2001), and there is little to suggest this is likely with sticklebacks. Given that interbreeding is likely to remain substantial, the likelihood of sympatric divergence of the Enos Lake hybrid swarm seems exceedingly small. This interpretation is supported, in part, by the observation in Second Lake on Texada Island, which was populated in the 1980s with hybrids from Paxton Lake and has shown no signs of reconstituting the original parental forms. In addition, initial speciation of all stickleback species pairs is thought to have occurred through a double-invasion process where populations were already partly differentiated from some time in allopatry, rather than through full sympatric speciation (Taylor and McPhail 2000).

Another possibility for recovering the Enos pair is to try and identify genetically pure benthics and limnetics with which to try and establish a captive breeding program and reintroduce fish to Enos Lake following restoration. In addition to controlling or eradicating the crayfish, this option would likely also require ridding the lake of hybrid sticklebacks. This in itself would be a considerable task, perhaps requiring piscicides (antimycin or rotenone) and partial or complete draining of the basin. There would undoubtedly be social barriers to this task.

There are also several other technical hurdles to establishing a captive breeding program, the most important being a source of fish. In 2004, D. Schluter led an effort to capture Enos Lake Benthic and Limnetic sticklebacks from the wild to establish a captive population for conservation purposes. Fish were caught during the breeding season using Gee traps, sorted by eye into putative limnetics and benthics and crosses made among the most limnetic-like fish and among the most benthic-like fish. The offspring were reared in the lab as separate families, while the parents were screened using a quantitative shape test based on historical morphological data, and genotyped using markers that had been used in the past to effectively diagnose individuals as limnetic or benthic (Gow *et al.* 2006). Several putative limnetic and benthic families were made. Few, if any, putative limnetics passed the quantitative shape test, and genetics confirmed that all were hybrids. The parents of several families of benthics passed both the shape and genetic tests, and 1,008 of the young were introduced into an experimental pond on the UBC campus, but the population failed to establish. There remained some skepticism that these were indeed pure benthics, because the genetic markers could no longer be expected to be truly diagnostic after collapse, and follow-up laboratory-based work on colour and shape indicated they were not pure benthics (D. Schluter, UBC Zoology Department, pers. comm., 2010). The emergency captive breeding program was terminated, and it seems there are no true limnetics or benthics left in Enos Lake, even though some look like the parental forms.

As noted earlier, a self-supporting population of Enos Lake Limnetic sticklebacks was established in a golf course pond in Murdo Frazer Park in 1988 and 1989 and these fish apparently continue to thrive. The population, however, has been in this different environment for more than 20 generations, and appears to have diverged from its limnetic ecotype at least morphologically (D. Schluter, UBC Zoology Department, pers. comm., 2011). Whether fish in this population can still be considered "Enos Lake Limnetic sticklebacks" is a matter of conjecture. There is no captive population of Enos Lake Benthic sticklebacks.

In summary, the cumulative probability of recovering a self-supporting, wild population of Enos Lake Benthic and Limnetic sticklebacks seems to be vanishingly small and beyond current technical feasibility.