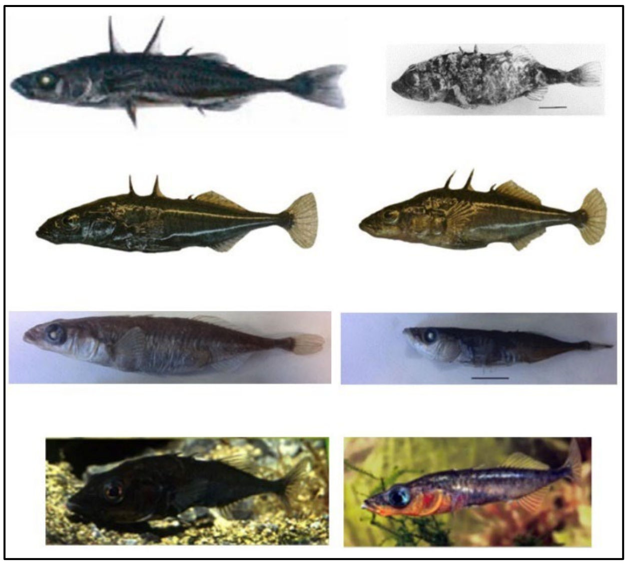
# COSEWIC
# Assessment and Status Report

on the

## Threespine Stickleback species bundle
## *Gasterosteus aculeatus*

Giant Threespine Stickleback, Unarmoured Threespine Stickleback, Misty Lake Lentic Threespine Stickleback, Misty Lake Lotic Threespine Stickleback, Paxton Lake Benthic Threespine Stickleback, Paxton Lake Limnetic Threespine Stickleback, Vananda Creek Benthic Threespine Stickleback, Vananda Creek Limnetic Threespine Stickleback, Little Quarry Lake Benthic Threespine Stickleback, Little Quarry Lake Limnetic Threespine Stickleback, Enos Lake Benthic Threespine Stickleback, Enos Lake Limnetic Threespine Stickleback

**in Canada**



**Giant Threespine Stickleback - SPECIAL CONCERN**
**Unarmoured Threespine Stickleback - ENDANGERED**
**Misty Lake Lentic Threespine Stickleback - ENDANGERED**
**Misty Lake Lotic Threespine Stickleback - ENDANGERED**
**Paxton Lake Benthic Threespine Stickleback - ENDANGERED**
**Paxton Lake Limnetic Threespine Stickleback - ENDANGERED**
**Vananda Creek Benthic Threespine Stickleback - ENDANGERED**
**Vananda Creek Limnetic Threespine Stickleback - ENDANGERED**
**Little Quarry Lake Benthic Threespine Stickleback - THREATENED**
**Little Quarry Lake Limnetic Threespine Stickleback - THREATENED**
**Enos Lake Benthic Threespine Stickleback - EXTINCT**
**Enos Lake Limnetic Threespine Stickleback - EXTINCT**
**2023**

**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:

COSEWIC. 2023. COSEWIC assessment and status report on the Threespine Stickleback species bundle *Gasterosteus aculeatus,* Giant Threespine Stickleback, Unarmoured Threespine Stickleback, Misty Lake Lentic Threespine Stickleback, Misty Lake Lotic Threespine Stickleback, Paxton Lake Benthic Threespine Stickleback, Paxton Lake Limnetic Threespine Stickleback, Vananda Creek Benthic Threespine Stickleback, Vananda Creek Limnetic Threespine Stickleback, Little Quarry Lake Benthic Threespine Stickleback, Little Quarry Lake Limnetic Threespine Stickleback, Enos Lake Benthic Threespine Stickleback and Enos Lake Limnetic Threespine Stickleback in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. lxiv + 103 pp. (https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html).

Previous report(s):

COSEWIC. 2013. COSEWIC assessment and status report on the Giant Threespine Stickleback *Gasterosteus aculeatus* and the Unarmoured Threespine Stickleback *Gasterosteus aculeatus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xiv + 62 pp. (https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html).

Reimchen, T.E. 1984. COSEWIC status report on the Charlotte Unarmoured Stickleback *Gasterosteus sp.* in Canada Committee on the Status of Endangered Wildlife in Canada. Ottawa. 13 pp.

Moodie, G.E.E. 1980. COSEWIC status report on the Giant Sticklebank *Gasterosteus sp.* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 6 pp.

COSEWIC. 2006. COSEWIC assessment and status report on the Misty Lake sticklebacks *Gasterosteus* sp. (Misty Lake lentic stickleback and Misty Lake lotic stickleback) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vii + 27 pp. (https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html).

COSEWIC. 2010. COSEWIC assessment and status report on the Paxton Lake Benthic and Limnetic Threespine Stickleback Species Pair *Gasterosteus aculeatus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xiv + 22 pp. (https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html).

COSEWIC. 2000. COSEWIC assessment and update status report on Paxton Lake Stickleback Species Pair *Gasterosteus* spp. in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vii + 13 pp. (https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html).

Hatfield, T. and J. Ptolemy 1999. Update COSEWIC status report on the Paxton Lake Stickleback Species Pair *Gasterosteus* spp. in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 1-11 pp.

Houston, J. 1998. COSEWIC status report on the Texada Island Stickleback Species Pair *Gasterosteus sp.* in Canada. Committee on the status of Endangered Wildlife in Canada. Ottawa. 11 pp.

COSEWIC. 2010. COSEWIC assessment and status report on the Vananda Creek Benthic and Limnetic Threespine Stickleback Species Pair *Gasterosteus aculeatus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xiv + 25 pp. (https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html).

COSEWIC. 2000. COSEWIC assessment and update status report on the Vananda Creek Stickleback Species Pair *Gasterosteus* spp. in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vi + 17 pp. (https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html).

Hatfield, T., and J. Ptolemy. 1999. COSEWIC update status report on the Vananda Creek Stickleback Species Pair *Gasterosteus* spp. in Canada, *in* COSEWIC assessment and update status report on the Vananda Creek stickleback species pair *Gasterosteus* spp. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 1-17 pp.

COSEWIC. 2015. COSEWIC assessment and status report on the Little Quarry Lake Benthic Threespine Stickleback and the Little Quarry Lake Limnetic Threespine Stickleback *Gasterosteus aculeatus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xiii + 37 pp. (https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html).

COSEWIC. 2012. COSEWIC assessment and status report on the Enos Lake Benthic and Limnetic Threespine Stickleback Species Pair *Gasterosteus aculeatus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xii + 30 pp. (https://www.canada.ca/en/environment-climate-change/services/species-risk-public-registry.html).

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.

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COSEWIC would like to acknowledge Jennifer Gow for writing the status report on the Threespine Stickleback species bundle, *Gasterosteus aculeatus*, in Canada, prepared under contract with Environment and Climate Change Canada. This status report was overseen and edited by Margaret Docker, Co-chair of the COSEWIC Freshwater Fishes Specialist Subcommittee.

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Également disponible en français sous le titre Évaluation et Rapport de situation du COSEPAC sur l'Ensemble d'espèces d'épinoches à trois épines (*Gasterosteus aculeatus*), Épinoche à trois épines géante, Épinoche à trois épines lisse, Épinoche à trois épines lentique du lac Misty, Épinoche à trois épines lotique du lac Misty, Épinoche à trois épines benthique du lac Paxton, Épinoche à trois épines limnétique du lac Paxton, Épinoche à trois épines benthique du ruisseau Vananda, Épinoche à trois épines limnétique du ruisseau Vananda, Épinoche à trois épines benthique du lac Little Quarry, Épinoche à trois épines limnétique du lac Little Quarry, Épinoche à trois épines benthique du lac Enos et Épinoche à trois épines limnétique du lac Enos au Canada.

Cover illustration/photo:
Threespine Stickleback species bundle — Giant Threespine Stickleback, Mayer Lake (top row left; copyright Bruce Deagle); Unarmoured Threespine Stickleback, Rouge Lake (top row right; copyright Reimchen); Misty Lake Lentic (second row left ) and Lotic (second row right) Threespine Stickleback (copyright Katja Räsänen); Little Quarry Lake Benthic (third row left) and Limnetic (third row right) Threespine Stickleback (copyright Eric B Taylor); Enos Lake Benthic (bottom row left) and Limnetic (bottom row right) Threespine Stickleback (copyright Ernie Cooper).


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# COSEWIC
# Assessment Summary

**Assessment Summary – December 2023**

**Common name**
Giant Threespine Stickleback

**Scientific name**
*Gasterosteus aculeatus*

**Status**
Special Concern

**Reason for designation**
This freshwater fish is a Canadian endemic, and it is unusual as it is nearly twice as long as other sticklebacks. It is currently known from only two small lakes on Haida Gwaii in British Columbia. The main threat to this species is introduction of invasive species, although the highly acidic waters of the lakes would likely make them unsuitable to most invasive predatory fishes. In addition, tadpole predation by native fish in these lakes might help slow the impact of invasive frogs that are already on the island. However, the potential long-term impacts of invasive species are uncertain, and this distinctive Canadian species could become Threatened if these threats are neither reversed nor managed effectively.

**Occurrence**
British Columbia

**Status history**
Designated Special Concern in April 1980. Status re-examined and confirmed in November 2013 and December 2023.

**Assessment Summary – December 2023**

**Common name**
Unarmoured Threespine Stickleback

**Scientific name**
*Gasterosteus aculeatus*

**Status**
Endangered

**Reason for designation**
This small freshwater fish is a Canadian endemic, and it is unusual as it has reduced bony plates and protective spines compared to other sticklebacks. It is currently known from only three very small lakes on Haida Gwaii in British Columbia. Its revised status reflects the increased risk of extinction following dramatic declines observed in one lake when it temporarily dried up, and in a second lake following the introduction of an invasive frog, the tadpoles of which may compete with the adult fish. These three lakes lack predatory fishes that could prey on the tadpoles. If these threats are not prevented or reversed, they could lead to the extinction of this distinctive Canadian species.

**Occurrence**
British Columbia

**Status history**
Designated Special Concern in April 1983. Status re-examined and confirmed in November 2013. Status re-examined and designated Endangered in December 2023.

**Assessment Summary – December 2023**

**Common name**
Misty Lake Lentic Threespine Stickleback

**Scientific name**
*Gasterosteus aculeatus*

**Status**
Endangered

**Reason for designation**
This small, lake-dwelling fish is endemic to Canada as part of a species pair restricted to a single small lake-stream complex on northern Vancouver Island. This fish could quickly become extinct if invasive non-native fishes that prey on the eggs and adults of this species are accidentally or deliberately introduced. Proximity of this species to a major highway and public access make an introduction more probable. Logging activities in the watershed could also negatively impact habitat quality. If these threats are not prevented or reversed, they could lead to the extinction of this distinctive Canadian species.

**Occurrence**
British Columbia

**Status history**
Designated Endangered in November 2006. Status re-examined and confirmed in December 2023.

**Assessment Summary – December 2023**

**Common name**
Misty Lake Lotic Threespine Stickleback

**Scientific name**
*Gasterosteus aculeatus*

**Status**
Endangered

**Reason for designation**
This small, lake-dwelling fish is endemic to Canada as part of a species pair restricted to a single small lake-stream complex on northern Vancouver Island. This fish could quickly become extinct if invasive non-native fishes that prey on the eggs and adults of this species are accidentally or deliberately introduced. Proximity of this species to a major highway and public access make an introduction more probable. Logging activities in the watershed could also negatively impact habitat quality. If these threats are not prevented or reversed, they could lead to the extinction of this distinctive Canadian species.

**Occurrence**
British Columbia

**Status history**
Designated Endangered in November 2006. Status re-examined and confirmed in December 2023.

**Assessment Summary – December 2023**

**Common name**
Paxton Lake Benthic Threespine Stickleback

**Scientific name**
*Gasterosteus aculeatus*

**Status**
Endangered

**Reason for designation**
This small, robust-bodied freshwater fish is part of an endemic species pair restricted to one small lake in coastal British Columbia (BC). It lives close to the bottom of the lake, while the other member of the pair lives in the open water. The main threat to this species is introduction of invasive species, which have caused the rapid extinction of similar species pairs in two other lakes in coastal BC, either through predation or hybridization resulting from habitat modification. Invasive species continue to spread in the region. Water extraction could also result in loss of habitat and increase the risk of hybridization. If these threats are not mitigated, they could lead to the extinction of this distinctive Canadian species.

**Occurrence**
British Columbia

**Status history**
Designated Threatened in April 1998. Status re-examined and confirmed in April 1999. Status re-examined and designated Endangered in May 2000. Status re-examined and confirmed in April 2010 and December 2023.

**Assessment Summary – December 2023**

**Common name**
Paxton Lake Limnetic Threespine Stickleback

**Scientific name**
*Gasterosteus aculeatus*

**Status**
Endangered

**Reason for designation**
This small, slender-bodied freshwater fish is part of an endemic species pair restricted to one small lake in coastal British Columbia (BC). It lives in the open water of the lake, while the other member of the pair lives close to the bottom. The main threat to this species is introduction of invasive species, which have caused the rapid extinction of similar species pairs in two other lakes in coastal BC, either through predation or hybridization resulting from habitat modification. Invasive species continue to spread in the region. Water extraction could also result in loss of habitat and increase the risk of hybridization. If these threats are not mitigated, they could lead to the extinction of this distinctive Canadian species.

**Occurrence**
British Columbia

**Status history**
Designated Threatened in April 1998. Status re-examined and confirmed in April 1999. Status re-examined and designated Endangered in May 2000. Status re-examined and confirmed in April 2010 and December 2023.

**Assessment Summary – December 2023**

**Common name**
Vananda Creek Benthic Threespine Stickleback

**Scientific name**
*Gasterosteus aculeatus*

**Status**
Endangered

**Reason for designation**
This small, robust-bodied freshwater fish is part of an endemic species pair restricted to three small, interconnected lakes in coastal British Columbia (BC). It lives close to the bottom of the lakes, while the other member of the pair lives in the open water. The main threat to this species is introduction of invasive species, which have caused the rapid extinction of similar species pairs in two other lakes in coastal BC, either through predation or hybridization resulting from habitat modification. Invasive species continue to spread in the region. Water extraction could also result in loss of habitat and increase the risk of hybridization. If these threats are not mitigated, they could lead to the extinction of this distinctive Canadian species.

**Occurrence**
British Columbia

**Status history**
Designated Threatened in April 1999. Status re-examined and designated Endangered in May 2000. Status re-examined and confirmed in April 2010 and December 2023.

**Assessment Summary – December 2023**

**Common name**
Vananda Creek Limnetic Threespine Stickleback

**Scientific name**
*Gasterosteus aculeatus*

**Status**
Endangered

**Reason for designation**
This small, slender-bodied freshwater fish is part of an endemic species pair restricted to three small, interconnected lakes in coastal British Columbia (BC). It lives in the open water of the lake, while the other member of the pair lives close to the bottom. The main threat to this species is introduction of invasive species, which have caused the rapid extinction of similar species pairs in two other lakes in coastal BC, either through predation or hybridization resulting from habitat modification. Invasive species continue to spread in the region. Water extraction could also result in loss of habitat and increase the risk of hybridization. If these threats are not mitigated, they could lead to the extinction of this distinctive Canadian species.

**Occurrence**
British Columbia

**Status history**
Designated Threatened in April 1999. Status re-examined and designated Endangered in May 2000. Status re-examined and confirmed in April 2010 and December 2023.

**Assessment Summary – December 2023**

**Common name**
Little Quarry Lake Benthic Threespine Stickleback

**Scientific name**
*Gasterosteus aculeatus*

**Status**
Threatened

**Reason for designation**
This small, robust-bodied freshwater fish is part of an endemic species pair restricted to one small lake in coastal British Columbia (BC). It lives close to the bottom of the lake, while the other member of the pair lives in the open water. The main threat to this species is introduction of invasive species, which have caused the rapid extinction of similar species pairs in two other lakes in coastal BC, either through predation or hybridization resulting from habitat modification. Although this lake on Nelson Island is relatively inaccessible, invasive species continue to spread in the region. If this threat is not prevented, it could lead to the extinction of this distinctive Canadian species.

**Occurrence**
British Columbia

**Status history**
Designated Threatened in November 2015. Status re-examined and confirmed in December 2023.

**Assessment Summary – December 2023**

**Common name**
Little Quarry Lake Limnetic Threespine Stickleback

**Scientific name**
*Gasterosteus aculeatus*

**Status**
Threatened

**Reason for designation**
This small, slender-bodied freshwater fish is part of an endemic species pair restricted to one small lake in coastal British Columbia (BC). It lives in the open water of the lake, while the other member of the pair lives close to the bottom. The main threat to this species is introduction of invasive species, which have caused the rapid extinction of similar species pairs in two other lakes in coastal BC, either through predation or hybridization resulting from habitat modification. Although this lake on Nelson Island is relatively inaccessible, invasive species continue to spread in the region. If this threat is not prevented, it could lead to the extinction of this distinctive Canadian species.

**Occurrence**
British Columbia

**Status history**
Designated Threatened in November 2015. Status re-examined and confirmed in December 2023.

**Assessment Summary – December 2023**

**Common name**
Enos Lake Benthic Threespine Stickleback

**Scientific name**
*Gasterosteus aculeatus*

**Status**
Extinct

**Reason for designation**
This small, robust-bodied freshwater fish was part of an endemic species pair restricted to one small lake in south coastal British Columbia. It lived close to the bottom of the lake, while the other member of the pair lived in the open water. However, the introduction of an invasive crayfish in this lake dramatically reduced aquatic vegetation, which likely had been important in preventing hybridization between the two species. As a consequence, the two species collapsed into a hybrid swarm resulting in the loss of the original two species. The revised status reflects the inability to find genetically non-hybridized individuals of this distinctive Canadian species despite repeated surveys. There is sufficient information to document that no individuals of the species remain.

**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. Status re-examined and designated Extinct in December 2023.

**Assessment Summary – December 2023**

**Common name**
Enos Lake Limnetic Threespine Stickleback

**Scientific name**
*Gasterosteus aculeatus*

**Status**
Extinct

**Reason for designation**
This small, slender-bodied freshwater fish was part of an endemic species pair restricted to one small lake in south coastal British Columbia. It lived in the open water of the lake, while the other member of the pair lived close to the bottom. However, the introduction of an invasive crayfish in this lake dramatically reduced aquatic vegetation, which had likely been important in preventing hybridization between the two species. As a consequence, the two species collapsed into a hybrid swarm resulting in the loss of the original two species. The revised status reflects the inability to find genetically non-hybridized individuals of this distinctive Canadian species despite repeated surveys. There is sufficient information to document that no individuals of the species remain.

**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 Limnetic Threespine Stickleback was designated Endangered. Status re-examined and confirmed in May 2012. Status re-examined and designated Extinct in December 2023.

## COSEWIC
## Executive Summary

### Threespine Stickleback species bundle
*Gasterosteus aculeatus*

**Wildlife Species Description and Significance**

The Threespine Stickleback is a small-bodied fish (approximately 40–80 mm long) recognized by the presence of three (sometimes two) isolated dorsal spines followed by a soft-rayed dorsal fin, well-armoured and calcified lateral plates, and pelvic spines. There are both freshwater and marine forms. The marine form is usually anadromous (returning to freshwater to reproduce), and it has given rise to isolated freshwater forms in numerous postglacial lakes and streams, including those within this Threespine Stickleback species bundle. This species bundle is composed of 12 genetically and evolutionarily distinct designatable units (DUs), including Giant and Unarmoured Threespine Stickleback, a parapatric lake-stream pair, and four sympatric Benthic-Limnetic pairs. These DUs exhibit dramatic adaptations in their morphology, including body shape and size, trophic morphology, and defensive armour.

Giant Threespine Stickleback are almost twice the length of other Threespine Stickleback. Unarmoured Threespine Stickleback are characterized by the loss of one or more spines in the majority of fish, and the lateral bony plates may also be reduced or absent. Misty Lake Lentic and Lotic Threespine Stickleback are the archetypal example of a parapatric lake-stream pair, where the Misty Lake Lentic (lake-form and outlet stream-form) Threespine Stickleback are more streamlined and have longer pelvic spines and more gill rakers than the Lotic (inlet stream-form) Threespine Stickleback. Sympatric Benthic-Limnetic stickleback pairs have been found in only five lakes (on four islands in southwestern British Columbia), despite the sampling of hundreds of coastal lakes in this region and globally. Benthic Threespine Stickleback have a greater overall body depth, shorter dorsal and anal fins, a smaller eye, and a shorter jaw that is more downward-oriented than their sympatric Limnetic counterparts.

Molecular genetic evidence strongly supports the independent evolution of each DU within this species bundle, including parallel evolution across lakes for those that occur at multiple sites. Furthermore, within each parapatric and sympatric pair, each DU is genetically distinct from the other, and hybridization between them occurs naturally in the wild at a low level, with the exception of the Benthic-Limnetic pair in Enos Lake, which has collapsed into a hybrid swarm. Thus, each DU is genetically and evolutionarily distinct. They uniquely contribute to Canada's biodiversity and have high scientific value, with each DU adding to our understanding of evolutionary processes, including phenotypic variation, genomic variation, speciation, and eco-evolutionary dynamics.

## Distribution

The global range of the Threespine Stickleback species bundle assessed by COSEWIC in this report is restricted to the west coast of British Columbia (BC). Giant and Unarmoured Threespine Stickleback are endemic to Graham Island, Haida Gwaii. Giant Threespine Stickleback have been confirmed in two lakes (Mayer and Drizzle lakes) and Unarmoured Threespine Stickleback in three lakes (Serendipity, Rouge, and Boulton lakes). Misty Lake Lotic and Lentic Threespine Stickleback are found only in the Misty Lake watershed on northern Vancouver Island. Sympatric pairs of Benthic and Limnetic Threespine Stickleback occur in four watersheds in southwestern coastal BC: one on Nelson Island in Little Quarry Lake, one on southeastern Vancouver Island in Enos Lake, and two on Texada Island—in the Vananda Creek watershed (comprised of the interconnected Emily, Priest, and Spectacle lakes) and in Paxton Lake. The species pair in Enos Lake has now formed a hybrid swarm consisting of only a single intermediate form (both morphologically and genetically) following invasion of the lake by the American Signal Crayfish. A fifth pair, in Hadley Lake on Lasqueti Island, BC, became extinct following predation by the unauthorized introduction of Brown Bullhead and is not included in this species bundle.

## Habitat

The Threespine Stickleback species bundle has shared habitat requirements that limit the size or viability of freshwater Threespine Stickleback populations in general. Broadly speaking, these needs likely include sustained productivity, the maintenance of habitat for nesting and rearing juveniles, the absence of invasive species, as well as sufficient cover, food and nutrient supply from a stable and productive riparian zone. Knowledge of habitat features that are essential to the persistence of specific DUs varies by DU.

Giant Threespine Stickleback are found in large, shallow, dystrophic lakes with low pH and heavily stained waters; few other macro-organisms are able to survive the acidic conditions of these lakes. Unarmoured Threespine Stickleback are found in small, shallow, and acidic lakes. Their loss of armour is probably closely linked to their lakes' small size and acidic conditions, which exclude predatory fishes and larger birds. The importance of other lake habitat features to the persistence of Giant and Unarmoured Threespine Stickleback awaits further investigation. The habitat requirements of sympatric pairs of Benthic and Limnetic Threespine Stickleback include features of the environment that provide pre-mating and extrinsic post-mating barriers that maintain reproductive isolation and low levels of hybridization. This likely includes adequate light transmission to enable mate recognition, and sufficient habitat complexity to maintain nesting site preferences. There remains some uncertainty as to which habitat components are needed to maintain reproductive isolation between Misty Lake Lotic and Lentic Threespine Stickleback. While sufficient habitat complexity to maintain pre-mating habitat isolation appears important, the role of stable light transmission in the dark-stained waters of the Misty Lake system is unknown.

## Biology

The biology of Giant Threespine Stickleback from Mayer Lake and Benthic and Limnetic Threespine Stickleback from Paxton and Enos lakes has been studied extensively. Less is known about the biology of Misty Lake Lotic and Lentic Threespine Stickleback, although more recent studies are yielding insights. There has been little direct study of the biology of Benthic and Limnetic Threespine Stickleback in Vananda Creek or Little Quarry Lake. They are assumed to be similar to the other Benthic-Limnetic pairs. Similarly, little is known about the biology of Unarmoured Threespine Stickleback, and it is assumed to be similar to that of other freshwater Threespine Stickleback.

The reproductive biology of the DUs within the Threespine Stickleback species bundle is broadly similar to that of other freshwater Threespine Stickleback with some striking deviations. One is the loss of typical male red nuptial coloration in the melanistic Enos Lake Benthic (prior to collapse into a hybrid swarm), Giant, and Misty Lake Lentic Threespine Sticklebacks. Giant and Unarmoured Threespine Stickleback on Haida Gwaii show differences in fecundity (producing larger but fewer eggs when corrected for body size), and they are older than typical at onset of reproduction. Giant Threespine Stickleback also have an exceptionally long lifespan. Within parapatric and sympatric Threespine Stickleback pairs, Misty Lake Lotic Threespine Stickleback and Limnetic Threespine Stickleback usually breed at an earlier age than their Lentic or Benthic counterparts. There is some spatial and temporal reproductive segregation within these pairs.

Giant Threespine Stickleback are thought to have evolved their distinct morphology at least in part as a result of adaptation to predation by gape-limited fish and birds. Little is known about predators in the Misty Lake system, although both Lentic and Lotic fish coexist with other fishes. In sharp contrast to these lakes, those containing Unarmoured Threespine Stickleback have no predatory fishes in them, and these Unarmoured Threespine Stickleback are thought to have evolved their distinguishing loss of defensive structures as a result of adaptation to avian and macroinvertebrate predation regimes. Similarly, a simple fish community appears to be a major ecological determinant of where Benthic and Limnetic Threespine Stickleback pairs are found.

Threespine Stickleback in general are capable of adapting to change, including anthropogenic disturbance. However, this adaptability is an underlying factor of vulnerability for the DUs within the Threespine Stickleback species bundle. Each DU has evolved in response to specific selective forces, deviations from which could threaten their persistence by leading to adaptive alterations that could result in loss of their distinguishing suites of morphological characteristics and, in the case of parapatric and sympatric pairs, to the breakdown of barriers that maintain reproductive isolation. In the context of maintaining their morphological and genetic distinctness, the DUs within the Threespine Stickleback species bundle are not resilient to environmental disturbance because of their adaptability.

## Population Sizes and Trends

Information about population sizes and trends is presented for each DU separately, including abundance estimates, sampling effort and methods, and fluctuations and trends.

## Threats and Limiting Factors

Potential threats to the DUs that comprise the Threespine Stickleback species bundle include aquatic invasive species (AIS), habitat alteration/degradation (including water level fluctuations and pollution), scientific collection, and climate change. Threat levels vary by DU, with some threats specific to particular DUs.

The most likely or imminent threat to all of the Threespine Stickleback species bundle DUs comes from the introduction of AIS. The Threespine Stickleback species are highly susceptible to extinction from AIS introductions, which may threaten their abundance directly or indirectly. In the case of parapatric and sympatric pairs, AIS that alter the habitat can potentially disrupt barriers that maintain reproductive isolation which could lead to increased hybridization. AIS have been linked to the rapid extinction of Benthic and Limnetic Threespine Stickleback pairs in at least two lakes (Hadley and Enos lakes) in recent decades. The imminence of this threat is uncertain, but the consequences would likely be disastrous.

## Protection, Status and Ranks

Giant and Unarmoured Threespine Stickleback are listed as Special Concern under Schedule 1 of the *Species at Risk Act* (SARA). Misty Lake Lotic and Lentic Threespine Stickleback and all Benthic and Limnetic Threespine Stickleback pairs are listed as Endangered under Schedule 1 of SARA in keeping with their previous COSEWIC designations, except for Benthic and Limnetic Threespine Stickleback from Little Quarry Lake, which are designated Threatened by COSEWIC, and are under consideration for addition to Schedule 1 of SARA. Critical Habitat Orders, which invoke the prohibition against the destruction of the identified Critical Habitat, have been issued for Misty Lake Lotic and Lentic Threespine Stickleback and Paxton Lake and Vananda Creek Benthic and Limnetic Threespine Stickleback.

All of the DUs in the Threespine Stickleback species bundle are “Red-listed” by the Conservation Data Centre and the BC Ministry of Environment. Most of the DUs are considered to be *Critically Imperilled* at global, national and provincial levels, meaning that they are thought to be at very high risk of extinction or collapse. Exceptions are Little Quarry Lake Benthic and Limnetic Threespine Stickleback, which have yet to be assigned a global rank but are also listed as *Critically Imperilled* nationally and provincially, and Enos Lake Benthic and Limnetic Threespine Stickleback, which are *Presumed Extinct*, meaning they have collapsed throughout their range.

## TECHNICAL SUMMARY - Giant Threespine Stickleback (DU1)

*Gasterosteus aculeatus*

Giant Threespine Stickleback

Épinoche à trois épines géante

Range of occurrence in Canada: British Columbia

**Demographic Information**

| | |
|---|---|
| Generation time (usually average age of parents in the population) | 3–5 yrs<br>3 yrs in Mayer Lake, 4–5 yrs in Drizzle Lake |
| Is there an [observed, inferred, or projected] continuing decline in number of mature individuals? | Unknown<br>Population size estimates were made only once several decades ago, but expert opinion based on general observations suggests that population size is stable (see **Abundance**). |
| Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations, whichever is longer up to a maximum of 100 years] | Unknown<br>Probably stable |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Unknown<br>Probably stable |
| [Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Unknown<br>Probably stable |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any period [10 years, or 3 generations, whichever is longer up to a maximum of 100 years], including both the past and the future. | Unknown<br>Probably stable |
| Are the causes of the decline a. clearly reversible and b. understood, and c. ceased? | Not applicable, no known decline |
| Are there extreme fluctuations in number of mature individuals? | No |

**Extent and Occupancy Information**

| | |
|---|---|
| Estimated extent of occurrence (EOO) | 63 $km^2$ |
| Index of area of occupancy (IAO)<br>(Always report 2x2 grid value). | 52 $km^2$, although the combined surface area of both lakes is only 7.4 $km^2$ |
| Is the population "severely fragmented" i.e., is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse? | No |

| | |
|---|---|
| Number of “locations” (use plausible range to reflect uncertainty if appropriate) | 2<br>Mayer and Drizzle lakes |
| Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? | No |
| Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of subpopulations? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of “locations”? | No |
| Is there an [observed, inferred, or projected] continuing decline in area, extent and/or quality of habitat? | Probably not<br>Invasive Northern Red-legged Frogs have been found in roadside ponds adjacent to Mayer Lake. They may already be present in Mayer Lake at low levels of abundance, but have not been observed in either lake yet. Expert opinion suggests that the lakes' native fish community (which would eat the tadpoles) could help reduce the negative effect of the Northern Red-legged Frog, and properties of the lakes (including their acidic waters, which would likely make them unsuitable to most invasive predatory fishes) would likely slow the impact of AIS on stickleback habitat. |
| Are there extreme fluctuations in number of subpopulations? | 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 subpopulation)**

| Subpopulations (give plausible ranges) | N Mature Individuals<br><br>Estimates are based on various techniques (mark-recapture, nest densities) used in 1985 and are considered approximate only. Notwithstanding these caveats, total adult population sizes are likely in excess of several tens of thousands per lake (see **Abundance**). |
|---|---|
| Drizzle Lake | 30,000 and 120,000 adults (95% CI, mean = 75,000) |
| Mayer Lake | > 100,000 |
| Total | > 175,000 |

**Quantitative Analysis**

| | |
|---|---|
| Is the probability of extinction in the wild at least [20% within 20 years or 5 generations whichever is longer up to a maximum of 100 years, or 10% within 100 years]? | Unknown<br>Analysis not conducted |

**Threats (direct, from highest impact to least, as per IUCN Threats Calculator)**

Was a threats calculator completed for this species? Yes (03 June 2022)
Overall threat impact = Low (Appendix 1)

Key threat was identified as:
8.1 Invasive & Other Problematic Species & Genes (low)

Unknown threats:
7.3 Other ecosystem modifications
11 Climate Change & Severe Weather

What additional limiting factors are relevant? Their biology and occurrence in two small lakes make them vulnerable to introduction of aquatic invasive species; abiotic and biotic properties of these lakes are likely to slow the impact of AIS compared to other stickleback populations, although there is uncertainty in the potential long-term impacts of invasive species on this fish.

**Rescue Effect (immigration from outside Canada)**

| | |
|---|---|
| Status of outside population(s) most likely to provide immigrants to Canada. | Not applicable<br>This species is endemic to Canada |
| Is immigration known or possible? | No, there are no populations elsewhere |
| Would immigrants be adapted to survive in Canada? | Not applicable |
| Is there sufficient habitat for immigrants in Canada? | Not applicable |
| Are conditions deteriorating in Canada? | Not applicable |
| Are conditions for the source (i.e., outside) population deteriorating? | Not applicable |
| Is the Canadian population considered to be a sink? | Not applicable |
| Is rescue from outside populations likely? | No, there are no populations elsewhere |

**Data Sensitive Species**

| | |
|---|---|
| Is this a data sensitive species? | No |

**Status History**

COSEWIC:
Designated Special Concern in April 1980. Status re-examined and confirmed in November 2013 and December 2023.

**Status and Reasons for Designation:**

| Status:<br>Special Concern | Alpha-numeric codes:<br>Not applicable |
|---|---|
| Reasons for designation:<br>This freshwater fish is a Canadian endemic, and it is unusual as it is nearly twice as long as other sticklebacks. It is currently known from only two small lakes on Haida Gwaii in British Columbia. The main threat to this species is introduction of invasive species, although the highly acidic waters of the lakes would likely make them unsuitable to most invasive predatory fishes. In addition, tadpole predation by native fish in these lakes might help slow the impact of invasive frogs that are already on the island. However, the potential long-term impacts of invasive species are uncertain, and this distinctive Canadian species could become Threatened if these threats are neither reversed nor managed effectively. | |

**Applicability of Criteria**

| |
|---|
| Criterion A (Decline in Total Number of Mature Individuals):<br>Not applicable. Insufficient data to reliably infer, project, or suspect population trends, although there is no reason to suspect significant declines. |
| Criterion B (Small Distribution Range and Decline or Fluctuation):<br>Not applicable. EOO (63 $km^2$) and IAO (52 $km^2$) below thresholds for Endangered, and the population occurs at only 2 locations, but no other subcriteria are met. |
| Criterion C (Small and Declining Number of Mature Individuals):<br>Not applicable. Number of mature individuals (> 175,000) exceeds threshold. |
| Criterion D (Very Small or Restricted Population):<br>Not applicable. Number of locations (2) is below threshold for Threatened, but magnitude of threats does not suggest that the population is likely vulnerable to rapid and substantial decline (not within 1–2 generations). |
| Criterion E (Quantitative Analysis): Not applicable. Analysis not conducted. |

## TECHNICAL SUMMARY - Unarmoured Threespine Stickleback (DU2)

*Gasterosteus aculeatus*

Unarmoured Threespine Stickleback

Épinoche à trois épines lisse

Range of occurrence in Canada: British Columbia

**Demographic Information**

| | |
|---|---|
| Generation time (usually average age of parents in the population) | 2 yrs |
| Is there an [observed, inferred, or projected] continuing decline in number of mature individuals? | Yes<br>The population in Rouge Lake was thought to have been eliminated when the lake dried up in 2017, although some survivors were found in 2019. A dramatic decline in mature individuals was seen in Boulton Lake following invasion by the Northern Red-legged Frog in 2009. Although the ratio of tadpoles to stickleback had decreased by 2017, it is unknown whether Northern Red-legged Frog abundance has declined or if Unarmoured Threespine Stickleback has recovered in this lake. Catastrophic declines due to invasive species are projected in Rouge and Serendipity lakes (see **Fluctuations and Trends**, **Threats**, and **Appendix 2**). |
| Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations, whichever is longer up to a maximum of 100 years] | Percent unknown<br>Rapid (2009–2013) and severe (~80%) decline in Boulton Lake due to AIS, and declines in Rouge and Serendipity Lake due to AIS are projected to be near 100%. However, with possible partial recovery in Boulton Lake, overall estimated percent unquantified. |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Percent unknown<br>Near 100% decline in Rouge Lake in 2017 due to temporary drought, but some recovery since; ~80% decline in Boulton Lake between 2009 and 2013 due to AIS; numbers may be increasing again, but this has not been confirmed or quantified. |
| [Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Percent unknown: some recovery may be occurring in Boulton Lake (0.18 $km^2$), but this has not been confirmed or quantified. Declines near 100% are projected in the much smaller Rouge and Serendipity lakes (0.02 $km^2$ each) due to AIS. From estimates in the 1980s, ~90% of Unarmoured Stickleback occur in Boulton Lake, so projecting percent change in total number of mature individuals is not possible without estimating net change in Boulton Lake. |

| | |
|---|---|
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any period [10 years, or 3 generations, whichever is longer up to a maximum of 100 years], including both the past and the future. | Percent unknown |
| Are the causes of the decline a. clearly reversible and b. understood, and c. ceased? | a. No; b. yes; c. no; some recovery suggested, but extent of recovery unknown and causes of decline still present |
| Are there extreme fluctuations in number of mature individuals? | No<br>Rouge Lake underwent an extreme population bottleneck event in 2017 with rapid and near 100% decline and some recovery since; ~80% decline in Boulton Lake in 2009 with some recovery possible by 2017; however, drastic changes in abundance are not known to occur frequently |

**Extent and Occupancy Information**

| | |
|---|---|
| Estimated extent of occurrence (EOO) | 124 km² |
| Index of area of occupancy (IAO)<br>(Always report 2x2 grid value). | 20 km², although the combined surface area of the three lakes is only 0.22 km² |
| Is the population “severely fragmented” i.e., is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse? | No |
| Number of “locations” (use plausible range to reflect uncertainty if appropriate) | 3<br>Boulton, Rouge, and Serendipity lakes |
| Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? | No |
| Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of subpopulations? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of “locations? | No |
| Is there an [observed, inferred, or projected] continuing decline in area, extent and/or quality of habitat? | Yes<br>Severe decline in extent and quality of habitat observed and projected due to changes in water level and presence of the invasive Northern Red-legged Frog whose tadpoles compete with stickleback; small lakes and a simple fish community make this population highly vulnerable to the impact of this frog. |
| Are there extreme fluctuations in number of subpopulations? | 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 subpopulation)**

| Subpopulations (give plausible ranges) | N Mature Individuals<br><br>Estimates are based on unspecified sampling techniques used in the late 1970s/early 1980s and are considered approximate only. Notwithstanding these caveats, total adult population sizes are likely > 10,000 per lake (see **Abundance**). |
|---|---|
| Boulton Lake | ~350,000 |
| Rouge Lake | ~17,500 |
| Serendipity Lake | ~22,000 |
| Total | ~389,500 |

**Quantitative Analysis**

| | |
|---|---|
| Is the probability of extinction in the wild at least 20% within 20 years or 10% within 100 years? | Unknown<br>Analysis not conducted |

**Threats (direct, from highest impact to least, as per IUCN Threats Calculator)**

Was a threats calculator completed for this species? Yes (03 June 2022)
Overall threat impact = High–Very High (Appendix 2)

Key threat was identified as:
8.1 Invasive & Other Problematic Species & Genes (high–very high)

Unknown threats:
11 Climate Change & Severe Weather

What additional limiting factors are relevant? Their biology (including reduced armour) and occurrence in three small lakes with simple fish communities make them vulnerable to introduction of aquatic invasive species.

**Rescue Effect (immigration from outside Canada)**

| | |
|---|---|
| Status of outside population(s) most likely to provide immigrants to Canada. | Not applicable<br>This species is endemic to Canada |
| Is immigration known or possible? | No, there are no populations elsewhere |
| Would immigrants be adapted to survive in Canada? | Not applicable |
| Is there sufficient habitat for immigrants in Canada? | Not applicable |
| Are conditions deteriorating in Canada? | Not applicable |
| Are conditions for the source (i.e., outside) population deteriorating? | Not applicable |

| | |
|---|---|
| Is the Canadian population considered to be a sink? | Not applicable |
| Is rescue from outside populations likely? | No, there are no populations elsewhere |

**Data Sensitive Species**

| | |
|---|---|
| Is this a data sensitive species? | No |

**Status History**

| |
|---|
| COSEWIC:<br>Designated Special Concern in April 1983. Status re-examined and confirmed in November 2013. Status re-examined and designated Endangered in December 2023. |

**Status and Reasons for Designation:**

| | |
|---|---|
| Status: Endangered<br>NOTE this is changed to current 'status' after a Wildlife Species Assessment Meeting when the report is finalized | Alpha-numeric codes:<br>B1ab(iii,v)+2ab(iii,v) |
| Reasons for designation:<br>This small freshwater fish is a Canadian endemic, and it is unusual as it has reduced bony plates and protective spines compared to other sticklebacks. It is currently known from only three very small lakes on Haida Gwaii in British Columbia. Its revised status reflects the increased risk of extinction following dramatic declines observed in one lake when it temporarily dried up, and in a second lake following the introduction of an invasive frog, the tadpoles of which may compete with the adult fish. These three lakes lack predatory fishes that could prey on the tadpoles. If these threats are not prevented or reversed, they could lead to the extinction of this distinctive Canadian species. | |

**Applicability of Criteria**

| |
|---|
| Criterion A (Decline in Total Number of Mature Individuals):<br>Not applicable. May meet Endangered A2ace+3bce+4abce, but insufficient data are available to reliably infer, project, or suspect that population declines exceed thresholds. Although there was a near 100% decline in Rouge Lake in 2017 due to drought, numbers may be increasing again, so the net decline over the last 10 years is unknown. A decline of about 80% was observed in Boulton Lake between 2009 and 2013 coinciding with the appearance of the non-native Northern Red-legged Frog; partial recovery may be occurring but has not been confirmed or quantified. Near 100% declines are projected within the next 10 years due to invasive species in Rouge and Serendipity lakes; however, total projected population decline could not be quantified. |
| Criterion B (Small Distribution Range and Decline or Fluctuation):<br>Meets Endangered, B1ab(iii,v)+2ab(iii,v). EOO (124 $km^2$) and IAO (20 $km^2$) are below thresholds for Endangered, the population is known to exist at only 3 locations, and it is experiencing an observed and projected decline in the quality of habitat and number of mature individuals due to drought and the introduction of the Northern Red-legged Frog. |
| Criterion C (Small and Declining Number of Mature Individuals):<br>Not applicable. Estimated number of mature individuals (likely > 10,000 per lake) exceeds threshold for Threatened. |
| Criterion D (Very Small or Restricted Population):<br>Meets Threatened, D2. IAO 20 $km^2$, number of locations 3, and prone to substantial decline from effects of human activities or stochastic events within 1–2 generations. |
| Criterion E (Quantitative Analysis): Not applicable. Analysis not conducted. |

## TECHNICAL SUMMARY - Misty Lake Lentic Threespine Stickleback (DU3)

*Gasterosteus aculeatus*

Misty Lake Lentic Threespine Stickleback

Épinoche à trois épines lentique du lac Misty

Range of occurrence in Canada: British Columbia

**Demographic Information**

| | |
|---|---|
| Generation time (usually average age of parents in the population) | 3 yrs<br>Most commonly breed at 2–4 yrs old |
| Is there an [observed, inferred, or projected] continuing decline in number of mature individuals? | Yes<br>Although researchers continue to readily trap Misty Lake Lentics, there has been no systematic monitoring of population trends, and a catastrophic decline is projected given the high impact threat from AIS, including predatory Brown Bullhead, which was responsible for extinction of the Hadley Lake pair, and Smallmouth Bass (see **Threats**). |
| Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations, whichever is longer up to a maximum of 100 years] | Unknown |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Unknown |
| [Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Projected to be nearly 100% if AIS enter the lake |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any period [10 years, or 3 generations, whichever is longer up to a maximum of 100 years], including both the past and the future. | Unknown |
| Are the causes of the decline a. clearly reversible and b. understood, and c. ceased? | a. No; b. yes; c. no |
| Are there extreme fluctuations in number of mature individuals? | No<br>Numbers may fluctuate somewhat with changes in water level, but extreme fluctuations not observed |

**Extent and Occupancy Information**

| | |
|---|---|
| Estimated extent of occurrence (EOO) | 12 km² |
| Index of area of occupancy (IAO)<br>(Always report 2x2 grid value). | 12 km², although the surface area of the lake and outlet stream is only ~0.36 km² |

| | |
|---|---|
| Is the population "severely fragmented" i.e., is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse? | No |
| Number of "locations" (use plausible range to reflect uncertainty if appropriate) | 1 |
| Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? | No |
| Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of subpopulations? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of "locations"? | No |
| Is there an [observed, inferred, or projected] continuing decline in area, extent and/or quality of habitat? | Yes, observed or inferred from logging activities; projected given the threat of AIS introduction |
| Are there extreme fluctuations in number of subpopulations? | 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 subpopulation)**

| Subpopulations (give plausible ranges) | N Mature Individuals<br><br>A rough estimate of the number of mature individuals is drawn from total population abundance estimate (males, females, and juveniles) from a single mark-recapture estimate from 2016, combined with the estimate of proportion of mature individuals in total population from Paxton Lake Benthic data from 2005 (approx. 20%) (see **Abundance**). |
|---|---|
| | 18,151–49,864 (95% CI, mean = 26,768) |
| Total | Same |

**Quantitative Analysis**

| | |
|---|---|
| Is the probability of extinction in the wild at least [20% within 20 years or 5 generations whichever is longer up to a maximum of 100 years, or 10% within 100 years]? | Unknown<br>Analysis not conducted |

**Threats (direct, from highest impact to least, as per IUCN Threats Calculator)**

| |
|---|
| Was a threats calculator completed for this species? Yes (06 June 2022)<br>Overall threat impact = Very High–Low (Assigned) (Appendix 3)<br><br>Key threats were identified as:<br>8.1 Invasive & Other Problematic Species & Genes (very high–high)<br>9.3 Agricultural & Forestry Effluents (low)<br><br>Unknown threats:<br>11 Climate Change & Severe Weather<br><br>What additional limiting factors are relevant? Their biology and occurrence in a single small watershed make them especially vulnerable to introduction of aquatic invasive species. |

**Rescue Effect (immigration from outside Canada)**

| | |
|---|---|
| Status of outside population(s) most likely to provide immigrants to Canada. | Not applicable<br>This species is endemic to Canada |
| Is immigration known or possible? | No, there are no populations elsewhere |
| Would immigrants be adapted to survive in Canada? | Not applicable |
| Is there sufficient habitat for immigrants in Canada? | Not applicable |
| Are conditions deteriorating in Canada? | Not applicable |
| Are conditions for the source (i.e., outside) population deteriorating? | Not applicable |
| Is the Canadian population considered to be a sink? | Not applicable |
| Is rescue from outside populations likely? | No, there are no populations elsewhere |

**Data Sensitive Species**

| | |
|---|---|
| Is this a data sensitive species? | No |

**Status History**

| |
|---|
| COSEWIC:<br>Designated Endangered in November 2006. Status re-examined and confirmed in December 2023. |

**Status and Reasons for Designation:**

| | |
|---|---|
| Status: Endangered<br>NOTE this is changed to current 'status' after a Wildlife Species Assessment Meeting when the report is finalized | Alpha-numeric codes:<br>B1ab(iii,v)+2ab(iii,v) |
| Reasons for designation:<br>This small, lake-dwelling fish is endemic to Canada as part of a species pair restricted to a single small lake-stream complex on northern Vancouver Island. This fish could quickly become extinct if invasive non-native fishes that prey on the eggs and adults of this species are accidentally or deliberately introduced. Proximity of this species to a major highway and public access make an introduction more probable. Logging activities in the watershed could also negatively impact habitat quality. If these threats are not prevented or reversed, they could lead to the extinction of this distinctive Canadian species. | |

**Applicability of Criteria**

| |
|---|
| Criterion A (Decline in Total Number of Mature Individuals):<br>Not applicable. Although the threat from invasive species is high and would likely rapidly drive the population to extinction, the likelihood of an invasive species being introduced within the next 10 years is unknown. |
| Criterion B (Small Distribution Range and Decline or Fluctuation):<br>Meets Endangered, B1ab(iii,v)+2ab(iii,v). EOO and IAO (both 12 $km^2$) are below thresholds for Endangered, the population is known to exist at a single location, and a decline in quality of habitat and number of mature individuals is projected given the threat of invasive species introduction. |
| Criterion C (Small and Declining Number of Mature Individuals):<br>Not applicable. Estimated number of mature individuals (18,15–49,864) exceeds threshold for Threatened. |
| Criterion D (Very Small or Restricted Population):<br>Meets Threatened, D2. IAO 12 $km^2$, number of locations 1, and prone to substantial decline from effects of human activities or stochastic events within 1–2 generations. |
| Criterion E (Quantitative Analysis): Not applicable. Analysis not conducted. |

## TECHNICAL SUMMARY - Misty Lake Lotic Threespine Stickleback (DU4)

*Gasterosteus aculeatus*

Misty Lake Lotic Threespine Stickleback

Épinoche à trois épines lotique du lac Misty

**Demographic Information**

| | |
|---|---|
| Generation time (usually average age of parents in the population) | Approx. 1.5 yrs<br>Most commonly breed at 1–2 yrs old |
| Is there an [observed, inferred, or projected] continuing decline in number of mature individuals? | Yes<br>Although researchers continue to readily trap Misty Lake Lotics, there has been no systematic monitoring of population trends, and a catastrophic decline is projected given the high impact threat from AIS, including predatory Brown Bullhead, which was responsible for extinction of the Hadley Lake pair, and Smallmouth Bass (see **Threats**). |
| Estimated percent of continuing decline in total number of mature individuals [5 years or 2 generations, whichever is longer up to a maximum of 100 years] | Unknown |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Unknown |
| [Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Projected near 100% if AIS enter the lake |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any period [10 years, or 3 generations, whichever is longer up to a maximum of 100 years], including both the past and the future. | Unknown |
| Are the causes of the decline a. clearly reversible and b. understood, and c. ceased? | a. No; b. yes; c. no |
| Are there extreme fluctuations in number of mature individuals? | No<br>Numbers may fluctuate somewhat with changes in water level, but extreme fluctuations not noted |

**Extent and Occupancy Information**

| | |
|---|---|
| Estimated extent of occurrence (EOO) | 12 km² |
| Index of area of occupancy (IAO)<br>(Always report 2x2 grid value). | 12 km², although the surface area of the inlet stream is only ~0.04 km² |

| | |
|---|---|
| Is the population "severely fragmented" i.e., is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse? | No |
| Number of "locations" (use plausible range to reflect uncertainty if appropriate) | 1 |
| Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? | No |
| Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of subpopulations? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of "locations? | No |
| Is there an [observed, inferred, or projected] continuing decline in area, extent and/or quality of habitat? | Yes, observed or inferred from logging activities; projected given the threat of AIS introduction |
| Are there extreme fluctuations in number of subpopulations? | 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 subpopulation)**

| Subpopulations (give plausible ranges) | N Mature Individuals<br><br>A rough estimate of the number of mature individuals is derived from total population abundance estimate (males, females, and juveniles) from a single mark-recapture estimate from 2016, combined with estimate of proportion of mature individuals in total population from Paxton Lake Benthic data from 2005 (approx. 20%) (see **Abundance**). |
|---|---|
| | 1,096–3,771 (95% CI, mean = 2,998) |
| Total | Same |

**Quantitative Analysis**

| | |
|---|---|
| Is the probability of extinction in the wild at least [20% within 20 years or 5 generations whichever is longer up to a maximum of 100 years, or 10% within 100 years]? | Unknown<br>Analysis not conducted |

**Threats (direct, from highest impact to least, as per IUCN Threats Calculator)**

| |
|---|
| Was a threats calculator completed for this species? Yes (06 June 2022)<br>Overall threat impact = Very High–Low (Assigned) (Appendix 3)<br><br>Key threats were identified as:<br>8.1 Invasive & Other Problematic Species & Genes (very high – high)<br>9.3 Agricultural & Forestry Effluents (low)<br><br>Unknown threats:<br>11 Climate Change & Severe Weather<br><br>What additional limiting factors are relevant? Their biology and occurrence in a single small watershed make them especially vulnerable to introduction of aquatic invasive species. |

**Rescue Effect (immigration from outside Canada)**

| | |
|---|---|
| Status of outside population(s) most likely to provide immigrants to Canada. | Not applicable<br>This species is endemic to Canada |
| Is immigration known or possible? | No, there are no populations elsewhere |
| Would immigrants be adapted to survive in Canada? | Not applicable |
| Is there sufficient habitat for immigrants in Canada? | Not applicable |
| Are conditions deteriorating in Canada? | Not applicable |
| Are conditions for the source (i.e., outside) population deteriorating? | Not applicable |
| Is the Canadian population considered to be a sink? | Not applicable |
| Is rescue from outside populations likely? | No, there are no populations elsewhere |

**Data Sensitive Species**

| | |
|---|---|
| Is this a data sensitive species? | No |

**Status History**

| |
|---|
| COSEWIC:<br>Designated Endangered in November 2006. Status re-examined and confirmed in December 2023. |

**Status and Reasons for Designation:**

| | |
|---|---|
| Status:<br>Endangered | Alpha-numeric codes:<br>B1ab(iii,v)+2ab(iii,v) |
| Reasons for designation:<br>This small, lake-dwelling fish is endemic to Canada as part of a species pair restricted to a single small lake-stream complex on northern Vancouver Island. This fish could quickly become extinct if invasive non-native fishes that prey on the eggs and adults of this species are accidentally or deliberately introduced. Proximity of this species to a major highway and public access make an introduction more probable. Logging activities in the watershed could also negatively impact habitat quality. If these threats are not prevented or reversed, they could lead to the extinction of this distinctive Canadian species. | |

**Applicability of Criteria**

| |
|---|
| Criterion A (Decline in Total Number of Mature Individuals):<br>Not applicable. Although the threat from invasive species is high and would likely rapidly drive the population to extinction, the likelihood of an invasive species being introduced within the next 10 years is unknown. |
| Criterion B (Small Distribution Range and Decline or Fluctuation):<br>Meets Endangered, B1ab(iii,v)+2ab(iii,v). EOO and IAO (both 12 $km^2$) are below thresholds for Endangered, the population is known to exist at a single location, and a decline in quality of habitat and number of mature individuals is projected given the threat of invasive species introduction. |
| Criterion C (Small and Declining Number of Mature Individuals):<br>Meets Threatened, C2a(ii). Number of mature individuals is estimated at 1,096–3,771(95% CI, mean = 2,998), with 100% in one subpopulation, and there is a projected continuing decline. |
| Criterion D (Very Small or Restricted Population):<br>Meets Threatened, D2. IAO 12 $km^2$, number of locations 1, and species is prone to substantial decline from effects of human activities or stochastic events within 1–2 generations. |
| Criterion E (Quantitative Analysis): Not applicable. Analysis not conducted. |

## TECHNICAL SUMMARY - Paxton Lake Benthic Threespine Stickleback (DU5)

*Gasterosteus aculeatus*

Paxton Lake Benthic Threespine Stickleback

Épinoche à trois épines benthique du lac Paxton

Range of occurrence in Canada: British Columbia

**Demographic Information**

| | |
|---|---|
| Generation time (usually average age of parents in the population) | Approx. 2.5 yrs<br>Usually mature at 2 yrs old and breed for several seasons |
| Is there an [observed, inferred, or projected] continuing decline in number of mature individuals? | Yes<br>No systematic monitoring of population trends, but declines may have occurred due to fluctuation in water level from human activities (see **Fluctuations and Trends**) and a catastrophic decline is projected given the high impact threat from AIS. |
| Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations, whichever is longer up to a maximum of 100 years] | Unknown |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Unknown |
| [Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Projected near 100% if AIS enter the lake, especially with other ecosystem modifications affecting reproductive isolation |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any period [10 years, or 3 generations, whichever is longer up to a maximum of 100 years], including both the past and the future. | Unknown |
| Are the causes of the decline a. clearly reversible and b. understood, and c. ceased? | a. No; b. yes; c. no |
| Are there extreme fluctuations in number of mature individuals? | No |

**Extent and Occupancy Information**

| | |
|---|---|
| Estimated extent of occurrence (EOO) | 8 km² |
| Index of area of occupancy (IAO)<br>(Always report 2x2 grid value). | 8 km², although the surface area of the lake is only 0.17 km² |

| | |
|---|---|
| Is the population “severely fragmented” i.e., is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse? | No |
| Number of “locations” (use plausible range to reflect uncertainty if appropriate) | 1 |
| Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? | No |
| Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of subpopulations? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of “locations”? | No |
| Is there an [observed, inferred, or projected] continuing decline in area, extent and/or quality of habitat? | Yes, observed or inferred due to changes in water level (water licences for Paxton Lake remain large relative to the volume of the lakes and size of the catchments); projected given the threat of AIS introduction, including AIS that impact littoral vegetation |
| Are there extreme fluctuations in number of subpopulations? | 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 subpopulation)**

| | |
|---|---|
| Subpopulations (give plausible ranges) | N Mature Individuals<br><br>An estimate of the number of mature individuals is derived from total population abundance estimate (males, females, and juveniles) a from single mark-recapture estimate from 2016, combined with estimate of proportion of mature individuals in total population from 2005 (approx. 20%) (see **Abundance**). |
| | 3,509–5,799 (95% CI, mean = 4,438) |
| Total | Same |

**Quantitative Analysis**

| | |
|---|---|
| Is the probability of extinction in the wild at least [20% within 20 years or 5 generations whichever is longer up to a maximum of 100 years, or 10% within 100 years]? | Unknown<br>Simple population viability analysis (PVA) calculations were done as part of Critical Habitat determinations (see Hatfield 2009), but this analysis did not use COSEWIC parameters or address the relationship between and genetic integrity of the pair (see **Fluctuations and Trends**). |

**Threats (direct, from highest impact to least, as per IUCN Threats Calculator)**

| |
|---|
| Was a threats calculator completed for this species? Yes (14 June 2022)<br>Overall threat impact = Very High–Medium (Assigned) (Appendix 4, 5)<br><br>Key threats were identified as:<br>8.1 Invasive & Other Problematic Species & Genes (very high)<br>7.3 Other Ecosystem Modifications (very high)<br>7.2 Dams & Water Management/Use (low)<br><br>Unknown threats:<br>11 Climate Change & Severe Weather<br><br>What additional limiting factors are relevant? Their biology and occurrence in a single small lake make them especially vulnerable to introduction of aquatic invasive species. |

**Rescue Effect (immigration from outside Canada)**

| | |
|---|---|
| Status of outside population(s) most likely to provide immigrants to Canada. | Not applicable<br>This species is endemic to Canada |
| Is immigration known or possible? | No, there are no populations elsewhere |
| Would immigrants be adapted to survive in Canada? | Not applicable |
| Is there sufficient habitat for immigrants in Canada? | Not applicable |
| Are conditions deteriorating in Canada? | Not applicable |
| Are conditions for the source (i.e., outside) population deteriorating? | Not applicable |
| Is the Canadian population considered to be a sink? | Not applicable |
| Is rescue from outside populations likely? | No, there are no populations elsewhere |

**Data Sensitive Species**

| | |
|---|---|
| Is this a data sensitive species? | No |

**Status History**

| |
|---|
| COSEWIC:<br>Designated Threatened in April 1998. Status re-examined and confirmed in April 1999. Status re-examined and designated Endangered in May 2000. Status re-examined and confirmed in April 2010 and December 2023. |

**Status and Reasons for Designation:**

| Status: Endangered | Alpha-numeric codes: B1ab(iii,v)+2ab(iii,v) |
|---|---|
| Reasons for designation: This small, robust-bodied freshwater fish is part of an endemic species pair restricted to one small lake in coastal British Columbia (BC). It lives close to the bottom of the lake, while the other member of the pair lives in the open water. The main threat to this species is introduction of invasive species, which have caused the rapid extinction of similar species pairs in two other lakes in coastal BC, either through predation or hybridization resulting from habitat modification. Invasive species continue to spread in the region. Water extraction could also result in loss of habitat and increase the risk of hybridization. If these threats are not mitigated, they could lead to the extinction of this distinctive Canadian species. | |

**Applicability of Criteria**

| |
|---|
| Criterion A (Decline in Total Number of Mature Individuals): Not applicable. Although introduction of invasive species would likely rapidly drive the population to extinction, the likelihood of such an introduction within the next 10 years is unknown. |
| Criterion B (Small Distribution Range and Decline or Fluctuation): Meets Endangered, B1ab(iii,v)+2ab(iii,v). EOO and IAO (both 8 $km^2$) are below thresholds for Endangered, the population is known to exist at a single location, and a decline in quality of habitat and number of mature individuals is projected given the threat of invasive species introduction and water extraction activities. |
| Criterion C (Small and Declining Number of Mature Individuals): Meets Threatened, C2a(ii). Number of mature individuals is estimated at 3,509–5,799 (95% CI, mean = 4,438), with 100% in one subpopulation, and there is a projected continuing decline. |
| Criterion D (Very Small or Restricted Population): Meets Threatened, D2. IAO 8 $km^2$, number of locations 1, and prone to substantial decline from effects of human activities or stochastic events within 1–2 generations. |
| Criterion E (Quantitative Analysis): Not applicable. Analysis did not use COSEWIC parameters or address the genetic integrity of the pair. |

## TECHNICAL SUMMARY - Paxton Lake Limnetic Threespine Stickleback (DU6)

*Gasterosteus aculeatus*

Paxton Lake Limnetic Threespine Stickleback

Épinoche à trois épines limnétique du lac Paxton

Range of occurrence in Canada: British Columbia

**Demographic Information**

| | |
|---|---|
| Generation time (usually average age of parents in the population) | 1 yr |
| Is there an [observed, inferred, or projected] continuing decline in number of mature individuals? | Yes<br>No systematic monitoring of population trends, but declines may have occurred due to fluctuation in water level from human activities (see **Fluctuations and Trends**) and a catastrophic decline is projected given the high impact threat from AIS. |
| Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations, whichever is longer up to a maximum of 100 years] | Unknown |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Unknown |
| [Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Projected to be nearly 100% if AIS enter the lake, especially with other ecosystem modifications affecting reproductive isolation |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any period [10 years, or 3 generations, whichever is longer up to a maximum of 100 years], including both the past and the future. | Unknown |
| Are the causes of the decline a. clearly reversible and b. understood, and c. ceased? | a. No; b. yes; c. no |
| Are there extreme fluctuations in number of mature individuals? | No |

**Extent and Occupancy Information**

| | |
|---|---|
| Estimated extent of occurrence (EOO) | 8 km² |
| Index of area of occupancy (IAO)<br>(Always report 2x2 grid value). | 8 km², although the surface area of the lake is only 0.17 km² |

| | |
|---|---|
| Is the population "severely fragmented" i.e., is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse? | No |
| Number of "locations" (use plausible range to reflect uncertainty if appropriate) | 1 |
| Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? | No |
| Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of subpopulations? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of "locations"? | No |
| Is there an [observed, inferred, or projected] continuing decline in area, extent and/or quality of habitat? | Yes, observed or inferred due to changes in water level (water licences for Paxton Lake remain large relative to the volume of the lakes and size of the catchments); projected given the threat of AIS introduction, including AIS that impact littoral vegetation |
| Are there extreme fluctuations in number of subpopulations? | 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 subpopulation)**

| Subpopulations (give plausible ranges) | N Mature Individuals<br><br>A rough estimate of the number of mature individuals is derived from total population abundance estimate (males, females, and juveniles) from a mark-recapture estimate from 2016 (which likely overestimates abundance due to schooling behaviour), combined with estimate of proportion of mature individuals in total population from Benthic data from 2005 (approx. 20%) (see **Abundance**). |
|---|---|
| | 47,227–168,504 (95% CI, mean = 73,777) |
| Total | Same |

**Quantitative Analysis**

| | |
|---|---|
| Is the probability of extinction in the wild at least [20% within 20 years or 5 generations whichever is longer up to a maximum of 100 years, or 10% within 100 years]? | Unknown<br>Simple population viability analysis (PVA) calculations were done as part of Critical Habitat determinations (see Hatfield 2009), but this analysis did not use COSEWIC parameters or address the relationship between and genetic integrity of the pair (see **Fluctuations and Trends**). |

**Threats (direct, from highest impact to least, as per IUCN Threats Calculator)**

| |
|---|
| Was a threats calculator completed for this species? Yes (14 June 2022)<br>Overall threat impact = Very High–Medium (Assigned) (Appendix 4, 5)<br><br>Key threats were identified as:<br>8.1 Invasive & Other Problematic Species & Genes (very high)<br>7.3 Other Ecosystem Modifications (very high)<br>7.2 Dams & Water Management/Use (low)<br><br>Unknown threats:<br>11 Climate Change & Severe Weather<br><br>What additional limiting factors are relevant? Their biology and occurrence in a single small lake make them especially vulnerable to introduction of aquatic invasive species. |

**Rescue Effect (immigration from outside Canada)**

| | |
|---|---|
| Status of outside population(s) most likely to provide immigrants to Canada. | Not applicable<br>This species is endemic to Canada |
| Is immigration known or possible? | No, there are no populations elsewhere |
| Would immigrants be adapted to survive in Canada? | Not applicable |
| Is there sufficient habitat for immigrants in Canada? | Not applicable |
| Are conditions deteriorating in Canada? | Not applicable |
| Are conditions for the source (i.e., outside) population deteriorating? | Not applicable |
| Is the Canadian population considered to be a sink? | Not applicable |
| Is rescue from outside populations likely? | No, there are no populations elsewhere |

**Data Sensitive Species**

| | |
|---|---|
| Is this a data sensitive species? | No |

**Status History**

| |
|---|
| COSEWIC:<br>Designated Threatened in April 1998. Status re-examined and confirmed in April 1999. Status re-examined and designated Endangered in May 2000. Status re-examined and confirmed in April 2010 and December 2023. |

**Status and Reasons for Designation:**

| Status:<br>Endangered | Alpha-numeric codes:<br>B1ab(iii,v)+2ab(iii,v) |
|---|---|
| Reasons for designation:<br>This small, slender-bodied freshwater fish is part of an endemic species pair restricted to one small lake in coastal British Columbia (BC). It lives in the open water of the lake, while the other member of the pair lives close to the bottom. The main threat to this species is introduction of invasive species, which have caused the rapid extinction of similar species pairs in two other lakes in coastal BC, either through predation or hybridization resulting from habitat modification. Invasive species continue to spread in the region. Water extraction could also result in loss of habitat and increase the risk of hybridization. If these threats are not mitigated, they could lead to the extinction of this distinctive Canadian species. | |

**Applicability of Criteria**

| |
|---|
| Criterion A (Decline in Total Number of Mature Individuals):<br>Not applicable. Although introduction of invasive species would likely rapidly drive the population to extinction, the likelihood of such an introduction within the next 10 years is unknown. |
| Criterion B (Small Distribution Range and Decline or Fluctuation):<br>Meets Endangered, B1ab(iii,v)+2ab(iii,v). EOO and IAO (both 8 $km^2$) are below thresholds for Endangered, the population is known to exist at a single location, and a decline in quality of habitat and number of mature individuals is projected given the threat of invasive species introduction and water extraction activities. |
| Criterion C (Small and Declining Number of Mature Individuals):<br>Not applicable. Number of mature individuals (47,227–168,504) exceeds threshold for Threatened. |
| Criterion D (Very Small or Restricted Population):<br>Meets Threatened, D2. IAO 8 $km^2$, number of locations 1, and prone to substantial decline from effects of human activities or stochastic events within 1–2 generations. |
| Criterion E (Quantitative Analysis): Not applicable. Analysis did not use COSEWIC parameters or address the genetic integrity of the pair. |

## TECHNICAL SUMMARY - Vananda Creek Benthic Threespine Stickleback (DU7)

*Gasterosteus aculeatus*

Vananda Creek Benthic Threespine Stickleback

Épinoche à trois épines benthique du ruisseau Vananda

Range of occurrence in Canada: British Columbia

**Demographic Information**

| | |
|---|---|
| Generation time (usually average age of parents in the population) | Approx. 2.5 yrs<br>Usually mature at 2 yrs old and breed for several seasons |
| Is there an [observed, inferred, or projected] continuing decline in number of mature individuals? | Yes<br>Although researchers continue to readily trap Benthics in Priest Lake, sampling from Spectacle Lake and Emily Lake remains sporadic, and a catastrophic decline is projected given the high impact threat from AIS and other ecosystem modifications. |
| Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations, whichever is longer up to a maximum of 100 years]. | Unknown |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Unknown |
| [Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Projected to be nearly 100% if AIS enter the lake, especially with other ecosystem modifications affecting reproductive isolation |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any period [10 years, or 3 generations, whichever is longer up to a maximum of 100 years], including both the past and the future. | Unknown |
| Are the causes of the decline a. clearly reversible and b. understood, and c. ceased? | a. No; b. yes; c. no |
| Are there extreme fluctuations in number of mature individuals? | No |

**Extent and Occupancy Information**

| | |
|---|---|
| Estimated extent of occurrence (EOO) | 16 km² |
| Index of area of occupancy (IAO)<br>(Always report 2x2 grid value). | 16 km², although the surface area of the three interconnected lakes is only 0.63 km² |

| | |
|---|---|
| Is the population "severely fragmented" i.e., is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse? | No |
| Number of "locations" (use plausible range to reflect uncertainty if appropriate) | 1<br>Interconnected lakes Emily, Priest, and Spectacle |
| Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? | No |
| Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of subpopulations? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of "locations"? | No |
| Is there an [observed, inferred, or projected] continuing decline in area, extent and/or quality of habitat? | Yes, observed or inferred due to changes in water level (water licences for the Vananda Creek watershed remains large relative to the volume of the lakes and size of the catchments; projected given the threat of AIS introduction, including AIS that impact littoral vegetation |
| Are there extreme fluctuations in number of subpopulations? | 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 subpopulation)**

| | |
|---|---|
| Subpopulations (give plausible ranges) | N Mature Individuals<br><br>A rough estimate of the number of mature individuals is derived from total population abundance estimate (males, females, and juveniles) from a single mark-recapture estimate from Priest Lake in 2016, extrapolating for Emily and Spectacle lakes based on lake perimeter, combined with estimate of proportion of mature individuals in total population from Paxton Lake Benthic data from 2005 (approx. 20%) (see **Abundance**). |
| | 37,873–52,823 (95% CI, mean = 44,116) |
| Total | Same |

**Quantitative Analysis**

| | |
|---|---|
| Is the probability of extinction in the wild at least [20% within 20 years or 5 generations whichever is longer up to a maximum of 100 years, or 10% within 100 years]? | Unknown<br>Simple population viability analysis (PVA) calculations were done as part of Critical Habitat determinations (see Hatfield 2009), but this analysis did not use COSEWIC parameters or address the relationship between and genetic integrity of the pair (see **Fluctuations and Trends**). |

**Threats (direct, from highest impact to least, as per IUCN Threats Calculator)**

Was a threats calculator completed for this species? Yes (14 June 2022)
Overall threat impact = Very High–High (Assigned) (Appendix 4, 5)

Key threats were identified as:
8.1 Invasive & Other Problematic Species & Genes (very high)
7.3 Other Ecosystem Modifications (very high)
7.2 Dams & Water Management/Use (medium–low)

Unknown threats:
11 Climate Change & Severe Weather

What additional limiting factors are relevant? Their biology and occurrence in three small, interconnected lakes make them especially vulnerable to introduction of aquatic invasive species.

**Rescue Effect (immigration from outside Canada)**

| | |
|---|---|
| Status of outside population(s) most likely to provide immigrants to Canada. | Not applicable<br>This species is endemic to Canada |
| Is immigration known or possible? | No, there are no populations elsewhere |
| Would immigrants be adapted to survive in Canada? | Not applicable |
| Is there sufficient habitat for immigrants in Canada? | Not applicable |
| Are conditions deteriorating in Canada? | Not applicable |
| Are conditions for the source (i.e., outside) population deteriorating? | Not applicable |
| Is the Canadian population considered to be a sink? | Not applicable |
| Is rescue from outside populations likely? | No, there are no populations elsewhere |

**Data Sensitive Species**

| | |
|---|---|
| Is this a data sensitive species? | No |

**Status History**

COSEWIC:
Designated Threatened in April 1999. Status re-examined and designated Endangered in May 2000. Status re-examined and confirmed in April 2010 and December 2023.

**Status and Reasons for Designation:**

| Status:<br>Endangered | Alpha-numeric codes:<br>B1ab(iii,v)+2ab(iii,v) |
|---|---|
| Reasons for designation:<br>This small, robust-bodied freshwater fish is part of an endemic species pair restricted to three small, interconnected lakes in coastal British Columbia (BC). It lives close to the bottom of the lakes, while the other member of the pair lives in the open water. The main threat to this species is introduction of invasive species, which have caused the rapid extinction of similar species pairs in two other lakes in coastal BC, either through predation or hybridization resulting from habitat modification. Invasive species continue to spread in the region. Water extraction could also result in loss of habitat and increase the risk of hybridization. If these threats are not mitigated, they could lead to the extinction of this distinctive Canadian species. | |

**Applicability of Criteria**

| |
|---|
| Criterion A (Decline in Total Number of Mature Individuals):<br>Not applicable. Although the threat from invasive species is very high and would likely rapidly drive the population to extinction, the likelihood of an invasive species being introduced within the next 10 years is unknown. |
| Criterion B (Small Distribution Range and Decline or Fluctuation):<br>Meets Endangered, B1ab(iii,v)+2ab(iii,v). EOO and IAO (both 16 $km^2$) are below thresholds for Endangered, the population is known to exist at a single location, and a decline in quality of habitat and number of mature individuals is projected given the threat of invasive species introduction and water extraction activities. |
| Criterion C (Small and Declining Number of Mature Individuals):<br>Not applicable. Estimated number of mature individuals (37,873–52,823) exceeds threshold for Threatened. |
| Criterion D (Very Small or Restricted Population):<br>Meets Threatened, D2. IAO 16 $km^2$, number of locations 1, and prone to substantial decline from effects of human activities or stochastic events within 1–2 generations. |
| Criterion E (Quantitative Analysis): Not applicable. Analysis did not use COSEWIC parameters or address the genetic integrity of the pair. |

## TECHNICAL SUMMARY - Vananda Creek Limnetic Threespine Stickleback (DU8)

*Gasterosteus aculeatus*

Vananda Creek Limnetic Threespine Stickleback

Épinoche à trois épines limnétique du ruisseau Vananda

Range of occurrence in Canada: British Columbia

**Demographic Information**

| | |
|---|---|
| Generation time (usually average age of parents in the population) | 1 yr |
| Is there an [observed, inferred, or projected] continuing decline in number of mature individuals? | Yes<br>Although researchers continue to readily trap Benthics in Priest Lake, sampling from Spectacle and Emily lakes remains sporadic, and a catastrophic decline is projected given the high impact threat from AIS and other ecosystem modifications. |
| Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations, whichever is longer up to a maximum of 100 years] | Unknown |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Unknown |
| [Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Projected to be nearly 100% if AIS enter the lake, especially with other ecosystem modifications affecting reproductive isolation |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any period [10 years, or 3 generations, whichever is longer up to a maximum of 100 years], including both the past and the future. | Unknown |
| Are the causes of the decline a. clearly reversible and b. understood, and c. ceased? | a. No; b. yes; c. no |
| Are there extreme fluctuations in number of mature individuals? | No |

**Extent and Occupancy Information**

| | |
|---|---|
| Estimated extent of occurrence (EOO) | 16 km² |
| Index of area of occupancy (IAO)<br>(Always report 2x2 grid value). | 16 km², although the surface area of the three interconnected lakes is only 0.63 km² |

| | |
|---|---|
| Is the population "severely fragmented" i.e., is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse? | No |
| Number of "locations" (use plausible range to reflect uncertainty if appropriate) | 1<br>Interconnected lakes Emily, Priest, and Spectacle |
| Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? | No |
| Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of subpopulations? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of "locations"? | No |
| Is there an [observed, inferred, or projected] continuing decline in area, extent and/or quality of habitat? | Yes, observed or inferred due to changes in water level (water licences for the Vananda Creek watershed remains large relative to the volume of the lakes and size of the catchments; projected given the threat of AIS introduction, including AIS that impact littoral vegetation |
| Are there extreme fluctuations in number of subpopulations? | 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 subpopulation)**

| | |
|---|---|
| Subpopulations (give plausible ranges) | N Mature Individuals<br><br>A rough estimate of the number of mature individuals is derived from total population abundance estimate (males, females, and juveniles) from a single mark-recapture estimate from Priest Lake in 2016, extrapolating for Emily Lake and Spectacle Lake based on lake area, combined with estimate of proportion of mature individuals in total population from Paxton Lake Benthic data from 2005 (approx. 20%) (see **Abundance**). |
| | 22,204–53,951 (95% CI, mean = 31,461) |
| Total | Same |

**Quantitative Analysis**

| | |
|---|---|
| Is the probability of extinction in the wild at least [20% within 20 years or 5 generations whichever is longer up to a maximum of 100 years, or 10% within 100 years]? | Unknown<br>Simple population viability analysis (PVA) calculations were done as part of Critical Habitat determinations (see Hatfield 2009), but this analysis did not use COSEWIC parameters or address the relationship between and genetic integrity of the pair (see **Fluctuations and Trends**). |

**Threats (direct, from highest impact to least, as per IUCN Threats Calculator)**

| |
|---|
| Was a threats calculator completed for this species? Yes (14 June 2022)<br>Overall threat impact = Very High–High (Assigned) (Appendix 4, 5)<br><br>Key threats were identified as:<br>8.1 Invasive & Other Problematic Species & Genes (very high)<br>7.3 Other Ecosystem Modifications (very high)<br>7.2 Dams & Water Management/Use (medium–low)<br><br>Unknown threats:<br>11 Climate Change & Severe Weather<br><br>What additional limiting factors are relevant?<br>Their biology and occurrence in three small, interconnected lakes make them especially vulnerable to introduction of aquatic invasive species. |

**Rescue Effect (immigration from outside Canada)**

| | |
|---|---|
| Status of outside population(s) most likely to provide immigrants to Canada. | Not applicable<br>This species is endemic to Canada |
| Is immigration known or possible? | No, there are no populations elsewhere |
| Would immigrants be adapted to survive in Canada? | Not applicable |
| Is there sufficient habitat for immigrants in Canada? | Not applicable |
| Are conditions deteriorating in Canada? | Not applicable |
| Are conditions for the source (i.e., outside) population deteriorating? | Not applicable |
| Is the Canadian population considered to be a sink? | Not applicable |
| Is rescue from outside populations likely? | No, there are no populations elsewhere |

**Data Sensitive Species**

| | |
|---|---|
| Is this a data sensitive species? | No |

**Status History**

| |
|---|
| COSEWIC:<br>Designated Threatened in April 1999. Status re-examined and designated Endangered in May 2000. Status re-examined and confirmed in April 2010 and December 2023. |

**Status and Reasons for Designation:**

| Status:<br>Endangered | Alpha-numeric codes:<br>B1ab(iii,v)+2ab(iii,v) |
|---|---|

| |
|---|
| Reasons for designation:<br>This small, slender-bodied freshwater fish is part of an endemic species pair restricted to three small, interconnected lakes in coastal British Columbia (BC). It lives in the open water of the lake, while the other member of the pair lives close to the bottom. The main threat to this species is introduction of invasive species, which have caused the rapid extinction of similar species pairs in two other lakes in coastal BC, either through predation or hybridization resulting from habitat modification. Invasive species continue to spread in the region. Water extraction could also result in loss of habitat and increase the risk of hybridization. If these threats are not mitigated, they could lead to the extinction of this distinctive Canadian species. |

**Applicability of Criteria**

| |
|---|
| Criterion A (Decline in Total Number of Mature Individuals):<br>Not applicable. Although the threat from invasive species is very high and would likely rapidly drive the population to extinction, the likelihood of an invasive species being introduced within the next 10 years is unknown. |
| Criterion B (Small Distribution Range and Decline or Fluctuation):<br>Meets Endangered, B1ab(iii,v)+2ab(iii,v). EOO and IAO (both 16 $km^2$) are below thresholds for Endangered, the population is known to exist at a single location, and a decline in quality of habitat and number of mature individuals is projected given the threat of invasive species introduction and water extraction activities. |
| Criterion C (Small and Declining Number of Mature Individuals):<br>Not applicable. Estimated number of mature individuals (22,204–53,951) exceeds threshold for Threatened. |
| Criterion D (Very Small or Restricted Population):<br>Meets Threatened, D2. IAO 16 $km^2$, number of locations 1, and prone to substantial decline from effects of human activities or stochastic events within 1–2 generations. |
| Criterion E (Quantitative Analysis): Not applicable. Analysis did not use COSEWIC parameters or address the genetic integrity of the pair. |

## TECHNICAL SUMMARY - Little Quarry Lake Benthic Threespine Stickleback (DU9)

*Gasterosteus aculeatus*

Little Quarry Lake Benthic Threespine Stickleback

Épinoche à trois épines benthique du lac Little Quarry

Range of occurrence in Canada: British Columbia

### Demographic Information

| | |
|---|---|
| Generation time (usually average age of parents in the population) | 2.5 yrs<br>Inferred from research on other Benthic-Limnetic Threespine Stickleback pairs; no specific data exists for Little Quarry Lake |
| Is there an [observed, inferred, or projected] continuing decline in number of mature individuals? | Yes<br>Although researchers continue to readily trap Quarry Lake Benthics, there has been no systematic monitoring of population trends and a catastrophic decline is projected given the high impact threat from AIS and other ecosystem modifications. |
| Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations, whichever is longer up to a maximum of 100 years] | Unknown |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Unknown |
| [Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Projected to be nearly 100% if AIS enter the lake, especially with other ecosystem modifications affecting reproductive isolation |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any period [10 years, or 3 generations, whichever is longer up to a maximum of 100 years], including both the past and the future. | Unknown |
| Are the causes of the decline a. clearly reversible and b. understood, and c. ceased? | a. No; b. yes; c. no |
| Are there extreme fluctuations in number of mature individuals? | No |

### Extent and Occupancy Information

| | |
|---|---|
| Estimated extent of occurrence (EOO) | 8 km² |
| Index of area of occupancy (IAO)<br>(Always report 2x2 grid value). | 8 km², although the surface area of the lake is only 0.22 km² |

| | |
|---|---|
| Is the population "severely fragmented" i.e., is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse? | No |
| Number of "locations" (use plausible range to reflect uncertainty if appropriate) | 1 |
| Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? | No |
| Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of subpopulations? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of "locations"? | No |
| Is there an [observed, inferred, or projected] continuing decline in area, extent and/or quality of habitat? | Yes, projected decline in extent and quality of habitat due to introduction of AIS, especially AIS that impact vegetation in the very limited littoral zone |
| Are there extreme fluctuations in number of subpopulations? | 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 subpopulation)**

| | |
|---|---|
| Subpopulations (give plausible ranges) | N Mature Individuals<br><br>A rough estimate of the number of mature individuals is derived from total population abundance estimate (males, females, and juveniles) from a single mark-recapture estimate from Paxton Lake in 2016, extrapolating for Little Quarry Lake based on lake perimeter, combined with estimate of proportion of mature individuals in total population from Paxton Lake Benthic data from 2005 (approx. 20%) (see **Abundance**). |
| | 4,007–6,620 (95% CI, mean = 5,068) |
| Total | Same |

**Quantitative Analysis**

| | |
|---|---|
| Is the probability of extinction in the wild at least [20% within 20 years or 5 generations whichever is longer up to a maximum of 100 years, or 10% within 100 years]? | Unknown<br>Analysis not conducted |

**Threats (direct, from highest impact to least, as per IUCN Threats Calculator)**

| |
|---|
| Was a threats calculator completed for this species? Yes (14 June 2022)<br>Overall threat impact = Very High–Medium (Assigned) (Appendix 4, 5)<br><br>Key threats were identified as:<br>8.1 Invasive & Other Problematic Species & Genes (very high)<br>7.3 Other Ecosystem Modifications (very high)<br><br>Unknown threats:<br>11 Climate Change & Severe Weather<br><br>What additional limiting factors are relevant? Their biology and occurrence in a single small lake make them especially vulnerable to introduction of aquatic invasive species. |

**Rescue Effect (immigration from outside Canada)**

| | |
|---|---|
| Status of outside population(s) most likely to provide immigrants to Canada. | Not applicable<br>This species is endemic to Canada |
| Is immigration known or possible? | No, there are no populations elsewhere |
| Would immigrants be adapted to survive in Canada? | Not applicable |
| Is there sufficient habitat for immigrants in Canada? | Not applicable |
| Are conditions deteriorating in Canada? | Not applicable |
| Are conditions for the source (i.e., outside) population deteriorating? | Not applicable |
| Is the Canadian population considered to be a sink? | Not applicable |
| Is rescue from outside populations likely? | No, there are no populations elsewhere |

**Data Sensitive Species**

| | |
|---|---|
| Is this a data sensitive species? | No |

**Status History**

| |
|---|
| COSEWIC:<br>Designated Threatened in November 2015. Status re-examined and confirmed in December 2023. |

**Status and Reasons for Designation:**

| Status: T<br>hreatened | Alpha-numeric codes:<br>D2 |
|---|---|
| Reason for designation:<br>This small, robust-bodied freshwater fish is part of an endemic species pair restricted to one small lake in coastal British Columbia (BC). It lives close to the bottom of the lake, while the other member of the pair lives in the open water. The main threat to this species is introduction of invasive species, which have caused the rapid extinction of similar species pairs in two other lakes in coastal BC, either through predation or hybridization resulting from habitat modification. Although this lake on Nelson Island is relatively inaccessible, invasive species continue to spread in the region. If this threat is not prevented, it could lead to the extinction of this distinctive Canadian species. | |

**Applicability of Criteria**

| |
|---|
| Criterion A (Decline in Total Number of Mature Individuals):<br>Not applicable. Although introduction of invasive species would likely rapidly drive the population to extinction, the likelihood of such an introduction within the next 10 years is unknown. |
| Criterion B (Small Distribution Range and Decline or Fluctuation):<br>Not applicable. EOO and IAO (both 8 $km^2$) are below thresholds for Endangered, and the population is known to exist at a single location, but no other subcriteria are met. |
| Criterion C (Small and Declining Number of Mature Individuals):<br>Not applicable. Number of mature individuals is estimated at 4,007–6,620 (95% CI, mean = 5,068), with 100% in one subpopulation, but there is no projected continuing decline. |
| Criterion D (Very Small or Restricted Population):<br>Meets Threatened, D2. IAO 8 $km^2$, number of locations 1, and prone to substantial decline from effects of human activities or stochastic events within 1–2 generations. |
| Criterion E (Quantitative Analysis): Not applicable. Analysis not conducted. |

## TECHNICAL SUMMARY - Little Quarry Lake Limnetic Threespine Stickleback (DU10)

*Gasterosteus aculeatus*

Little Quarry Lake Limnetic Threespine Stickleback

Épinoche à trois épines limnétique du lac Little Quarry

Range of occurrence in Canada: British Columbia

**Demographic Information**

| | |
|---|---|
| Generation time (usually average age of parents in the population) | 1 yr<br>Inferred from research on other Benthic Limnetic Threespine Stickleback pairs; no specific data exists for Little Quarry Lake |
| Is there an [observed, inferred, or projected] continuing decline in number of mature individuals? | Yes<br>Although researchers continue to readily trap Quarry Lake Limnetics, there has been no systematic monitoring of population trends and a catastrophic decline is projected given the high impact threat from AIS and other ecosystem modifications. |
| Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations, whichever is longer up to a maximum of 100 years] | Unknown |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Unknown |
| [Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Projected to be nearly 100% if AIS enter the lake, especially with other ecosystem modifications affecting reproductive isolation |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any period [10 years, or 3 generations, whichever is longer up to a maximum of 100 years], including both the past and the future. | Unknown |
| Are the causes of the decline a. clearly reversible and b. understood, and c. ceased? | a. No; b. yes; c. no |
| Are there extreme fluctuations in number of mature individuals? | No |

**Extent and Occupancy Information**

| | |
|---|---|
| Estimated extent of occurrence (EOO) | 8 km² |
| Index of area of occupancy (IAO)<br>(Always report 2x2 grid value). | 8 km², although the surface area of the lake is only 0.22 km² |

| | |
|---|---|
| Is the population “severely fragmented” i.e., is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse? | No |
| Number of “locations” (use plausible range to reflect uncertainty if appropriate) | 1 |
| Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? | No |
| Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of subpopulations? | No |
| Is there an [observed, inferred, or projected] continuing decline in number of “locations”? | No |
| Is there an [observed, inferred, or projected] continuing decline in area, extent and/or quality of habitat? | Yes, projected decline in extent and quality of habitat due to introduction of AIS, especially AIS that impact vegetation in the very limited littoral zone |
| Are there extreme fluctuations in number of subpopulations? | 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 subpopulation)**

| | |
|---|---|
| Subpopulations (give plausible ranges) | N Mature Individuals<br><br>A rough estimate of the number of mature individuals is derived from a total population abundance estimate (males, females, and juveniles) from a single mark-recapture estimate from Paxton Lake in 2016 (which likely overestimates abundance due to schooling behaviour), extrapolating for Little Quarry Lake based on lake area, combined with estimate of proportion of mature individuals in total population from Paxton Lake Benthic data from 2005 (approx. 20%) (see **Abundance**). |
| | 125,658–448,341 (95% CI, mean = 196,300) |
| Total | Same |

| | |
|---|---|
| Is the probability of extinction in the wild at least [20% within 20 years or 5 generations whichever is longer up to a maximum of 100 years, or 10% within 100 years]? | Unknown<br>Analysis not conducted |

**Threats (direct, from highest impact to least, as per IUCN Threats Calculator)**

| |
|---|
| Was a threats calculator completed for this species? (14 June 2022)<br>Overall threat impact = Very High–Medium (Assigned) (Appendix 4, 5)<br><br>Key threats were identified as:<br>8.1 Invasive & Other Problematic Species & Genes (very high)<br>7.3 Other Ecosystem Modifications (very high)<br><br>Unknown threats:<br>11 Climate Change & Severe Weather<br><br>What additional limiting factors are relevant? Their biology and occurrence in a single small lake make them especially vulnerable to introduction of aquatic invasive species. |

**Rescue Effect (immigration from outside Canada)**

| | |
|---|---|
| Status of outside population(s) most likely to provide immigrants to Canada. | Not applicable<br>This species is endemic to Canada |
| Is immigration known or possible? | No, there are no populations elsewhere |
| Would immigrants be adapted to survive in Canada? | Not applicable |
| Is there sufficient habitat for immigrants in Canada? | Not applicable |
| Are conditions deteriorating in Canada? | Not applicable |
| Are conditions for the source (i.e., outside) population deteriorating? | Not applicable |
| Is the Canadian population considered to be a sink? | Not applicable |
| Is rescue from outside populations likely? | No, there are no populations elsewhere |

**Data Sensitive Species**

| | |
|---|---|
| Is this a data sensitive species? | No |

**Status History**

| |
|---|
| COSEWIC:<br>Designated Threatened in November 2015. Status re-examined and confirmed in December 2023. |

**Status and Reasons for Designation:**

| Status:<br>Threatened | Alpha-numeric codes:<br>D2 |
|---|---|
| Reasons for designation:<br>This small, slender-bodied freshwater fish is part of an endemic species pair restricted to one small lake in coastal British Columbia (BC). It lives in the open water of the lake, while the other member of the pair lives close to the bottom. The main threat to this species is introduction of invasive species, which have caused the rapid extinction of similar species pairs in two other lakes in coastal BC, either through predation or hybridization resulting from habitat modification. Although this lake on Nelson Island is relatively inaccessible, invasive species continue to spread in the region. If this threat is not prevented, it could lead to the extinction of this distinctive Canadian species. | |

**Applicability of Criteria**

| |
|---|
| Criterion A (Decline in Total Number of Mature Individuals):<br>Not applicable. Although introduction of invasive species would likely rapidly drive the population to extinction, the likelihood of such an introduction within the next 10 years is unknown. |
| Criterion B (Small Distribution Range and Decline or Fluctuation):<br>Not applicable. EOO and IAO (both 8 $km^2$) are below thresholds for Endangered, and the population is known to exist at a single location, but no other subcriteria are met. |
| Criterion C (Small and Declining Number of Mature Individuals):<br>Not applicable. Estimated number of mature individuals (125,658–448,341) exceeds threshold for Threatened. |
| Criterion D (Very Small or Restricted Population):<br>Meets Threatened, D2. IAO 8 $km^2$, number of locations 1, and prone to substantial decline from effects of human activities or stochastic events within 1–2 generations. |
| Criterion E (Quantitative Analysis): Not applicable. Analysis not conducted. |

## TECHNICAL SUMMARY - Enos Lake Benthic Threespine Stickleback (DU11)

*Gasterosteus aculeatus*

Enos Lake Benthic Threespine Stickleback

Épinoche à trois épines benthique du lac Enos

Range of occurrence in Canada: British Columbia

**Demographic Information**

| | |
|---|---|
| Generation time (usually average age of parents in the population) | Approx. 2.5 yrs<br>Usually mature at 2 yrs old and breed for several seasons |
| Is there an [observed, inferred, or projected] continuing decline in number of mature individuals? | No, decline has already occurred. Following introduction of a non-native crayfish in 1990 and significant subsequent reduction in macrophyte cover, there are thought to be no pure Enos Benthics in the wild or in captivity, with only a hybrid swarm remaining (see **Abundance, Population Sizes and Trends**). |
| Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations, whichever is longer up to a maximum of 100 years] | Not applicable, none left |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Not applicable, none left |
| [Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Not applicable, none left |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any period [10 years, or 3 generations, whichever is longer up to a maximum of 100 years], including both the past and the future. | Not applicable, none left |
| Are the causes of the decline a. clearly reversible and b. understood, and c. ceased? | a. No; b. yes; c. no |
| Are there extreme fluctuations in number of mature individuals? | No, none left |

**Extent and Occupancy Information**

| | |
|---|---|
| Estimated extent of occurrence (EOO) | 8 km² |
| Index of area of occupancy (IAO)<br>(Always report 2x2 grid value). | 8 km², although the surface area of the lake is only 0.17 km² |

| | |
|---|---|
| Is the population "severely fragmented" i.e., is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse? | No, none left |
| Number of "locations" (use plausible range to reflect uncertainty if appropriate) | 1 |
| Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? | No, none left |
| Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy? | No, none left |
| Is there an [observed, inferred, or projected] continuing decline in number of subpopulations? | No, none left |
| Is there an [observed, inferred, or projected] continuing decline in number of "locations"? | No, none left |
| Is there an [observed, inferred, or projected] continuing decline in area, extent and/or quality of habitat? | No<br>Habitat has been substantially altered by introduced crayfish and alteration is ongoing, with submergent macrophytes now covering only 0.1% of Enos Lake, a fraction of the abundance found in other lakes with Benthic and Limnetic Threespine Stickleback pairs (see **Threats**, **Invasive Species [8.1, 7.3]**), but there are no pure Enos Benthics left |
| Are there extreme fluctuations in number of subpopulations? | No, none left |
| Are there extreme fluctuations in number of "locations"? | No, none left |
| Are there extreme fluctuations in extent of occurrence? | No, none left |
| Are there extreme fluctuations in index of area of occupancy? | No, none left |

**Number of Mature Individuals (in each subpopulation)**

| Subpopulations (give plausible ranges) | N Mature Individuals |
|---|---|
| | Unknown, possibly 0<br><br>There are thought to be no Enos Benthics in the wild or in captivity (see **Population Sizes and Trends**). |
| Total | Same |

**Quantitative Analysis**

| | |
|---|---|
| Is the probability of extinction in the wild at least [20% within 20 years or 5 generations whichever is longer up to a maximum of 100 years, or 10% within 100 years]? | There are thought to be no Enos Benthics in the wild or in captivity |

**Threats (direct, from highest impact to least, as per IUCN Threats Calculator**

| |
|---|
| Was a threats calculator completed for this species? Yes (14 June 2022)<br>Overall threat impact = Very High (Appendix 4, 5)<br><br>Key threats were identified as:<br>8.1 Invasive & Other Problematic Species & Genes (very high)<br>7.3 Other Ecosystem Modifications (very high)<br><br>Unknown threats:<br>11 Climate Change & Severe Weather<br><br>What additional limiting factors are relevant? Their biology and occurrence in a single small lake make them especially vulnerable to introduction of aquatic invasive species. |

**Rescue Effect (immigration from outside Canada)**

| | |
|---|---|
| Status of outside population(s) most likely to provide immigrants to Canada. | Not applicable<br>This species is endemic to Canada |
| Is immigration known or possible? | No, there are no populations elsewhere |
| Would immigrants be adapted to survive in Canada? | Not applicable |
| Is there sufficient habitat for immigrants in Canada? | Not applicable |
| Are conditions deteriorating in Canada? | Not applicable |
| Are conditions for the source (i.e., outside) population deteriorating? | Not applicable |
| Is the Canadian population considered to be a sink? | Not applicable |
| Is rescue from outside populations likely? | No, there are no populations elsewhere |

**Data Sensitive Species**

| | |
|---|---|
| Is this a data sensitive species? | No |

**Status History**

| |
|---|
| COSEWIC: 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. Status re-examined and designated Extinct in December 2023. |

**Status and Reasons for Designation:**

| | |
|---|---|
| Status:<br>Extinct | Alpha-numeric codes:<br>Not applicable |
| Reasons for designation:<br>This small, robust-bodied freshwater fish was part of an endemic species pair restricted to one small lake in south coastal British Columbia. It lived close to the bottom of the lake, while the other member of the pair lived in the open water. However, the introduction of an invasive crayfish in this lake dramatically reduced aquatic vegetation, which likely had been important in preventing hybridization between the two species. As a consequence, the two species collapsed into a hybrid swarm resulting in the loss of the original two species. The revised status reflects the inability to find genetically non-hybridized individuals of this distinctive Canadian species despite repeated surveys. There is sufficient information to document that no individuals of the species remain. | |

**Applicability of Criteria**

| |
|---|
| Criterion A (Decline in Total Number of Mature Individuals):<br>Not applicable. |
| Criterion B (Small Distribution Range and Decline or Fluctuation):<br>Not applicable. |
| Criterion C (Small and Declining Number of Mature Individuals):<br>Not applicable. |
| Criterion D (Very Small or Restricted Population):<br>Not applicable. |
| Criterion E (Quantitative Analysis):<br>Not applicable. |

## TECHNICAL SUMMARY - Enos Lake Limnetic Threespine Stickleback (DU12)

*Gasterosteus aculeatus*

Enos Lake Limnetic Threespine Stickleback

Épinoche à trois épines limnétique du lac Enos

Range of occurrence in Canada: British Columbia

### Demographic Information

| | |
|---|---|
| Generation time (usually average age of parents in the population) | 1 yr |
| Is there an [observed, inferred, or projected] continuing decline in number of mature individuals? | No, decline has already occurred. Following introduction of a non-native crayfish in 1990 and significant subsequent reduction in macrophyte cover, there are thought to be no pure Enos Limetics in the wild or in captivity, with only a hybrid swarm remaining (see **Population Sizes and Trends**). |
| Estimated percent of continuing decline in total number of mature individuals within [5 years or 2 generations, whichever is longer up to a maximum of 100 years] | Not applicable, none left |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over the last [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Not applicable, none left |
| [Projected or suspected] percent [reduction or increase] in total number of mature individuals over the next [10 years, or 3 generations, whichever is longer up to a maximum of 100 years]. | Not applicable, none left |
| [Observed, estimated, inferred, or suspected] percent [reduction or increase] in total number of mature individuals over any period [10 years, or 3 generations, whichever is longer up to a maximum of 100 years], including both the past and the future. | Not applicable, none left |
| Are the causes of the decline a. clearly reversible and b. understood, and c. ceased? | a. No; b. yes; c. no |
| Are there extreme fluctuations in number of mature individuals? | No, none left |

### Extent and Occupancy Information

| | |
|---|---|
| Estimated extent of occurrence (EOO) | 8 km² |
| Index of area of occupancy (IAO)<br>(Always report 2x2 grid value). | 8 km², although the surface area of the lake is only 0.17 km² |

| | |
|---|---|
| Is the population “severely fragmented” i.e., is >50% of its total area of occupancy in habitat patches that are (a) smaller than would be required to support a viable population, and (b) separated from other habitat patches by a distance larger than the species can be expected to disperse? | No |
| Number of “locations” (use plausible range to reflect uncertainty if appropriate) | 1 |
| Is there an [observed, inferred, or projected] continuing decline in extent of occurrence? | No, none left |
| Is there an [observed, inferred, or projected] continuing decline in index of area of occupancy? | No, none left |
| Is there an [observed, inferred, or projected] continuing decline in number of subpopulations? | No, none left |
| Is there an [observed, inferred, or projected] continuing decline in number of “locations”? | No, none left |
| Is there an [observed, inferred, or projected] continuing decline in area, extent and/or quality of habitat? | No<br>Substantial habitat alteration due to introduced crayfish has occurred and is ongoing, with submergent macrophytes now covering only 0.1% of Enos Lake, a fraction of the abundance found in other lakes with Benthic and Limnetic Threespine Stickleback pairs (see **Threats, Invasive Species [8.1, 7.3]**), but there are no pure Enos Limentics left |
| Are there extreme fluctuations in number of subpopulations? | No, none left |
| Are there extreme fluctuations in number of “locations”? | No, none left |
| Are there extreme fluctuations in extent of occurrence? | No, none left |
| Are there extreme fluctuations in index of area of occupancy? | No, none left |

**Number of Mature Individuals (in each subpopulation)**

| Subpopulations (give plausible ranges) | N Mature Individuals |
|---|---|
| | There are thought to be no Enos Limnetics in the wild or in captivity (see **Population Sizes and Trends**). |
| Total | Same |

**Quantitative Analysis**

| | |
|---|---|
| Is the probability of extinction in the wild at least [20% within 20 years or 5 generations whichever is longer up to a maximum of 100 years, or 10% within 100 years]? | There are thought to be no Enos Limnetics in the wild or in captivity. |

**Threats (direct, from highest impact to least, as per IUCN Threats Calculator**

| |
|---|
| Was a threats calculator completed for this species? Yes (14 June 2022)<br>Overall threat impact = Very High (Appendix 4, 5)<br><br>Key threats were identified as:<br>8.1 Invasive & Other Problematic Species & Genes (very high)<br>7.3 Other Ecosystem Modifications (very high)<br><br>Unknown threats:<br>11 Climate Change & Severe Weather<br><br>What additional limiting factors are relevant? Their biology and occurrence in a single small lake make them especially vulnerable to introduction of aquatic invasive species. |

**Rescue Effect (immigration from outside Canada)**

| | |
|---|---|
| Status of outside population(s) most likely to provide immigrants to Canada. | Not applicable<br>This species is endemic to Canada |
| Is immigration known or possible? | No, there are no populations elsewhere |
| Would immigrants be adapted to survive in Canada? | Not applicable |
| Is there sufficient habitat for immigrants in Canada? | Not applicable |
| Are conditions deteriorating in Canada? | Not applicable |
| Are conditions for the source (i.e., outside) population deteriorating? | Not applicable |
| Is the Canadian population considered to be a sink? | Not applicable |
| Is rescue from outside populations likely? | No, there are no populations elsewhere |

**Data Sensitive Species**

| | |
|---|---|
| Is this a data sensitive species? | No |

**Status History**

| |
|---|
| COSEWIC: 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 Limnetic Threespine Stickleback was designated Endangered. Status re-examined and confirmed in May 2012. Status re-examined and designated Extinct in December 2023. |

**Status and Reasons for Designation:**

| | |
|---|---|
| Status:<br>Extinct | Alpha-numeric codes:<br>Not applicable |
| Reasons for designation:<br>This small, slender-bodied freshwater fish was part of an endemic species pair restricted to one small lake in south coastal British Columbia. It lived in the open water of the lake, while the other member of the pair lived close to the bottom. However, the introduction of an invasive crayfish in this lake dramatically reduced aquatic vegetation, which had likely been important in preventing hybridization between the two species. As a consequence, the two species collapsed into a hybrid swarm resulting in the loss of the original two species. The revised status reflects the inability to find genetically non-hybridized individuals of this distinctive Canadian species despite repeated surveys. There is sufficient information to document that no individuals of the species remain. | |

**Applicability of Criteria**

| |
|---|
| Criterion A (Decline in Total Number of Mature Individuals):<br>Not applicable. |
| Criterion B (Small Distribution Range and Decline or Fluctuation):<br>Not applicable. |
| Criterion C (Small and Declining Number of Mature Individuals):<br>Not applicable. |
| Criterion D (Very Small or Restricted Population):<br>Not applicable. |
| Criterion E (Quantitative Analysis):<br>Not applicable. |

## PREFACE

All 12 DUs within the Threespine Stickleback species bundle have been previously assessed by COSEWIC:

- Giant and Unarmoured Threespine Stickleback were designated as Special Concern in April 1980 (Moodie 1980) and 1983 (Reimchen 1984b), respectively; their status was re-examined and confirmed in November 2013 (COSEWIC 2013).
- Misty Lake Lotic and Lentic Threespine Stickleback were designated as Endangered in November 2006 (COSEWIC 2006).
- Enos Lake Benthic and Limnetic Threespine Stickleback were designated as Threatened in April 1988 (McPhail 1988). Their status was re-examined and designated Endangered in November 2002 (COSEWIC 2002) and again re-examined and confirmed as Endangered in April 2012 (COSEWIC 2012).
- Paxton Lake Benthic and Limnetic Threespine Stickleback were designated as Threatened in April 1998 (Houston 1998), a status that was re-examined and confirmed in April 1999 (Hatfield and Ptolemy 1999a). Vananda Creek Benthic and Limnetic Threespine Stickleback were designated as Threatened in April 1999 (Hatfield and Ptolemy 1999b). Status of both pairs was again re-examined in May 2000 and resulted in Endangered designations (COSEWIC 2000a, b). The status of both pairs was re-examined again and confirmed as Endangered in April 2010 (COSEWIC 2010a, b).
- Little Quarry Lake Benthic and Limnetic Threespine Stickleback were designated as Threatened in November 2015 (COSEWIC 2015a).

As a result of their Endangered listing under Schedule 1 of the *Species at Risk Act* (SARA), the *Recovery Strategy for Paxton Lake, Enos Lake, and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada* was published on the Species at Risk Public Registry in 2007 (National Recovery Team for Stickleback Species Pairs 2007). Progress towards recovery strategy implementation was addressed in the *Report on the Progress of Recovery Strategy Implementation for the Paxton Lake, Enos Lake, and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada for the Period 2007 – 2015* (DFO 2016a) and the *Report on the Progress of Recovery Strategy Implementation for the Paxton Lake and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada for the Period 2016 to 2021* (DFO 2022a). The recovery strategy was amended in 2019 to include updates to the biology, recovery feasibility assessment, threats, population abundance, population and distribution objectives, and identification of Critical Habitat (DFO 2019). While it found recovery for Benthic and Limnetic Threespine Stickleback from Paxton Lake and Vananda Creek to be biologically and technically feasible, it determined recovery for Benthic and Limnetic Threespine Stickleback from Enos Lake to be biologically and/or technically infeasible (DFO 2019). An *Action Plan for the Paxton Lake and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada [Proposed]* was first published in 2016 (DFO 2016b) and was amended in 2020 (DFO 2020a) to reflect changes to the amended recovery strategy (DFO 2019).

As a result of their Endangered listing under Schedule 1 of SARA, the *Recovery Strategy for the Misty Lake Sticklebacks (Gasterosteus aculeatus) in Canada* was published on the Species at Risk Public Registry in 2018 (DFO 2018b), followed by an Action Plan in 2020 (DFO 2020b). The Recovery Strategy found recovery for Misty Lake Lotic and Lentic Threespine Stickleback to be biologically and technically feasible and identified critical habitat (DFO 2018b).

As a result of their Special Concern listing under Schedule 1 of SARA, the *Management Plan for the Giant and Unarmoured Threespine Sticklebacks (*Gasterosteus aculeatus*) in Canada [Proposed]* was published on the Species at Risk Public Registry in 2022 (DFO 2022b)".

Little Quarry Lake Benthic and Limnetic Stickleback are currently under consideration for listing as Threatened under Schedule 1 of SARA. A recovery potential assessment was completed in 2018 (DFO 2018a).

This status report consolidates information provided in the previous status reports for the DUs within the Threespine Stickleback species bundle, as well as the recovery strategies and action plans that are available for a subset of the DUs. While the extent of occurrence and area of occupancy have remained the same for each DU, this report adds and interprets updated information on population sizes, trends, and threats.

## 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
## (2023)

| | |
|---|---|
| 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 is 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 and Climate Change Canada
Canadian Wildlife Service

Environnement et Changement climatique Canada
Service canadien de la faune

Canada

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

# COSEWIC Status Report

on the

# Threespine Stickleback species bundle
*Gasterosteus aculeatus*

Giant Threespine Stickleback, Unarmoured Threespine Stickleback, Misty Lake Lentic Threespine Stickleback, Misty Lake Lotic Threespine Stickleback, Paxton Lake Benthic Threespine Stickleback, Paxton Lake Limnetic Threespine Stickleback, Vananda Creek Benthic Threespine Stickleback, Vananda Creek Limnetic Threespine Stickleback, Little Quarry Lake Benthic Threespine Stickleback, Little Quarry Lake Limnetic Threespine Stickleback, Enos Lake Benthic Threespine Stickleback, Enos Lake Limnetic Threespine Stickleback

**in Canada**

2023

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# WILDLIFE SPECIES DESCRIPTION AND SIGNIFICANCE

## Name and Classification

Phylum: Chordata
Class: Actinopterygii (ray-finned fishes)
Order: Gasterosteiformes
Family: Gasterosteidae
Genus: Gasterosteus
Giant Species: *Gasterosteus aculeatus*
Unarmoured Species: *Gasterosteus aculeatus*
Lentic (Lake) Species: *Gasterosteus aculeatus*
Lotic (Stream) Species: *Gasterosteus aculeatus*
Limnetic Species: *Gasterosteus aculeatus*
Benthic Species: *Gasterosteus aculeatus*

Common Name
English:
Giant Threespine Stickleback
Unarmoured Threespine Stickleback
Misty Lake Lentic Threespine Stickleback
Misty Lake Lotic Threespine Stickleback
Paxton Lake Benthic Threespine Stickleback
Paxton Lake Limnetic Threespine Stickleback
Vananda Creek Benthic Threespine Stickleback
Vananda Creek Limnetic Threespine Stickleback
Little Quarry Lake Benthic Threespine Stickleback
Little Quarry Lake Limnetic Threespine Stickleback
Enos Lake Benthic Threespine Stickleback
Enos Lake Limnetic Threespine Stickleback

French:
Épinoche à trois épines géante
Épinoche à trois épines lisse
Épinoche à trois épines lentique du lac Misty
Épinoche à trois épines lotique du lac Misty
Épinoche à trois épines benthique du lac Paxton
Épinoche à trois épines limnétique du lac Paxton

Épinoche à trois épines benthique du ruisseau Vananda
Épinoche à trois épines limnétique du ruisseau Vananda

Épinoche à trois épines benthique du lac Little Quarry Épinoche à trois épines limnétique du lac Little Quarry

Épinoche à trois épines benthique du lac Enos

Épinoche à trois épines limnétique du lac Enos

## Morphological Description

Threespine Stickleback are small-bodied fish, typically between 40 and 60 mm in total length and rarely exceeding 80 mm (Baker 1994). They are easily recognized by the presence of three (sometimes two) isolated dorsal spines, followed by a soft-rayed dorsal fin. In addition to these dorsal spines, they are usually well-armoured with calcified lateral plates and pelvic spines (Scott and Crossman 1973; Wooton 1976; Reimchen 1994). Those inhabiting marine waters tend to have the most armour; the extent of armour varies among the freshwater species (Reimchen 1994). The caudal fin is truncate and the pectoral fins are fan-shaped and located about halfway up the side of the body. Body colour varies from silvery to mottled green and brown, with sexually mature males typically developing bright red throats during the breeding season, although males in a few freshwater populations turn completely black instead (McPhail 1969; Reimchen 1989).

The marine form is usually anadromous, meaning it returns to fresh water to reproduce, and has given rise to isolated freshwater forms in numerous postglacial lakes and streams, including those within this Threespine Stickleback species bundle (Schluter and McPhail 1992; McPhail 1994, 2007; McKinnon and Rundle 2002). While marine Threespine Stickleback look similar throughout their range, freshwater Threespine Stickleback are diverse (Bell 1976). The Threespine Stickleback within this species bundle exhibit dramatic adaptation in their morphology, including body shape and size, trophic morphology, and defensive armour.

Found in coastal British Columbia (BC), Misty Lake Lotic (inlet stream-form) and Lentic (lake-form and outlet stream-form) Threespine Stickleback, and Giant Threespine Stickleback and its parapatric stream counterparts, are the archetypal examples of parapatric lake-stream Threespine Stickleback pairs. Parapatric lake-stream pairs show somewhat similar patterns of morphological divergence across lake systems, with lake fish generally more streamlined and having more gill rakers than stream fish (Kaeuffer *et al.* 2012; Haines *et al.* 2020; Paccard *et al.* 2020). While the magnitude of these differences varies, stream-forms and lake-forms of Threespine Stickleback within each pair are morphologically more similar to other stream-forms and lake-forms of Threespine Stickleback from different pairs than they are to one another (Lavin and McPhail 1993; Berner *et al.* 2008; Kaeuffer *et al.* 2012; Paccard *et al.* 2020). Stream forms and lake forms of Threespine Stickleback can also differ in several other traits, such as coloration and defensive armour, but the direction of divergence in these traits is not consistent across pairs (Kaeuffer *et al.* 2012).

Misty Lake Lotic and Lentic Threespine Stickleback represent one of the most extreme cases of morphological divergence among parapatric lake-stream pairs of Threespine Stickleback (Kaeuffer *et al.* 2012; Haines *et al.* 2020; Paccard *et al.* 2020). Within this system, there is a steep cline in morphology across a narrow contact zone, with more streamlined Lentic Threespine Stickleback that have longer pelvic spines and more gill rakers in the lake transitioning to more robust-bodied Lotic Threespine Stickleback with shorter pelvic spines and fewer gill rakers in the inlet (Lavin and McPhail 1993; Haenel *et al.* 2021). In addition, Lotic Threespine Stickleback tend to be mottled brown compared with the more melanistic coloration of Lentic Threespine Stickleback (Lavin and McPhail 1993).

As its name suggests, the Giant Threespine Stickleback has a large body size, which is an exceptional and defining morphological feature. Mean adult standard length exceeded 75 mm in two confirmed instances of occurrence  (Gambling and Reimchen 2012). It is almost twice the length of its typical parapatric stream counterpart (Gambling and Reimchen 2012), a unique feature among known parapatric lake-stream pairs of Threespine Stickleback (Figure 3 in COSEWIC 2013). In addition to being streamlined and having more gill rakers than its parapatric stream counterpart, Giant Threespine Stickleback are also more heavily armoured, with longer pelvic spines and more lateral plates (Moodie 1972a,b; Reimchen *et al.* 1985). Giant Threespine Stickleback have an unusual melanistic coloration similar to Misty Lake Lentic Threespine Stickleback (Moodie 1972a; Reimchen *et al.* 1985).

The distinguishing suites of morphological characteristics of Misty Lake Lotic and Lentic Threespine Stickleback and Giant Threespine Stickleback appear to have evolved as adaptations to divergent foraging, predation, and breeding environments (Moodie 1972a,b; Reimchen *et al.* 1985; Lavin and McPhail 1993; Hendry *et al.* 2002; Spoljaric and Reimchen 2007; Reimchen *et al.* 2013; Haenel *et al.* 2021).

In addition to parapatric lake-stream Threespine Stickleback pairs, sympatric Benthic and Limnetic Threespine Stickleback pairs also inhabit several lakes in coastal BC. Benthic and Limnetic Threespine Stickleback are morphologically distinct from one another; the most notable shifts in the benthic species relative to  the limnetic species include a greater overall body depth, shorter dorsal and anal fins, a smaller eye, and a shorter jaw that is more downward-oriented (McPhail 1984, 1992, 1993; Gow *et al.* 2008). These divergent suites of morphological traits are considered adaptations to the species' divergent feeding patterns: Benthic Threespine Stickleback eat mainly benthic invertebrates in the littoral zone while Limnetic Threespine Stickleback feed primarily on plankton in open water (Schluter and McPhail 1992; McGee *et al.* 2013).

Benthic and Limnetic Threespine Stickleback are remarkably similar in shape across the lakes they inhabit; the magnitude of the morphological shifts between Benthic and Limnetic Threespine Stickleback within lakes is closely correlated between all three intact pairs in Little Quarry Lake, Paxton Lake, and Vananda Creek (Gow *et al.* 2008). A fourth pair of Benthic and Limnetic Threespine Stickleback—those found in Enos Lake (McPhail 1984) —has undergone a collapse into a hybrid swarm over the past three decades, such that distinct morphological (and genetic) groupings of Benthic and Limnetic Threespine Stickleback are now absent in this lake (Kraak *et al.* 2001; Taylor *et al.* 2006; Behm *et al.* 2010; Taylor and Piercey 2018).

Benthic and Limnetic Threespine Stickleback can also differ in several other traits, such as coloration and defensive armour, but the direction of divergence in these traits is not consistent across pairs. Prior to the collapse of the Enos Lake pair, the breeding colour of male Limnetic fish (red throats and blue backs) was typical for Threespine Stickleback, while breeding male Benthic fish were black (McPhail 1984). A notable morphological difference between Benthic and Limnetic Threespine Stickleback from Paxton Lake and Little Quarry Lake is that the vast majority of Benthic individuals lack a pelvic girdle (McPhail 1992; Gow *et al.* 2008). This unusual characteristic of partial or complete loss of pelvic structures among Threespine Stickleback has only been documented in two dozen or so freshwater Threespine Stickleback populations globally (Bell 1987). Selection forces thought to contribute to the evolution of pelvic reduction in Threespine Stickleback populations include the absence of gape-limited predatory fishes, limited calcium availability, and predation by grasping insects (Reimchen 1980; Giles 1983; Bell *et al.* 1993; Marchinko 2009).

Unarmoured Threespine Stickleback are another exceptional example of Threespine Stickleback as they exhibit partial or complete loss of body armour (spines and/or lateral plates). They are characterized by the loss of one or more spines in the majority of fish, and the lateral bony plates may also be reduced or absent (Reimchen 1984a). Other aspects of their morphology are unremarkable (Moodie and Reimchen 1976). Their body shape is typical of freshwater Threespine Stickleback that inhabit small, shallow, stained lakes (Spoljaric and Reimchen 2007).

Patterns of reduced body armour vary across the three lakes where Unarmoured Threespine Stickleback are found. In Serendipity Lake, the vast majority of fish (97%) lack pelvic spines and all fish lack lateral plates (Reimchen 1984a). Dorsal spines are usually present but are reduced to vestigial projections (Reimchen 1984a). In Rouge Lake, the loss of the first dorsal spine (in 31% of fish) and the third dorsal spine (in 63% of fish) is common, while the pelvic spines are present (Reimchen 1984a). Lateral plates are missing in 50% of fish (Reimchen 1984a). In Boulton Lake, the absence of the second dorsal spine and at least one pelvic spine is common (in 80% of fish; Reimchen 1980). The vast majority of Boulton Lake fish (96%) lack at least one pelvic spine and/or the second dorsal spine, with only 4% of fish in this lake fully spined (Reimchen 1980). Unusually, there is variation in these defence structures in Boulton Lake, with polymorphism (including sexual dimorphism) in both the number and symmetry of pelvic spines (Moodie and Reimchen 1976; Reimchen 1980; Reimchen and Nosil 2002, 2004) and lateral plates (Reimchen and

Nosil 2001a).

The distinguishing loss of defensive structures in Unarmoured Threespine Stickleback appears to have evolved as a result of adaptation to their predation regimes; birds and macroinvertebrates are the predominant predators in these lakes which have no predatory fishes (Moodie and Reimchen 1976; Reimchen 1980, 1984a, 1994). Specifically, research on the Unarmoured Threespine Stickleback from Boulton Lake provides strong evidence that while the dorsal and pelvic spines are a defensive adaptation against avian predators, which are gape-limited, they are detrimental and are selected against by grasping macroinvertebrate predators (Reimchen 1980). Thus, intrapopulation variability in spine number appears to be a functional adaptation to spatial and temporal variability in the two predator groups (Reimchen 1980; Reimchen and Nosil 2002).

## Population Spatial Structure and Variability

The freshwater Threespine Stickleback found along the coastal areas of the Pacific Islands and in the Pacific National Freshwater Biogeographic Zone evolved recently following the rapid post-Wisconsinan glacial colonization of freshwater habitats by their marine ancestors within the last 10,000 years (Bell and Foster 1994). Genetic analyses of mitochondrial DNA (mtDNA) consistently support such an origin for the DUs within the Threespine Stickleback species bundle (Giant and Unarmoured Threespine Stickleback: Gach and Reimchen 1989; O'Reilly *et al.* 1993; Deagle *et al.* 1996; Thompson *et al.* 1997; Misty Lake Lotic and Lentic Threespine Stickleback: Thompson *et al.* 1997; Benthic and Limnetic Threespine Stickleback pairs: Taylor and McPhail 1999).

Freshwater Threespine Stickleback inhabiting different coastal watersheds in this area are generally considered to be independently derived from the marine forms (Bell 1976). There is genetic evidence that supports the independent origin of Threespine Stickleback within this species bundle, including parallel evolution across lakes for species that occur in multiple areas. Threespine Stickleback within this species bundle (and between lakes for those that occur in multiple locations) are genetically differentiated from one another (Giant Threespine Stickleback: mtDNA, Thompson *et al.* 1997, Marques *et al.* 2022; single nucleotide polymorphisms (SNPs), Deagle *et al.* 2012, 2013, Marques *et al.* 2022; Misty Lake Lotic and Lentic Threespine Stickleback: mtDNA, Thompson *et al.* 1997; microsatellites, Hendry *et al.* 2002; Hendry and Taylor 2004; Berner *et al.* 2009; Unarmoured Threespine Stickleback: mtDNA, Marques *et al.* 2022; SNPs, Jones *et al.* 2012, Marques *et al.* 2022; Benthic and Limnetic Threespine Stickleback pairs: mtDNA, Taylor and McPhail 1999; microsatellite, Taylor and McPhail 2000; SNPs, Jones *et al.* 2012), and from other Threespine Stickleback.

Furthermore, genetic evidence reveals genetic differentiation within each of the parapatric or sympatric pairs within this Threespine Stickleback species bundle. A genome-wide SNP analysis reveals significant differentiation between Giant Threespine Stickleback and its stream counterparts within both the Drizzle and Mayer Lake watersheds (Deagle *et al.* 2012), as well as between Misty Lake Lotic and Lentic Threespine Stickleback (Haenel *et al.* 2021). Microsatellite DNA analyses also show significant differentiation between Misty

Lake Lotic and Lentic Threespine Stickleback (Hendry *et al.* 2002; Hendry and Taylor 2004). Several genetic analyses show the strong genetic distinction between Benthic and Limnetic Threespine Stickleback within Paxton Lake, Little Quarry Lake, and Vananda Creek (allozymes, McPhail 1992; microsatellites, Taylor *et al.* 2006; Gow *et al.* 2008; SNPs, Jones *et al.* 2012). Historically, Enos Lake Benthic and Limnetic Threespine Stickleback were also genetically differentiated (allozymes, McPhail 1984; mtDNA, Taylor and McPhail 1999; microsatellites, Taylor and McPhail 2000). However, microsatellite analyses conducted since the late 1990s show that Enos Lake Benthic and Limnetic Threespine Stickleback are no longer genetically distinct, having collapsed into a hybrid swarm (Gow *et al.* 2006; Taylor *et al.* 2006; Taylor and Piercey 2018).

The origin of each of the parapatric or sympatric pairs within this Threespine Stickleback species bundle is unresolved; it remains unknown whether their genetic differentiation reflects a single colonization with secondary modifications (parapatric/sympatric divergence) or multiple, independent divergence events with secondary contact (allopatric divergence). It is difficult to tease this apart for recently derived species (Endler 1982). Nevertheless, the clustering of Giant Threespine Stickleback with its stream counterpart in each watershed supports the plausibility of a postglacial ecological parapatric divergence along the lake-stream ecotone (mtDNA, Thompson *et al.* 1997; SNPs, Deagle *et al.* 2012). In contrast, there is evidence of an allopatric divergence between Misty Lake Lotic and Lentic Threespine Stickleback; different mtDNA clades predominating in the inlet and lake have likely originated from historical isolation in two separate glacial refugia (Thompson *et al.* 1997; Hendry *et al.* 2002).

Details of the origin of each Benthic and Limnetic Threespine Stickleback pair are still not well understood. Earlier geological evidence suggested that two temporally spaced postglacial marine submergences inundated coastal BC lakes in the vicinity of the Benthic-Limnetic Threespine Stickleback pairs (Mathews *et al.* 1970). This contributed to the idea that the same marine Threespine Stickleback species had colonized each lake twice at intervals (the “double invasion hypothesis”; Schluter and McPhail 1992; McPhail 1993; Taylor and McPhail 1999, 2000). More exhaustive geological analysis, however, does not support this scenario for the origin of the pairs, finding that a second postglacial sea level rise in this region would have been of insufficient magnitude to enable a second invasion (Hutchinson *et al.* 2004). Furthermore, the genetic evidence that supports the double invasion hypothesis cannot be disentangled from other possibilities, such as differences in effective population sizes between Benthic and Limnetic Threespine Stickleback (Taylor and McPhail 1999, 2000; Jones *et al.* 2012). The most recent and extensive genetic study to date does, however, suggest that allopatric adaptive divergence (such as could be generated by separate invasions of coastal lake habitats by marine Threespine Stickleback) and reuse of standing genetic variation has played a role in the repeated evolution of the Benthic and Limnetic pairs (Jones *et al.* 2012).

## Designatable Units

Table 1 provides an overview of the 12 designatable units (DUs) and their distributions. Each of the 12 populations within the Threespine Stickleback species bundle were recognized as separate DUs within *Gasterosteus aculeatus* in their previous status reports; an assemblage of inherited traits and genetic data support the view that each DU is genetically distinct from other Threespine Stickleback, and each DU is significant, existing within an ecological and evolutionary setting in which their associated divergent adaptations are crucial to their persistence (COSEWIC 2011). They also satisfy COSEWIC's new "discreteness" and "significance" criteria (COSEWIC 2020), where discrete means that there is currently very little transmission of heritable (cultural or genetic) information from other such units, and evolutionarily significant means that the unit harbours heritable adaptive traits or an evolutionary history not found elsewhere in Canada. Loss of any one of these DUs would eliminate a significant aspect of the morphological diversity of *G. aculeatus* as a whole.

**Table 1. Summary of Designatable Units (DUs) and their distributions, including Extent of Occurrence (EOO) and Area of Occupancy (IAO), and whether Critical Habitat Orders are in effect.**

| DU # | Common Name of Threespine Stickleback DU | Co-occurrence with another DU? | Range in BC | # Locations | Lake surface area ($km^2$) | EOO ($km^2$) | IAO ($km^2$) | Critical Habitat Order |
|---|---|---|---|---|---|---|---|---|
| 1 | Giant | No | Graham Island, Haida Gwaii | 2 | 6.27 + 1.12 = 7.39 (COSEWIC 2013) | 63 | 52 | No |
| 2 | Unarmoured | No | Graham Island, Haida Gwaii | 3 | 0.18 + 0.02 + 0.02 = 0.22 (COSEWIC 2013) | 124 | 20 | No |
| 3 | Misty Lake Lentic (Lake Form) | Parapatric pair | Northern Vancouver Island | 1 | 0.36 $km^2$ lake + ~0.007 $km^2$ of outlet stream (total length of outlet 2.3 km × mean wetted width 3 m) (COSEWIC 2006) | 12 | 12 | Yes |
| 4 | Misty Lake Lotic (Stream Form) | | | 1 | ~0.04 $km^2$ of inlet stream (total length of inlet stream 20 km × mean wetted width 2 m) (COSEWIC 2006) | 12 | 12 | Yes |
| 5 | Paxton Lake Benthic | Sympatric pair | Texada Island | 1 | 0.17 (Ormond *et al.* 2011) | 8 | 8 | Yes |
| 6 | Paxton Lake Limnetic | | | 1 | 0.17 (Ormond *et al.* 2011) | 8 | 8 | Yes |
| 7 | Vananda Creek Benthic | Sympatric pair | Texada Island | 1 | 0.072 + 0.442 + 0.115 = 0.63 (COSEWIC 2010) | 16 | 16 | Yes |
| 8 | Vananda Creek Limnetic | | | 1 | 0.072 + 0.442 + 0.115 = 0.63 (COSEWIC 2010) | 16 | 16 | Yes |
| 9 | Little Quarry Lake Benthic | Sympatric pair | Nelson Island | 1 | 0.22 (Ormond *et al.* 2011) | 8 | 8 | No |
| 10 | Little Quarry Lake Limnetic | | | 1 | 0.22 (Ormond *et al.* 2011) | 8 | 8 | No |
| 11 | Enos Lake Benthic | Sympatric pair | Southeastern Vancouver Island | 1 | 0.17 (Ormond *et al.* 2011) | 8 | 8 | No |
| 12 | Enos Lake Limnetic | | | 1 | 0.17 (Ormond *et al.* 2011) | 8 | 8 | No |

In terms of discreteness, all the DUs satisfy *Criterion D1*, with evidence of heritable traits and markers that clearly distinguish each putative DU from other DUs. Genetic markers show each DU to be at least partially reproductively isolated from the others (see below), and the traits that distinguish them have a heritable basis. For example, studies show a large genetic component to body shape in the Giant Threespine Stickleback from Mayer and Drizzle lakes. Only about 10% of the total variation in body shape among Threespine Stickleback on Haida Gwaii is attributable to plasticity (Spoljaric and Reimchen 2007), and experimental breeding and common garden experiments have shown a strong genetic basis to morphology in the Misty Lake system (e.g., Sharpe *et al.* 2008).

In terms of significance, a variety of genetic studies (see below) show that individual Threespine Stickleback populations evolved independently from one another as a diversity of freshwater habitats emerged when the ice sheets retreated some12,000 years ago (Moodie and Reimchen 1976). Thus, each DU has been on an independent evolutionary trajectory for an evolutionarily significant period (~2,400–12,000 generations), satisfying *Criterion S1*. Considerable evidence also exists showing that the selective regime of each population has given rise to DU-wide local adaptations that could not be practically reconstituted if lost (*criterion S2*).

<u>Details of the studies supporting these criteria are outlined below for each population.</u>

*Giant Threespine Stickleback (DU1)*

Genetic analyses (mtDNA and SNPs) demonstrate that Giant Threespine Stickleback are genetically distinct from other Threespine Stickleback, including their typical parapatric stream counterpart (see **Population Spatial Structure and Variability**). This genetic distinctness corresponds to a distinct suite of morphological characteristics (see **Morphological Description**), which has a large inherited component that is driven at least in part by additive genetic variation (McPhail 1977; Lavin and McPhail 1993; Hendry *et al.* 2002; Spoljaric and Reimchen 2007; Sharpe *et al.* 2008; Berner *et al.* 2011). Furthermore, morphological and genetic analyses suggest that there is very little hybridization between Giant Threespine Stickleback and its parapatric stream counterpart (Moodie 1972a; Reimchen *et al.* 1985; Gach and Reimchen 1989; Deagle *et al.* 2012). This is supported by mate preference tests which provide evidence of positive assortative mating between Giant Threespine Stickleback and its stream counterpart (Moodie 1972a; Stinson 1983).

Giant Threespine Stickleback are evolutionarily significant, representing the most extreme cases of gigantism known among parapatric lake-stream pairs of Threespine Stickleback (see **Morphological Description**) and in *G. aculeatus* despite the sampling of hundreds of coastal lakes (see **Distribution**). The persistence of the morphological character complex that sets Giant Threespine Stickleback apart from other Threespine Stickleback is the result of evolutionary adaptations (to foraging, predation, and breeding environments) that are crucial to their persistence in parapatry with their typical stream counterpart (see **Morphological Description**). Loss of Giant Threespine Stickleback would, therefore, restrict the range of morphological variability in *G. aculeatus* as a whole.

However, it should be noted that Giant Threespine Stickleback may constitute more than one DU. First, there is evidence that the two recognized Giant subpopulations (Mayer and Drizzle lakes) evolved independently (see **Population Spatial Structure and Variability**), and the similar morphologies are the result of convergent evolution in similar habitats. Marques *et al.* (2022) resequenced single genomes from one marine and 28 freshwater subpopulations of Threespine Stickleback on Haida Gwaii (Giant, Unarmoured, and typical forms) and found 89 likely targets of parallel selection in the genome that are enriched for old (shared) standing genetic variation. Candidate genes and genotype-environment correlations were found to correspond to three major environmental axes: predation regime, light environment, and ecosystem size. Additional research is required to understand the extent to which each subpopulation possesses local adaptations that could not be reconstituted if lost. Furthermore, recent search efforts have found large-bodied Threespine Stickleback in other watersheds on Haida Gwaii (see **Search Effort**). Although not as large as those in Mayer and Drizzle lakes, there are at least seven other lakes with Threespine Stickleback > 75 mm adult standard length: Coates Lake, Eden Lake, Escarpment Lake, Laurel Pond, Skidegate Lake, Solstice Lake, and Spence Lake (Gambling and Reimchen 2012; Marques *et al.* 2022). These deserve further study.

*Unarmoured Threespine Stickleback (DU2)*

Genetic analyses (mtDNA and SNPs) demonstrate that Unarmoured Threespine Stickleback are genetically distinct from other Threespine Stickleback (see **Population Spatial Structure and Variability**). This distinctness is reinforced by the strong genetic basis of the morphological trait (spine loss) that distinguishes Unarmoured Threespine Stickleback (Reimchen 1984a), which is controlled by major genes (Shapiro *et al.* 2004; Chan *et al.* 2010). The divergent patterns of spine reduction among the three known lakes with Unarmoured Threespine Stickleback (see **Morphological Description**) supports the independent, parallel evolution of their distinct unarmoured morphology.

Unarmoured Threespine Stickleback are evolutionarily significant, representing some of the most extreme cases of reduced armour that have been described among the many hundreds of Threespine Stickleback populations that have been sampled across the global range of *G. aculeatus* (see **Distribution**). The persistence of the loss of defensive structures that characterize Unarmoured Threespine Stickleback is the result of evolutionary adaptations to divergent predation regimes that are crucial to their persistence (see **Morphological Description**). Loss of Unarmoured Threespine Stickleback would, therefore, eliminate a significant aspect of the morphological diversity of *G. aculeatus* as a whole.

As already mentioned, however, Unarmoured Threespine Stickleback may constitute more than one DU. Genetic evidence shows that the three known Unarmoured Threespine Stickleback subpopulations (from Boulton, Rouge, and Serendipity lakes) evolved independently, and are found in two highly divergent mitochondrial DNA lineages (see **Population Spatial Structure and Variability**). Unarmoured forms were included in the Marques *et al.* (2022) study which found likely targets of parallel selection in the genome of

Haida Gwaii Threespine Stickleback that are enriched for old standing genetic variation (see above), including Threespine Stickleback reported as unarmoured from three additional locations: Branta Pond, Skokun Lake, and Solstice Lake. As in the case of Giant Threespine Stickleback, further study is required to understand the extent to which each subpopulation possesses local adaptations that could not be reconstituted if lost.

*Misty Lake Lotic (DU4) and Lentic (DU3) Threespine Stickleback*

The basis for the recognition of Misty Lake Lotic and Lentic Threespine Stickleback as separate DUs is their parapatric occurrence. It is therefore appropriate and important to consider the status of both DUs together. Genetic analyses (mtDNA and microsatellites) demonstrate that Misty Lake Lotic and Lentic Threespine Stickleback are genetically distinct from each other and from other Threespine Stickleback, including other parapatric lake-stream pairs (see **Population Spatial Structure and Variability**). This distinctness matches the morphological differences between them, which have a strong inherited basis that is driven at least in part by additive genetic variation (Lavin and McPhail 1993; Hendry *et al.* 2002; Hendry and Taylor 2004; Sharpe *et al.* 2008; Berner *et al.* 2011). While neither positive assortative mating (Raeymaekers *et al.* 2010; Räsänen *et al.* 2012; Hanson *et al.* 2016a) nor selection against lake-stream hybrids (Hendry *et al.* 2002; Räsänen and Hendry 2014; Hanson *et al.* 2016a) appear to be substantial reproductive barriers (Raeymaekers *et al.* 2010; Räsänen *et al.* 2012; Hanson *et al.* 2016a), a combination of several (probably weak and asymmetric) extrinsic barriers to reproduction appear to be working together to constrain gene flow to a narrow contact zone, and to achieve strong reproductive isolation between Lotic and Lentic Threespine Stickleback (Räsänen and Hendry 2014; Hanson *et al.* 2016a; Lackey and Boughman 2017; Haenel *et al.* 2021).

Misty Lake Lotic and Lentic Threespine Stickleback are evolutionarily significant. While other instances of parapatric lake-stream pairs of Threespine Stickleback can be found elsewhere (see **Distribution**), there is considerable non-parallel divergence among these pairs (Paccard *et al.* 2020). Misty Lake Lotic and Lentic Threespine Stickleback represent one of the most extreme cases of morphological divergence among parapatric lake-stream pairs of Threespine Stickleback (see **Morphological Description**). Their persistence is the result of evolutionary adaptations (to foraging, predation, and breeding environments) that are crucial to their persistence in parapatry (see **Morphological Description**). Loss of this pair would, therefore, eliminate a significant aspect of the morphological diversity of *G. aculeatus* as a whole.

*Benthic and Limnetic Threespine Stickleback (DUs 5 -12)*

The recognition of each Benthic and each Limnetic Threespine Stickleback as a separate DU is based on the sympatric occurrence of each pair; hence it is appropriate and important to assess the status of both DUs of each pair together. Genetic analyses (allozymes, mtDNA, microsatellites, and SNPs) demonstrate that each Benthic and Limnetic Threespine Stickleback pair is genetically distinct from every other pair and from other Threespine Stickleback, including other sympatric Benthic and Limnetic Threespine Stickleback pairs (see **Population Spatial Structure and Variability**). Their genetic distinctness is reinforced by the strong genetic basis of the morphological differences between Benthic and Limnetic Threespine Stickleback, which is driven at least in part by additive genetic variation (McPhail 1984, 1992).

Furthermore, within each intact pair, genetic analyses also indicate a low level of interbreeding between Benthic and Limnetic Threespine Stickleback that is remarkably similar across pairs; few individuals from Little Quarry Lake, Paxton Lake, and Vananda Creek carry a signature of hybridization (i.e., direct mating between a Benthic and a Limnetic) or admixture (i.e., genetic blending resulting from generations of mixed mating between the two DUs and their hybrids; Gow *et al.* 2008). This evidence of strong reproductive isolation between Benthic and Limnetic Threespine Stickleback is reinforced by the strong positive assortative mating that Benthic and Limnetic fish have demonstrated in the laboratory and in the wild (Ridgway and McPhail 1984; Nagel and Schluter 1998; Boughman 2001), and by the ecologically mediated selection against the intermediate trophic morphology of hybrids (Schluter 1995; Hatfield and Schluter 1999; Gow *et al.* 2007; Thompson *et al.* 2022). In fact, a combination of several extrinsic pre-mating and post-mating barriers contribute to the strong reproductive isolation between Benthic and Limnetic Threespine Stickleback, including strong pre-mating sexual and habitat isolation barriers and post-mating selection against hybrids (reviewed in Lackey and Boughman 2017).

The Enos Lake Benthic and Limnetic Threespine Stickleback pair is an exception to these low levels of interbreeding. As with the other sympatric pairs, these fish were morphologically and genetically distinct from each other and from other Threespine Stickleback (see **Morphological Description** and **Population Spatial Structure and Variability**). There was a strong genetic basis to the morphological differences between them (McPhail 1984), and several extrinsic pre-mating and post-mating barriers helped to maintain reproductive isolation between them (Lackey and Boughman 2017). As in the case of other Benthic and Limnetic Threespine Stickleback pairs, hybridization between the Enos Lake Stickleback species pair occurred naturally in the wild at a low rate (McPhail 1984; Schluter and McPhail 1992). However, since the invasion of the lake by the American Signal Crayfish (*Pascifasticus leniusculus*) in the late 1990s, genetic (microsatellites) and morphological data have shown that Enos Lake Benthic and Limnetic Threespine Stickleback are no longer distinct from each other and have collapsed into a hybrid swarm (Kraak *et al.* 2001; Gow *et al.* 2006; Taylor *et al.* 2006; Behm *et al.* 2010; Taylor and Piercey 2018), which means that pre-mating and post-mating isolation has broken down (Behm *et al.* 2010; Lackey and Boughman 2017). As a result, Enos Lake Benthic and Limnetic Threespine Stickleback no longer occur in the wild as distinct populations (see **Search Effort**), and their unique combinations of characters no longer exist in Enos Lake (Taylor and Piercey 2018).

Benthic and Limnetic Threespine Stickleback are evolutionarily significant. Despite the sampling of hundreds of coastal lakes in this region and globally, only four existing instances (occurring in four different watersheds on three different islands) of these sympatric pairs of *G. aculeatus* have been found (see **Distribution**). All four pairs have evolved independently from one another (see **Population Spatial Structure and**

**Variability**). The persistence of these divergent pairs is the result of evolutionary adaptations (to foraging, predation, and breeding environments) that are crucial to their persistence in sympatry (see **Morphological Description**). The loss of any of these discrete sympatric populations would eliminate a unique aspect of the morphological diversity of *G. aculeatus* as a whole.

## Special Significance

The significance of the DUs within the Threespine Stickleback species bundle is primarily attributable to their unique contribution to Canada's biodiversity and their scientific value. While the Threespine Stickleback species bundle has no direct commercial value, its DUs have intrinsic value as a significant prey item in their respective ecosystems (see **Interspecific Interactions**).

Each DU within the Threespine Stickleback species bundle contributes uniquely to the range of morphological variation displayed by *G. aculeatus* as a whole. Giant Threespine Stickleback and Benthic and Limnetic Threespine Stickleback are highly endemic, having a known global range of just two lakes and four lakes in BC, respectively. The three lakes harbouring Unarmoured Threespine Stickleback represent a distinctive and significant proportion of the Stickleback fish that feature reduced body armour (loss of one or more spines in the majority of fish) in the Canadian and global range of Threespine Stickleback. Misty Lake Lotic and Lentic Threespine Stickleback represent one of the most extreme cases of divergence within the circumboreal complex of parapatric lake-stream Threespine Stickleback pairs. What is exceptional about the Misty Lake Stickleback is the nature of the transition between the two members of the pair; they essentially act like two separate species, with massive genomic turnover (including many fixed differences) on the scale of only about 100 m (Haenel *et al.* 2021; Hendry pers. comm. 2022).

The Threespine Stickleback species bundle provides an important scientific model, with each DU continuing to contribute to our understanding of evolutionary processes including phenotypic variation, genomic variation, speciation, and eco-evolutionary dynamics (Hendry *et al.* 2013).

Since the first description of Benthic and Limnetic Threespine Stickleback, these sympatric pairs have become an important example of recent parallel evolution in nature. They are widely held as scientific treasures; their repeated patterns of population divergence along similar environmental gradients offer some of the strongest evidence that population divergence has been driven by natural selection. These sympatric pairs are now among the most extensively studied systems of ecological speciation in nature, giving insight into the processes that give rise to biological diversity (reviewed in Rundle and Nosil 2005; Nosil and Schluter 2011; Seehausen *et al.* 2014).

Parapatric lake-stream Threespine Stickleback pairs have emerged as another important model in the study of parallel evolution, and they are used frequently to study the repeatability of adaptive divergence (reviewed in Paccard *et al.* 2020). Misty Lake Lotic and Lentic Threespine Stickleback, in particular, are extensively studied, and continue to yield new insights into the evolution of adaptive divergence and reproductive isolation (Räsänen and Hendry 2014; Hanson *et al.* 2016a; Lackey and Boughman 2017; Stuart *et al.* 2017; Haenel *et al.* 2021).

The distinct morphological characteristics of Giant and Unarmoured Threespine Stickleback have provided rare opportunities to investigate the ecological and evolutionary causes of morphological variance (e.g., Bergstrom and Reimchen 2000, 2003; Reimchen and Nosil 2001a; Spoljaric and Reimchen 2007, 2008; Reimchen and Bergstrom 2009). In addition, Giant Threespine Stickleback have given insight into selection (linked to predation) on body size and armour (see **Morphological Description**), while Unarmoured Threespine Stickleback have been useful in studies examining spatial and temporal variation in ecological selection (from divergent predation regimes) on armour (e.g., Reimchen 1980; Reimchen and Nosil 2002).

More recently, the Threespine Stickleback has been used as a genomic model to address questions in evolution, and each DU within the Threespine Stickleback species bundle continues to yield significant insights into population and speciation genomics (reviewed in Seehausen *et al.* 2014), particularly the genetic basis of adaptation (e.g., Chan *et al.* 2010; Jones *et al.* 2012; Bay *et al.* 2017; Marques *et al.* 2018, 2022; Xie *et al.* 2019; Kirch *et al.* 2021) and genome evolution (reviewed in Ahmad *et al.* 2022).

## DISTRIBUTION

### Global Range

Table 1 provides an overview of the 12 DUs and their distributions. The global range of the Threespine Stickleback species bundle is restricted to coastal BC (Figure 1).

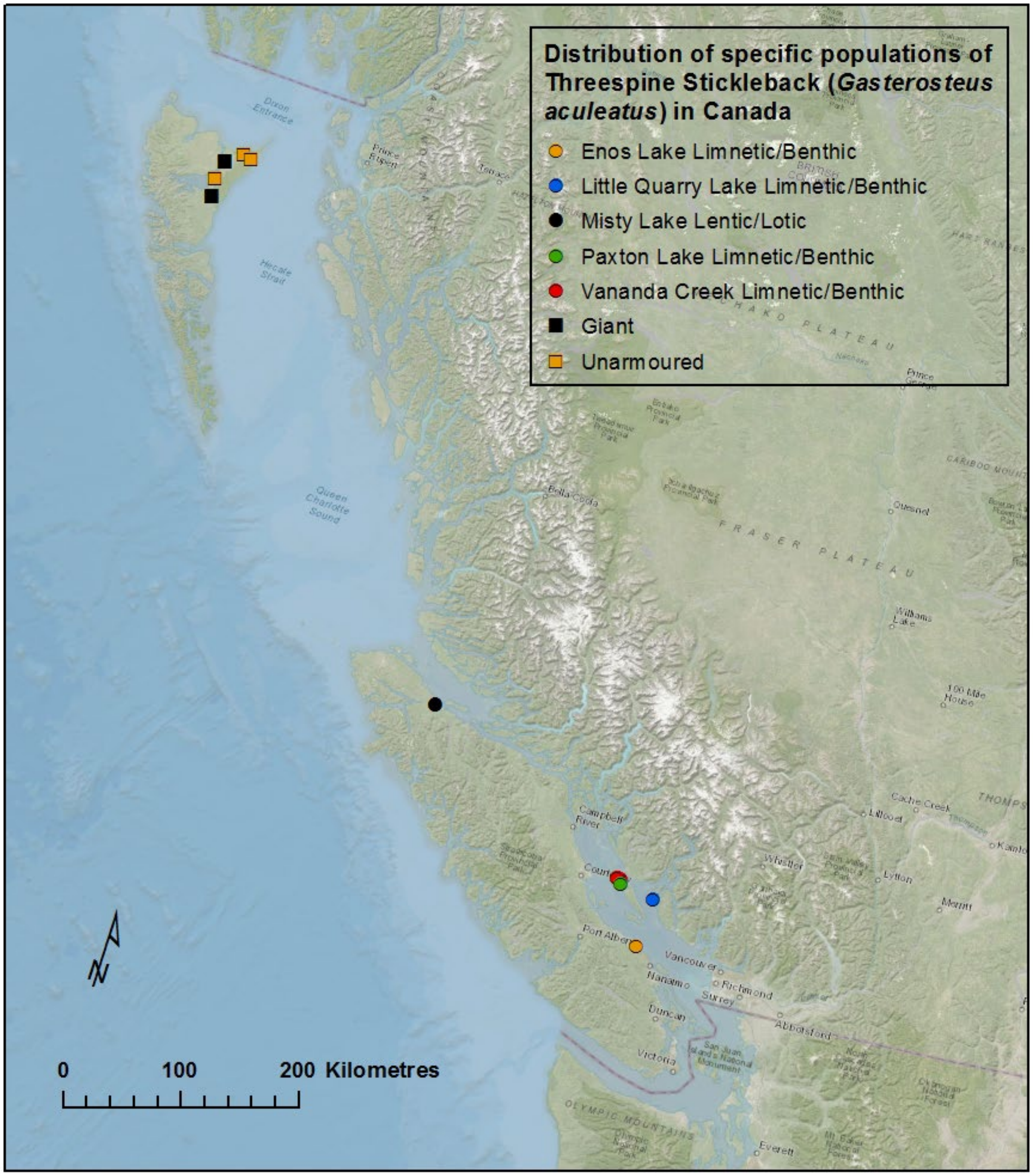


Figure 1. Distribution map showing the global range of the 12 DUs included in this Threespine Stickleback species bundle.

The archetypal parapatric pairs of lake-stream Threespine Stickleback (Misty Lake Lotic and Lentic Threespine Stickleback, and Giant Threespine Stickleback) are part of a larger circumboreal complex of Threespine Stickleback that exhibit morphological divergence between lake forms and stream forms, including some elsewhere on Vancouver Island and Quadra Island, BC (Hendry and Taylor 2004; Berner *et al.* 2008, 2009) and in eastern Canada (Paccard *et al.* 2020), Alaska (Aguirre 2009), and Europe (Reusch *et al.* 2001; Berner *et al.* 2010; Lucek *et al.* 2013; Ravinet *et al.* 2013; Paccard *et al.* 2020).

The known ranges of the archetypal parapatric pairs of lake-stream Threespine Stickleback are restricted to the west coast of BC, occurring only in the Pacific Islands National Freshwater Biogeographic Zone. Giant Threespine Stickleback are endemic to Haida Gwaii on the west coast of BC, having been confirmed from just two lakes: Mayer Lake in the Mayer River watershed and Drizzle Lake in the Sangan River watershed, both located in the northeastern portion of Graham Island, the most northerly island of Haida Gwaii (Moodie 1972a, 1984; Moodie and Reimchen 1973, 1976; Reimchen 1984a; Reimchen *et al.* 1985; Figure 2). Misty Lake Lotic and Lentic Threespine Stickleback are found only in the Misty Lake watershed in the Keogh River system on northern Vancouver Island, BC (Figure 3). There is sufficient gene flow (Hendry *et al.* 2002; Hendry and Taylor 2004; Moore and Hendry 2005) between Misty Lake Lentic Threespine Stickleback and those found in the outlet stream (known limit 2.3 km downstream, Oke *et al.* 2017) to consider Threespine Stickleback in the outlet stream part of the Lentic DU (COSEWIC 2006, 2018b). Misty Lake Lotic Threespine Stickleback are restricted to the inlet stream, and co-occur with Lentic Threespine Stickleback in the narrow, swampy transition zone between lake and inlet (COSEWIC 2006; Oke *et al.* 2017; DFO 2018b). They do not occupy the entire length of the inlet stream, and the upper limit of their distribution remains uncertain. It likely lies beyond their known extent, but no systematic survey has been completed (COSEWIC 2006; Oke *et al.* 2017; DFO 2018b). The distributions of Misty Lake Lotic and Lentic Threespine Stickleback and Giant Threespine Stickleback have not changed since their discovery several decades ago. It remains possible that additional highly divergent lake-stream pairs, including other Giant Threespine Stickleback, may yet be described (see **Search Effort**).

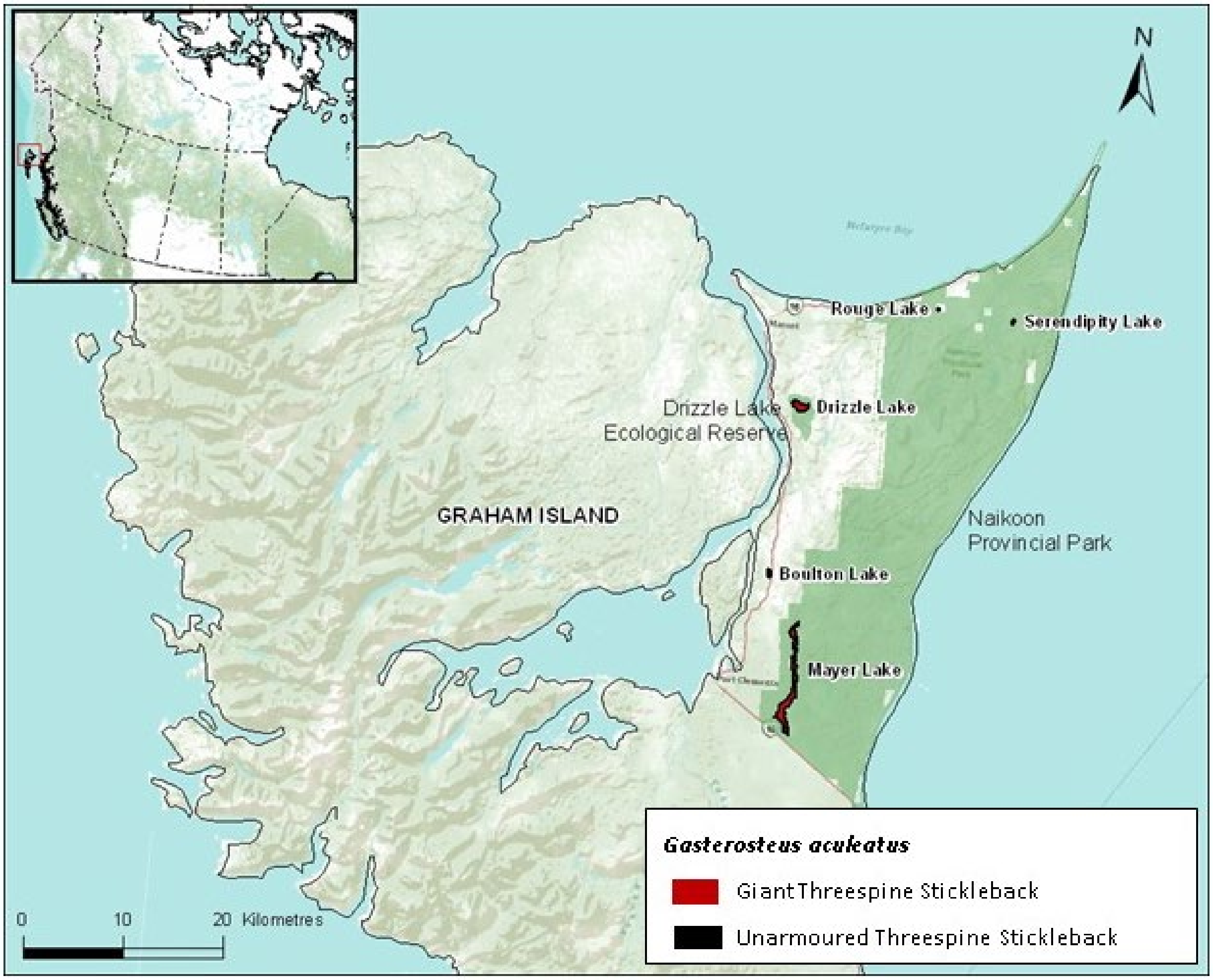


Figure 2. Distribution map of Giant and Unarmoured Threespine Stickleback. Current and historical distributions are identical, as are the global and Canadian ranges (COSEWIC 2013: data from Moodie 1984; Reimchen 1984; Reimchen *et al.* 1985).

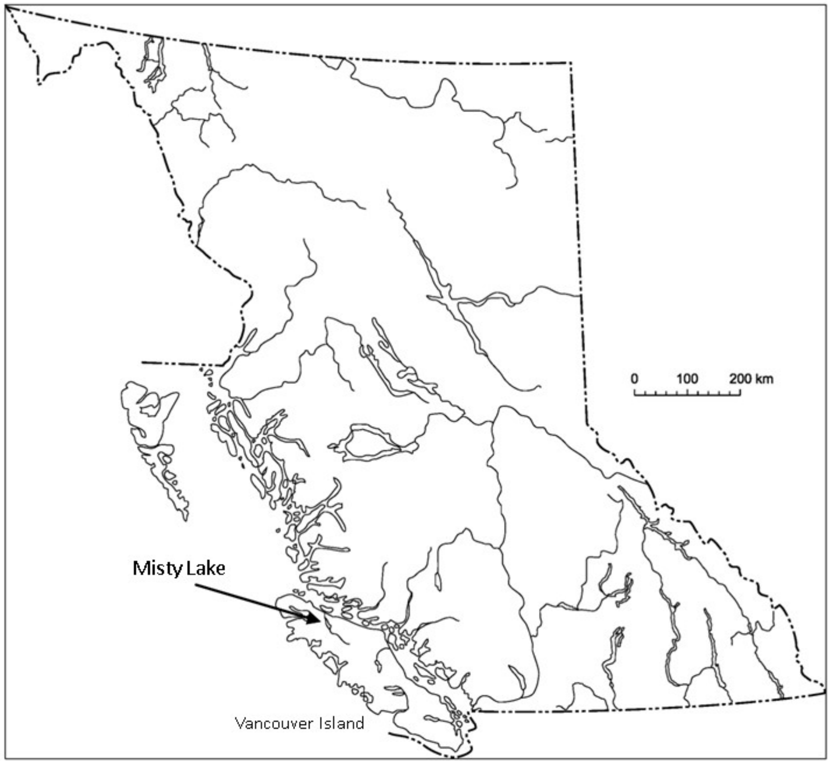


Figure 3. Distribution map of Misty Lake Lotic and Lentic Threespine Stickleback on northern Vancouver Island. Current and historical distributions are identical, as are the global and Canadian ranges. Arrow indicates location of Misty Lake watershed (COSEWIC 2006).

Unarmoured Threespine Stickleback are part of a larger circumboreal complex of Threespine Stickleback populations that contain a majority of fish lacking one or more spines. Other such populations displaying partial or complete loss of the pelvic skeleton have been described elsewhere in coastal BC (McPhail 1993; Reimchen and Nosil 2006; Gow *et al.* 2008), and in Quebec (Edge and Coad 1983), Alaska (Bell *et al*. 1993), Scotland (Bell 1987), and Iceland (Shapiro *et al.* 2004). In this report, Unarmoured Threespine Stickleback refers to the unarmoured form found specifically on Haida Gwaii on the west coast of BC (Figure 2). They are restricted to the Pacific Islands National Freshwater Biogeographic Zone, and known from three lakes in separate watersheds on Graham Island: Serendipity, Rouge, and Boulton lakes (Moodie and Reimchen 1976; Reimchen 1980, 1984a). The distribution of Unarmoured Threespine Stickleback has not changed since their discovery on Haida Gwaii several decades ago, although it is possible that other populations may yet be described (see **Search Effort**).

Sympatric pairs of Benthic-Limnetic Threespine Stickleback have a restricted geographic distribution, occurring in four watersheds in southwestern coastal BC: one pair in Little Quarry Lake on Nelson Island (Figure 4), one in Enos Lake on southeastern Vancouver Island (Figure 5), and two on Texada Island: one in the Vananda Creek watershed (comprised of the interconnected Emily, Priest, and Spectacle lakes, Figure 6) and the other in Paxton Lake (Figure 7). Little Quarry Lake on Nelson Island lies in the Pacific National Freshwater Biogeographic Zone; the other three pairs occur in the Pacific Islands National Freshwater Biogeographic Zone. The known distribution of Limnetic-Benthic Threespine Stickleback pairs has changed somewhat over the past few decades; Little Quarry Lake Benthic and Limnetic Threespine Stickleback were described most recently (Gow *et al.* 2008), while a fifth pair in Hadley Lake on Lasqueti Island, BC, became extinct following predation associated with the unauthorized introduction of Brown Bullhead (*Ameirus nebulosus*) to the lake (Hatfield 2001). It remains possible that additional Limnetic-Benthic Threespine Stickleback pairs may yet be described in the Pacific Islands and Pacific National Freshwater Biogeographic Zones (see **Search Effort**).

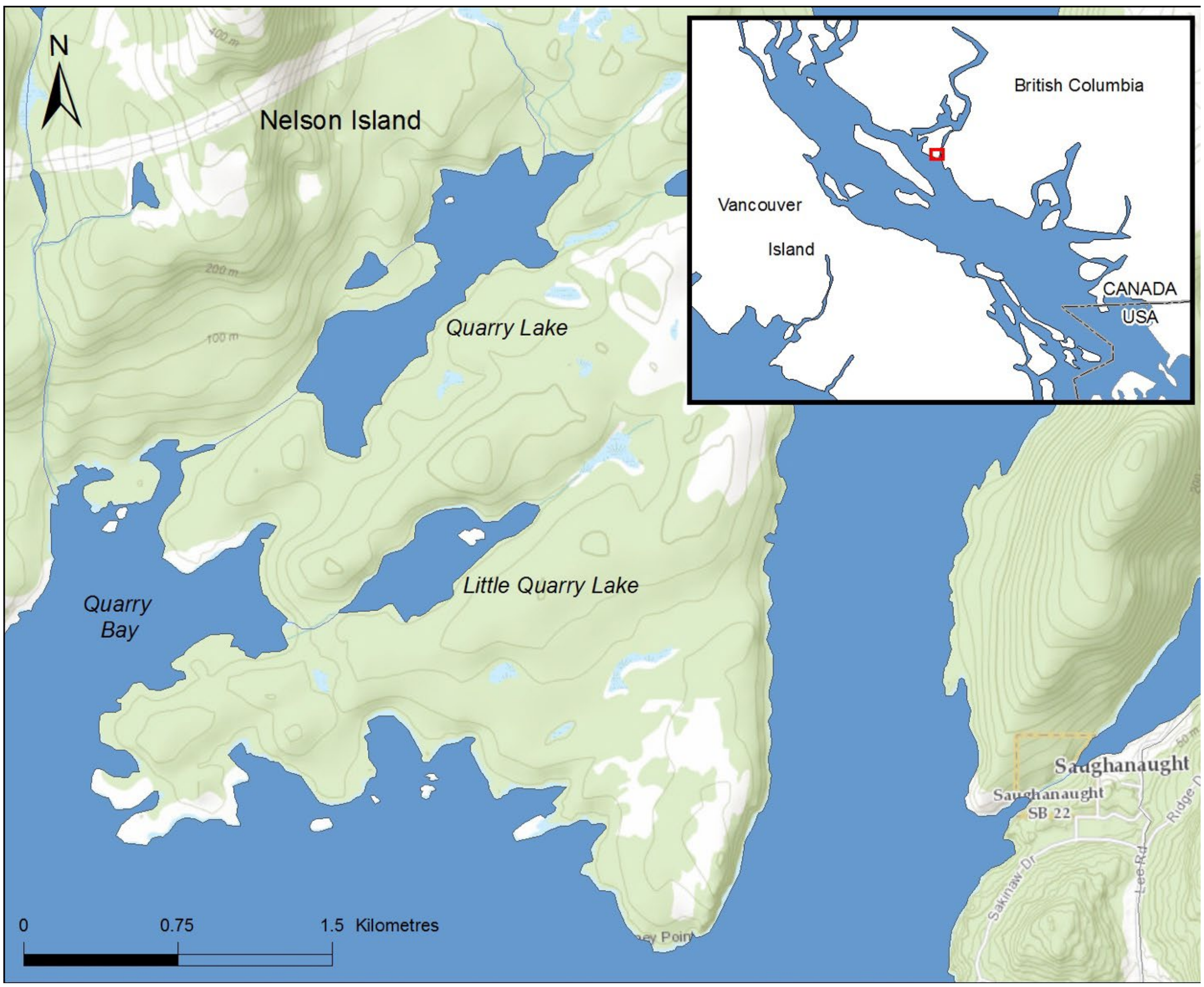


Figure 4. Distribution map of Little Quarry Lake Benthic and Limnetic Threespine Stickleback on Nelson Island. Current and historical distributions are identical, as are the global and Canadian ranges (COSEWIC 2015a: data from Gow *et al.* 2008).

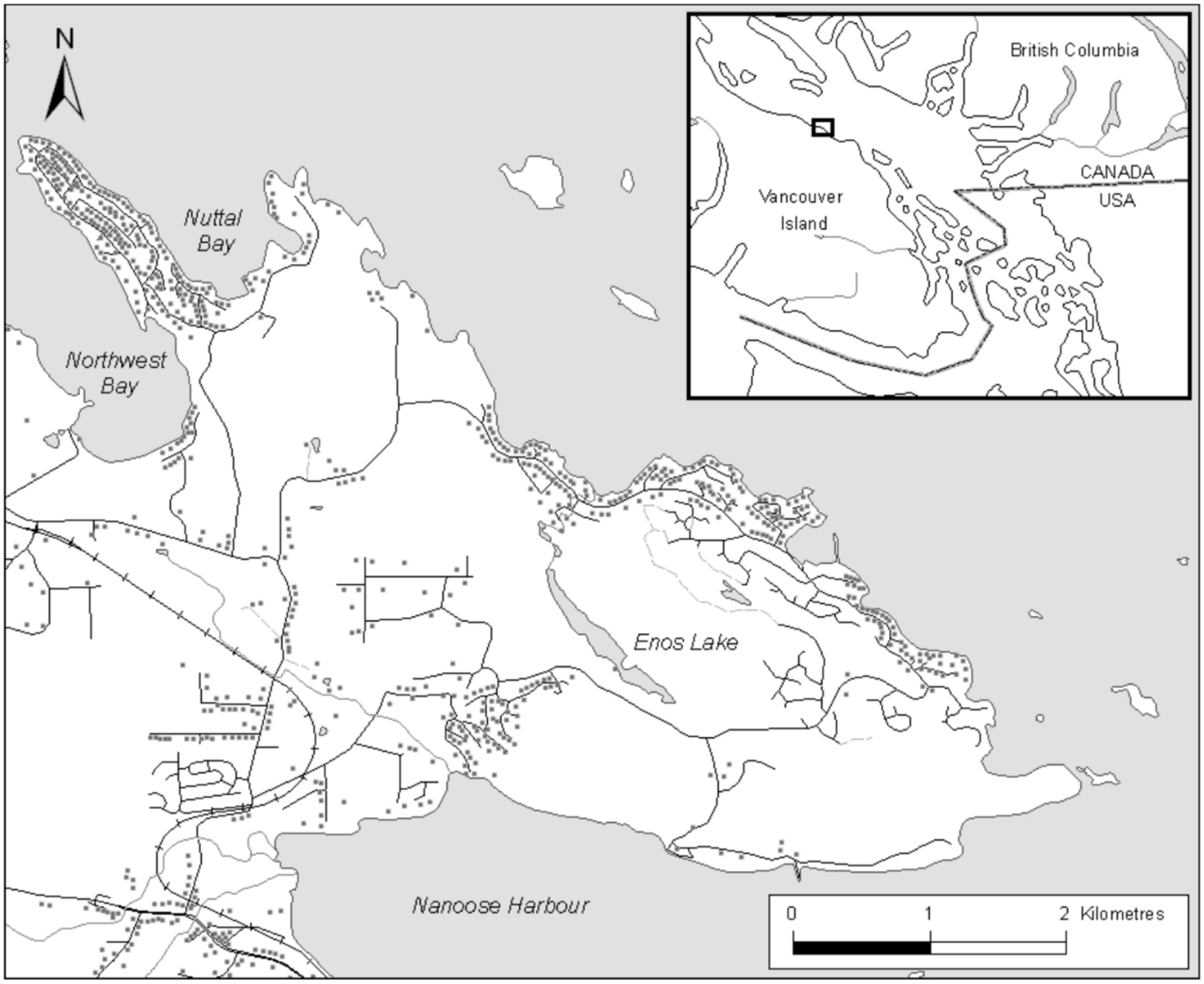


Figure 5. Distribution map of Enos Lake Benthic and Limnetic Threespine Stickleback on southeastern Vancouver Island. Global and Canadian ranges are identical, as are the current and historical distributions (although the pair have now collapsed into a hybrid swarm; COSEWIC 2012).

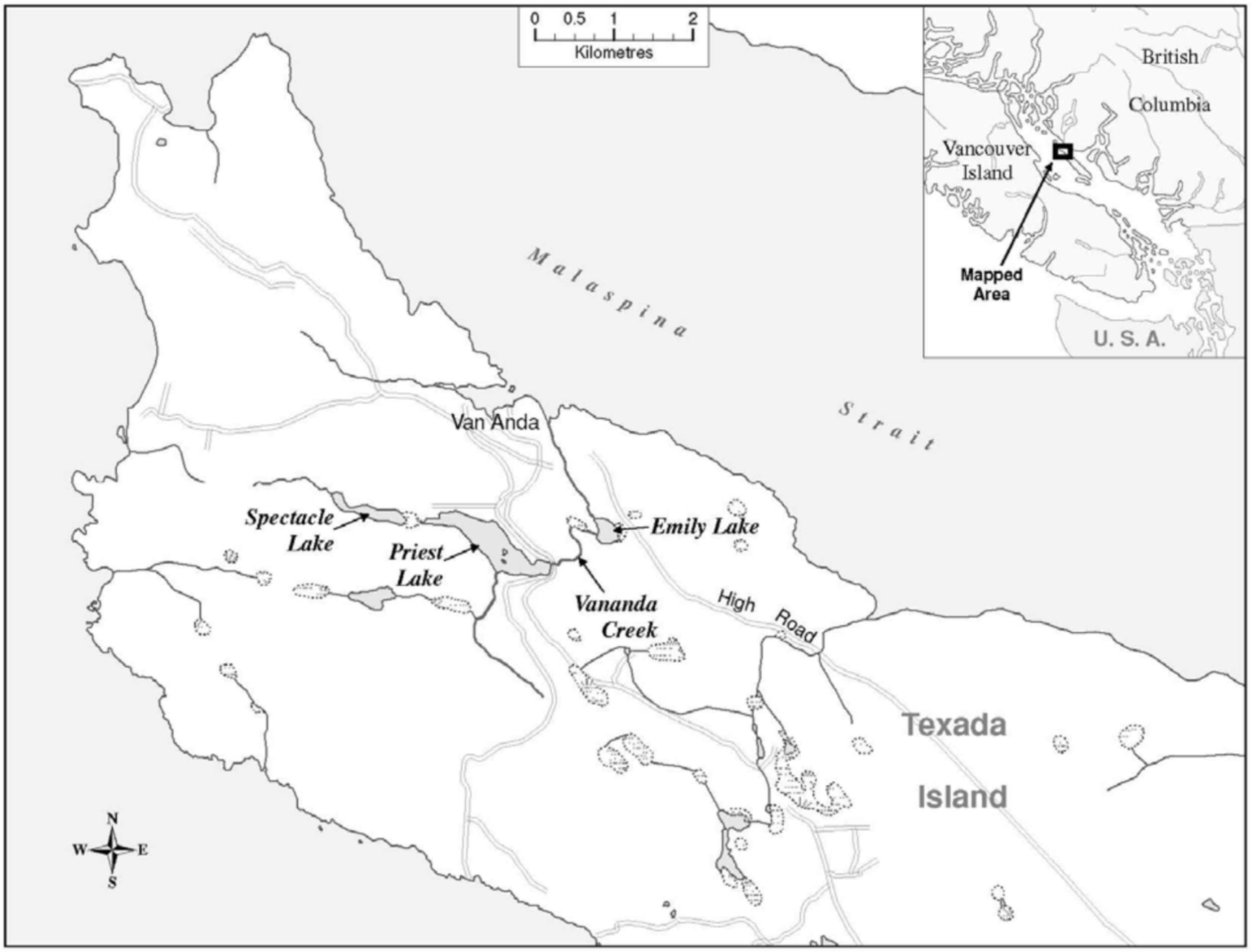


Figure 6. Distribution map of Vananda Creek Benthic and Limnetic Threespine Stickleback in three lakes on Texada Island. Current and historical distributions are identical, as are the global and Canadian ranges (COSEWIC 2010b).

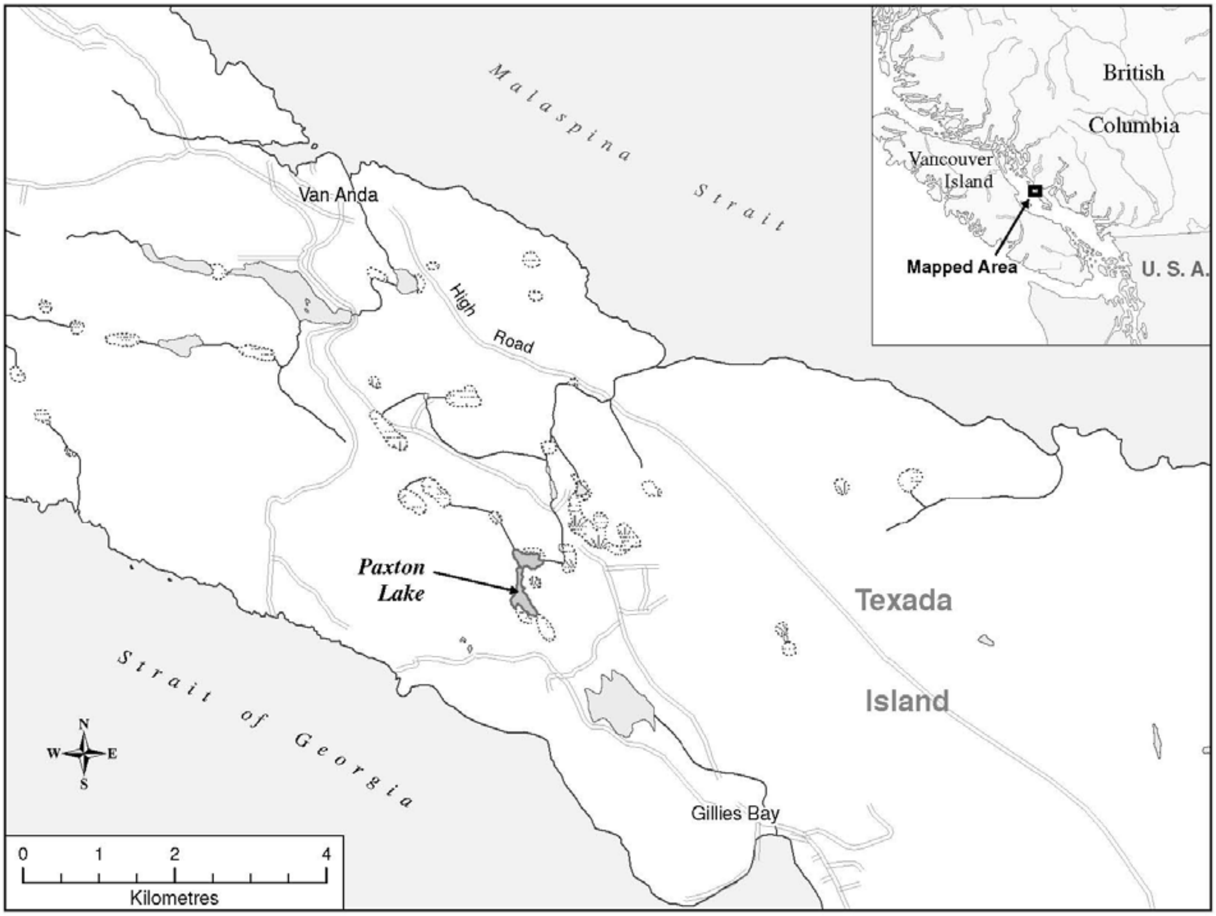


Figure 7. Distribution map of Paxton Lake Benthic and Limnetic Threespine Stickleback on Texada Island. Current and historical distributions are identical, as are the global and Canadian ranges (COSEWIC 2010a).

## Canadian Range

Because all of the DUs within the Threespine Stickleback species bundle are endemic to Canada, the Canadian and global ranges for each DU are identical.

## Extent of Occurrence and Area of Occupancy

The extent of occurrence (EOO) and index of area of occupancy (IAO) were estimated for each DU in the Threespine Stickleback species bundle according to the COSEWIC guidelines (i.e., using the minimum convex polygon method for EOO and an overlaid grid of 2 km × 2 km cells for IAO), and checked by COSEWIC Science Support (Filion pers. comm. 2021).

Table 1 provides an overview of the EOO and IAO for each of the DUs, along with lake surface area estimates for comparison. The EOO for Giant Threespine Stickleback is estimated to be 63 $km^2$ and the IAO, 52 $km^2$ (COSEWIC 2013). Note, however, that these values exceed the actual size of the sympatric pairs of Benthic-Limnetic Threespine Stickleback and the parapatric pair of Misty Lake Lotic and Lentic Threespine Stickleback, EOO was calculated to be less than the IAO value. Therefore, based on the convention that the EOO cannot be less than the IAO, the EOO and IAO are reported as being the same for each of these pairs. They are estimated to be 8 $km^2$ for the Benthic-Limnetic Threespine Stickleback pairs found in Little Quarry Lake (COSEWIC 2015a), Enos Lake (COSEWIC 2012), and Paxton Lake (COSEWIC 2010a), and 16 $km^2$ for the pair found in Vananda Creek (COSEWIC 2010a). They are estimated to be 12 $km^2$ for Misty Lake Lotic and Lentic Threespine Stickleback, calculated using the entire inlet watershed for the Lotic DU, and the entire lake and 2.3 km of the outlet stream watershed (the known limit of Threespine Stickleback) for the Lentic DU. As with the Giant and Unarmoured Threespine Stickleback, actual surface area for these water bodies (0.17–0.63 $km^2$ for the lakes and only about 0.04 $km^2$ for the Misty Lake inlet stream) is considerably less than EOO and IAO (Table 1).

## Search Effort

As a species, Threespine Stickleback is common in coastal marine and fresh waters throughout the Northern Hemisphere. Physically and reproductively isolated populations exist in numerous low-elevation lakes. Many hundreds of these lakes have been surveyed for Threespine Stickleback along the coasts of BC, Washington, and Alaska, and many more throughout their global range (e.g., Bell and Foster 1994; Reusch *et al.* 2001; Berner *et al.* 2010). Within Canada alone, extensive surveys have been conducted particularly along coastal BC over more than four decades (e.g., Moodie and Reimchen 1976; Reimchen *et al.* 1985; McPhail 1994; Reimchen 1994; Spoljaric and Reimchen 2007; Berner *et al.* 2009; Gambling and Reimchen 2012).

Despite global surveys, sympatric Benthic and Limnetic Threespine Stickleback pairs have been discovered in just five lakes, all within a highly confined geographic area in southwestern BC (McPhail 1994; Gow *et al.* 2008). The Little Quarry Lake pair is the one that was most recently discovered within this area, specifically during a targeted survey of lakes on Nelson Island, BC (Gow *et al.* 2008).

The Enos Lake species pair has been well studied. Morphological and genetic studies (using five microsatellite loci) showed two distinct clusters in 1977 and 1988. A sample collected in 1994 indicated that two genetically distinct populations were still present; however, by 1997, only a single genetic population was found (Taylor et al. 2006). Additional samples from 2000 and 2002 confirmed the breakdown of reproductive isolation (morphological and genetic); a single cluster was found along with strong signals of “hybrids” which were genetically intermediate to parental genotypes. Follow-up multi-season sampling conducted in 2013 and 2014 also failed to find a single pure benthic or limnetic stickleback. More than 1,200 fish were sampled from all areas of the lake and across life-history stages, but researchers consistently found only a single (intermediate) morphological group and a single genetic group (Taylor and Pierce (2018). They also failed to find any Limnetic Threespine Stickleback in the open water portions of the lake during daytime surveys over a total of 9 days and during three nighttime searches that replicated the timing and methods that McPhail (1984) had used to sample limnetics. Threespine Stickleback are still present in the lake, but the distinct Benthic and Limnetic DUs have suffered "extinction by hybridization" (Rhymer and Simberloff 1996). Habitat is inadequate for reproductive isolation and, even if crayfish were removed and benthic and limnetic forms evolved in the future (e.g., by re-segregation of "benthic" and "limnetic" alleles), they would not be the same as the DUs that existed before the hybrid swarm.

Extensive surveys of lakes on Haida Gwaii have identified Giant Threespine Stickleback from only two lakes on Graham Island, Haida Gwaii, BC (Moodie and Reimchen 1976; Reimchen *et al.* 1985; Reimchen 1989, 1994; Spoljaric and Reimchen 2007). Given the inaccessible nature of this area, it is possible that there may be more instances of these fish yet to be described. Notably, more recent, extensive surveys of lakes on Haida Gwaii, have found other specimens of "large-bodied" Threespine Stickleback (adult standard length > 75 mm) in several other watersheds; however, the morphological data sets are currently inadequate to calculate mean adult standard length (Gambling and Reimchen 2012; Reimchen *et al.* 2013). Consequently, the Threespine Stickleback found in these lakes on Haida Gwaii and other lakes in adjacent areas (e.g., the Banks-Estevan archipelago, Reimchen and Nosil 2006) cannot be confirmed to be Giant Threespine Stickleback without having the data required to evaluate their average size and other characteristics.

Despite extensive surveys, it is possible that more instances of Unarmoured Threespine Stickleback exist on Haida Gwaii but have not yet been found owing to the inaccessible nature of this area. As with parapatric pairs of lake-stream Threespine Stickleback, populations of Threespine Stickleback that contain a majority of fish lacking one or more spines occur across its circumboreal range (see **Global Range**). One such population has been found in the Banks-Estevan archipelago, 120 km to the east of Haida Gwaii (Reimchen and Nosil 2006). Therefore, Threespine Stickleback with reduced armour are not endemic to Haida Gwaii in this region.

# HABITAT

## Habitat Requirements

Collectively, the habitat requirements of the Threespine Stickleback species bundle likely include some universal features that may limit the size or viability of freshwater Threespine Stickleback populations in general. These needs likely include sustained productivity, the maintenance of habitat for nesting and rearing juveniles, as well as the absence of invasive species. Knowledge of the specific habitat features that are essential to the persistence of each DU within the Threespine Stickleback species bundle varies.

In the case of Unarmoured and Giant Threespine Stickleback, earlier work suggested a relatively close concordance between lake habitat/ecosystem characteristics and Threespine Stickleback morphology on Haida Gwaii (Reimchen 1994). This research showed that Unarmoured Threespine Stickleback were restricted to shallow bog ponds with no predatory fish and few predatory birds, whereas Giant Threespine Stickleback were restricted to larger dystrophic lakes (i.e., having brown acidic water that is low in oxygen and supports little life, owing to high levels of dissolved humus) with predatory fishes and avian piscivores (Reimchen 1994). More recent discoveries, however, describe the occurrence of other “large-bodied” Threespine Stickleback in a diversity of lake habitats that range from relatively large, clear oligotrophic mountain lakes through to a smaller, shallow dystrophic pond (Reimchen and Nosil 2006; Gambling and Reimchen 2012). The importance of lake habitat features to the persistence of Giant and Unarmoured Threespine Stickleback currently awaits further investigation.

Brief overviews given here of the characteristics of the two Giant Threespine Stickleback lakes and the three Unarmoured Threespine Stickleback lakes are drawn from the detailed descriptions given in COSEWIC (2013) and references therein. The three lakes with Unarmoured Threespine Stickleback are all small, shallow, and acidic. Each lake has thick layers of organic substrate in all or part of the lake, and floating vegetation is common. The loss of armour in these fish is probably closely linked to the small size and acidic conditions of these lakes because these attributes exclude other fishes and large avian predators (see **Interspecific Interactions**). While adult females remain primarily limnetic in spring and summer, adult males stay nearer shore during the nesting season.

The two large shallow dystrophic lakes with Giant Threespine Stickleback have low pH and heavily stained waters. Much of the littoral habitat in Mayer Lake is gentle slopes of sand or pebbles, with scattered patches of vegetation. The bottom substrate of Drizzle Lake consists mostly of sand and gravel, with some pebble beaches and aquatic vegetation present around the shoreline. While Giant Threespine Stickleback are adapted to a generally pelagic life, they prefer to nest in clumps in the shallower littoral zone in vegetation stands on gently sloping sandy or gravel substrate. As a result, they tend to spend spring and summer near the shore to spawn, but they overwinter in deeper water. Although it is not known how performance of reproductive functions varies quantitatively with habitat availability, the extent of littoral habitat is a possible limiting factor for Giant Threespine Stickleback (see **Threats and Limiting Factors**).

Parapatric and sympatric Threespine Stickleback pairs are particularly sensitive to habitat changes (see **Physiology and Adaptability**). As evolutionary young DUs, they are not intrinsically reproductively isolated from one another and can still produce viable hybrids. The persistence of their morphological and genetic distinctness instead depends on pre-mating and extrinsic post-mating barriers that maintain reproductive isolation (Lackey and Boughman 2017). The potential for habitat changes to disrupt these barriers and increase hybridization has long been appreciated in fishes (Hubbs 1955), and habitat changes could cause the breakdown of a parapatric or sympatric pair into a hybrid swarm. The collapse of the sympatric pair in Enos Lake into a hybrid swarm (Gow *et al.* 2006; Taylor *et al.* 2006) is associated with a qualitatively observed reduction in macrophyte abundance (National Recovery Team for Stickleback Species Pairs 2007; Rosenfeld *et al.* 2008a), which now covers only 0.1% of the lake (Ormond *et al.* 2011). This habitat change may be implicated in the breakdown of reproductive barriers and the collapse of the pair into a hybrid swarm, as it may have affected pre-mating habitat and sexual isolation (Lackey and Boughman 2017).

Consequently, in addition to features that may limit the size or viability of any Threespine Stickleback population or subpopulation, the habitat requirements of sympatric pairs of Benthic and Limnetic Threespine Stickleback and the parapatric pair of Misty Lake Lotic and Lentic Threespine Stickleback also include environmental features that maintain pre-mating and extrinsic post-mating barriers that ensure reproductive isolation and prevent hybridization (Hatfield 2009; DFO 2018b, 2019). This includes features that help to maintain low levels of hybridization (i.e., direct mating between a Benthic and a Limnetic) and admixture (i.e., genetic blending resulting from generations of mixed mating between the two DUs and their hybrids; Gow *et al.* 2008). Key elements in maintaining reproductive isolation likely include adequate light transmission to enable mate recognition, and sufficient habitat complexity to maintain nesting site preferences.

The specific factors that limit the distribution of Benthic and Limnetic Threespine Stickleback pairs are not fully understood. The lakes harbouring Threespine Stickleback sympatric pairs share some physical attributes; they are all low lying (< 100 m elevation), relatively shallow (< 30 m) and small (< 45 ha), each connected to the sea by a high gradient stream that is inaccessible to anadromous marine Threespine Stickleback (Ormond *et al.* 2011; COSEWIC 2015a). Despite these similarities, these lakes do not appear to have a specific suite of abiotic factors that sets them apart from other freshwater lakes. For example, lake size, relative littoral area, and water chemistry do not differ significantly between these lakes and comparable lakes with solitary Threespine Stickleback (those composed of just a single form, Ormond *et al.* 2011). Similarly, there is no significant difference in the habitat structure; for example, emergent and submerged macrophyte abundance does not vary substantially between sympatric pair and comparable non-sympatric pair lakes (Ormond *et al.* 2011).

While the specific limiting factors for the distribution of Benthic and Limnetic Threespine Stickleback pairs are not fully understood, considerable insight into the specific habitat features that are essential to their persistence has been gained largely through studies of the sympatric pairs in Paxton Lake and Enos Lake (prior to its collapse). These features are outlined in detail in the *Recovery Strategy for Paxton Lake, Enos Lake, and Vananda Creek Stickleback Species Pairs (*Gasterosteus *spp.) in Canada* (DFO 2019) and references therein, and they are summarized here. Limnetic adults feed on zooplankton in the pelagic zone, and Benthic adults feed on benthic invertebrates in the littoral zone. During the spring breeding season, shallow littoral areas of the lakes form the spawning habitat for both Benthic and Limnetic adults; however, there is microspatial segregation of nesting sites. The Limnetic adult requires open nesting sites on gravel, rock, or submerged logs, while the Benthic adult requires the cover of aquatic vegetation or other structures. These habitat requirements play a role in maintaining pre-mating barriers that ensure their reproductive isolation (Lackey and Boughman 2017). Little is known about the habitat requirements of early life stages, although both Benthic and Limnetic fry are known to use the littoral zone, where macrophyte beds provide food and refuge from predators. Habitat partitioning between Benthic and Limnetic fishes increases in later life stages, with Limnetic juveniles commonly found along steep, rocky, unvegetated littoral shorelines, and Benthic juveniles more often sheltering around macrophytes in littoral areas. Limnetic juveniles eventually move offshore to feed in pelagic areas. During the fall and winter, both Benthic and Limnetic fishes overwinter in deep-water habitats.

Based on this current knowledge of their life-cycle processes (mating, spawning, nursery, rearing, foraging, and overwintering), the habitat needs of sympatric pairs of Benthic and Limnetic Threespine Stickleback that relate to maintaining reproductive isolation, as well as abundance and distribution, include stable water levels and quality (including water clarity and light transmission, and nutrient levels that sustain littoral and pelagic productivity); a stable floral and faunal community free of invasive species; and maintenance of habitat complexity and substrate (including gently sloping sediment beaches and natural littoral macrophyte beds to maintain nesting site preferences). Critical to these requirements is sufficient cover, food, and nutrient supply from a stable and productive riparian zone. These habitat requirements are outlined in detail in the *Recovery Strategy for Paxton Lake, Enos Lake, and Vananda Creek Stickleback Species Pairs (*Gasterosteus *spp.) in Canada* (section 8.1.3 and Table 8, DFO 2019), in which they are assessed in order to identify the Critical Habitat needed to provide the functions and features necessary to support Benthic and Limnetic Threespine Stickleback life-cycle processes, and to maintain their distribution and abundance in Paxton Lake and Vananda Creek (see also **Habitat Protection and Ownership**).

There have been few direct studies of the habitat requirements of Benthic and Limnetic Threespine Stickleback in Vananda Creek and Little Quarry Lake. While they are assumed to be ecologically and behaviourally similar to the other Benthic-Limnetic pairs, differences in physical and chemical attributes may give rise to some differences in habitat use. For example, the relative littoral area is significantly smaller in Little Quarry Lake than in other lakes with Benthic-Limnetic Threespine Stickleback pairs (Ormond *et al.* 2011). This habitat is vital for the mating, spawning, nursery, and rearing of both Benthic and Limnetic Threespine Stickleback, as well as the foraging of Benthic fish. Although it is not known how performance of reproductive functions varies quantitatively with habitat availability, the extent of littoral habitat in Little Quarry Lake is a possible limiting factor (DFO 2018a; see **Threats and Limiting Factors**).

The habitat needs of the parapatric pair of Misty Lake Lotic and Lentic Threespine Stickleback that are related to maintain barriers that ensure reproductive isolation, as well as abundance and distribution, are based largely on the current knowledge of the life-cycle processes of sympatric pairs of Benthic and Limnetic Threespine Stickleback described above, and those of stream-dwelling Threespine Stickleback from Little Campbell River in the BC Lower Mainland, as well as a growing body of direct research on Misty Lake Lotic and Lentic Threespine Stickleback and other parapatric pairs of lake-stream Threespine Stickleback (COSEWIC 2006; Hatfield 2009; DFO 2018b). These habitat requirements are outlined in detail in the *Recovery Strategy for the Misty Lake Sticklebacks (*Gasterosteus aculeatus*) in Canada* (section 7.1.3 and Table 3, DFO 2018b), where they have been considered in order to identify the Critical Habitat needed to provide the functions and features necessary to support Misty Lake Lotic and Lentic Threespine Stickleback life-cycle processes and to maintain their distribution and abundance (see also **Habitat Protection and Ownership**).

Based on current knowledge of their life-cycle processes (mating, spawning, nursery, rearing, foraging, and overwintering), the habitat needs of Misty Lake Lentic and Lotic Threespine Stickleback include stable water levels and quality (including water clarity and transmission), and oligotrophic (nutrient poor) conditions that sustain lake and stream productivity. A combination of fast-flowing sections and low-velocity pools are necessary to maintain a healthy stream ecosystem that supports the needs of Lotic Threespine Stickleback (Lotic Threespine Stickleback likely occupy low-velocity areas, while the fast riffle habitats are required for their prey production). A stable floral and faunal community free of invasive species is also required. This includes overhanging riparian and vegetation cover for Lotic Threespine Stickleback, macrophyte beds for Lentic Threespine Stickleback, and emergent vegetation in the swampy transition zone between Lotic and Lentic Threespine Stickleback. Physical structural complexity is needed, including sufficient littoral habitat with sand substrate and pelagic water of adequate depths in the lake, shallow pools with muddy substrate as well as faster flowing areas in the streams, and dynamic littoral habitat structure within the swampy transition zone that can provide different microhabitats to Lotic and Lentic Threespine Stickleback. Sufficient shade, cover, food, and nutrient supply from a physically stable riparian zone are a critical part of these requirements.

New research indicates that a combination of several barriers is responsible for achieving strong reproductive isolation between Lotic and Lentic Threespine Stickleback (Räsänen and Hendry 2014; Hanson *et al.* 2016a; Haenel *et al.* 2021), with some notable differences from Benthic and Limnetic Threespine Stickleback (Lackey and Boughman 2017). While the strength and balance of these barriers is being uncovered, there remains some uncertainty as to which habitat components are needed to maintain reproductive isolation between Lotic and Lentic Threespine Stickleback. Pre-mating sexual isolation barriers appear weaker between the parapatric pair in Misty Lake, and the role that stable light transmission plays in the dark-stained waters of Misty Lake and its inlet is unknown, while pre-mating habitat isolation appears strong. In light of current knowledge, it remains important to ensure that the selective regime is not altered, to ensure persistence of this parapatric pair.

## Habitat Trends

Water quality samples collected from Enos Lake between 2006 and 2014 provide data for most years that give very good coverage of typical mixed conditions over the course of the seasons (PGL Environmental Consultants 2016). PGL Environmental Consultants (2016) describes in detail the thermal stratification typical of lakes occupied by Benthic-Limnetic Threespine Stickleback pairs, and it characterizes Enos Lake with high confidence as generally clear regardless of season. Other parameters have been relatively consistent over this time period, and they meet BC water quality guidelines. However, these data postdate the collapse of this Benthic and Limnetic Threespine Stickleback pair into a hybrid swarm. PGL Environmental Consultants (2016) outlines a water quality monitoring program for Enos Lake as of 2017, with many parameters being sampled quarterly and sampling repeated annually. The developer of the residential area (FW Enterprises Ltd.) within this watershed is responsible for implementing this program until at least one year post build-out.

Trends in habitat quantity and quality for lakes occupied by other DUs within the Threespine Stickleback species bundle can be assessed only qualitatively because there has been no collation of long-term sampling data from these habitats. Short-term monthly monitoring of water quality in Paxton Lake and Priest Lake (part of Vananda Creek system) in the late 2000s found no significant changes in surface temperature and pH, temperature and oxygen profiles, Secchi depth, or turbidity (COSEWIC 2010a, b). The baseline data collected by Ormond *et al.* (2011) from lakes with Benthic and Limnetic Threespine Stickleback pairs can enable future monitoring of habitat trends in these lakes. While there are no data to quantify the rate of habitat change over the last decade, qualitative information gives some insight into habitat trends.

Water levels in habitats occupied by many of the Threespine Stickleback species bundle DUs have been subject to human influence through the construction of dams and the extraction of water. In the case of Benthic and Limnetic Threespine Stickleback, the most severe fluctuations in water level are known to have occurred in Paxton Lake due to damming of the outlet stream and water extraction as part of historical mining operations (Larson 1976). However, industrial use of water has declined substantially during the last 40 years due to a shift in mining operations (COSEWIC 2010a). Lakes in the Vananda Creek watershed have also been subjected to damming of outlet streams (Priest and Emily lakes) and water extraction for mining (Emily Lake, COSEWIC 2010b). Existing water licences for Paxton Lake and the Vananda Creek watershed continue to be large relative to the volume of the lakes and size of the catchments (Government of British Columbia 2021). The community of Van Anda depends on water extraction from the Vananda Creek watershed for its drinking water supply and for firefighting (COSEWIC 2010b). The dams on Little Quarry Lake and Enos Lake outlets, which have also been in place for decades, are believed to have raised the water level by 1–1.5 m (COSEWIC 2012, 2015a). Although the impact of historical and current use of water is uncertain, a higher historical rate of hybridization between Benthic and Limnetic Threespine Stickleback in Paxton Lake (Taylor and McPhail 1999) is thought to be consistent with the higher rate of historical perturbations, including drawdowns from water extraction, in this lake (COSEWIC 2010a).

The watersheds (Misty, Drizzle, and Mayer lakes) that are home to Lotic and Lentic Threespine Stickleback and Giant Threespine Stickleback are natural ecosystems that appear to have been relatively stable over recent decades (Moodie 1984; Reimchen 1994; COSEWIC 2006; DFO 2018b). Conversely, the three lakes inhabited by Unarmoured Threespine Stickleback had their outlet streams blocked in the 1970s by the activities of the introduced North American Beaver (*Castor canadensis,* Reimchen 1984a), which caused lake levels to rise as much as 1 m. In Rouge Lake, the rise in water level eliminated most of the shallow sandy littoral beaches (Reimchen 1984a), which may have impacted

Threespine Stickleback reproduction (by potentially disrupting mate recognition and nesting habitat) and interspecific interactions (by potentially increasing lake use by predatory birds such as loons). Since that time, however, water levels appear to have stabilized in Serendipity and Boulton lakes (Reimchen 1984a; Reimchen pers. comm. 2022). The beavers in Rouge Lake disappeared about 2017, the year the lake temporarily dried up (Reimchen pers. comm. 2022).

Land-based activities can also negatively affect within-lake habitats. For example, forest harvesting, road building, or other types of construction can increase sedimentation and reduce riparian function. With regard to Benthic and Limnetic Threespine Stickleback, extensive mining and forest harvesting have been carried out in the Vananda Creek and Paxton Lake watersheds, but the ongoing influence of these land uses on Threespine Stickleback and their habitats have not been quantified (COSEWIC 2010a,b). The land surrounding Little Quarry Lake is relatively free from human activities; it is forested, with no roads or other development (COSEWIC 2015a). There are some houses on recreational lots around the oceanside of nearby Quarry Bay, downstream of Little Quarry Lake (COSEWIC 2015a). The shoreline of Enos Lake remains mostly undisturbed, but sediment-laden runoff from nearby residential development has caused concern in the recent past (COSEWIC 2012). The biggest habitat change in this lake is a qualitatively observed reduction in the abundance of aquatic plants (National Recovery Team for Stickleback Species Pairs 2007; Rosenfeld *et al.* 2008a). Submerged macrophytes now cover only 0.1% of Enos Lake, a fraction of the abundance found in the other lakes with Benthic and Limnetic Threespine Stickleback pairs (Ormond *et al.* 2011). Given the known impact of American Signal Crayfish on macrophyte abundance in Enos Lake (Rosenfeld *et al.* 2008a) and elsewhere (e.g., Rosenthal et al. 2006; Gherardi and Acquistapace 2007), and the fact that crayfish were not historically present in Enos Lake (Taylor *et al.* 2006; Rosenfeld *et al.* 2008a), this habitat change is likely a consequence of the appearance of American Signal Crayfish in Enos Lake (Rosenfeld *et al.* 2008a). This change in habitat may be implicated in the weak pre-mating isolation now found between this sympatric pair and their collapse into a hybrid swarm (Lackey and Boughman 2017).

The main land use activity in the Misty Lake area is forestry and road building, although there have been no apparent long-term impacts to date from previous forestry operations in the watershed (COSEWIC 2006; DFO 2018b). However, loss of canopy cover has been associated with increased algal growth in the inlet (COSEWIC 2006). Aside from dam operations on the two lakes, the habitat of Giant and Unarmoured Threespine Stickleback does not appear to have undergone significant habitat alteration due to land use (COSEWIC 2013).

## BIOLOGY

The biology of Giant Threespine Stickleback from Mayer Lake, along with Benthic and Limnetic Threespine Stickleback from Paxton and Enos lakes, has been studied extensively; the latter in the wild, experimental ponds, and the laboratory (reviewed in COSEWIC, 2013, 2010a, 2012). Less is known about the biology of Misty Lake Lotic and Lentic Threespine Stickleback, although more recent studies are yielding insights. Little is known about the biology of Unarmoured Threespine Stickleback; it is assumed that much of its biology is similar to that of other freshwater Threespine Stickleback. Similarly, there has been little direct study of the biology of Benthic and Limnetic Threespine Stickleback in Vananda Creek or Little Quarry Lake. They are assumed to be ecologically and behaviourally similar to the other Benthic-Limnetic pairs, although their biology may vary in some regards; for example, differences in physical and chemical attributes between Little Quarry Lake and the other Benthic-Limnetic pair lakes (see **Habitat Requirements**) may give rise to differences in habitat use.

Knowledge of the biology of the DUs within the Threespine Stickleback species bundle is presented in detail in their previous COSEWIC status reports (COSEWIC 2006, 2013, 2010a,b, 2012, 2015a), and is briefly summarized here. For sake of brevity, only more recent, relevant research since the publication of their respective COSEWIC status reports is cited in this section; please refer to COSEWIC 2006, 2013, 2010a,b, 2012, 2015a and references therein for more details.

### Life Cycle and Reproduction

The reproductive biology of the DUs within the Threespine Stickleback species bundle is broadly similar to that of other freshwater Threespine Stickleback. Males construct nests, within which spawning occurs, and they guard and defend the nests until fry are about a week old. Eggs take up to one week to hatch, depending on temperature, and the larvae are free-swimming after another three to five days. Within a breeding season, female Threespine Stickleback typically complete multiple breeding cycles, and males may mate with multiple females, and may nest more than once. There are, however, some striking deviations in reproductive biology within the Threespine Stickleback species bundle from most other populations of Threespine Stickleback.

One deviation is the loss of typical male red nuptial coloration in the melanistic Enos Lake Benthic Threespine Stickleback (prior to collapse into a hybrid swarm), Giant Threespine Stickleback, and Misty Lake Lentic Threespine Stickleback (see **Morphological Description**). Another difference is fecundity. As egg size and count are positively correlated with standard length of adult fish in *Gasterosteus*, Giant Threespine Stickleback tend to produce more eggs that are larger than others (Oravec and Reimchen 2013). When corrected for body size, they do in fact tend to produce larger but fewer eggs relative to Threespine Stickleback from continental regions (Oravec and Reimchen 2013). This extreme example seems to reflect a more general pattern among freshwater Threespine Stickleback on Haida Gwaii, including Unarmoured Threespine Stickleback. Relative to other Threespine Stickleback, they produce larger but fewer eggs when corrected for body size (Oravec and Reimchen 2013). In the case of Giant Threespine Stickleback, cool temperatures, low aquatic productivity and community diversity, predation regime, and spawning over multiple years may contribute to this shift (Oravec and Reimchen 2013).

There is also some variation in the age of reproduction. Threespine Stickleback are typically short-lived, reaching reproductive age at approximately 12 or 24 months with a typical maximum lifespan of 2–3 years. Remarkably, “large-bodied” Threespine Stickleback of Haida Gwaii (including Giant Threespine Stickleback) reach maximum lifespans that range from 4 to 8 years old or older in Mayer and Drizzle lakes, respectively (Gambling and Reimchen 2012). Breeding males appear to be at least 2 or 3 years old at first reproduction in Mayer and Drizzle lakes, respectively, but they appear to remain reproductive throughout their extended life. The modal age of parents is about 4–5 years in Drizzle Lake and about 3 years in Mayer Lake (Reimchen pers. comm. 2022). Therefore, although generation time for the Giant Threespine Stickleback is given as 2–3 years in COSEWIC (2013), it is likely closer to 3–5 years. Factors contributing to these exceptional lifespan values remain speculative, although low productivity habitats and refuge against gape-limited piscivores (each theoretically predicting a reduced rate of senescence) may play a role. Unarmoured Threespine Stickleback reach sexual maturity in their third year; generation time is about 2 years.

There is also variation in age of reproduction within both the parapatric and sympatric Threespine Stickleback pairs. Misty Lake Lotic Threespine Stickleback breed at an earlier age (most commonly between 1 and 2 years old) than Lentic Threespine Stickleback (most commonly between 2 and 4 years old). Limnetic Threespine Stickleback also usually mature earlier than Benthic Threespine Stickleback, typically maturing at 1 year old and rarely living beyond a single breeding season. Benthic Threespine Stickleback have delayed sexual maturation relative to Limnetic Threespine Stickleback, with many not mating until they are 2 years old and going on to mate across several breeding seasons.

There are other notable differences within parapatric and sympatric Threespine Stickleback pairs. In the Misty Lake parapatric pair, Lotic Threespine Stickleback begin reproducing earlier in the breeding season than Lentic Threespine Stickleback, although there is considerable overlap (Hanson *et al.* 2016b). While their eggs are similar in size, Lotic females have greater reproductive effort (clutch mass) and larger clutches (more eggs) than Lentic females (Baker *et al.* 2013). Lentic nests are bulkier than Lotic nests, and they are more often placed on sand as opposed to gravel substrate (Raeymaekers *et al.* 2009).

Within sympatric Benthic and Limnetic Threespine Stickleback pairs, there is some spatial and temporal segregation. While shallow littoral areas of the lakes serve as spawning habitat for both Benthic and Limnetic adults, there is microspatial segregation of nesting sites. The Limnetic adult requires open nesting sites on gravel, rock, or submerged logs, while the Benthic adult requires the cover of aquatic vegetation or other structures. Benthic Threespine Stickleback begin reproducing earlier in spring than Limnetic Threespine Stickleback, although there is considerable overlap in spawning times. The shorter-lived Limnetic females produce more clutches in quick succession within the breeding season than the longer-lived Benthic females. There is also strong assortative mating between them. Both Benthic and Limnetic fry use inshore areas, where there is abundant food and cover from predators. Limnetic Threespine Stickleback eventually move offshore to feed in pelagic areas, whereas Benthic Threespine Stickleback remain in littoral areas throughout their life. Benthic and Limnetic Threespine Stickleback in Enos Lake are the exception, as pre-mating and post-mating isolation has broken down (Lackey and Boughman 2017), and the pair has collapsed into a hybrid swarm (Taylor and Piercey 2018).

## Physiology and Adaptability

As a species, Threespine Stickleback occurs in a wide array of environments, and is known to have high tolerance of many water quality characteristics (e.g., turbidity, water velocity, temperature, depth, pH, alkalinity, calcium and total hardness, salinity, conductivity). For example, within the Threespine Stickleback species bundle, Giant Threespine Stickleback are tolerant of the low calcium levels, low pH, and heavily stained waters which characterize the dystrophic lakes it inhabits (see **Habitat Requirements**). Unarmoured Threespine Stickleback are also tolerant of acidic waters. Their tolerance to the acidic waters in Serendipity Lake is considered unparalleled for fish survival.

While Threespine Stickleback are known to be generally sensitive to stress from environmental contaminants, and they are proving to be a useful model in ecotoxicological research, they can generally adapt to change, including anthropogenic disturbance (e.g., Kitano *et al.* 2008; Barrett *et al.* 2011; Leaver and Reimchen 2012; Taylor and Piercey 2018). Threespine Stickleback are easily reared artificially, and the DUs within the Threespine Stickleback species bundle would likely survive transplantation (either as artificially reared or wild fish) to lakes with similar physical and chemical characteristics to their lakes of origin. Wild Benthic and Limnetic Threespine Stickleback translocated to experimental ponds remain viable across generations, and wild Giant Threespine Stickleback transplanted to nearby ponds have persisted over decades.

Non-intuitively, the adaptability of the DUs within the Threespine Stickleback species bundle may be an underlying factor of vulnerability for them. Each has evolved in response to specific selective forces (including divergent feeding lifestyles, predation, and breeding environments; see **Morphological Description**, **Habitat Requirements** and **Interspecific Interactions**). Rather than physiological tolerance per se, deviations from natural conditions could instead pose a greater threat to their persistence by altering their selection regimes; this could lead to adaptive alterations in phenotype that could result in loss of their distinguishing suites of morphological characteristics and, in the case of parapatric and sympatric pairs, to the breakdown of barriers to reproductive isolation (Haenel *et al.* 2021).

The collapse of the Enos Lake sympatric pair bears testament to the potential catastrophic consequences of altered lake conditions (see **Habitat Requirements**). The potential for morphological change is also reflected in alterations that have been observed following transplantations. Transplanted Benthic and Limnetic Threespine Stickleback do not maintain reproductive isolation when paired together in experimental ponds. Furthermore, shifts in body shape from the source population were observed in a population of Limnetic Threespine Stickleback transplanted from Enos Lake to a small, shallow pond in the late 1980s. Despite their Limnetic ancestry, these fish are now morphologically very similar to Benthic fish from Enos Lake (Taylor and Piercey 2018). Differences in body shape have also been detected after just one generation between source Giant Threespine Stickleback (from a large dystrophic lake with a diverse vertebrate predator regime) and a transplanted subpopulation in an ecologically divergent habitat (an adjacent fishless eutrophic pond with macroinvertebrate piscivores). Furthermore, abrupt changes in defence and trophic morphology within eight generations after this transplantation were consistent with predictions based on the change in habitat (Leaver and Reimchen 2012).

These studies strongly suggest that the characteristics of lake environments are critical factors influencing the evolution and maintenance of morphological variation in the Threespine Stickleback species bundle DUs, and that habitat change can result in rapid morphological change. The maintenance of their distinct suites of morphological characteristics and their genetic integrity most likely depends on stable selective pressures in their habitat, including interspecific competition and predator-prey interactions (see **Interspecific Interactions**), as well as the physical and chemical attributes of the lakes.

Even if Threespine Stickleback from within this species bundle are transplanted to superficially similar lakes, the success of this undertaking can in no way be assured, given that our understanding of the specific lake habitat features that are essential to their persistence is incomplete (see **Habitat Requirements**). The historical forces and the present-day factors that contribute to the presence of Benthic-Limnetic pairs in some lakes rather than others are not fully understood. Each DU is highly endemic, and not found in other lakes close by that appear to be at least superficially similar but instead harbour more typical freshwater forms of Threespine Stickleback.

Collectively, this evidence strongly suggests that, in the context of maintaining their morphological and genetic distinctness, the DUs within the Threespine Stickleback species bundle are not resilient to environmental disturbance because of their adaptability.

## Dispersal and Migration

The DUs within the Threespine Stickleback species bundle are geographically isolated from one another and from other *G. aculeatus*. Each undertakes short-distance seasonal movements within their respective lakes associated with spawning, feeding, and overwintering.

Benthic and Limnetic Threespine Stickleback do not migrate beyond the limits of Paxton Lake, Little Quarry Lake, Enos Lake, or the watercourses in the Vananda Creek watershed. A few individuals dispersing into outlet streams would be lost to the populations; however, such losses are likely to be of little consequence to general population dynamics. Within the Vananda Creek watershed, there may be some movement between the three lakes (Emily, Priest, and Spectacle) via connecting streams.

Similar to Benthic and Limnetic Threespine Stickleback systems, Giant Threespine Stickleback lakes (Drizzle and Mayer) are connected to marine waters via their outlets. While migration of Giant Threespine Stickleback to marine waters, as well as gene flow to its stream counterparts, could occur, this DU is largely confined to its lakes. Extensive sampling has not found any Giant Threespine Stickleback in the outlet streams; only recent sampling has captured some from Mayer Lake inlets. Similarly, stream-form fish have only been detected near the stream mouths in Mayer Lake. While these fish presumably have ecological interactions when they do occasionally occur in sympatry, morphometric and genetic analyses have revealed no indication of introgression or hybridization.

In the case of Unarmoured Threespine Stickleback, all three lakes lack an inlet and are maintained by groundwater seepage. The outlet from Boulton Lake is intermittent and too steep to support Threespine Stickleback. Emigration from Rouge and Serendipity lakes is possible. A molecular and morphological cline suggests that there is downstream gene flow from Rouge Lake into the outlet. However, molecular evidence suggests there is little to no upstream gene flow from the outlet stream into Rouge Lake (Deagle *et al.* 1996).

Research continues to modify our understanding of the dispersal of Lotic and Lentic Threespine Stickleback within the Misty Lake system. Lentic Threespine Stickleback extend 2.3 km downstream of the lake but have not been found beyond that, indicating that there is little or no migration into or out of the Misty Lake system (Oke *et al.* 2017). Although there is some gene flow between Lotic and Lentic Threespine Stickleback within a narrow contact zone, reproductive isolation between them remains strong (reviewed in DFO 2018b and see **Designatable Units**).

## Interspecific Interactions

Interspecific competition and predation

Giant Threespine Stickleback are thought to have evolved their distinct morphology at least in part as a result of adaptation to predation by gape-limited fish and birds. Coastal Cutthroat Trout (*Oncorhynchus clarkii clarkii*) is its major fish predator, with Giant Threespine Stickleback their major food item. Prickly Sculpin *(Cottus asper)* have also been found to prey on Threespine Stickleback in one lake it inhabits (Mayer Lake), and they can likely also access the other one (Drizzle Lake). Non-resident salmonids, specifically Coho Salmon (*Oncorhynchus kisutch*) and Dolly Varden (*Salvelinus malma*), do not appear to prey on Giant Threespine Stickleback. Avian predators of Threespine Stickleback, including nine species of piscivorous birds, can be numerous on these lakes. The Common Loon (*Gavia immer*) accounts for the majority of fish consumed by birds and, alongside Coastal

Cutthroat Trout, is thought to exert significant evolutionary pressure on the morphology of Threespine Stickleback. The two predominant predators vary in the size and location of Threespine Stickleback that they tend to consume, with the Coastal Cutthroat Trout being the primary predator of juvenile and subadult Threespine Stickleback in the littoral zone, and the Common Loon, the primary predator of subadults and adults in the limnetic zone. Little is known about predators in the Misty Lake system, although both Lentic and Lotic fish coexist with Coastal Cutthroat Trout, Coho Salmon, and Dolly Varden, and Lentic fish also coexist with Rainbow Trout (*Oncorhynchus mykiss*) and Prickly Sculpin.

Unlike lakes supporting lake-stream pairs, those containing Unarmoured Threespine Stickleback have no predatory fishes. Instead, Unarmoured Threespine Stickleback are thought to have evolved their distinguishing loss of defensive structures as a result of adaptation to avian and macroinvertebrate predation regimes. Similar to Unarmoured Threespine Stickleback, Benthic and Limnetic Threespine Stickleback pairs inhabit lakes with have a simple fish community. This appears to be a major ecological determinant of where these sympatric pairs are found. Relatively low to absent interspecific competition and predation are likely key to their diversification into, and their persistence as, sympatric pairs. This is underscored by the rapid extinction of a fifth pair in Hadley Lake following the unauthorized introduction of Brown Bullhead (Hatfield 2001). In Hadley Lake, nocturnal nest predation by Brown Bullhead was the likely mechanism, and the extinction was "alarmingly rapid" (Hatfield 2001). Brown Bullhead abundance increased until stickleback recruitment failed, leaving only the adult population, which went extinct within a few years. The only other fish species that lives in lakes with Benthic-Limnetic Threespine Stickleback pairs is the Coastal Cutthroat Trout. It is found in all of these lakes except for Little Quarry Lake, which does not seem to hold any other fish species. The construction of an outlet dam over 50 years ago may have blocked a now extinct population of Coastal Cutthroat Trout from accessing spawning habitat.

In addition to Coastal Cutthroat Trout, lakes with Benthic-Limnetic Threespine Stickleback pairs are inhabited by numerous invertebrates that may feed on young Threespine Stickleback, and several species of piscivorous birds regularly visit. Adult Threespine Stickleback may also prey on Threespine Stickleback eggs and young. At current levels of abundance, none of this predation is considered a threat to the persistence of Benthic-Limnetic Threespine Stickleback pairs. Regarding interspecific competitors, studies showing competition and character displacement between Benthic and Limnetic Threespine Stickleback indicate that they are, in fact, their own greatest competitors.

Trophic resources

Within each of the sympatric and parapatric pairs of Threespine Stickleback, each DU utilizes contrasting prey resources. Misty Lake Lotic Threespine Stickleback forage mainly on benthic macroinvertebrates, whereas Lentic Threespine Stickleback feed on zooplankton and insect larvae in the surface waters of the lake (Berner *et al.* 2008; Kaeuffer *et al.* 2012). Benthic Threespine Stickleback eat mainly benthic invertebrates in the littoral zone, while Limnetic Threespine Stickleback primarily exploit plankton in open water (see **Morphological Description**). Although there is no significant difference in the abundance of trophic resources between the lakes with Benthic and Limnetic Threespine Stickleback pairs and comparable lakes without Benthic-Limnetic pairs, the biomass of zooplankton and benthic invertebrates in Little Quarry Lake is significantly lower than that in the other extant Benthic-Limnetic pair lakes (Ormond *et al.* 2011). Giant Threespine Stickleback are presumed to forage predominantly on zooplankton (COSEWIC 2013 and see **Morphological Description**). Unarmoured Threespine Stickleback prey on a mixture of benthic, pelagic, and surface invertebrate resources (Reimchen and Nosil 2001b).

Parasite/host interactions

Two unusual fish parasites are associated with Unarmoured Threespine Stickleback. In Rouge Lake, the Threespine Stickleback have a symbiotic relationship with an unusual taxon of dinoflagellate parasite that is apparently unique to Threespine Stickleback. In addition, the cestode *Cyathocephalus* is common in some of the Haida Gwaii Threespine Stickleback populations, including the Boulton Lake population, yet it is rare elsewhere in western Canada.

Macrophytes

Submerged macrophytes are considered crucial to maintaining mate recognition and reproductive barriers between Benthic and Limnetic Threespine Stickleback. Macrophyte coverage is much lower in Little Quarry Lake (0.5% of lake surface area) and Priest Lake (within the Vananda Creek system; 0.9%) than in Paxton Lake (7.6%). Qualitative observations indicate that there has been a reduction in aquatic plant abundance in Enos Lake (National Recovery Team for Stickleback Species Pairs 2007; Rosenfeld *et al.* 2008a), with submerged macrophytes now covering only 0.1% of the lake (Ormond *et al.* 2011). This habitat change may be implicated in the breakdown of reproductive barriers between Benthic and Limnetic Threespine Stickleback, and the collapse of the pair into a hybrid swarm.

# POPULATION SIZES AND TRENDS

## Sampling Effort and Methods

Quantitative abundance data have been collected for some of the DUs within the Threespine Stickleback species bundle using mark-recapture methods (Giant Threespine Stickleback, Misty Lake Lotic and Lentic Threespine Stickleback, and Paxton, Vananda Creek (Priest Lake), and Enos Lake Benthic and Limnetic Threespine Stickleback). Visual counts of nests have also been used to estimate nest densities and number of breeding males for Giant Threespine Stickleback. For other DUs within the Threespine Stickleback species bundle, abundance estimates rely on crude estimates derived using, for example, unspecified sampling techniques (Unarmoured Threespine Stickleback) and extrapolation from other systems (Benthic and Limnetic Threespine Stickleback from Little Quarry Lake and some of the Vananda Creek system). See **Abundance** for details.

## Abundance

The abundance of Giant Threespine Stickleback in Drizzle Lake was estimated from a mark-recapture study and nest density estimates in 1985 (Reimchen 1990). In spring 1985, 17,033 adult Threespine Stickleback were marked and released, of which 3,803 were recaptured in summer (Reimchen 1990). Based on mark-recapture results, the number of adults was estimated to be between 30,000 and 120,000 (mean = 75,000). At the same time, nest densities (and number of breeding males) were estimated at 10,000–60,000 nests based on visual counts of nests in different littoral areas in the lake over the three-month breeding season. With an average clutch size of 395 eggs/nest, total annual fry recruitment to the lake was estimated to be 24,000 from 30,000 nests, assuming 0.2% survival (based on an estimate of survival of fry to adulthood from another lake in coastal BC, Hyatt and Ringler 1989). With an average age of approximately 4 years old (Gambling and Reimchen 2012), about four annual recruitment events will accumulate to form a population of about 96,000 adults. The estimates of adult abundance obtained with both methodologies are, therefore, in broad agreement (Reimchen 1990). No population estimates have been made for Giant Threespine Stickleback in Mayer Lake. The adult population in this larger lake was estimated to exceed 100,000 through expert opinion taking into account general observations of adult Threespine Stickleback in littoral zones (Moodie 1984; Reimchen 2004).

The abundance of Unarmoured Threespine Stickleback has been crudely estimated to be 350,000 for Boulton Lake, 17,500 for Rouge Lake, and 22,000 for Serendipity Lake, using several unspecified sampling techniques (Reimchen 1984a). Unarmoured Threespine Stickleback from Boulton and Rouge lakes exhibit very low heterozygosity, which is, in fact, amongst the lowest levels recorded for the freshwater populations surveyed across the *G. aculeatus* range (Jones *et al.* 2012). Given the small size of these lakes (< 20 ha), it is very likely that small effective population size, perhaps as well as demographic histories involving bottlenecks during colonization, have contributed to this low genetic diversity.

The abundance of Misty Lake Lotic and Lentic Threespine Stickleback was estimated from a mark-recapture study in 2016 (Oke *et al.* 2017). In spring 2016, 6,078 adult Threespine Stickleback were marked and released from 27 sites in the Misty Lake watershed, of which 545 were recaptured in summer (Oke *et al.* 2017). The mark-recapture approach estimated total Threespine Stickleback population abundance (males, females, and juveniles) to be 123,991 (95% confidence interval: 86,169–227,717) in the lake, 14,991 (95% confidence intervals: 5,481–18,855) in the inlet, and 9,851 (95% confidence interval: 4,586–21,604) in the outlet (Oke *et al.* 2017). These estimates agree with the general observation that lake Threespine Stickleback are much more numerous than stream Threespine Stickleback in parapatric lake-stream pairs (Hendry and Taylor 2004). These quantitative estimates update previous estimates that were based on expert knowledge and genetic data, and they suggest that population sizes are larger than previously believed (reviewed in Hatfield 2009, DFO 2018b). To estimate the total number of mature individuals, the totals derived above were multiplied by 20%, the proportion of mature individuals in the Paxton Lake Benthic population in 2005 (Hatfield 2009; COSEWIC 2010a; see below).

Empirically derived population estimates do not exist for all of the Benthic and Limnetic Threespine Stickleback pairs. A 2016 mark-recapture study estimated total population abundance (males, females, and juveniles) of Threespine Stickleback in Paxton Lake to be 22,191 (95% confidence interval: 17,544–28,991) Benthic fish and 368,885 (95% confidence interval: 236,137–842,518) Limnetic fish (Schluter *et al.* 2017). This was based on 51 and 29 adult Benthic and Limnetic Threespine Stickleback, respectively, recaptured from 882 and 4,401 marked adult Benthic and Limnetic Threespine Stickleback, respectively, in spring 2016 (Schluter *et al.* 2017). The estimates used trap data, but the Limnetic population estimate may be artificially high due to schooling behaviour (Schluter *et al.* 2017). If traps containing more than 40 individuals are excluded from the analysis to reduce this influence, the Limnetic population estimate remains above 150,000 (Schluter *et al.* 2017). While the size of the Limnetic population in Paxton Lake is considered very large, its exact size remains uncertain (Schluter *et al.* 2017).

This updates previous estimates from an earlier mark-recapture study (Nomura 2005). The 2016 study did not distinguish mature males from other individuals and so does not yield insight into the number of mature individuals. While the estimates from 2005 were based on relatively small samples, and low capture success of Limnetic fish contributed to highly uncertain estimates (Hatfield 2009; COSEWIC 2010a), the number of mature male Benthic fish gives some insight into the proportion of mature individuals. Approximately 10% of Paxton Lake Benthic Threespine Stickleback were estimated to be mature males (Nomura 2005), leading to the expectation that 20% of the population represents reproductively mature individuals, assuming an equal sex ratio of reproductive individuals (Hatfield 2009; COSEWIC 2010a). This yields an estimate of 4,438 mature Benthic fish in Paxton Lake based on the total population estimates from the 2016 mark-recapture study.

The first direct total population abundance estimate of Vananda Creek Benthic and Limnetic Threespine Stickleback comes from a 2016 mark-recapture study; it estimated total population abundance within Priest Lake to be 118,058 (95% confidence interval: 101,351–141,358) Benthic fish and 110,612 (95% confidence interval: 78,068–189,684) Limnetic fish (Schluter *et al.* 2017). This was based on the recapture of 227 and 37 adult Benthic and Limnetic Threespine Stickleback, respectively, out of 4,458 and 2,211 marked adult Benthic and Limnetic Threespine Stickleback, respectively, in spring 2016 (Schluter *et al.* 2017). Numbers of Benthic and Limnetic Threespine Stickleback are similarly high in Priest Lake. In comparison, numbers of Benthic fish are estimated to be much lower than Limnetic fish in Paxton Lake (Schluter *et al.* 2017). Anecdotally, differences in native Cutthroat Trout between the lakes or underestimates of Limnetic fish in the Priest Lake mark-recapture study may contribute to these differences (Schluter *et al.* 2017).

These direct estimates of population size can be compared to indirect measures of effective population size ($N_e$) from genetic studies. $N_e$ is the number of individuals that effectively participate in producing the next generation. It is, generally, considerably less than the census population size ($N$), averaging 0.1 across wildlife species (Frankham 1995). Comparing $N_e$:$N$ ratios for Threespine Stickleback can give insight into the congruence between these independent data sets, and hence our confidence in the abundance estimates. $N_e$ has been estimated as 1,160 for Paxton Benthic fish and 1,334 for Priest Benthic fish (Gow *et al.* 2006). Assuming there are 4,438 mature Benthic fish in Paxton Lake and 23,612 mature Benthic fish in Priest Lake, the $N_e$:$N$ ratios are 0.26 and 0.06 for Paxton and Priest lakes, respectively, which are similar to the average $N_e$:$N$ of 0.1 (Frankham 1995). Despite the potential limitations of both approaches, the congruence between the estimates from mark-recapture and $N_e$ calculations give some confidence to the abundance estimates of adult Benthic Threespine Stickleback in Paxton and Priest lakes.

There are no direct population estimates from other parts of the Vananda Creek range, including Emily and Spectacle lakes (DFO 2019). Estimates can be extrapolated from mark-recapture data according to lake area and perimeter differences (Hatfield 2009). For Vananda Creek, estimates from the 2016 mark-recapture study (Schluter *et al.* 2017) in Priest Lake (44.3 ha, 3,868 m perimeter) can be extrapolated to the other two lakes in this system, Emily (7.2 h, 1,091 m lake perimeter) and Spectacle (11.5 ha, 2,268 m perimeter) lakes. Based on this, total population sizes are estimated to be 220,580 Benthic fish and 157,304 Limnetic fish in Vananda Creek. Nevertheless, given the uncertainty associated with the mark-recapture studies, extreme caution must be exercised when considering the accuracy of such rudimentary extrapolations (Hatfield 2009).

There are also no direct population estimates for Benthic and Limnetic Threespine Stickleback from Little Quarry Lake (COSEWIC 2015a). Estimates of abundance extrapolated from the 2005 and 2016 mark-recapture studies of Paxton Lake (Nomura 2005; Schluter *et al.* 2017) according to lake area and perimeter differences (Hatfield 2009) yield total population size estimates between 25,339 (2017) and 33,548 (2005) for Benthic fish, and between 156,400 (2005) and 981,497 (2017) for Limnetic fish in Little Quarry Lake (Hatfield 2009; COSEWIC 2015a). In addition to the need to exercise caution owing to the uncertainties inherent in the mark-recapture studies (Nomura 2005; Schluter *et al.* 2017), important differences between lakes (e.g., productivity, available habitat, predation) may undermine attempts at extrapolation. For example, lower abiotic indicators of productivity, along with zooplankton and benthic invertebrate biomass, as well as a smaller littoral zone and amount of macrophytes in Little Quarry Lake compared to Paxton Lake (Ormond *et al.* 2011), may contribute to relatively lower abundance of both Benthic and Limnetic Threespine Stickleback in Little Quarry Lake (COSEWIC 2015a). The difference in ratios of Benthic and Limnetic Threespine Stickleback observed between Paxton and Priest lakes from the mark-recapture studies highlights the extreme caution that is required when extrapolating population sizes between lakes.

In Enos Lake, McPhail (1989) suggested that population sizes were on the order of 100,000 for both Benthic and Limnetic Threespine Stickleback prior to their collapse, but this was not a direct estimate based on captures. Also, more recent data suggest this may be generous, at least for individuals ≥1 year old. Matthews *et al.* (2001) estimated population sizes in Enos Lake using mark-recapture methods, but these were confounded by identification problems due to the substantial hybridization between Benthic and Limnetic Threespine Stickleback that had already occurred by that time. When the sample is pooled, the population estimate is 26,630 ± 8,240 (2.5% and 97.5% quantiles), which gives an indication of the total number of Threespine Stickleback in the lake ≥ 1 year old

(Matthews *et al.* 2001; COSEWIC 2012). Extrapolating from the 2005 and 2017 mark-recapture studies of Paxton Lake (Nomura 2005; Schluter *et al.* 2017), according to lake area and perimeter differences (Hatfield 2009), yields population estimates between 85,050 (2005) and 533,565 (2017) for Enos Lake Limnetic fish, and between 15,681 (2017) and 20,761 (2005) for Enos Lake Benthic fish, or between 105,811 (2005) and 391,076 (2017) for both DUs combined (Hatfield 2009; COSEWIC 2012). Given the uncertainty associated with the mark-recapture studies, and the difference in ratios of Benthic and Limnetic Threespine Stickleback observed between Paxton and Priest lakes from these data, extreme caution must be exercised when considering the accuracy of such rudimentary extrapolations (Hatfield 2009).

Prior to the collapse of the Benthic and Limnetic Threespine Stickleback in Enos Lake, a population of Limnetic Threespine Stickleback from Enos Lake was established in a pond in Murdo-Frazer Park in North Vancouver in 1988 and 1989 (Taylor and Piercey 2018). Morphological analyses of subsequent generations of these pond fish found they quickly became more similar to the Benthic Threespine Stickleback (Taylor and Piercey 2018). As such, this subpopulation does not represent Limnetic Threespine Stickleback from Enos Lake and cannot be considered for use as a rescue population. A captive breeding program was started at the University of British Columbia but was discontinued in 2015 because genetic and morphological evidence suggested too much hybridization had occurred prior to capturing individuals (DFO 2019).

## Fluctuations and Trends

There has been no systematic abundance monitoring of any of the DUs within the Threespine Stickleback species bundle, hence there are no quantitative estimates of population abundance trends (Giant and Unarmoured Threespine Stickleback: COSEWIC 2013; Misty Lake Lotic and Lentic Threespine Stickleback: COSEWIC 2006; DFO 2018b; Benthic and Limnetic Threespine Stickleback, Paxton Lake: COSEWIC 2010a; DFO 2019; Vananda Creek: COSEWIC 2010b; DFO 2019; Enos Lake: COSEWIC 2012; Little Quarry Lake: COSEWIC 2015a; DFO 2018a). Abundance estimates for Paxton Lake from 2005 (Nomura 2005) and 2016 (Schluter *et al.* 2017) indicate some variability in total population sizes; however, large confidence intervals associated with these estimates mean trends cannot be inferred at this time (DFO 2022).

Qualitatively, population declines may have occurred in Benthic and Limnetic Threespine Stickleback due to fluctuation in water level from human activities in Priest and Paxton lakes (including damming and water extraction; reviewed in COSEWIC 2010a, b). Numbers of Misty Lake Lotic and Lentic Threespine Stickleback may also fluctuate somewhat with annual changes in water level (COSEWIC 2006). However, in recent decades, researchers have continued to trap Misty Lake Lotic and Lentic Threespine Stickleback (COSEWIC 2006; Oke *et al.* 2017; DFO 2018b), as well as the extant Benthic and Limnetic Threespine Stickleback pairs. However, sampling in Spectacle and Emily lakes, the other two lakes in the Vananda Creek watershed, remains sporadic (COSEWIC 2010a, b, 2012, 2015a; Schluter *et al.* 2017; DFO 2019, 2022).

Simple population viability analyses have been conducted for Benthic Threespine Stickleback from Paxton Lake; they were conservatively selected over Limnetic Threespine Stickleback because of their lower reproductive potential (Hatfield 2009). This population modelling using an age-structured model indicated that their high intrinsic rate of population growth gives Benthic Threespine Stickleback resilience to environmental perturbations that cause small to moderate population reductions (Hatfield 2009). However, evidence suggests this survival may come at the expense of morphological changes and genetic integrity if the perturbations shift the selective regime in their habitats (see **Physiology and Adaptability** section).

Although Threespine Stickleback remain easy to trap in Enos Lake, this species pair has formed a hybrid swarm consisting of only a single intermediate form since invasion of the lake by the American Signal Crayfish in 1990 (see **Morphological Description** and **Population Spatial Structure and Variability**), such that genetically pure DUs are thought to no longer exist. In contrast, based on the extent and quality of existing critical habitat and the understanding that Paxton Lake, Vananda Creek, and Misty Lake are natural ecosystems which have been relatively stable over the longer term, there is no *a priori* reason to expect that historical abundance was significantly greater than it is at present (Hatfield 2009). While Critical Habitat will only be identified for Little Quarry Lake Benthic and Limnetic Threespine Stickleback if the pair is listed as Endangered or Threatened under SARA (see **Legal Protection and Status**), there is also no reason to believe they are in decline (DFO 2018a).

The 2016 abundance estimates for the Threespine Stickleback within Misty Lake, Paxton Lake, and Vananda Creek are thought to be near historical levels and self-sustaining (DFO 2018b, 2019). Developing and implementing long-term population monitoring programs in Paxton Lake, Vananda Creek, and Misty Lake has been identified as a high priority measure in their action plans (DFO 2020a,b) to provide a method to assess their response to management activities and/or threats outlined in their recovery strategy (DFO 2018b, 2019).

General observations of adult Giant Threespine Stickleback in littoral zones of Mayer Lake during the reproductive season provide no evidence of changes in abundance from the late 1960s to 2003 (Moodie 1984; Reimchen 2004; COSEWIC 2013). Population density is likely regulated, at least in part, by the availability of spawning sites and by predator abundance (Moodie 1984). Since there has been no account of change in either of these factors (see **Habitat Trends** and **Interspecific Interactions** sections), the limited available information suggests that the population size is stable.

While there were no obvious changes in abundance of Unarmoured Threespine Stickleback during earlier sampling periods (Boulton 1970–1981; Rouge 1976–1981; Serendipity 1979–1981) based on trap success (number of fish per trap hour; Reimchen 1984a), population trends in more recent decades remain largely unknown (COSEWIC 2013). The lakes underwent a period of habitat alteration due to beaver activity around the earlier sampling time (see **Habitat Trends**; Reimchen 1984a, Reimchen pers. comm. 2022). The impact of these changes on fish abundance have not been documented, but rising water levels have the potential to change recruitment rates by decreasing nesting areas, disrupting mate recognition, and increasing lake use by predatory birds such as loons (Reimchen 1984a). Because water levels appear to have stabilized since then in Serendipity and Boulton lakes (Reimchen 1984a, pers. comm. 2022), this limited information suggests that population size is stable (COSEWIC 2013). In contrast, Rouge Lake temporarily dried up in 2017, around the same time beavers disappeared from the lake (Reimchen pers. comm. 2022). Unarmoured Threespine Stickleback were presumed not to have survived this drought, but they have since been sampled in 2019 as lake water levels rebound (Reimchen pers. comm. 2022). It is thought that they survived this extreme bottleneck event in 1 m deep, small bog pools immediately adjacent to the lake (Reimchen pers. comm. 2022).

There has been one other notable decline in Unarmoured Threespine Stickleback abundance observed during the last decade or so. A rapid, dramatic decline in Unarmoured Threespine Stickleback abundance was observed in the years following the first observation of the Northern Red-legged Frog (*Rana aurora*) in Boulton Lake in 2009. While Unarmoured Threespine Stickleback abundance seemed to be increasing in 2017, it is unknown whether this indicates a recovery(Reimchen pers. comm. 2022). Over this time, a shift in morphology, including defensive structures, has also been observed: Unarmoured Threespine Stickleback in this lake have shifted towards a shape more typically associated with limnetic habitat, where there is selection pressure from predatory birds (Reimchen pers. comm. 2022).

## Rescue Effect

The concept of rescue effect does not apply to any of the DUs within this Threespine Stickleback species bundle; each DU has a global range that is restricted to one, two, or three lakes within BC, Canada.

# THREATS AND LIMITING FACTORS

## Threats

Threats to Paxton Lake and Vananda Creek Benthic and Limnetic Threespine Stickleback and Misty Lake Lotic and Lentic Threespine Stickleback have been identified and described in detail in their respective recovery strategies (DFO 2018b, 2019). In the *Recovery Strategy for Paxton Lake, Enos Lake, and Vananda Creek Stickleback Species Pairs (Gasterosteus aculeatus) in Canada* (DFO 2019), threats to Enos Lake Benthic and Limnetic Threespine Stickleback were not considered since the survival and recovery of this sympatric pair was deemed to be infeasible. The threats to Giant and Unarmoured Sticklebacks are also described in the *Management Plan for the Giant and Unarmoured Threespine Sticklebacks (*Gasterosteus aculeatus*) in Canada [Proposed]* (DFO 2022b). For the other DUs within the Threespine Stickleback species bundle, threat descriptions are outlined in their respective COSEWIC assessment and status reports (COSEWIC 2012, 2013, 2015a). All of these threats assessments are based largely on professional opinion and, due to an absence of information on the effects of different threats on population vital rates (e.g., hybridization rates, growth, survival, reproductive success), quantitative risk assessment is not yet possible for any of the DUs.

The types of threats to the Threespine Stickleback species bundle are broadly similar across the DUs. The main threats are outlined below in the approximate order of most to least significant threats (identified by the IUCN threat category), with details pertaining to specific DUs noted under each category where available.

Completion of the IUCN Threat Calculator (IUCN 2021) resulted in the following overall threat impacts being assigned: Low for Giant Threespine Stickleback (DU1; Appendix 1); Very High–High for Unarmoured Stickleback (DU2; Appendix 2); Very High–Low for Misty Lake Lotic and Lentic Threespine Stickleback (DUs 3 and 4; Appendix 3); Very High–High for Vananda Creek Benthic and Limnetic Threespine Stickleback; Very High–Medium for Paxton Lake and Little Quarry Lake Benthic and Limnetic Threespine Stickleback (DUs 5,6 and 9,10, respectively; Appendix 5); and Very High for Enos Lake Threespine Stickleback (DUs 11 and 12; Appendix 5). The threat scores for all DUs are summarized in Appendix 5.

Invasive Species (8.1, 7.3)

The most likely or imminent threat to all Threespine Stickleback species bundle DUs comes from the introduction of aquatic invasive species (AIS), the leading driver of biotic change in freshwater systems globally (Sala *et al.* 2000). The persistence of the Threespine Stickleback species appears to depend on stable floral and faunal communities (see **Habitat Requirements**), and they are highly susceptible to extinction from AIS introductions which may threaten their abundance directly (e.g., via predation, competition for food resources, or displacement from nesting habitat leading to recruitment failure or disease; IUCN category 8.1) or indirectly (e.g., through ecosystem modifications that alter the selective regime of their habitat; IUCN category 7.3). In the case of parapatric and sympatric pairs, AIS that alter the habitat can potentially disrupt barriers maintaining reproductive isolation and lead to increased hybridization (DFO 2018b). These pairs are also susceptible to extinction through the loss of their genetic integrity due to the presence of AIS. Body armour is an important adaptation to post-capture defence. DUs with reduced or absent body armour (Unarmoured Threespine Stickleback and Benthic Threespine Stickleback from Paxton Lake and Little Quarry Lake) therefore face a particular threat from the introduction of gape-limited predators (COSEWIC 2013). Meanwhile, ecologically similar species, such as crayfish and benthic and spiny-rayed fishes, are considered to be the biggest threat to Misty Lake Lotic and Lentic Threespine Stickleback (DFO 2018b).

Qualitative risk assessments concluded that for most regions of BC, the probability of invasive fish species becoming established after release is high or very high, and the likely magnitude of ecological impact in small water bodies is very high (Bradford *et al.* 2008a,b; Tovey *et al.* 2008). Given the growing number of AIS, particularly in southwestern BC, the threat of AIS introduction is likely high. In addition to Brown Bullhead, two other bullhead species, Yellow Bullhead and Black Bullhead (*A. natalis* and *A. melas*, respectively), have been introduced into BC, either inadvertently in shipments of game fish intentionally released by the province during stocking efforts or intentionally through transfers made by anglers or other private citizens (Tovey *et al.* 2008). Another AIS, Smallmouth Bass (*Micropterus dolomieu*), has been introduced in dozens of waterbodies throughout BC (including more than 50 on Vancouver Island; Tovey *et al.* 2008). It has already caused the loss of Threespine Stickleback, through predation, from some small lowland lakes on southern Vancouver Island (Tovey *et al.* 2008). The Largemouth Bass (*Micropterus salmoides*) is also of concern; the presence of these fish have been confirmed in > 90 waterbodies in BC. So far, Largemouth Bass have mostly been introduced into waterbodies on the BC mainland (with fewer confirmed occurrences in lakes on Vancouver Island). However, because the greatest concern is intentional transfer by anglers rather than natural spread, the source population does not necessarily have to be geographically proximate to a stickleback lake. The introduction of any of these species could directly threaten DUs within the Threespine Stickleback species bundle, with the Misty Lake Lotic and Lentic Threespine Stickleback in northern Vancouver Island likely at highest risk.

Accessibility can increase the risk of an AIS introduction. Misty Lake is highly accessible, being right next to a major highway with an adjacent rest stop. Priest Lake (within the Vananda Creek system) is also highly accessible to the public, with two well-used island roads running along its shores (Guzek and Wilson 2022). The other two lakes in the Vananda Creek system are less accessible, via nearby dirt roads (Guzek and Wilson 2022). Paxton Lake has limited accessibility, with permission needed from the local quarry company to use access roads (Guzek and Wilson 2022). With no ferry service to Nelson Island and no road access, Little Quarry Lake is comparatively remote and inaccessible compared to these other watersheds. While the occurrences of Giant and Unarmoured Threespine Stickleback on Haida Gwaii are relatively remote, the large island is readily accessible by ferry and the islands are serviced by a commercial airline. Mayer Lake has vehicle access and is the most accessible lake on Haida Gwaii. Boulton Lake is also easily accessible from a major highway. While remoteness and inaccessibility may afford some protection from the risk of AIS introductions, it is important to remember that inaccessibility did not prevent the introduction of the exotic Brown Bullhead in Hadley Lake on Lasqueti Island (Hatfield 2001), and it has not prevented the introduction of the Northern Red-legged Frog in Boulton Lake.

The impact that AIS can have on the DUs within the Threespine Stickleback species bundle has been seen first-hand. AIS are associated with the rapid extinction of Benthic and Limnetic Threespine Stickleback pairs in at least two lakes in recent decades. Following the introduction of the Brown Bullhead, the Hadley Lake pair swiftly became extinct through predation (Hatfield 2001). Bullhead are known to occur on Vancouver Island (in the south), and they likely pose the greatest risk to Misty Lake Lentic and Lotic Threespine Stickleback (located in the north of the island; Appendix 3).

The collapse of the Enos Lake pair into a hybrid swarm (Kraak *et al.* 2001; Taylor *et al.* 2006; Taylor and Piercey 2018) occurred alongside the appearance of the American Signal Crayfish, which was not historically present in this lake (Rosenfeld *et al.* 2008a). American Signal Crayfish may play an important role as a keystone consumer in pond ecosystems, and this species may have strong and complex trophic effects (direct and indirect) on littoral communities (Nystrom *et al*. 1996). Consequently, the American Signal Crayfish is implicated as the main factor driving this collapse through altered environmental conditions, potentially including impacts on pre-mating isolation and post-mating isolation (Behm *et al.* 2010; Velema *et al.* 2012; Lackey and Boughman 2017). The collapse of this sympatric pair highlights the sensitivity to habitat degradation or loss of the DUs within the Threespine Stickleback species bundle—the leading threat to freshwater fishes in Canada (Taylor 2004; Dextrase and Mandrak 2006).

The Northern Red-legged Frog is an AIS that is now threatening the Giant and Unarmoured Threespine Stickleback DUs on Haida Gwaii due to direct competition from its tadpoles. While it is known to be native to the range of the other DUs in the Threespine Stickleback species bundle, the Northern Red-legged Frog has more recently been observed in Haida Gwaii, with its range expanding since it was first documented in 2001 (COSEWIC 2015b; Gamlen-Greene 2022). First observed in Boulton Lake in 2009, its tadpoles already exceeded the number of Unarmoured Threespine Stickleback trapped during scientific trap collections in 2011 (Reimchen pers. comm. 2022). By 2013, there was a dramatic decline in Threespine Stickleback abundance, with a ratio of 25:1 tadpoles to Unarmoured Threespine Stickleback trapped (Reimchen pers. comm. 2022). This ratio had declined by 2017, with fewer tadpoles and more Threespine Stickleback, but it is unknown whether this indicates a decline in Northern Red-legged Frog abundance or recovery of Unarmoured Threespine Stickleback (Reimchen pers. comm. 2022). Over this time, a shift in morphology, including defensive structures, was also observed: Unarmoured Threespine Stickleback in this lake have shifted towards a shape more typically associated with limnetic habitat, where there is selection pressure from predatory birds (Reimchen pers. comm. 2022). The Northern Red-legged Frog has not yet been found in other lakes containing Unarmoured or Giant Threespine Stickleback, but they have been found on the road adjacent to Mayer Lake, and it is considered only a matter of time before they reach all of these lakes (Reimchen pers. comm. 2022). Although it is native to the range of the other DUs in the Threespine Stickleback species bundle, the threat from the Northern Red-legged Frog is considered particularly serious for Unarmoured Threespine Stickleback because they inhabit relatively simple aquatic communities without other native fishes that would prey on the tadpoles and frogs (see Appendix 2).

These examples highlight the extreme vulnerability of the DUs to AIS. While there may be some uncertainty around the imminence of the threat for some DUs, the consequences can be rapid and disastrous.

For Invasive Non-Native/Alien Species/Diseases (8.1), the IUCN Threat Calculator (IUCN 2021) assigned the following threat impacts: Low for Giant Threespine Stickleback (DU1; Appendix 1); Very High–High for Unarmoured Stickleback (DU2; Appendix 2); Very High–High for Misty Lake Lotic and Lentic Threespine Stickleback (DUs 3 and 4; Appendix 3); Very High for Paxton Lake, Vananda Creek, and Little Quarry Lake Benthic and Limnetic Threespine Stickleback (DUs 5 and 6, 7 and 8, and 9 and 10, respectively; Appendix 5); and Very High for Enos Lake Threespine Stickleback (DUs 11 and 12; Appendix 5).

Natural System Modifications: Dams & Water Management/Use (7.2)

Water levels in habitats occupied by many of the Threespine Stickleback species bundle DUs have been subject to human influence through the construction of dams and the extraction of water. The most severe fluctuations have been observed in Paxton Lake (see **Habitat Trends**). Existing water licences for the Paxton Lake and Vananda Creek watersheds continue to be large relative to the volume of the lakes and size of the catchments (see **Habitat Trends**). Although the impact of historical and current use of water is uncertain, the higher historical rate of hybridization between Benthic and Limnetic Threespine Stickleback in Paxton Lake (Taylor and McPhail 1999) is thought to be consistent with the higher rate of historical perturbations at this lake (COSEWIC 2010a). Consequently, large fluctuations in water levels should be avoided to minimize changes to the lake water volume and littoral zone habitat required by the DUs within the Threespine Stickleback species bundle for foraging, spawning and juvenile rearing.

For Natural System Modifications, the IUCN Threat Calculator (IUCN 2021) assigned the following threat impacts: Very High for all four Benthic and Limnetic Threespine Stickleback pairs (DUs 5–12; Appendix 5).

Pollution (9)

Water-borne pollution from land-based runoff can threaten the Threespine Stickleback species bundle DUs. Historically, potential non-point sources of nutrient, toxic chemical, and/or sediment runoff have included industrial mining in the watersheds of Paxton Lake and Vananda Creek, and forestry effluents in the watersheds of Paxton Lake, Vananda Creek, Little Quarry Lake, and Misty Lake (COSEWIC 2015a; DFO 2018b, 2019). These potential sources of pollution can impact water quality (e.g., turbidity and clarity (especially in the case of Benthic and Limnetic Threespine Stickleback sympatric pairs), oxygen, temperature, pH, clarity, and nutrient loads (DFO 2018b, 2019). However, there have been no apparent long-term impacts to date as a result of pollution from mining or forestry activities in the Threespine Stickleback species bundle DUs. Mining activities are still being carried out in the Paxton Lake watershed and there is potential for future forestry operations in all regions, except for Serendipity Lake and Drizzle Lake (see **Habitat Protection and Ownership**). Misty Lake and its inlet are also susceptible to runoff from Highway 19 (which runs closely along the southern shore of the lake and crosses the inlet) and a rest stop (which is buffered from the lake by approximately 50 m; DFO 2018b). These have been identified as point sources of hydrocarbons and pesticides for this lake (British Columbia Ministry of Environment – Parks 2003). Other pollutants of concern include sewage seeping from the outhouses at the rest stop. The threat of non-point source pollution will depend on the extent, severity, and proximity of pollution inputs to the lakes and streams, and the application of sedimentation and pollution prevention and mitigation measures.

Point source pollution also has the capacity to affect water quality and degrade aquatic habitat. Given their proximity to Highway 19 and an adjacent rest stop, Misty Lake and its inlet are also susceptible to point source pollution such as spills from vehicles, including herbicides and fertilizers (DFO 2018b). The extent and severity of this threat to Misty Lake Lotic and Lentic Threespine Stickleback ranges from low to high owing to the risk of a harmful spill or accident occurring along this stretch of highway or at the rest stop. The highway adjacent to Boulton Lake and Mayer Lake, and vehicle access to Mayer Lake, may also make Unarmoured and Giant Threespine Stickleback inhabiting them, respectively, susceptible to point source pollution.

For pollution from Agricultural & Forestry Effluents, the IUCN Threat Calculator (IUCN 2021) assigned the following threat impact: Low for Misty Lake Lotic and Lentic Threespine Stickleback (DUs 3 and 4; Appendix 3).

Climate Change & Severe Weather (11)

Scientific evidence clearly indicates that animal and plant distributions are responding to climate change (Parmesan and Yohe 2003; Rosenzweig *et al.* 2008). Because climate affects precipitation, water flow and water temperature in many ways, it has the potential to affect the abundance and distribution of the Threespine Stickleback species bundle DUs. For example, the onset and duration of breeding in Threespine Stickleback are strongly influenced by temperature; therefore, the timing of reproduction and length of breeding season can be expected to change in response to altered water temperatures (DFO 2018b). Additionally, changes to water flow and water level may impact the spawning habitat of Threespine Stickleback in the littoral zone of the lake and quiescent areas within the streams (DFO 2018b). Climate change may also alter the average number of days with ice cover on the lake, the frequency of major forest fires and numerous other ecological cycles (DFO 2018b).

One of the lakes inhabited by Unarmoured Threespine Stickleback, Serendipity Lake, is susceptible to climate-induced coastal erosion owing to its relatively low elevation (20 m, Johnson and Taylor 2004) and close proximity to the eastern beaches of Graham Island, which are known to be vulnerable to erosion caused by climate change and sea-level rise (Walker *et al.* 2007). In the future, there is a risk of drainage of Serendipity Lake associated with coastal erosion (COSEWIC 2013).

Another of the lakes inhabited by Unarmoured Threespine Stickleback, Rouge Lake, temporarily dried up in 2017 (Reimchen pers. comm. 2022). Unarmoured Threespine Stickleback were presumed not to have survived this drought but have since been sampled as lake water levels rebounded (see **Population Sizes and Trends**). This event highlights the vulnerability of the Unarmoured Threespine Stickleback and the small lakes they inhabit to drought events.

Biological Resource Use: Fishing & Harvesting Aquatic Resources (5.4)

Scientific research collection activities have likely been a leading source of non-natural mortality of adult fish in Paxton Lake and Vananda Creek Benthic and Limnetic Threespine Stickleback (Rosenfeld *et al.* 2008b). Given the uncertainty around the abundance estimates for Benthic and Limnetic Threespine Stickleback, a precautionary approach has been taken toward sampling activities in their lakes. Collection guidelines recommend limits for lethal and non-lethal sampling, and they restrict all sampling to half of the lakes (Rosenfeld *et al.* 2008b). They also prohibit the use of hybrids or AIS in any in situ studies (see **Protection, Status and Ranks** for details). While the guidelines were developed for Benthic and Limnetic Threespine Stickleback, their rationale may be relevant to other listed fish species in the province (Rosenfeld *et al.* 2008b), and it may be appropriate to apply them to the other DUs in the Threespine Stickleback species bundle. Publication of updates to these guidelines is pending (see **Protection, Status and Ranks**).

It is considered that the removal of less than 5% of Lotic and Lentic Threespine Stickleback from Misty Lake is an allowable harm unlikely to impact long-term persistence (DFO 2018b). While abundance estimates are uncertain, past and current collections (which are permit controlled) are considered to fall within this allowable limit (DFO 2018b). Unpermitted or excessive removal of specimens beyond these allowable limits could threaten the Misty Lake DUs (DFO 2018b). There is, however, no evidence of such activities taking place.

In addition to the collection of Threespine Stickleback from the DUs within this species bundle for scientific research, harvesting of other fish in their lakes could impact some of its DUs. For example, a decline in Coastal Cutthroat Trout from overfishing and/or the Common Loon from recreational disturbance could exert a decline in predation pressure on Giant Threespine Stickleback. This is considered a significant threat to this DU (COSEWIC 2013) as these predators form an important part of their selective regime (see **Limiting Factors** below). Similarly, any non-conforming recreational use of Misty Lake Ecological Reserve (e.g., consumptive resource uses such as fishing) are also recognized to potentially impact Misty Lake Lotic and Lentic Threespine Stickleback (COSEWIC 2006).

Logging (5.3) and Mining (3.2)

Physical alteration of the riparian zone has the capacity to directly alter aquatic habitat. For example, previous loss of riparian cover associated with forest harvesting adjacent to Misty Lake inlet has been associated with an increase in algal growth (COSEWIC 2006). While this can potentially affect Threespine Stickleback habitat use, the replacement of lost riparian vegetation along small coastal streams may be rapid, and so the impact may be temporary (COSEWIC 2006). Runoff can also threaten Threespine Stickleback habitat through physical alteration, including shoreline erosion, substrate changes (for example, smothering of nesting areas) and, in the case of Misty Lake Lotic Threespine Stickleback, stream water velocity changes (DFO 2018b, 2019). The persistence of the Threespine Stickleback species bundle DUs in spite of forestry activities conducted in the past, and, in some cases, industrial mining activities, suggests that impacts from riparian vegetation removal is a low risk.

## Limiting Factors

Threespine Stickleback in general can adapt readily to change and are resilient to environmental perturbations (Candolin 2009; Hatfield 2009). Nevertheless, their endemicity and short lifespan contribute to their vulnerability to specific threats. In summary, each DU most likely depends on (see **Habitat Requirements** for details):

- sustained productivity (littoral, pelagic, and/or stream); and
- sufficient littoral habitat with gently sloping sediment (e.g., silt, sand, gravel) beaches and natural littoral macrophytes for nesting and juvenile rearing (or structural complexity with riparian and vegetation cover in the stream habitat of Misty Lake Lotic Threespine Stickleback)

While Giant Threespine Stickleback likely depend on selective pressure exerted by gape-limited predators (predominantly Coastal Cutthroat Trout and loons), Unarmoured and Benthic and Limnetic Threespine Stickleback pairs require simple ecological communities for their persistence in an environment where there is little to no interspecific competition and predation (see **Habitat Requirements** and **Interspecific Interactions**).

Benthic and Limnetic Threespine Stickleback pairs, and Misty Lake Lotic and Lentic Threespine Stickleback are likely considerably more sensitive to habitat and environmental changes than their solitary freshwater counterparts. Because they have the capacity to interbreed when pre-mating barriers to reproductive isolation are removed, they are vulnerable to changes that disrupt these barriers. As a result, the environmental specificity of the Benthic and Limnetic Threespine Stickleback pairs and Misty Lake Lotic and Lentic Threespine Stickleback includes features of the environment that prevent hybridization, as well as those features needed to maintain a viable population (DFO 2018b, 2019). In summary, their habitat needs probably also include:

- natural light transmission to enable mate recognition; and
- maintenance of both gently sloping sediment beaches and natural littoral macrophytes to provide segregated nesting and juvenile rearing habitats.

While the specific limiting factors for the Threespine Stickleback species bundle DUs remain poorly understood, it would be prudent to maintain the lakes they inhabit within the current ranges of abiotic and biotic variables, including those that contribute to reproductive isolation.

## Number of Locations

For each of the Threespine Stickleback species bundle DUs confined to a single watershed (lake, creek, and/or associated inlets and outlets), the probable extent of any of these threats corresponds to the entire lake or creek since any change in water quality or habitat will likely affect most, if not all, of each of these DUs. Because the term “location” defines a geographically or ecologically distinct area in which a single threatening event can rapidly affect all individuals of the taxon present, there is a single location for Little Quarry Lake, Paxton Lake, and Vananda Creek Benthic and Limnetic Threespine Stickleback DUs, as well as for Misty Lake Lotic and Lentic Threespine Stickleback, where the most serious plausible threat is the introduction of aquatic invasive species. For Giant and Unarmoured Threespine Stickleback, each of which occupy lakes in different watersheds, threats would act independently across each lake. Consequently, two locations are recognized for Giant Threespine Stickleback, and three locations for Unarmoured Threespine Stickleback.

# PROTECTION, STATUS AND RANKS

## Legal Protection and Status

Under the *Species at Risk Act* (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 shall 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)].

All Benthic and Limnetic Threespine Stickleback pairs are listed as Endangered under Schedule 1 of SARA in keeping with their COSEWIC designations, except for Benthic and Limnetic Threespine Stickleback from Little Quarry Lake:

- COSEWIC designated Enos Lake Benthic and Limnetic Threespine Stickleback as Threatened in April 1988 (McPhail 1988). Their status was re-examined and designated Endangered in November 2002 (COSEWIC 2002). This status was subsequently re-examined and confirmed in April 2012 (COSEWIC 2012). Status was re-examined and designated Extinct in December 2023. They have been listed as Endangered under Schedule 1 of SARA since 2005.
- COSEWIC designated Paxton Lake Benthic and Limnetic Threespine Stickleback as Threatened in April 1998 (Houston 1998), a status that was re-examined and confirmed in April 1999 (Hatfield and Ptolemy 1999a). Status was re-examined and designated Endangered in May 2000. This status was re-examined and confirmed in April 2010 and December 2023. Similarly, COSEWIC designated Vananda Creek Benthic and Limnetic Threespine Stickleback as Threatened in April 1999 (Hatfield and Ptolemy 1999b). Status of both pairs was again re-examined in May 2000 and resulted in an Endangered designation (COSEWIC 2000a,b). This status was subsequently re-examined and confirmed in April 2010 (COSEWIC 2010a,b) and again in December 2023. The Paxton Lake and Vananda Creek Stickleback Species Pairs have been listed as Endangered under Schedule 1 of SARA since 2003.

- COSEWIC designated Little Quarry Lake Benthic and Limnetic Threespine Stickleback as Threatened in November 2015 (COSEWIC 2015a). Status was re-examined and confirmed in December 2023. They are currently under consideration for addition on Schedule 1 of SARA.

As a result of their Endangered listing under Schedule 1 of SARA, the *Recovery Strategy for Paxton Lake, Enos Lake, and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada* was published on the Species at Risk Public Registry in 2007 (National Recovery Team for Stickleback Species Pairs 2007). The recovery strategy was amended in 2019 to include updates to the biology, recovery feasibility assessment, threats, population abundance, population and distribution objectives, and identification of Critical Habitat (DFO 2019). While recovery for Benthic and Limnetic Threespine Stickleback from Paxton Lake and Vananda Creek was found to be biologically and technically feasible, recovery for Benthic and Limnetic Threespine Stickleback from the hybrid swarm in Enos Lake was deemed to be biologically and/or technically infeasible (DFO 2019). An *Action Plan for the Paxton Lake and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada [Proposed]* was first published in 2016 (DFO 2016b) and a new proposed action plan was published in 2018 (DFO 2018c) to reflect the amended recovery strategy. The final action plan was published in 2020 (DFO 2020a).

Progress towards recovery strategy implementation is discussed in the *Report on the Progress of Recovery Strategy Implementation for the Paxton Lake, Enos Lake, and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada for the Period 2007 – 2015* (DFO 2016a) and the *Report on the Progress of Recovery Strategy Implementation for the Paxton Lake and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada for the Period 2016 to 2021* (DFO 2022a). During this time period, progress has been made in the identification and protection of critical habitat (see **Habitat Protection and Ownership**), as well as in the following areas: the development of a preliminary report outlining an AIS plan, updates to scientific collection guidelines, consideration of Benthic-Limnetic pairs in the Texada Island Official Community Plan, and continued research into understanding the mechanism(s) underlying speciation and genetic divergence (DFO 2022a). While there has been measurable progress in implementing the broad strategies and research and management approaches outlined in the recovery strategy, priority next steps to support the survival and recovery of Paxton Lake and Vananda Creek Benthic and Limnetic Threespine Stickleback are outlined in Section 4 of the progress report (DFO 2022a).

COSEWIC designated Misty Lake Lotic and Lentic Threespine Stickleback as Endangered in November 2006 (COSEWIC 2006). Their status was re-examined and confirmed in December 2023. They have been listed as Endangered under Schedule 1 of SARA since 2010. Accordingly, the *Recovery Strategy for the Misty Lake Sticklebacks (Gasterosteus aculeatus) in Canada* was published on the Species at Risk Public Registry in 2018 (DFO 2018b), followed by an action plan in 2020 (DFO 2020b). The recovery strategy indicates that recovery for Misty Lake Lotic and Lentic Threespine Stickleback is biologically and technically feasible and identifies Critical Habitat (DFO 2018b). Further measures to be taken to address threats to Misty Lake Lotic and Lentic Threespine Stickleback and monitor its recovery are outlined in Section 1.2 of the action plan (DFO 2020b).

COSEWIC designated both Giant Threespine Stickleback and Unarmoured Threespine Stickleback as Special Concern in April 1980 (Moodie 1980) and 1983 (Reimchen 1984b), and re-examined and confirmed their status in November 2013 (COSEWIC 2013). The status of Giant Threespine Stickleback was re-examined and confirmed in December 2023. The status of Unarmoured Threespine Stickleback was re-examined and designated Endangered in December 2023. They have both been listed as Special Concern under Schedule 1 of SARA since 2019. Pursuant to SARA, a species listed as Special Concern must be managed to prevent it from becoming Endangered or Threatened, and a management plan must be prepared for the species and its habitat within three years after the wildlife species is listed as Special Concern. Accordingly, the *Management Plan for the Giant and Unarmoured Threespine Sticklebacks (*Gasterosteus aculeatus*) in Canada [Proposed]* was published on the Species at Risk Public Registry in 2022 (DFO 2022b)".

The Canadian *Fisheries Act* (section 35) provides protection for all fish and fish habitat, including the DUs within the Threespine Stickleback species bundle. The *Fisheries Act* (Department of Justice Canada 1996) delegates authority to the provinces and territories to establish and enforce fishing regulations. In accordance with this Act, the *BC Sport Fishing Regulations* stipulate that it is illegal to fish for, or catch and retain, any fish that are considered to be at risk in Canada and are legally protected by federal statutes. This includes Benthic and Limnetic Threespine Stickleback from Enos Lake, Paxton Lake and Vananda Creek, and Misty Lake Lotic and Lentic Threespine Stickleback (British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development 2021).

The DUs within the Threespine Stickleback species bundle are also afforded some protection in BC under the provincial *Wildlife Act*, which enables authorities to license anglers and angling guides, and to regulate scientific fish collection permits.

## Non-Legal Status and Ranks

Giant Threespine Stickleback, Misty Lake Lotic and Lentic Threespine Stickleback, and Benthic and Limnetic Threespine Stickleback from Paxton Lake and Vananda Creek all have a Global Conservation Status rank of Critically Imperilled (G1), meaning that they are considered to be “at very high risk of extinction or collapse due to very restricted range, very few populations or occurrences, very steep declines, very severe threats, or other factors” (NatureServe 2021). They are all also listed as Critically Imperilled nationally in Canada (N1) and subnationally in BC (S1), meaning that they are considered to be at “at very high risk of extirpation in the jurisdiction due to very restricted range, very few populations or occurrences, very steep declines, severe threats, or other factors” (NatureServe 2021). While Little Quarry Lake Benthic and Limnetic Threespine Stickleback have yet to be assigned a Global Conservation Status rank, they are listed as Critically Imperilled nationally in Canada (N1) and subnationally in BC (S1).

Unarmoured Threespine Stickleback have a Infraspecific Taxon Global Conservation Status rank of Critically Imperilled population (T1, NatureServe 2021). They are also listed as Critically Imperilled nationally in Canada (N1) and subnationally in BC (S1; NatureServe 2021).

Enos Lake Benthic and Limnetic Threespine Stickleback have a Global Conservation Status rank of Presumed Extinct (GX), which means they have “collapsed throughout its range, due to loss of key dominant and characteristic taxa and/or elimination of the sites and ecological processes on which the type depends” (NatureServe 2021). They are also listed as Presumed Extirpated nationally in Canada (NX) and subnationally in BC (SX), which means they are “believed to be extirpated from the jurisdiction (i.e., nation, or state/province)” (NatureServe 2021).

All of the DUs in the Threespine Stickleback species bundle are “Red-listed” by the Conservation Data Centre and the BC Ministry of Environment (BCCDC 2021).

The former Recovery Team for Non-Game Freshwater Fish Species in BC developed collection guidelines with the aim of limiting impacts from collection activities on Benthic and Limnetic Threespine Stickleback (Rosenfeld *et al.* 2008b). These guidelines restrict all sampling (lethal or capture and release) of Benthic and Limnetic Threespine Stickleback pairs to half of the lake. They also recommend limiting lethal and non-lethal sampling, such that scientific collections should constitute less than 10% of the mature fish population, as measured in spring and summer seasons. It is also recommended that a 5% mortality rate for “non- lethal” sampling should be factored into overall permitting levels. Other recommendations cover sampling methods and in situ scientific studies and include prevention of the spread of invasive species and disease organisms by sterilizing sampling equipment (traps, seines, boats, boots, etc.); prohibition on the use of non-wild, native hybrids in experimental in situ studies; and prohibitions on translocation of Threespine Stickleback, or any plant or animal that does not occur naturally in the lake, to Little Quarry Lake. While the guidelines were developed for the Benthic and Limnetic Threespine Stickleback pairs, they note that the rationale may be relevant to other listed fish species in the province (Rosenfeld *et al.* 2008b), and that it may be appropriate to consider applying the guidelines to the other DUs in the Threespine Stickleback species bundle.

DFO applied these guidelines to scientific research permitting processes under section 73 of the *Species at Risk Act* from 2008 to 2014. In 2015, the BC Ministry of Environment and DFO initiated updates to the guidelines (DFO 2016a); publication of these updates is pending (Woodruff *et al.* 2022).

Prevention and early detection of AIS will be key to the long-term conservation of the Threespine Stickleback species bundle. An "Exotic Species Prevention and Monitoring Plan for Threespine Stickleback Species Pair Lakes of Texada Island" (Guzek and Wilson 2022) is in preparation and will provide direction on the decisions and actions required to prevent the introduction of and conduct monitoring for exotic species in the Paxton Lake and Vananda Creek drainages. It may be appropriate to consider this plan as a guide for future AIS prevention and early detection in the watersheds of the other DUs in the Threespine Stickleback species bundle.

## Habitat Protection and Ownership

A 242-ha Wildlife Habitat Area was established in 2013 to protect and conserve the Vananda Creek Benthic and Limnetic Threespine Stickleback pair (British Columbia Ministry of Environment 2013). The *Recovery Strategy for Paxton Lake, Enos Lake, and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada* (DFO 2019) identified Critical Habitat as the entirety of Paxton, Spectacle, Priest and Emily lakes (each with a 15 m wide riparian buffer surrounding their wetted perimeters), along with the stream and marsh between Emily and Priest lakes, and the shallow marsh between Spectacle and Priest lakes (each with a 30 m wide riparian buffer surrounding their wetted perimeters). These areas identified as Critical Habitat are now protected from destruction through four SARA Critical Habitat Orders issued in 2020, which invoke the prohibition against the destruction of the identified Critical Habitat.

There are no habitat protection provisions specifically for the aquatic habitat of Little Quarry Lake Benthic and Limnetic Threespine Stickleback. Almost all lands surrounding Little Quarry Lake are Crown land, so they have some protection from the *BC Forest and Range Practices Act*, which has provisions to protect fish habitat from forestry activities (COSEWIC 2015a). The provincial *Riparian Areas Protection Regulation* also provides some protection for the riparian area around this lake. Little Quarry Lake Benthic and Limnetic Threespine Stickleback are under consideration for listing as Threatened under Schedule 1 of SARA. If they are so listed, their Critical Habitat will be identified.

There are no habitat protection provisions specifically for the aquatic habitat of Enos Lake. The Recovery Team for Stickleback Species Pairs previously identified Critical Habitat for Enos Lake Benthic and Limnetic Threespine Stickleback and recommended Critical Habitat identification of the entire wetted area of Enos Lake, plus a riparian buffer (Hatfield 2009). However, given the collapse of the pair into a hybrid swarm, their survival and recovery are not considered feasible based on current knowledge, and Critical Habitat has not been recognized (DFO 2019). All lands adjacent to Enos Lake are privately owned (COSEWIC 2012).

The *Recovery Strategy for the Misty Lake Sticklebacks (Gasterosteus aculeatus) in Canada* DFO 2018b) identified Critical Habitat as follows: the entirety of Misty Lake; the length of the inlet and outlet streams to the extent currently known to be occupied by Misty Lake Threespine Stickleback; the swampy transition zones between the lake and the inlet and outlet streams; a riparian area of 15 m surrounding the lake perimeter, and; a 30 m riparian area surrounding the swampy transition zones and the inlet and outlet streams adjacent to the currently known occupied extent. These areas identified as Critical Habitat are now protected from destruction through two SARA Critical Habitat Orders issued in 2018, which invoke the prohibition against the destruction of the identified Critical Habitat. In addition, Misty Lake and a small area around it is located within the Misty Lake Ecological Reserve. However, much of the watershed is not included in the reserve, including all of the outlet stream and most of the inlet stream habitat, so long-term protection of the whole lake-stream complex is not assured (COSEWIC 2006).

Most of the habitat of Giant Threespine Stickleback is afforded some level of protection from development. The Drizzle Lake watershed (837 ha) was established as an Ecological Reserve in 1973, principally to maintain the ecosystem for research on Giant Threespine Stickleback and their associated predators (BCMWLAP 2004). Consumptive uses such as hunting, fishing, camping and grazing, and removal of materials, plants or animals are prohibited. Mayer Lake and much of its watershed occurs within the boundaries of Naikoon Provincial Park. Although camping and recreational fishing are allowed here, rural and industrial development, such as real estate development and logging activities, are prohibited. There is one lakeside area of privately-owned land adjacent to Mayer Lake (COSEWIC 2013).

Rouge and Serendipity Lake watersheds, which contain Unarmoured Threespine Stickleback, are also located within the boundaries of Naikoon Provincial Park. Rouge Lake is located on a 130-ha private holding within this park. A 70-ha ecological reserve has been proposed for Boulton Lake watershed (PMT HG/QCI LUPP 2006). As it is located on Crown land, its fish habitat has some protection from forestry activities under the *BC Forest and Range Practices Act*.

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## INFORMATION SOURCES


Aguirre, W.E. 2009. Microgeographical diversification of threespine stickleback: body shape-habitat correlations in a small ecologically diverse Alaskan drainage. Biological Journal of the Linnean Society 98:139-151.

Ahmad, S.F., M. Jehangir, K. Srikulnath, and C. Martins. 2022. Fish genomics and its impact on fundamental and applied research of vertebrate biology. Reviews in Fish Biology and Fisheries 32:357-385.

Baker, J.A. 1994. Life history variation in female threespine stickleback. Pp. 144-187, *in* M.A. Bell and S.A. Foster (eds.). The evolutionary biology of the Threespine stickleback, Oxford University Press, New York.

Baker, J.A., K. Räsänen, J.-S. Moore, and A.P. Hendry. 2013. Genetic and plastic contributions to trait divergence between threespine habitats: female life-history traits in threespine stickleback from the Misty Lake system. Evolutionary Ecology Research 15:473-487.

Barrett, R.D., A. Paccard, T.M. Healy, S. Bergek, P.M. Schulte, D. Schluter, and S.M. Rogers. 2011. Rapid evolution of cold tolerance in stickleback. Proceedings of the Royal Society B: Biological Sciences 278:233-238.

Bay, R.A., M.E. Arnegard, G.L Conte, J. Best, N.L Bedford, S.R McCann, M.E Dubin, Y.F. Chan, F.C Jones, D.M Kingsley, D. Schluter, and K. Peichel. 2017. Genetic coupling of female mate choice with polygenic ecological divergence facilitates stickleback speciation. Current Biology 27:3344-3349.

BCCDC (British Columbia Conservation Data Centre). 2021. British Columbia Species and Ecosystems Explorer. Website: http://a100.gov.bc.ca/pub/eswp/ [accessed 5 November 2021].

BCMWLAP (British Columbia Ministry of Water, Land and Air Protection) Environmental Stewardship Division. 2004. Management Direction Statement for Drizzle Lake Ecological Reserve.

Behm, J., A.R. Ives, and J.W. Boughman. 2010. Breakdown in postmating isolation and the collapse of a species pair through hybridization. The American Naturalist 175:11-26.

Bell, M.A. 1976. Evolution of phenotypic diversity in *Gasterosteus aculeatus* superspecies on the Pacific Coast of North America. Systematic Zoology 25:211-227.

Bell, M.A. 1987. Interacting evolutionary constraints in pelvic reduction of threespine sticklebacks, *Gasterosteus aculeatus* (Pisces, Gasterosteidae). Biological Journal of the Linnean Society 31:347-382.

Bell, M.A., and S.A. Foster (eds.). 1994. The Evolutionary Biology of the Threespine Stickleback. Oxford University Press, Oxford, UK.

Bell, M.A., G. Ortí, J.A. Walker, and J.P. Koenings. 1993. Evolution of pelvic reduction in threespine stickleback fish: a test of competing hypotheses. Evolution 47:906-914.

Bergstrom, C.A., and T.E. Reimchen. 2000. Functional implications of fluctuating asymmetry among endemic populations of *Gasterosteus aculeatus*. Behaviour 137:1097-1112.

Bergstrom, C.A., and T.E. Reimchen. 2003. Asymmetry in structural defenses: insights into selective predation in the wild. Evolution 57:2128-2138.

Berner, D., D.C. Adams, A.C. Grandchamp, and A.P. Hendry. 2008. Natural selection drives patterns of lake-stream divergence in stickleback foraging morphology. Journal of Evolutionary Biology 21:1653-1665.

Berner, D., A.C. Grandchamp, and A.P. Hendry. 2009. Variable progress toward ecological speciation in parapatry: stickleback across eight lake-stream transitions. Evolution 63:1740-1753.

Berner, D., R. Kaeuffer, A.C. Grandchamp, J.A.M. Raeymaekers, K. Räsänen, and A.P. Hendry. 2011. Quantitative genetic inheritance of morphological divergence in a lake-stream stickleback ecotype pair: implications for reproductive isolation. Journal of Evolutionary Biology 24:1975-1983.

Berner, D., M. Roesti, A.P. Hendry, and W. Salzburger. 2010. Constraints on speciation suggested by comparing lake-stream stickleback divergence across two continents. Molecular Ecology 19:4963-4978.

Boughman, J.W. 2001. Divergent sexual selection enhances reproductive isolation in sticklebacks. Nature 411:944-947.

Bradford, M.J., C.P. Tovey, and L.M. Herborg. 2008a. Biological risk assessment for yellow perch (*Perca flavescens*) in British Columbia. Canadian Science Advisory Secretariat (CSAS) Research Document 2008/073.

Bradford, M.J., C.P. Tovey, and L.M. Herborg. 2008b. Biological risk assessment for Northern pike (*Esox lucius*), Pumpkinseed (*Lepomis gibbosus*), and Walleye (*Sander vitreus*) in British Columbia. Canadian Science Advisory Secretariat (CSAS) Research Document 2008/074.

British Columbia Ministry of Environment. 2013. Order – Wildlife Habitat Area: 2-250. Website: https://www.env.gov.bc.ca/cgi-bin/apps/faw/wharesult.cgi?search=number&select=2&number=250&submit2=Search [accessed June 10, 2022].

British Columbia Ministry of Environment – Parks. 2003. Misty Lake Ecological Reserve purpose statement.

British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development. 2021. Freshwater Fishing Regulations Synopsis. Website: https://www2.gov.bc.ca/gov/content/sports-culture/recreation/fishing-hunting/fishing/fishing-regulations [accessed May 22, 2022].

Candolin, U. 2009. Population responses to anthropogenic disturbance: lessons from three-spined sticklebacks *Gasterosteus aculeatus* in eutrophic habitats. Journal of Fish Biology 75:2108-2121.

Chan, Y.F., M.E. Marks, F.C. Jones, G. Jr. Villarreal, M.D. Shapiro, S.D. Brady, A.M. Southwick, D.M. Absher, J. Grimwood, J. Schmutz, R.M. Myers, D. Petrov, B. Jónsson, D. Schluter, M.A. Bell, D.M. Kingsley. 2010. Adaptive evolution of pelvic reduction in sticklebacks by recurrent deletion of a *Pitx1* enhancer. Science 327:302-305.

COSEWIC. 2000a. COSEWIC assessment and update status report on Paxton Lake Stickleback Species Pair *Gasterosteus* spp. in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vii + 13 pp.

COSEWIC. 2000b. COSEWIC assessment and update status report on the Vananda Creek Stickleback Species Pair *Gasterosteus* spp. in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vi + 17 pp.

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.

COSEWIC. 2006. COSEWIC assessment and status report on the Misty Lake sticklebacks *Gasterosteus* sp. (Misty Lake lentic stickleback and Misty Lake lotic stickleback) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vii + 27 pp.

COSEWIC. 2010a. COSEWIC assessment and status report on the Paxton Lake Benthic and Limnetic Threespine Stickleback Species Pair *Gasterosteus aculeatus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xiv + 22 pp.

COSEWIC. 2010b. COSEWIC assessment and status report on the Vananda Creek Benthic and Limnetic Threespine Stickleback Species Pair *Gasterosteus aculeatus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xiv + 25 pp.

COSEWIC. 2012. COSEWIC assessment and status report on the Enos Lake Benthic and Limnetic Threespine Stickleback Species Pair *Gasterosteus aculeatus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xii + 30 pp.

COSEWIC. 2013. COSEWIC assessment and status report on the Giant Threespine Stickleback *Gasterosteus aculeatus* and the Unarmoured Threespine Stickleback *Gasterosteus aculeatus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xiv + 62 pp.

COSEWIC. 2015a. COSEWIC assessment and status report on the Little Quarry Lake Benthic Threespine Stickleback and the Little Quarry Lake Limnetic Threespine Stickleback *Gasterosteus aculeatus* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xiii+37pp.

COSEWIC. 2015b. COSEWIC assessment and status report on the Red-legged Frog *Rana aurora* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xii + 69 pp.

COSEWIC. 2020. Status Reports: Guidelines for Recognizing Designatable Units, Government of Canada. Website: https://cosewic.ca/index.php/en/reports/preparing-status-reports/guidelines-recognizing-designatable-units.html [accessed October 30, 2021].

Deagle, B.E., F.C. Jones, D.M. Absher, D.M. Kingsley, and T.E. Reimchen. 2013. Phylogeography and adaptation genetics of stickleback from the Haida Gwaii archipelago revealed using genome-wide single nucleotide polymorphism genotyping. Molecular Ecology 22:1917-1932.

Deagle, B.E., F.C. Jones, Y.F. Chan, D.M. Absher, D.M. Kingsley, and T.E. Reimchen. 2012. Population genomics of parallel phenotypic evolution in stickleback across stream-lake ecological transitions. Proceedings of the Royal Society of London Series B 279:1277-1286.

Deagle, B.E., T.E. Reimchen, and D.B. Levin. 1996. Origins of endemic stickleback from the Queen Islands: mitochondrial and morphological evidence. Canadian Journal of Zoology 74:1045-1056.

Department of Justice Canada. 1996. British Columbia Sport Fishing Regulations, 1996 (SOR/96-137). Website: https://laws-lois.justice.gc.ca/eng/regulations/SOR-96-137/index.html [accessed November 5, 2021].

Dextrase, A., and N.E. Mandrak. 2006. Impacts of alien invasive species on freshwater fauna at risk in Canada. Biological Invasions 8: 3-24.

DFO (Fisheries and Oceans Canada). 2016a. Report on the Progress of Recovery Strategy Implementation for the Paxton Lake, Enos Lake, and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada for the Period 2007 – 2015. Species at Risk Act Recovery Strategy Report Series. Fisheries and Oceans Canada, Ottawa. iii + 23 pp.

DFO (Fisheries and Oceans Canada). 2016b. Action Plan for the Paxton Lake and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada [Proposed]. *Species at Risk Act* Action Plan Series. Fisheries and Oceans Canada, Ottawa. v + 40 pp.

DFO (Fisheries and Oceans Canada). 2018a. Recovery potential assessment for the Little Quarry Lake Stickleback species pair (*Gasterosteus aculeatus*). DFO Canadian Science Advisory Secretariat Science Response Report. 2018/006.

DFO (Fisheries and Oceans Canada). 2018b. Recovery Strategy for the Misty Lake Sticklebacks (*Gasterosteus aculeatus*) in Canada. Species at Risk Act Recovery Strategy Series. Fisheries and Oceans Canada, Ottawa. vi + 41 pp.

DFO (Fisheries and Oceans Canada). 2018c. Action Plan for the Paxton Lake and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada [Proposed]. Species at Risk Act Action Plan Series. Fisheries and Oceans Canada, Ottawa. v + 24 pp.

DFO (Fisheries and Oceans Canada). 2019. Recovery Strategy for Paxton Lake, Enos Lake, and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada. Species at Risk Act Recovery Strategy Series. Fisheries and Oceans Canada, Ottawa. ix + 45 pp.

DFO (Fisheries and Oceans Canada). 2020a. Action Plan for the Paxton Lake and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada. *Species at Risk Act* Action Plan Series. Fisheries and Oceans Canada, Ottawa. v + 24 pp.

DFO (Fisheries and Oceans Canada). 2020b. Action Plan for the Misty Lake Sticklebacks (*Gasterosteus aculeatus*) in Canada. *Species at Risk Act* Action Plan Series. Fisheries and Oceans Canada, Ottawa. iv + 13 pp.

DFO (Fisheries and Oceans Canada). 2022a. Report on the Progress of Recovery Strategy Implementation for the Paxton Lake and Vananda Creek Stickleback Species Pairs (*Gasterosteus aculeatus*) in Canada for the Period 2016 to 2021. *Species at Risk Act* Recovery Strategy Report Series. Fisheries and Oceans Canada, Ottawa. iv+ 16 pp.

DFO (Fisheries and Oceans Canada). 2022b. Management Plan for the Giant and Unarmoured Threespine Sticklebacks (*Gasterosteus aculeatus*) in Canada [Proposed]. *Species at Risk Act* Management Plan Series. Fisheries and Oceans Canada, Ottawa. v + 23 pp.

Edge, T.A., and B.W. Coad. 1983. Reduction of the pelvic skeleton in the Threespine stickleback *Gasterosteus aculeatus* in two lakes of Québec. Canadian Field-Naturalist 97:334-336.

Endler, J.A. 1982. Problems in distinguishing historical from ecological factors in biogeography. American Zoologist 22:441-452.

Filion, A., pers. comm. 2021. *Email correspondence with J. Gow.* December 2021. Scientific and GIS Project Officer, COSEWIC Science Support, Canadian Wildlife Service, Environment and Climate Change Canada, Gatineau, Quebec.

Frankham, R. 1995. Effective population size/adult population size ratios in wildlife: a review. Genetical Research 66:95-107.

Gach, M.H., and T.E. Reimchen. 1989. Mitochondrial DNA patterns among endemic stickleback from the Queen Charlotte Islands: a preliminary survey. Canadian Journal of Zoology 67:1324-1328.

Gambling, S.J., and T.E. Reimchen. 2012. Prolonged life span among endemic *Gasterosteus* populations. Canadian Journal of Zoology 90:284-290.

Gamlen-Greene, R. 2022. The ecology, distribution and population genetics of amphibians on Haida Gwaii, British Columbia. Ph.D. dissertation, University of British Columbia, Vancouver, British Columbia. 257 pp.

Gherardi, F., and Acquistapace, P. 2007. Invasive crayfish in Europe: the impact of *Procanbarus clarkia* on the littoral community of a Mediterranean lake. Freshwater Biology 52:1249-1259.

Giles, N. 1983. The possible role of environmental calcium levels during the evolution of phenotypic diversity in Outer Hebridean populations of the Three-spined stickleback, *Gasterosteus aculeatus*. Journal of Zoology 199:535-544.

Government of British Columbia. 2021. Water Licences Report. Website: http://a100.gov.bc.ca/pub/wtrwhse/water_licences.input [accessed December 16, 2021].

Gow, J.L., C.L. Peichel, and E.B. Taylor. 2006. Contrasting hybridization rates between sympatric three-spined sticklebacks highlight the fragility of reproductive barriers between evolutionarily young species. Molecular Ecology 15:739-752.

Gow, J.L., C.L. Peichel, and E.B. Taylor. 2007. Ecological selection against hybrids in natural populations of sympatric threespine sticklebacks. Journal of Evolutionary Biology 20:2173-2180.

Gow, J.L., S.M. Rogers, M. Jackson, and D. Schluter. 2008. Ecological predictions lead to the discovery of a benthic-limnetic sympatric species pair of threespine stickleback in Little Quarry Lake, British Columbia. Canadian Journal of Zoology 86:564-571.

Guzek, E., and G. Wilson. 2022. *Draft* Exotic Species Prevention and Monitoring Plan for Threespine Stickleback Species Pair (*Gasterosteus* spp.) Lakes of Texada Island. November 16, 2022 draft report No. RD xxx, Aquatic Ecosystems Branch, Province of British Columbia Ministry of Land, Waters and Resource Stewardship. Report prepared for British Columbia Ministry of Land, Waters and Resource Stewardship, and Fisheries and Oceans Canada. 65 pp.

Haenel, Q., K.B. Oke, T.G. Laurentino, A.P. Hendry, and D. Berner. 2021. Clinal genomic analysis reveals strong reproductive isolation across a steep habitat transition in stickleback fish. Nature Communications 12:4850.

Haines, G.E., Y.E. Stuart, D. Hanson, T. Tasneem, D.I. Bolnick, H.C.E. Larsson, and A.P. Hendry. 2020. Adding the third dimension to studies of parallel evolution of morphology and function: an exploration based on parapatric lake-stream stickleback. Ecology and Evolution 10:13297-13311.

Hanson, D., J.-S. Moore, E.B. Taylor, R.D.H. Barrett, and A.P. Hendry. 2016a. Assessing reproductive isolation using a contact zone between parapatric lake-stream stickleback ecotypes. Journal of Evolutionary Biology 29:2491-2501.

Hanson, D., R. Barrett, and A.P. Hendry. 2016b. Testing for parallel allochronic isolation in lake-stream stickleback. Journal of Evolutionary Biology 29:47-57.

Hatfield, T. 2001. Status of the stickleback species pair, *Gasterosteus* spp., in Hadley Lake, Lasqueti Island, British Columbia. Canadian Field-Naturalist 115:579-583.

Hatfield, T. 2009. Identification of critical habitat for sympatric Stickleback species pairs and the Misty Lake parapatric stickleback species pair. DFO Canadian Science Advisory Secretariat Research Document 2009/056.

Hatfield, T., and J. Ptolemy. 1999a. Update COSEWIC status report on the Paxton Lake Stickleback Species Pair *Gasterosteus* spp. in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 11 pp.

Hatfield, T., and J. Ptolemy, 1999b. COSEWIC update status report on the Vananda Creek Stickleback Species Pair *Gasterosteus* spp. in Canada, *in* COSEWIC assessment and update status report on the Vananda Creek stickleback species pair *Gasterosteus* spp. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 17 pp.

Hatfield, T., and D. Schluter. 1999. Ecological speciation in sticklebacks: environment-dependent hybrid fitness. Evolution 53:866-873.

Hendry, A. P., pers. comm. 2022. *Email correspondence with M. Docker.* July 2022. Professor, Department of Biology, McGill University, Montreal, Quebec.

Hendry, A.P., C.L. Peichel, B. Matthews, J.W. Boughman, and P. Nosil. 2013. Stickleback research: the now and the next. Evolutionary Ecology Research 15:111-141.

Hendry, A.P., and E.B. Taylor. 2004. How much of the variation in adaptive divergence can be explained by gene flow? An evaluation using lake-stream stickleback pairs. Evolution 58:2319-2331.

Hendry, A.P., E.B. Taylor, and J.D. McPhail. 2002. Adaptive divergence and the balance between selection and gene flow: lake and stream stickleback in the Misty system. Evolution 56:199-1216.

Houston, J. 1998. COSEWIC status report on the Texada Island Stickleback Species Pair *Gasterosteus* sp. in Canada. Committee on the status of Endangered Wildlife in Canada. Ottawa. 11 pp.

Hubbs, C.L. 1955. Hybridization between fish species in nature. Systematic Zoology 4:1-20.

Hutchinson, I., T. James, J. Clague, J.V. Barrie, and K. Conway. 2004. Reconstruction of late Quaternary sea-level change in southwestern British Columbia from sediments in isolation basins. Boreas 33:183-194.

Hyatt, K.D., and N.H. Ringler. 1989. Role of nest raiding and egg predation in regulating population density of threespine sticklebacks (*Gasterosteus aculeatus*) in a coastal British Columbia Lake. Canadian Journal of Fisheries and Aquatic Sciences 46:372-383.

IUCN. 2021. Threats classification scheme (version 3.2). International Union for the Conservation of Nature. Website: [https://www.iucnredlist.org/resources/threat-classification-scheme](https://www.iucnredlist.org/resources/threat-classification-scheme) [accessed November25, 2021].

Johnson, L.J., and E.B. Taylor. 2004. The distribution of divergent mitochondrial DNA lineages of threespine stickleback (*Gasterosteus aculeatus*) in the northeastern Pacific Basin: postglacial dispersal and lake accessibility. Journal of Biogeography 31:1073-1083.

Jones, F.C., Y.F. Chan, J. Schmutz, J. Grimwood, S.D. Brady, A.M. Southwick, D.M. Absher, R.M. Myers, T.E. Reimchen, B.E. Deagle, D. Schluter, and D.M. Kingsley. 2012. A genome-wide SNP genotyping array reveals patterns of global and repeated species pair divergence in sticklebacks. Current Biology 22:83-90.

Kaeuffer, R., C.L. Peichel, D.I. Bolnick, A.P. Hendry. 2012. Parallel and nonparallel aspects of ecological, phenotypic, and genetic divergence across replicate population pairs of lake and stream stickleback. Evolution 66:402-418.

Kirch, M., A. Romundset, M.T.P. Gilbert, F.C. Jones, A.D. Foote. 2021. Ancient and modern stickleback genomes reveal the demographic constraints on adaptation Current Biology 31:2027-2036.

Kitano, J., D.I. Bolnick, D.A. Beauchamp, M.M. Mazur, S. Mori, T. Nakano, C.L. Peichel. 2008. Reverse evolution of armor plates in the threespine stickleback. Current Biology 18:769-774.

Kraak, S.B.M., B. Mundwiler, and P.J.B. Hart. 2001. Increased number of hybrids between benthic and limnetic three-spined sticklebacks in Enos Lake, Canada; the collapse of a species pair? Journal of Fish Biology 58:1458-1464.

Lackey, A.C.R., and J.W. Boughman. 2017. Evolution of reproductive isolation in stickleback fish. Evolution 71:357-372.

Larson, G.L. 1976. Social behavior and feeding ability of two phenotypes of *Gasterosteus aculeatus* in relation to their spatial and trophic segregation in a temperate lake. Canadian Journal of Zoology 54:107-121.

Lavin, P.A., and J.D. McPhail. 1993. Parapatric lake and stream sticklebacks on northern Vancouver Island: disjunct distribution or parallel evolution? Canadian Journal of Zoology 71:11-17.

Leaver, S.D., and T.E. Reimchen. 2012. Abrupt changes in defence and trophic morphology of the giant threespine stickleback (*Gasterosteus* sp.) following colonization of a vacant habitat. Biological Journal of the Linnean Society 107:494-509.

Lucek, K., A. Sivasundar, D. Roy, O. Seehausen. 2013. Repeated and predictable patterns of ecotypic differentiation during a biological invasion: lake–stream divergence in parapatric Swiss stickleback. Journal of Evolutionary Biology 26:2691-2709.

Marchinko, K.B. 2009. Predation's role in repeated phenotypic and genetic divergence of armor in threespine stickleback. Evolution 63:127-138.

Marques, D.A, F.C. Jones, F. Di Palma, D. Kingsley, and T.E. Reimchen. 2018. Experimental evidence for rapid genomic adaptation to a new niche in an adaptive radiation. Nature Ecology & Evolution 2:1128-1138.

Marques, D.A, F.C. Jones, F. Di Palma, D. Kingsley, and T.E. Reimchen. 2022. Genomic mechanisms underlying repeated niche shifts in an adaptive radiation. Evolution doi:10.1111/evo.14490.

Mathews, W.H., J.G. Fyles, and H.W. Nasmith. 1970. Post-glacial crustal movements in southwestern British Columbia and adjacent Washington state. Canadian Journal of Earth Sciences 7:690-702.

Matthews, B., P. Ramsay, and K. Tienhaara. 2001. Population estimation and recovery planning for stickleback species pairs. An excerpt and adaptation from an undergraduate honours thesis at the University of British Columbia.

McGee, M.D., D. Schluter, and P.C. Wainwright. 2013. Functional basis of ecological divergence in sympatric stickleback. BMC Evolutionary Biology 13:277.

McKinnon, J.S., and H.D. Rundle. 2002. Speciation in nature: the threespine stickleback model systems. Trends in Ecology & Evolution 17:480-488.

McPhail, J.D. 1969. Predation and the evolution of a stickleback (*Gasterosteus*). Journal of the Fisheries Research Board of Canada 26:3183-3208.

McPhail, J.D. 1977. Inherited interpopulation differences in size at first reproduction in threespine stickleback, *Gasterosteus aculeatus* L. Heredity 38:53-60.

McPhail, J.D. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): morphological and genetic evidence for a species pair in Enos Lake, British Columbia. Canadian Journal of Zoology 62:1402-1408.

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.

McPhail, J. D. 1989. Status of the Enos Lake stickleback species pair, *Gasterosteus* spp. Canadian Field-Naturalist 103:216-219.

McPhail, J.D. 1992. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): evidence for a species-pair in Paxton Lake, Texada Island, British Columbia. Canadian Journal of Zoology 70:361-369.

McPhail, J.D. 1993. Ecology and evolution of sympatric sticklebacks (*Gasterosteu*s): origin of the species pairs. Canadian Journal of Zoology 71:515-523.

McPhail, J.D. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of southwestern British Columbia. Pp. 399-437, *in* M.A. Bell and S.A. Foster (eds.). The Evolutionary Biology of the Threespine Stickleback. Oxford University Press, Oxford, UK.

McPhail, J.D. 2007. The Freshwater Fishes of British Columbia. University of Alberta Press, Edmonton, AB.

Moodie, G.E.E. 1972a. Morphology, life history, and ecology of an unusual stickleback (*Gasterosteus aculeatus*) in the Queen Charlotte Islands, Canada. Canadian Journal of Zoology 50:721–732.

Moodie, G.E.E. 1972b. Predation, natural selection and adaptation in an unusual threespine stickleback. Heredity 28:155-167.

Moodie, G.E.E. 1980. COSEWIC status report on the Giant Stickleback *Gasterosteus sp.* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 6 pp.

Moodie, G.E.E. 1984. Status of the Giant (Mayer Lake) Stickleback, *Gasterosteus* sp., on the Queen Islands, British Columbia. Canadian Field-Naturalist 98:115-119.

Moodie, G.E.E., and T.E. Reimchen. 1973. Endemism and conservation of sticklebacks in the Queen Charlotte Islands. The Canadian Field-Naturalist 87:173-175.

Moodie, G.E.E., and T.E. Reimchen. 1976. Phenetic variation and habitat differences in *Gasterosteus* populations of the Queen Charlotte Islands. Systematic Zoology 25:49-61.

Moore, J.S., and A.P. Hendry. 2005. Both selection and gene flow are necessary to explain adaptive divergence: evidence from clinal variation in stream stickleback. Evolutionary Ecology Research 7:871-886.

Nagel, L., and D. Schluter. 1998. Body size, natural selection, and speciation in sticklebacks. Evolution 52:209-218.

National Recovery Team for Stickleback Species Pairs. 2007. Recovery Strategy for Paxton Lake, Enos Lake, and Vananda Creek Stickleback Species Pairs (*Gasterosteus* spp.) in Canada. Species at Risk Act Recovery Strategy Series. Fisheries and Oceans Canada, Ottawa. v + 31 pp.

NatureServe. 2021. NatureServe Explorer [web application]. NatureServe, Arlington, Virginia. Website: https://explorer.natureserve.org/ [accessed November 5, 2021].

Nomura, M. 2005. Population study of Paxton Lake stickleback species pair – 2005. unpublished data report.

Nosil, P., and D. Schluter. 2011. The genes underlying the process of speciation. Trends in Ecology and Evolution 26:160-167.

Nyström, P., C. Brönmark, W. Granéli. 1996. Patterns in benthic food webs: a role for omnivorous crayfish? Freshwater Biology 36:631-646.

Oke, K.B., M. Lu, and A.P. Hendry. 2017. Population size and geographical extent of Misty Lake lotic and lentic threespine stickleback. A report prepared for Fisheries and Oceans Canada and the Province of British Columbia.

Oravec, T.J., and T.E. Reimchen. 2013. Divergent reproductive life histories in Haida Gwaii stickleback (*Gasterosteus* spp.). Canadian Journal of Zoology 91:17-24.

O'Reilly, P., T.E. Reimchen, R. Beech, and C. Strobeck. 1993. Mitochondrial DNA in Gasterosteus and Pleistocene glacial refugium on the Queen Charlotte Islands, British Columbia. Evolution 47:678-684.

Ormond, C.I., J.S. Rosenfeld, and E.B. Taylor. 2011. Environmental determinants of threespine stickleback species pair evolution and persistence. Canadian Journal of Fisheries and Aquatic Science 68:1983-1997.

Paccard, A., D. Hanson, Y.E. Stuart, F.A. von Hippel, M. Kalbe, T. Klepaker, S. Skúlason, B.K. Kristjánsson, D.I. Bolnick, A.P. Hendry, and R.D.H. Barrett. 2020. Repeatability of adaptive radiation depends on spatial scale: regional versus global replicates of stickleback in lake versus stream habitats. Journal of Heredity 111:43-56.

Parmesan, C., and G. Yohe. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37-42.

PGL Environmental Consultants. 2016. Enos Lake Protection and Monitoring Program. PGL File: 4675-01.01. v + 36 pp.

PMT HG/QCI LUPP (Process Management Team for the Haida Gwaii / Queen Charlotte Islands Land Use Planning Process). 2006. Haida Gwaii Queen Charlotte Islands Land Use Plan Recommendations Report. Community Planning Forum, Haida Gwaii.1-xx pp.

Raeymaekers, J.A.M., L. Delaire, and A.P. Hendry. 2009. Genetically-based differences in nest characteristics between lake, inlet, and hybrid threespine stickleback from the Misty system, British Columbia, Canada. Evolutionary Ecology Research 11:905-919.

Raeymaekers, J.A.M., M. Boisjoly, L. Delaire, D. Berner, D., K. Räsänen, and A.P. Hendry. 2010. Testing for mating isolation between ecotypes: laboratory experiments with lake, stream and hybrid stickleback. Journal of Evolutionary Biology 23:2694-2708.

Räsänen, K., M. Delcourt, L.J. Chapman, A.P. Hendry. 2012. Divergent selection and then what not: the conundrum of missing reproductive isolation in Misty Lake and stream stickleback. International Journal of Ecology 2012:1-14.

Räsänen, K., and A.P. Hendry. 2014. Asymmetric reproductive barriers and mosaic reproductive isolation: insights from Misty lake-stream stickleback. Ecology and Evolution 4:1166-1175.

Ravinet, M., P.A. Prodöhl, and C. Harrod. 2013. Parallel and nonparallel ecological, morphological and genetic divergence in lake-stream stickleback from a single catchment. Journal of Evolutionary Biology 26:86-204.

Reimchen, T.E. 1980. Spine deficiency and polymorphism in a population of *Gasterosteus aculeatus*: an adaptation to predators? Canadian Journal of Zoology 58:1232-1244.

Reimchen, T.E. 1984a. Status of unarmoured and spine-deficient populations (unarmoured stickleback) of threespine stickleback, *Gasterosteus* sp., on the Queen Charlotte Islands, British Columbia. Canadian Field-Naturalist 98:120-126.

Reimchen, T.E. 1984b. COSEWIC status report on the Charlotte Unarmoured Stickleback *Gasterosteus* sp. in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 13 pp.

Reimchen, T.E. 1989. Loss of nuptial color in threespine sticklebacks (*Gasterosteus aculeatus*). Evolution 43: 450-460.

Reimchen, T.E. 1990. Size-structured mortality in a threespine stickleback (*Gasterosteus aculeatus*) – cutthroat trout (*Oncorhynchus clarki*) community. Canadian Journal of Fisheries and Aquatic Sciences 47: 1194-1205.

Reimchen, T.E. 1994. Predators and evolution in threespine stickleback. Pp. 240-273. *in* M.A. Bell and S.A. Foster (eds.). The Evolutionary Biology of the Threespine Stickleback, Oxford University Press, New York.

Reimchen, T.E. 2004. Update status report on giant (Mayer Lake) stickleback, *Gasterosteus aculeatus* [Unpublished Interim Draft Report]. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 1-17 pp.

Reimchen, T.E., pers. comm. 2022. *Meeting correspondence with J. Gow.* June 2022. Professor, Department of Biology, University of Victoria, Victoria, British Columbia.

Reimchen, T.E., and C.A. Bergstrom. 2009. The ecology of asymmetry in stickleback defense structures. Evolution 63:115-126.

Reimchen, T.E., C.A. Bergstrom, and P. Nosil. 2013. Natural selection and the adaptive radiation of Haida Gwaii stickleback. Evolutionary Ecology Research 15:241-269.

Reimchen, T.E., and P. Nosil. 2001a. Lateral plate asymmetry, diet and parasitism in threespine stickleback. Journal of Evolutionary Biology 14:632-645.

Reimchen, T.E., and P. Nosil. 2001b. Dietary differences between symmetrical and asymmetrical pelvic phenotypes in stickleback. Canadian Journal of Zoology 79:533-539.

Reimchen, T.E., and P. Nosil. 2002. Temporal variation in divergent selection on spine number in a population of threespine stickleback. Evolution 56:2472-2483.

Reimchen, T.E., and P. Nosil. 2004. Variable predation regimes predict the evolution of sexual dimorphism in a population of threespine stickleback. Evolution 58:1274-1281.

Reimchen, T.E., and P. Nosil. 2006. Replicated ecological landscapes and the evolution of morphological diversity among *Gasterosteus* populations from an archipelago on the west coast of Canada. Canadian Journal of Zoology 84:643-654.

Reimchen, T.E., E.M. Stinson, and J.S. Nelson. 1985. Multivariate differentiation of parapatric and allopatric populations of threespine stickleback in the Sangan River watershed, Queen Charlotte Islands. Canadian Journal of Zoology 63:2944-2951.

Reusch, T.B.H., K.M. Wegner, and M. Kalbe. 2001. Rapid genetic divergence in postglacial populations of threespine stickleback (*Gasterosteus aculeatus*): the role of habitat type, drainage and geographical proximity. Molecular Ecology 10:2435-2445.

Rhymer, J.M., and D. Simberloff. 1996. Extinction by hybridization and introgression. Annual Review of Ecology and Systematics 27: 83-109.

Ridgway, M.S., and J.D. McPhail. 1984. Ecology and evolution of sympatric sticklebacks (*Gasterosteus*): mate choice and reproductive isolation in the Enos Lake species pair. Canadian Journal of Zoology 62:1813-1818.

Rosenfeld, J., K. Campbell, E. Leung, and J. Bernhardt. 2008a. Effects of alien crayfish on macrophytes and benthic invertebrates in Enos Lake: implications for hybridization of limnetic and benthic stickleback species pairs. Interim Report for BC Forest Science Program Project Y081209.

Rosenfeld, J., D. Sneep, T. Hatfield, D. McPhail, J. Richardson, D. Schluter, E. Taylor, and P. Wood. 2008b. Guidelines for the Collection and In Situ Scientific Study of Stickleback Species Pairs (*Gasterosteus* spp.). Report prepared for Fisheries and Oceans Canada, Vancouver, British Columbia. 6 pp.

Rosenthal, S. K., S.S. Stevens, and D.M. Lodge. 2006. Whole-lake effects of invasive crayfish (*Orconectes* spp.) and the potential for restoration. Canadian Journal of Fisheries and Aquatic Sciences 63:1276-1285.

Rosenzweig, C., D. Karoly, M. Vicarelli, P. Neofotis, Q. Wu, G. Casassa, A. Menzel, T.L. Root, N. Estrella, B. Seguin, P. Tryjanowski, C. Liu, S. Rawlins, and A. Imeson. 2008. Attributing physical and biological impacts to anthropogenic climate change. Nature 453:353-358.

Rundle, H.D., and P. Nosil. 2005. Ecological speciation. Ecology Letters 8:336-352.

Sala, O.E., F.S. Chapin III, J.J. Armesto, E. Berlow, J. Bloomfield, R. Dirzo, E. Huber-Sanwald, L.F. Huenneke, R.B. Jackson, A. Kinzig, R. Leemans, D.M. Lodge, H.A. Mooney, M. Oesterheld, N.L. Poff, M.T. Sykes, B.H. Walker, M. Walker, and D.H. Wall. 2000. Global biodiversity scenarios for the year 2100. Science 287:1770-1774.

Schluter, D. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. Ecology 76:82-90.

Schluter, D., and J.D. McPhail. 1992. Ecological character displacement and speciation in sticklebacks. The American Naturalist 140:85-108.

Schluter, D., M. Roesti, and T. Veen. 2017. Mark-recapture estimates of stickleback population sizes in Paxton and Priest Lakes in 2016. V5.1. Unpublished Report Prepared for the B.C. Ministry of Environment and Fisheries and Oceans Canada.

Scott, W.B., and E.J. Crossman. 1973. Freshwater Fishes of Canada. Bulletin of the Fisheries Research Board of Canada 184.

Seehausen, O., R.K. Butlin, I. Keller, C.E. Wagner, J.W. Boughman, P.A. Hohenlohe, C.L. Peichel, G.P. Saetre, C. Bank, A. Brännström, A. Brelsford, C.S. Clarkson, F. Eroukhmanoff, J.L. Feder, M.C. Fischer, A.D. Foote, P. Franchini, C.D. Jiggins, F.C. Jones, A.K. Lindholm, K. Lucek, M.E. Maan, D.A. Marques, S.H. Martin, B. Matthews, J.I. Meier, M. Möst, M.W. Nachman, E. Nonaka, D.J. Rennison, J. Schwarzer, E.T. Watson, A.M. Westram, A. Widmer. 2014. Genomics and the origin of species. Nature Reviews Genetics 15:176-192.

Shapiro, M.D., M.E. Marks, C.L. Peichel, K. Nereng, B.K. Blackman, B. Jonsson, D. Schluter, and D.M. Kingsley. 2004. Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. Nature 428:717-723.

Sharpe, D.M.T., K. Räsänen, D. Berner, and A.P. Hendry. 2008. Genetic and environmental contributions to the morphology of lake and stream stickleback: implications for gene flow and reproductive isolation. Evolutionary Ecology Research 10:849-866.

Spoljaric, M.A., and T.E. Reimchen. 2007. 10 000 years later: Evolution of body shape in Haida Gwaii threespine stickleback. Journal of Fish Biology 70:1484-1503.

Spoljaric, M.A., and T.E. Reimchen. 2008. Habitat-dependent reduction of sexual dimorphism in geometric body shape of Haida Gwaii threespine stickleback. Biological Journal of the Linnean Society 95:505-516.

Stinson, E.M. 1983. Threespine stickleback (*Gasterosteus aculeatus*) in Drizzle Lake and its inlet, Queen Charlotte Islands: ecological and behavioural relationships and their relevance to reproductive isolation. Master’s thesis, University of Alberta, Edmonton, AB, Canada.

Stuart, Y., T. Veen, J.N. Weber, D. Hanson, M. Ravinet, B.K. Lohman, C.J. Thompson, T. Tasneem, A. Doggett, R. Izen, N. Ahmed, R.D.H. Barrett, A.P. Hendry, C.L. Peichel, and D.I. Bolnick. 2017. Contrasting effects of environment and genetics generate a continuum of parallel evolution. Nature Ecology and Evolution 1:0158.

Taylor, E.B. 2004. An analysis of homogenization and differentiation of Canadian freshwater fish faunas with an emphasis on British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 61:68-79.

Taylor, E.B., J.W. Boughman, M. Groenenboom, M. Sniatynski, D. Schluter, and J.L. Gow. 2006. Speciation in reverse: morphological and genetic evidence of the collapse of a three-spined stickleback (*Gasterosteus aculeatus*) species pair. Molecular Ecology 15:343-355.

Taylor, E.B. and J.D. McPhail. 1999. Evolutionary history of an adaptive radiation in species pairs of threespine sticklebacks (*Gasterosteus*): insights from mitochondrial DNA. Biological Journal of the Linnean Society 66:271-291.

Taylor, E.B., and J.D. McPhail. 2000. Historical contingency and ecological determinism interact to prime speciation in sticklebacks, *Gasterosteus*. Proceedings of the Royal Society of London, Series B 267:2375-2384.

Taylor, E.B., and R.S. Piercey. 2018. Going, going, gone: evidence for loss of an endemic species pair of threespine sticklebacks (*Gasterosteus aculeatus*) with implications for protection under species-at-risk legislation. Conservation Genetics 19:297-308.

Thompson, K.A., C.L. Peichel, D.J. Rennison, M.D. McGee, A.Y.K. Albert, T.H. Vines, A.K. Greenwood, A.R. Wark, Y. Brandvain, M. Schumer, and D. Schluter. 2022. Analysis of ancestry heterozygosity suggests that hybrid incompatibilities in threespine stickleback are environment dependent. PLoS Biology 20:e3001469.

Thompson, C.E., E.B. Taylor, and J.D. McPhail. 1997. Parallel evolution of lake-stream pairs of threespine stickleback (*Gasterosteus aculeatus*) inferred from mitochondrial DNA variation. Evolution 51:1955-1965.

Tovey, C.P., M.J. Bradford, and M.L. Herborg. 2008. Biological Risk Assessment for Smallmouth Bass (*Micropterus dolomieu*) and Largemouth Bass (*Micropterus salmoides*) in British Columbia. Canadian Science Advisory Secretariat Research Document 2008/075.

Velema, G.J., J.R. Rosenfeld, E.B. Taylor. 2012. Effects of invasive American signal crayfish (*Pacifastacus leniusculus*) on the reproductive behaviour of threespine stickleback (*Gasterosteus aculeatus*) sympatric species pairs. Canadian Journal of Zoology 90:1328-1338.

Walker, I.J, J.V. Barrie, A.H. Dolan, Z. Gedalof, G. Manson, D. Smith, and S. Wolfe. 2007. Coastal vulnerability to climate change and sea-level rise, Northeast Graham Island, Haida Gwaii (Queen Charlotte Islands), British Columbia. Ottawa, Ontario: Prepared for the Climate Change Impacts and Adaptation Directorate, Natural Resources Canada.

Woodruff, P., M. Nantel, and G. Wilson. 2022. *Draft* Guidelines for the Collection and In Situ Scientific Study of Stickleback Species Pairs (*Gasterosteus* spp.) in British Columbia, 2022 Update. July 27, 2022 draft. Aquatic Ecosystems Branch, Province of British Columbia Ministry of Land, Waters and Resource Stewardship. Report prepared for British Columbia Ministry of Land, Waters and Resource Stewardship, and Fisheries and Oceans Canada. 18 pp.

Wootton, R.J. 1976. The Biology of the Sticklebacks. Academic Press, London.

Xie, K.T., G. Wang, A.C. Thompson, J.I. Wucherpfennig, T.E. Reimchen, A.D.C. MacColl, D. Schluter, M.A. Bell, K.M. Vasquez, and D.M. Kingsley. 2019. DNA fragility in the parallel evolution of pelvic reduction in stickleback fish. Science 363:81-84.

## BIOGRAPHICAL SUMMARY OF REPORT WRITER(S)

Jennifer Gow, M. Res, Ph.D., has worked in the field of conservation biology and molecular ecology for more than a decade. Jennifer began applying her expertise in molecular ecology to Threespine Stickleback native to British Columbia in 2003, when she started postdoctoral work at the University of British Columbia. Her research gave insight into the ecological and evolutionary forces that shape patterns of genetic diversity in these and other freshwater fishes native to Canada. Jennifer has written extensively on the Threespine Stickleback species bundle in peer-reviewed scientific papers and reports.

## COLLECTIONS EXAMINED

No collections were examined for this report.

## Appendix 1 - Threats Assessment Worksheet DU1

| | | | | |
|---|---|---|---|---|
| **Species or Ecosystem Scientific Name** | Giant Threespine Stickleback (*Gasterosteus aculeatus*) DU1 | | | |
| **Element ID** | | **Elcode** | | |
| **Date:** | June 3, 2022 | | | |
| **Assessor(s):** | Dwayne Lepitzki, Margaret Docker, Jennifer Gow, Jennifer Diment, Trevor Pitcher, Mark Ridgway, Dolph Schluter, Cassandra Silverio, Greg Wilson, Joanna James, Marie-Eve Corbin | | | |
| **References:** | COSEWIC Draft Status Report, February 2022 | | | |

| Overall Threat Impact calculation Help: | | Level 1 Threat Impact Counts | |
|---|---|---|---|
| **Threat Impact** | | **high range** | **low range** |
| A | Very High | 0 | 0 |
| B | High | 0 | 0 |
| C | Medium | 0 | 0 |
| D | Low | 1 | 1 |
| **Calculated Overall Threat Impact:** | | Low | Low |

| | |
|---|---|
| **Assigned Overall Threat Impact:** | **D = Low** |
| **Impact Adjustment Reasons:** | |
| **Overall Threat Comments** | Remote locations on Haida Gwaii, BC, and within ecological reserve and provincial park offer some security for subpopulations. Threat of introduction of aquatic invasive species (AIS) is low; likelihood is high, but consequences would likely be slight. Generation time ~4-5 years in Drizzle Lake and 3 years in Mayer Lake. Based on estimates from the 1980s, ~43% of total population in Drizzle Lake; ~57% in Mayer Lake. Previous assessment was also D = Low, although Red-legged Frog was not a factor in previous assessment. |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 1 | Residential & Commercial Development | | | | | | Drizzle Lake threat considered negligible; potential threat for Mayer Lake unknown. |
| 1.1 | Housing & urban areas | | | | | | Development proposal on Mayer Lake in Naikoon Provincial Park. The 100-acre private lot was clear-cut 3 years ago, and owners would like to remove an additional 3,000 cubic metres of wood and subdivide/sell 30–35 recreational lots. However, property owners cannot barge anything up the lake and, if they want to use the road/parking lot/boat launch, they will have to apply for a park use permit, which would be adjudicated by the Haida Nation and the Province. Discussions are in progress about the feasibility of acquisition and/or having the owners donate the land; unless it is acquired, recreational development is a possibility/threat to Mayer Lake (Lucy Stefanyk, Area Supervisor Naikoon Park, by email). |
| 1.2 | Commercial & industrial areas | | | | | | |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 1.3 | Tourism & recreation areas | | | | | | Boat launch/campground/picnic at end of road on south end of Mayer Lake, but no known plans for expansion within 10–15 years |
| 2 | Agriculture & Aquaculture | | | | | | |
| 2.1 | Annual & perennial non-timber crops | | | | | | |
| 2.2 | Wood & pulp plantations | | | | | | |
| 2.3 | Livestock farming & ranching | | | | | | |
| 2.4 | Marine & freshwater aquaculture | | | | | | |
| 3 | Energy Production & Mining | | | | | | |
| 3.1 | Oil & gas drilling | | | | | | |
| 3.2 | Mining & quarrying | | | | | | |
| 3.3 | Renewable energy | | | | | | |
| 4 | Transportation & Service Corridors | | | | | | |
| 4.1 | Roads & railroads | | | | | | Both lakes close to current road, with road access to Mayer Lake, but no known plans for expansion of current road or for new road or culvert/bridge replacement directly in habitat; pollution from roads under 9.1 |
| 4.2 | Utility & service lines | | | | | | |
| 4.3 | Shipping lanes | | | | | | |
| 4.4 | Flight paths | | | | | | Noise from Helijet under 9.6 |
| 5 | Biological Resource Use | | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| 5.1 | Hunting & collecting terrestrial animals | | | | | | |
| 5.2 | Gathering terrestrial plants | | | | | | |
| 5.3 | Logging & wood harvesting | | | | | | Although Mayer Lake has extensive watershed catchment in Naikoon Provincial Park, forestry operations could impact some drainages that flow into the lake. However, direct impact from logging on shoreline of Mayer Lake (e.g., debris falling directly into habitat) unlikely. Pollution (e.g., sedimentation) from forestry under 9.3 |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 5.4 | Fishing & harvesting aquatic resources | | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | Scientific collection permit approved for lethal sampling of 100 adults from each location for at least 2 years. Effect of removing 100 adults negligible with 75,000 and > 100,000 adults total in Mayer and Drizzle Lake, respectively. Not used as bait. Overfishing of Cutthroat Trout (a predator), which could decrease selective pressures to retain large form, under 7.3. Non-lethal research under 6.3 |
| 6 | Human Intrusions & Disturbance | | | | | | |
| 6.1 | Recreational activities | | | | | | No direct mortality from boats on lake; boats can be dragged up on shore at Mayer Lake or Drizzle Lake (no dock), but no or negligible direct impact |
| 6.2 | War, civil unrest & military exercises | | | | | | |
| 6.3 | Work & other activities | | | | | | No known plans for research that does not kill or permanently remove stickleback, but effect would be negligible; no known plans for research on other species (e.g., loons, Raccoon, Red-legged Frog) that would have direct impact on stickleback |
| 7 | Natural System Modifications | | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |
| 7.1 | Fire & fire suppression | | | | | | Area not prone to forest fires; water bucketing not expected |
| 7.2 | Dams & water management/use | | | | | | |
| 7.3 | Other ecosystem modifications | | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | Introduced Beaver (present, widespread across Graham Island, including Mayer and Drizzle lakes) could alter limnology and habitat structure (shoreline extent); Beaver impact seen on riparian vegetation despite fences around trees to reduce effect; influence on allochthonous input not negligible, bigger at Mayer Lake, minimal effect likely at Drizzle Lake. Changes in predation pressure from trout and loons pervasive; effect expected if population sizes of these native predators change (see Threat 9). Reimchen and Douglas (2021; Canadian Field-Naturalist 135: 28-38) shows recent increase and decrease, respectively, in population estimates of Common and Red-throated Loons; potential impacts from reduced biomass/nutrient input to these nutrient-deprived lakes if there is a decline in abundance of anadromous salmonids, but severity of threat is unknown. |
| 8 | Invasive & Other Problematic Species & Genes | D | Low | Large (31-70%) | Slight (1-10%) | High (Continuing) | |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 8.1 | Invasive non-native/alien species/diseases | D | Low | Large (31-70%) | Slight (1-10%) | High (Continuing) | Threat from introduction of AIS is considered low; likelihood is high, but consequences would likely be slight because acidic conditions of habitat likely unsuitable to invasive predatory fishes. Also, native fish community likely to control impact of Red-legged Frog (RLF). RLF found in roadside ponds adjacent to Mayer Lake; not yet observed in either lake, although it is probably already in Mayer Lake (numbers are probably low and it is not known whether it is breeding there). Its appearance in both lakes within 10 years is considered inevitable, given its rapid spread on Graham Island. Probably would not be direct predation of RLF on Giant Threespine Stickleback, but there is competition for sticklebacks by tadpoles (modification to the habitat/ecosystem by AIS would be scored under 7.3). Scope 31-70% and timing high because RLF are likely already in Mayer Lake or invasion is imminent. Severity slight because, even though 80% decline in Unarmoured Threespine Stickleback due to RLF in Boulton Lake, no other fishes in Boulton Lake; in Mayer and Drizzle lakes, with other fishes/natural predators that eat tadpoles, RLF would likely be less problematic. In the course of their research, Andrew Hendry's group from McGill can monitor for invasive species. |
| 8.2 | Problematic native species/diseases | | | | | | |
| 8.3 | Introduced genetic material | | | | | | |
| 8.4 | Problematic species/diseases of unknown origin | | | | | | |
| 8.5 | Viral/prion-induced diseases | | | | | | |
| 8.6 | Diseases of unknown cause | | | | | | |
| 9 | Pollution | | | | | | |
| 9.1 | Domestic & urban wastewater | | | | | | Could be a potential threat if development of Mayer Lake subdivisions go ahead (see 1.1) |
| 9.2 | Industrial & military effluents | | | | | | Oil, chemicals not being transported here by road, so little concern regarding spills from road transportation. Possibly some pollution from recreational users at Mayer Lake road access (e.g., engine oil). Increased Helijet use over both lakes, possibility of collision with loons; possibility of pollution from Helijet crashing into lake, which could have a large ecological impact (see 9.6) |
| 9.3 | Agricultural & forestry effluents | | | | | | No sedimentation from forestry |
| 9.4 | Garbage & solid waste | | | | | | Potential for some pollution from recreational users at Mayer Lake road access (e.g., garbage) |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 9.5 | Air-borne pollutants | | | | | | |
| 9.6 | Excess energy | | | | | | Both lakes under flight paths of Helijets. Low flight paths have dramatic impact on soundscapes that could potentially displace loons, as well as risk of collision with them (e.g., up to 50 loons in a flock flying perpendicular to flight path pose a considerable risk of collision) |
| 10 | Geological Events | | | | | | |
| 10.1 | Volcanoes | | | | | | |
| 10.2 | Earthquakes/tsunamis | | | | | | Major previous earthquake on Haida Gwaii was about 70 years ago, although impact assumed to be small; not in tsunami zone |
| 10.3 | Avalanches/landslides | | | | | | |
| 11 | Climate Change & Severe Weather | | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |
| 11.1 | Habitat shifting & alteration | | | | | | |
| 11.2 | Droughts | | | | | | |
| 11.3 | Temperature extremes | | | | | | Not noticeably warmer water on Haida Gwaii according to Tom Reimchen who has been working on these lakes since the 1970s. Mayer Lake would be more susceptible to impact from warming water as it is shallower and with more littoral zone than the more bowl-like Drizzle Lake. In other stickleback lakes, Dolph Schluter observed high mortality of nesting males when temperatures turned warm; effect of heat waves is seen when spawning males are probably already physiologically stressed. However, male stickleback move offshore on warmer days, which might mitigate some of the impact. In future work Andrew Hendry intends to use lake monitors to assess possible effects of climate change |
| 11.4 | Storms & flooding | | | | | | |
| 11.5 | Other impacts | | | | | | |
| Classification of Threats adopted from IUCN-CMP, Salafsky et al. (2008). | | | | | | | |

## Appendix 2 - Threats Assessment Worksheet DU2

| | | | | |
|---|---|---|---|---|
| **Species or Ecosystem Scientific Name** | Unarmoured Threespine Stickleback (*Gasterosteus aculeatus*) (DU2) | | | |
| **Element ID** | | | **Elcode** | |
| **Date:** | 2022-06-03 | | | |
| **Assessor(s):** | Dwayne Lepitzki, Margaret Docker, Jennifer Gow, Jennifer Diment, Trevor Pitcher, Mark Ridgway, Dolph Schluter, Cassandra Silverio, Greg Wilson, Joanna James, Marie-Eve Corbin | | | |
| **References:** | COSEWIC Draft Status Report, February 2022. | | | |
| | | | **Level 1 Threat Impact Counts** | |
| | **Threat Impact** | | **high range** | **low range** |
| | A | Very High | 1 | 0 |
| | B | High | 0 | 1 |
| | C | Medium | 0 | 0 |
| | D | Low | 0 | 0 |
| **Calculated Overall Threat Impact:** | | | Very High | High |
| **Assigned Overall Threat Impact:** | | | **AB = Very High - High** | |
| **Impact Adjustment Reasons:** | | | | |
| **Overall Threat Comments** | | | Remote locations on Haida Gwaii, BC, and some portion of range within provincial park offer some security for populations. Threat of introduction of aquatic invasive species (AIS) is very high, with an introduction in at least one lake (Boulton) now known to have had serious consequences. Generation time 1-2 years. Three lakes, no inlets, groundwater seepage: based on estimates from the 1980s, Boulton (89.9% of total population), Rouge (4.5%), Serendipity (5.6%). | |

| **Threat** | | **Impact (calculated)** | | **Scope (next 10 Yrs)** | **Severity (10 Yrs or 3 Gen.)** | **Timing** | **Comments** |
|---|---|---|---|---|---|---|---|
| 1 | Residential & Commercial Development | | | | | | Although Serendipity Lake's entire catchment is within Naikoon Provincial Park, Boulton and Rouge Lake catchments are not protected and could be at increased risk from human activities, e.g., real estate. However, no known current or future land development near or on Boulton or Rouge Lake. |
| 1.1 | Housing & urban areas | | | | | | Private property adjacent to Rouge Lake; development proposal (including property and cattle) denied as Parks would not allow access through park. |
| 1.2 | Commercial & industrial areas | | | | | | |
| 1.3 | Tourism & recreation areas | | | | | | No boat launch at lakes; no trout in lakes, so no fishing interest. Boulton Lake is next to road; it is the only road-accessible of the three lakes. |
| 2 | Agriculture & Aquaculture | | | | | | Although Serendipity Lake's entire catchment is within Naikoon Provincial Park, Boulton and Rouge Lake catchments are not protected and could be at increased risk from human activities, e.g., agriculture. Sedimentation/pollution from agriculture, if any, would be under 9.3. |
| 2.1 | Annual & perennial non-timber crops | | | | | | |
| 2.2 | Wood & pulp plantations | | | | | | |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 2.3 | Livestock farming & ranching | | | | | | Private property adjacent to Rouge Lake; development proposal (including property and cattle) denied as Parks would not allow access through park. |
| 2.4 | Marine & freshwater aquaculture | | | | | | |
| 3 | Energy Production & Mining | | | | | | |
| 3.1 | Oil & gas drilling | | | | | | |
| 3.2 | Mining & quarrying | | | | | | |
| 3.3 | Renewable energy | | | | | | |
| 4 | Transportation & Service Corridors | | | | | | |
| 4.1 | Roads & railroads | | | | | | Boulton Lake next to road, but no known plans for expansion of road into aquatic habitat or culvert/bridge replacement. Pollution from roads under 9.1. |
| 4.2 | Utility & service lines | | | | | | |
| 4.3 | Shipping lanes | | | | | | |
| 4.4 | Flight paths | | | | | | |
| 5 | Biological Resource Use | | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| 5.1 | Hunting & collecting terrestrial animals | | | | | | |
| 5.2 | Gathering terrestrial plants | | | | | | |
| 5.3 | Logging & wood harvesting | | | | | | Although Serendipity Lake's entire catchment is within Naikoon Provincial Park, Boulton and Rouge Lake catchments are not protected and could be at increased risk from human activities, e.g., logging, but no known effect of debris falling directly into habitat. Pollution (e.g., sedimentation) from forestry, if any, under 9.3. |
| 5.4 | Fishing & harvesting aquatic resources | | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | Scientific collection permit approved for lethal sampling of 100 adults from each location for at least 2 years. Effect of removing 100 adults negligible out of approx. 350,000 (Boulton Lake), 17,500 (Rouge Lake), and 22,000 (Serendipity Lake) adults total. Not used as bait (no predatory fishes); non-lethal research under 6.3. |
| 6 | Human Intrusions & Disturbance | | | | | | |
| 6.1 | Recreational activities | | | | | | Boulton only accessible lake and none of the lakes have salmonids or other predatory fishes; no recreation |
| 6.2 | War, civil unrest & military exercises | | | | | | |
| 6.3 | Work & other activities | | | | | | No known plans for research that does not kill or permanently remove stickleback, but would be permitted only if negligible effect |
| 7 | Natural System Modifications | | | | | | |
| 7.1 | Fire & fire suppression | | | | | | |
| 7.2 | Dams & water management/use | | | | | | |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 7.3 | Other ecosystem modifications | | | | | | Small lakes make them susceptible to ecosystem modification; Beaver introduced in 1970s impacted habitat structure in Boulton and Serendipity lakes (i.e., shoreline extent, although now stable), but it has not had an observable impact on stickleback. No inlet streams in these lakes, and Beaver at outlet streams and without much influence on water level. Serendipity Lake is already at its maximum size given the contour of the land; it has almost tripled in size since 1970s when no Beaver. Concern in 1970s re flooding surrounding the bog, but it hasn't influenced stickleback numbers. A paper in progress by Tom Reimchen is looking at Serendipity Lake since the 1970s to present; there are no obvious changes. Introduced Beaver at Rouge Lake died out around the time the lake dried up (2017); a connection between these events is possible. Red-legged Frog is now present in Boulton Lake (since 2009); it presents a competitive threat, which is scored under 8.1. |
| 8 | Invasive & Other Problematic Species & Genes | AB | Very High - High | Pervasive (71-100%) | Extreme - Serious (31-100%) | High (Continuing) | |
| 8.1 | Invasive non-native/alien species/diseases | AB | Very High - High | Pervasive (71-100%) | Extreme - Serious (31-100%) | High (Continuing) | Threat of introduction of aquatic invasive species (AIS) is very high, with an introduction (Red-legged Frog, RLF) in at least one lake now known to have had serious consequences; RLF in Boulton Lake decreased stickleback numbers by 80% due to direct competition with tadpoles. In 2009, RLF was adjacent to Boulton Lake and adults were present, but no tadpoles were detected. By about 2013, there were 25:1 tadpoles:stickleback in traps, although tadpoles are now reduced compared to their peak numbers. As of 2017, Tom Reimchen indicated that there were no RLF in Serendipity Lake, but it is inevitable that they will get there, and a more severe impact is expected than at Boulton Lake. Incredibly serious in Rouge and Serendipity (especially the latter because it is very small and shallow); they have no room for buffering. Effect of tadpoles would be pretty dramatic. Possibly also indirect effects of RLF: shift in morphology, with defensive structures changing, and possible shift from littoral to open water, away from RLF but into open water with more predatory birds. Also concerns re introduction of salmonids, especially in Boulton Lake, which has road access; could result in extirpation or switch to armoured form. In the course of their research, Andrew Hendry's group from McGill will monitor for invasive species, including possible effects of RLF |
| 8.2 | Problematic native species/diseases | | | | | | |
| 8.3 | Introduced genetic material | | | | | | |
| 8.4 | Problematic species/diseases of unknown origin | | | | | | |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 8.5 | Viral/prion-induced diseases | | | | | | |
| 8.6 | Diseases of unknown cause | | | | | | |
| 9 | Pollution | | | | | | All three lakes have no inlet and are maintained by groundwater seepage, so little threat from stream-fed pollution or overland pollution. |
| 9.1 | Domestic & urban waste water | | | | | | Boulton and Rouge Lakes could experience some residential developments in watershed, resulting in possible pollution from septic tanks? |
| 9.2 | Industrial & military effluents | | | | | | Rouge Lake has some industrial developments. |
| 9.3 | Agricultural & forestry effluents | | | | | | Boulton Lake has forestry potential in watershed and Rouge Lake has some agricultural development, but because they are groundwater fed, there is little effect of overland/surface stream pollution. Boulton L: water is relatively clear, rain percolates through the ground, tannins are lost before reaching the lake, resulting in little influence of adjacent logging |
| 9.4 | Garbage & solid waste | | | | | | |
| 9.5 | Air-borne pollutants | | | | | | |
| 9.6 | Excess energy | | | | | | |
| 10 | Geological Events | | | | | | |
| 10.1 | Volcanoes | | | | | | |
| 10.2 | Earthquakes/tsunamis | | | | | | None of these lakes have historically been prone to effects from tsunamis. |
| 10.3 | Avalanches/landslides | | | | | | |
| 11 | Climate Change & Severe Weather | | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |
| 11.1 | Habitat shifting & alteration | | | | | | Serendipity Lake likely not susceptible to coastal erosion from climate change and sea level rise since it' is many km from coast (Tom Reimchen). Serendipity Lake represents < 6% of Canadian population |
| 11.2 | Droughts | | | | | | **Small lakes potentially susceptible to drought; Rouge Lake dried up in 2017, at least in part due to drought. However, unknown severity because, even though Rouge Lake dried up once, stickleback survived. Lake maintained by groundwater, and fish recolonized lake as it refilled (Andrew Hendry will be performing genetic analysis on samples collected before and after collapse due to drought. Tom Reimchen: Rouge Lake stickleback are monomorphic and most divergent from ancestral stickleback; evidence of an extreme bottleneck, due to drying out in the past?)** |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 11.3 | Temperature extremes | | | | | | Small lakes potentially susceptible to warming |
| 11.4 | Storms & flooding | | | | | | |
| 11.5 | Other impacts | | | | | | |
| Classification of Threats adopted from IUCN-CMP, Salafsky et al. (2008). | | | | | | | |

## Appendix 3 - Threats Assessment Worksheet DU3 and DU4

| | | | |
|---|---|---|---|
| **Species or Ecosystem Scientific Name** | Misty Lake Lotic and Lentic Threespine Stickleback (*Gasterosteus aculeatus*) (DU3, DU4) | | |
| **Element ID** | | **Elcode** | |
| **Date:** | 2022-06-06 | | |
| **Assessor(s):** | Dwayne Lepitzki, Margaret Docker, Jennifer Gow, Jennifer Diment, Trevor Pitcher, Cassandra Silverio, Maggie Boothroyd, Greg Wilson, Joanna James, Marie-Eve Corbin | | |
| **References:** | COSEWIC Draft Status Report, February 2022. | | |

| | | | **Level 1 Threat Impact Counts** | |
|---|---|---|---|---|
| | **Threat Impact** | | **high range** | **low range** |
| | A | Very High | 1 | 0 |
| | B | High | 0 | 1 |
| | C | Medium | 0 | 0 |
| | D | Low | 1 | 1 |
| **Calculated Overall Threat Impact:** | | | Very High | High |
| **Assigned Overall Threat Impact:** | | | **AB = Very High - High** | |
| **Impact Adjustment Reasons:** | | | Very High - Low is the most plausible range but was not an available choice. Although the threat impact from invasive species is high and would likely rapidly drive the population to extinction (as demonstrated in other stickleback species pairs), the likelihood of invasion within the next 10 years is unknown. | |
| **Overall Threat Comments** | | | The threat of introduction of aquatic invasive species (AIS) is high and the consequences would likely be severe. Single lake, generation time 2-4 years for Lentic (DU3) and 1-2 years Lotic (DU4), so timeframe for severity and timing is 10 years. Lentic DU also includes those in the outlet stream, while Lotic only includes those in the inlet stream, and Lotic co-occur with Lentic in narrow, swampy transition zone between lake and inlet. While Misty Lake and a small area around it is within the Misty Lake Ecological Reserve, much of the watershed is not included in the reserve, including all of the outlet stream and most of the inlet stream habitat. Both DUs are exposed to the same general threats, although in some cases with slightly different nuances in terms of impacts (e.g., in case of drought, the stream will dry out before lake). | |

| **Threat** | | **Impact (calculated)** | | **Scope (next 10 Yrs)** | **Severity (10 Yrs or 3 Gen.)** | **Timing** | **Comments** |
|---|---|---|---|---|---|---|---|
| 1 | Residential & Commercial Development | | | | | | No land development permitted in Misty Lake Ecological Reserve; although the reserve only covers about 1/3 to 1/2 of shoreline. No big land development in watershed and riparian buffer zone should be maintained as part of Misty Lake Critical Habitat Order. |
| 1.1 | Housing & urban areas | | | | | | |
| 1.2 | Commercial & industrial areas | | | | | | |
| 1.3 | Tourism & recreation areas | | | | | | No boat launches on or recreational developments on/near Misty Lake. |
| 2 | Agriculture & Aquaculture | | | | | | |
| 2.1 | Annual & perennial non-timber crops | | | | | | |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 2.2 | Wood & pulp plantations | | | | | | |
| 2.3 | Livestock farming & ranching | | | | | | |
| 2.4 | Marine & freshwater aquaculture | | | | | | |
| 3 | Energy Production & Mining | | | | | | |
| 3.1 | Oil & gas drilling | | | | | | |
| 3.2 | Mining & quarrying | | | | | | |
| 3.3 | Renewable energy | | | | | | |
| 4 | Transportation & Service Corridors | | | | | | |
| 4.1 | Roads & railroads | | | | | | No known plans for new roads, bridges, or culverts; any new stream crossings would need to be reviewed, with buffers in place. Point-source pollution from road runoff and rest stop scored under 9. |
| 4.2 | Utility & service lines | | | | | | |
| 4.3 | Shipping lanes | | | | | | |
| 4.4 | Flight paths | | | | | | |
| 5 | Biological Resource Use | | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| 5.1 | Hunting & collecting terrestrial animals | | | | | | Bait ban in effect. |
| 5.2 | Gathering terrestrial plants | | | | | | |
| 5.3 | Logging & wood harvesting | | | | | | Loss of riparian zone around lake due to forest harvest resulted in increased algal growth, but regrowth of riparian zone is rapid, so impact may be temporary. No known debris from logging falling into aquatic habitat. Sedimentation from logging considered under 9.3. |
| 5.4 | Fishing & harvesting aquatic resources | | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | Lethal sampling is regulated under scientific collection guidelines and permit. Assumed that some lethal sampling planned for next 10 years. Based on new population estimates, Province is updating allowable harm levels (which would permit larger take), although numbers taken for lethal sampling (e.g., genetics) are quite low even by current regulations. Most research is non-lethal (under 6.3). |
| 6 | Human Intrusions & Disturbance | | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| 6.1 | Recreational activities | | | | | | Watercraft (e.g., canoes, floats) can be dragged from rest area to lake (about 40-50m), need to go through reserve, but there is awareness of concerns for habitat. |
| 6.2 | War, civil unrest & military exercises | | | | | | |
| 6.3 | Work & other activities | | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | Non-lethal mark-recapture regulated under scientific collection guidelines and permit. Transplants prohibited. No research on other species known |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 7 | Natural System Modifications | | Negligible | Pervasive (71-100%) | Negligible (<1%) | Moderate - Low | |
| 7.1 | Fire & fire suppression | | | | | | Water bucketing possible but not known from the past. |
| 7.2 | Dams & water management/use | | | | | | No known licensed water users on Misty Lake or its inlet and outlet streams. Future demand for water from Misty Lake is unknown, but it is unlikely to be problematic in the near future due to the presence of the Misty Lake Ecological Reserve and the abundance of alternative potential water sources. |
| 7.3 | Other ecosystem modifications | | Negligible | Pervasive (71-100%) | Negligible (<1%) | Moderate - Low | Small lake system makes it susceptible to potential land use changes in the watershed such as sedimentation, eutrophication, and water level fluctuations. Crayfish, milfoil would modify ecosystem. Crayfish appears to have entered/been increasing in system since early 2000s. Change in stream water velocity (erosion, sedimentation) would affect Lotic DU. Some of inlet not covered by Critical Habitat Order. Loss of riparian cover associated with logging, but vegetation recovery was rapid and impact was temporary. Recovery in <9 years, so severity appears negligible over 10 years. |
| 8 | Invasive & Other Problematic Species & Genes | AB | Very High - High | Pervasive (71-100%) | Extreme - Serious (31-100%) | Moderate - Low | |
| 8.1 | Invasive non-native/alien species/diseases | AB | Very High - High | Pervasive (71-100%) | Extreme - Serious (31-100%) | Moderate - Low | The easy access to Misty Lake by the existing highway rest stop from Highway 19 at the southwest corner of the lake makes the likelihood of an exotic species invasion or introduction high. Risk from bait buckets is low since there is no fishing in Misty Lake. There are no reports yet of AIS in Misty Lake. Risk assessments concluded that for most regions of BC, the probability of invasive fish species becoming established after release is high or very high, and the likely magnitude of ecological impact in small water bodies is very high, although risk is low under current conditions. Bass are not yet close to Misty Lake, but bullhead are close (on Lower Island). However, some uncertainty re risk: e.g., Lasqueti Island is remote, and bullhead still ended up there; it's a chance event (it only takes one person). Also uncertainty of impact because the Misty Lake community is not as simple as in lakes with Unarmoured Sticklebacks, and the impact will also depend on type of AIS. For example, bass, perch are so voracious and predatory even with native species that they would be a big problem. Bullheads are benthic and wipe out a lot of fish by eating eggs in nests, thus affecting recruitment. In other systems, introduced Brown Trout wiped out sticklebacks within a year. Benthic and spiny-rayed fishes would eat stickleback; Brown and Black Bullheads occur in both stream and lake habitats so they would affect both Lentic and Lotic sticklebacks if present in system. |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 8.2 | Problematic native species/diseases | | | | | | Lentic and Lotic coexist with Coastal Cutthroat Trout, Coho Salmon, and Dolly Varden; Lentic also exposed to Rainbow Trout and Prickly Sculpin, but doesn't appear to be anything that is enhancing native predatory fishes |
| 8.3 | Introduced genetic material | | | | | | |
| 8.4 | Problematic species/diseases of unknown origin | | | | | | |
| 8.5 | Viral/prion-induced diseases | | | | | | |
| 8.6 | Diseases of unknown cause | | | | | | |
| 9 | Pollution | D | Low | Pervasive (71-100%) | Slight (1-10%) | Moderate - Low | |
| 9.1 | Domestic & urban waste water | | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | Dumping from RVs possible, but risk minimal given not known from past and 40-50m buffer to lake. Lots of traffic passes by, stuff comes off tires (recent study shows tire bits more toxic than we thought), and buffer probably doesn't do a great job of preventing input. However, population level declines very small for non-point-source pollution, if measurable. Spill right at inlet could be catastrophic (affecting both Lotic and Lentic), but probability low; other points would be less pervasive. Small chronic impact. Non-point-source pollution ongoing threat, but it hasn't happened in 60 years; lots of flow-through results in dilution. |
| 9.2 | Industrial & military effluents | | Not Calculated (outside assessment timeframe) | Pervasive (71-100%) | Extreme - Serious (31-100%) | Low (Possibly in the long term, >10 yrs/3 gen) | Highway crosses inlet and runs close along southern lake shore. Chances of spill low (small portion of road goes by lake), but impact could be catastrophic, particularly if toxic spill into inlet. e.g., similar spill happened in the past: an oil truck crashed beside Goldstream River (Goldstream Provincial Park, Victoria); it was largely contained, but could have large negative impact, but unlikely within 10 years. |
| 9.3 | Agricultural & forestry effluents | D | Low | Pervasive (71-100%) | Slight (1-10%) | Moderate - Low | The main land use activity in the area is forest harvesting, including the associated construction, use, and maintenance of logging roads. Some of the inlet is not covered by the Critical Habitat Order. Critical habitat includes the inlet stream up to the extent currently known to be occupied by the Misty Lake Stickleback, but there is potential for effluents to flow into critical habitat from works upstream. Cumulative logging impacts may be a concern, particularly to inlet habitat, from erosion, sedimentation, altered flows, and changes to the benthic community caused by riparian vegetation removal. However, topography is not that steep and there have been no apparent long-term impacts to date from previous forest harvesting activity in the watershed. Therefore, severity should be negligible or unknown, especially with current mitigation, unless increased intensity of these activities is expected to occur in the future. Effects to Lentic and Lotic not negligible, but followed by quick recovery. |
| 9.4 | Garbage & solid waste | | | | | | |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 9.5 | Air-borne pollutants | | | | | | |
| 9.6 | Excess energy | | | | | | |
| 10 | Geological Events | | | | | | |
| 10.1 | Volcanoes | | | | | | |
| 10.2 | Earthquakes/tsunamis | | | | | | At > 70 m elevation, Misty Lake is outside tsunami hazard zone. |
| 10.3 | Avalanches/landslides | | | | | | |
| 11 | Climate Change & Severe Weather | | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | Is Vancouver Island / Misty Lake currently experiencing effects of climate change? Heat dome of 2021? |
| 11.1 | Habitat shifting & alteration | | | | | | |
| 11.2 | Droughts | | | | | | Generally, a wet area, although there can be dry years; inlet relatively small, potential for drying affecting Lentic DU. |
| 11.3 | Temperature extremes | | | | | | |
| 11.4 | Storms & flooding | | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | Big floods in fall 2021. |
| 11.5 | Other impacts | | | | | | |

Classification of Threats adopted from IUCN-CMP, Salafsky et al. (2008).

## Appendix 4 - Threats Assessment Worksheet DU5 to DU12

| | | | | |
|---|---|---|---|---|
| **Species or Ecosystem Scientific Name** | Benthic and Limnetic Threespine Stickleback (*Gasterosteus aculeatus*) species pairs (DU5-12) | | | |
| **Element ID** | | **Elcode** | | |
| **Date:** | 2022-06-14 | | | |
| **Assessor(s):** | Dwayne Lepitzki, Margaret Docker, Jennifer Gow, Jennifer Diment, Mark Ridgway, Maggie Boothroyd, Jocelyn Nelson, Julie Perrault, Greg Wilson, Joanna James, Marie-Eve Corbin | | | |
| **References:** | COSEWIC Draft Status Report, February 2022. | | | |
| **Overall Threat Impact Calculation Help:** | | **Level 1 Threat Impact Counts** | | |
| | **Threat Impact** | | **high range** | **low range** |
| | A | Very High | 2 | 2 |
| | B | High | 0 | 0 |
| | C | Medium | 0 | 0 |
| | D | Low | 0 | 0 |
| **Calculated Overall Threat Impact:** | | | Very High | Very High |
| **Assigned Overall Threat Impact:** | | | **AC = Very High - Medium** | |
| **Impact Adjustment Reasons:** | | | Very High for Enos (DU11,12), Very High to High for Vananda (DU7,8), and Very High-Medium for Paxton (DU5,6) and Little Quarry (DU9,10). Although the threat impact from invasive species is high, the likelihood of invasion within the next 10 years is unknown (and is lower for Paxton and Little Quarry). Difference in comparison to Misty Lake (Very High-Low) is due to the sympatric vs. parapatric nature of these pairs, and a less complex fish community structure compared to Misty Lake. | |
| **Overall Threat Comments** | | | Vananda Creek pair (DU7,8) used as template. Scores and comments here are for Vananda Creek pair (DU7,8), with additional comments for other Benthic/Limnetic pairs where relevant. Overall threat impacts and scores/significant comments for each IUCN category for all 12 DUs are summarized in Appendix.5.<br><br>The threat of introduction of aquatic invasive species (AIS) is high and the consequences would likely be severe (as demonstrated in other stickleback species pairs). Generation time 2-3 years for Benthic, 1-2 years for Limnetic; time frame for severity and timing is 10 years. Vananda Creek watershed: 3 lakes (Emily, Priest, Spectacle lakes), no breakdown of numbers in each of the three lakes; Paxton L: 1 lake; Little Quarry L: 1 lake; Enos L: 1 lake, collapsed hybrid swarm associated with the appearance of American Signal Crayfish. | |

| **Threat** | | **Impact (calculated)** | | **Scope (next 10 Yrs)** | **Severity (10 Yrs or 3 Gen.)** | **Timing** | **Comments** |
|---|---|---|---|---|---|---|---|
| 1 | Residential & Commercial Development | | | | | | |
| 1.1 | Housing & urban areas | | | | | | There have been numerous historical land-based development activities in the watersheds of Vananda Creek (forestry, mining, road building, pipeline construction, and housing development). Magnitude of historical impact unknown. The threat from current land-based activities is unknown. Riparian buffer zone should be maintained as part of Vananda Creek Critical Habitat Order; pollution goes under 9.1. |

| Threat | | Impact (calculated) | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|
| 1.2 | Commercial & industrial areas | | | | | Riparian buffer zone should be maintained as part of Vananda Creek Critical Habitat Order; pollution from industry under 9.2. |
| 1.3 | Tourism & recreation areas | | | | | No known new or expansion of current boat launches, development in/at water's edge. |
| 2 | Agriculture & Aquaculture | | | | | |
| 2.1 | Annual & perennial non-timber crops | | | | | |
| 2.2 | Wood & pulp plantations | | | | | |
| 2.3 | Livestock farming & ranching | | | | | No livestock access to water that could trample eggs/fish/habitat; pollution, sedimentation from these activities under 9.3. |
| 2.4 | Marine & freshwater aquaculture | | | | | |
| 3 | Energy Production & Mining | | | | | |
| 3.1 | Oil & gas drilling | | | | | Magnitude of historical impact from pipeline construction in Vananda Creek watershed unknown and threat from current land-based activities is unknown. However, riparian buffer zone should be maintained as part of Vananda Creek Critical Habitat Order. Pollution from spills under 9.2. |
| 3.2 | Mining & quarrying | | | | | Magnitude of historical impact from mining in Vananda Creek watershed unknown and threat from current land-based activities is unknown, but riparian buffer zone should be maintained as part of Vananda Creek Critical Habitat Order. The Lafarge Mine (a limestone quarry mine), located beside Paxton Lake, partially drains(ed) to Priest Lake via Van Anda Creek. There are water quality monitoring stations on it, and DFO currently has a permit for the Vananda Creek Stickleback Species Pair (lethal sampling of 5 of each species) to support the development of a selenium bioaccumulation model for Priest Lake, with a goal of increasing understanding of how metal loadings (from LaFarge's quarry operations) are conveyed to Vananda Creek and Priest Lake. Pollution from spills under 9.2. |
| 3.3 | Renewable energy | | | | | |
| 4 | Transportation & Service Corridors | | | | | |
| 4.1 | Roads & railroads | | | | | Magnitude of historical impact from road building in Vananda Creek watershed unknown and threat from current land-based activities is unknown. However, riparian buffer zone should be maintained as part of Vananda Creek Critical Habitat Order. Not likely that there will be new roads/expansion of roads (including logging), culvert/bridge replacements directly in habitat. Pollution from roads (e.g., sedimentation, tire pieces, oil) under 9.1; toxic spills from transport of chemicals (roads/railroads) under 9.2. |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 4.2 | Utility & service lines | | | | | | |
| 4.3 | Shipping lanes | | | | | | |
| 4.4 | Flight paths | | | | | | |
| 5 | Biological Resource Use | | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| 5.1 | Hunting & collecting terrestrial animals | | | | | | |
| 5.2 | Gathering terrestrial plants | | | | | | |
| 5.3 | Logging & wood harvesting | | | | | | No known logging debris falling into aquatic habitat, especially with buffers in place. Sedimentation scored under 9.3. |
| 5.4 | Fishing & harvesting aquatic resources | | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | Scientific collections are regulated by permit and specific guidelines. Bait ban in effect province-wide. Illegal use or transport of bait dependent on access to water body. Lethal sampling and potential removal of live specimens for lab/field experiments, but small numbers (e.g., see 3.2). |
| 6 | Human Intrusions & Disturbance | | | | | | |
| 6.1 | Recreational activities | | | | | | Types/amounts of recreation dependent on access and if there is fishing, but boat launch should prevent much of this. |
| 6.2 | War, civil unrest & military exercises | | | | | | |
| 6.3 | Work & other activities | | | | | | Non-lethal (e.g., mark-recapture) regulated under scientific collection guidelines and permit. Transplants prohibited. Other research on habitat discussed here not likely to be significant. |
| 7 | Natural System Modifications | A | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate (Possibly in the short term, < 10 yrs/3 gen) | |
| 7.1 | Fire & fire suppression | | | | | | Vananda Creek is water supply for firefighting. |
| 7.2 | Dams & water management/use | CD | Medium - Low | Pervasive (71-100%) | Moderate - Slight (1-30%) | High (Continuing) | Lakes in the Vananda Creek watershed have been subjected to damming of outlet streams (Priest and Emily Lakes) and water extraction for mining (Emily Lake). Existing water licenses for Vananda Creek remain large relative to the volume of the lakes and size of the catchments. Priest Lake is the main water supply for the township of Vananda. The impact of historical and current use of water is uncertain. Not much population growth projected for Van Anda (0.5%, with a population projection of 275 people in 2011 to 296 in 2027). Some DUs have dams, which have increased water levels, but they have been there a long time. No known plans for expansion, heightening of dams. Water extraction (all purposes) scored here too. |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 7.3 | Other ecosystem modifications | A | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate (Possibly in the short term, < 10 yrs/3 gen) | Small lake areas make them susceptible to potential land use changes in the watershed such as sedimentation, eutrophication, and water level fluctuations. Introduction of species that affect habitat (e.g., crayfish, milfoil, and beaver) scored here. Changes to riparian scored here, but quick recovery of riparian shown for other DUs. |
| 8 | Invasive & Other Problematic Species & Genes | A | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate (Possibly in the short term, < 10 yrs/3 gen) | |
| 8.1 | Invasive non-native/alien species/diseases | A | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate (Possibly in the short term, < 10 yrs/3 gen) | The threat of introduction of AIS is likely high. Risk assessments concluded that for most regions of BC, the probability of invasive fish species becoming established after release is high or very high, and the likely magnitude of ecological impact in small water bodies is very high. This includes introduction of species that predate on stickleback or are in direct competition (e.g., tadpoles). Severity is dependent on type of species introduced and waterbody structure. Timing dependent on access to water body, e.g., whether road access. Calculated impact based on upper range of timing score if a range is used. Bullfrogs are abundant on Texada Island now and probably wiped out native frogs, but they seem to have no impact on stickleback (Dolph Schluter) |
| 8.2 | Problematic native species/diseases | | | | | | Augmentation of native species (e.g., hatchery releases) not known. |
| 8.3 | Introduced genetic material | | | | | | |
| 8.4 | Problematic species/diseases of unknown origin | | | | | | |
| 8.5 | Viral/prion-induced diseases | | | | | | |
| 8.6 | Diseases of unknown cause | | | | | | |
| 9 | Pollution | | Negligible | Large (31-70%) | Negligible (<1%) | High (Continuing) | |
| 9.1 | Domestic & urban wastewater | | Negligible | Large (31-70%) | Negligible (<1%) | High (Continuing) | Pollution from sewage, septic systems, dumping of RV tanks, oil, salt, sediments, tire pieces from roads likely with negligible effect. |
| 9.2 | Industrial & military effluents | | Not Calculated (outside assessment timeframe) | Large (31-70%) | Moderate - Slight (1-30%) | Low - Insignificant/ Negligible | Road runs alongside some of shore. Chances of spill low (considering type and volume of road traffic), but impact could be significant. Could result in catastrophic toxic spills from road/railroads, industry, but not likely within time frame. |
| 9.3 | Agricultural & forestry effluents | | | | | | Sedimentation, pollution from forestry and agriculture not significant. |
| 9.4 | Garbage & solid waste | | | | | | |
| 9.5 | Air-borne pollutants | | | | | | |

| Threat | | Impact (calculated) | | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 9.6 | Excess energy | | | | | | |
| 10 | Geological Events | | | | | | No history of such events. |
| 10.1 | Volcanoes | | | | | | |
| 10.2 | Earthquakes/tsunamis | | | | | | |
| 10.3 | Avalanches/landslides | | | | | | |
| 11 | Climate Change & Severe Weather | | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | Effect of increasing droughts, temperature extremes (e.g., heat dome), storms and flooding being experienced in these areas of BC unknown. |
| 11.1 | Habitat shifting & alteration | | | | | | |
| 11.2 | Droughts | | | | | | All species pairs susceptible, exacerbated with water extraction in some DUs. Droughts impacting littoral shoreline will most directly impact Limnetic breeding fish, but will indirectly impact Benthics by reducing segregated breeding sites and increasing chance of hybridization. |
| 11.3 | Temperature extremes | | | | | | High adult male mortality at nest observed at Paxton Lake following extreme heat days; all pairs susceptible. Dolph Schluter reported deaths of many males in shallow water when the weather turned hot rapidly, no doubt associated with stress of reproduction; additional males observed setting up new territories in the same locations at the same time. Climate change will likely bring hot days more often, which could impact reproduction if males die while tending nests in the shallows (<= 1 m depth); likely mainly affecting Limnetics, since Benthic nests are deeper. |
| 11.4 | Storms & flooding | | | | | | |
| 11.5 | Other impacts | | | | | | |
| Classification of Threats adopted from IUCN-CMP, Salafsky et al. (2008). | | | | | | | |

## Appendix 5 - Threats Assessment Worksheet DU5, DU6, DU9-12

| **Species or Ecosystem Scientific Name** | | | Benthic and Limnetic Threespine Stickleback (*Gasterosteus aculeatus*) species bundle: Summary of threat scores for all DUs and threat scores and comments for three Benthic/Limnetic species pairs for which full threats calculator not done (Paxton, Little Quarry, Enos; DU5,6,9-12). | | |
|---|---|---|---|---|---|
| | | | **Calculated overall threat impact** | **Assigned overall threat impact** | **Current COSEWIC status** |
| DU 1 | Giant | Drizzle and Mayer lakes | Low | Low | SC 2013: could become EN with intro of invasives |
| DU 2 | Unarmoured | Serendipity, Rouge, Boulton lakes | Very High - High | Very High - High | SC 2013: could become EN with intro of invasives |
| DU 3,4 | Misty Lake | Lotic and Lentic pair | Very High - High | Very High - Low | EN 2006: A3e, potential intro of invasives, next to highway, public access, logging, highway use & maintenance |
| DU 5,6 | Paxton Lake | Benthic-Limnetic sympatric pair | Very High - Very High | Very High - Medium | EN 2021, A3e: potential intro of invasives, habitat loss & degradation water extraction & land use activities) (Fig. 7), 1 lake; historical severe water level fluctuations due to dam on outlet stream for mining; past forestry extensive |
| DU 7,8 | Vananda Creek (Emily, Priest, Spectacle lakes) | Benthic-Limnetic sympatric pair | Very High - Very High | Very High - High | EN 2021, A3e: potential intro of invasives, habitat loss & degradation water extraction & land use activities) (Fig. 6), 3 lakes, no breakdown of numbers in each of the three lakes, Priest & Emily outlets dammed for water extraction & mining, historical mining & forestry extensive |
| DU 9,10 | Little Quarry Lake | Benthic-Limnetic sympatric pair | Very High - Very High | Very High - Medium | TH D2 2015, potential intro of invasives (Fig 4), 1 lake, dam on outlet raised water level; land surrounding relatively free of human activities, forested, no roads, residences, or other development |
| DU 11,12 | Enos Lake | Benthic-Limnetic sympatric pair | Very High - Very High | Very High | EN 2012: a2ace: B1ab(iii,v)+2ab(iii,v); C2a(ii), presence of invasive crayfish resulted in collapse into hybrids swarm (Fig. 5), 1 lake, collapsed hybrid swarm due to introduction of American Signal Crayfish causing loss of reproductive barriers; dam on outlet raised water level |
| DU 1 | Giant | Drizzle and Mayer lakes | **Overall Threat Comments** | Remote locations on Haida Gwaii, BC, and within ecological reserve and provincial park offer some security for subpopulations. Threat of introduction of aquatic invasive species (AIS) is low; likelihood is high, but consequences would likely be slight | |
| DU 2 | Unarmoured | Serendipity, Rouge, Boulton lakes | **Overall Threat Comments** | Remote locations on Haida Gwaii, BC, and some portion of range within provincial park offer some security for populations. Threat of introduction of aquatic invasive species (AIS) is very high, with an introduction in at least one lake (Boulton) now known to have had serious consequences. | |
| DU 3,4 | Misty Lake | Lotic and Lentic pair | **Overall Threat Comments** | The threat of introduction of aquatic invasive species (AIS) is high and the consequences would likely be severe (as demonstrated in other stickleback species pairs). While Misty Lake and a small area around it is within the Misty Lake Ecological Reserve, much of the watershed is not included in the reserve, including all of the outlet stream and most of the inlet stream habitat. Both DUs are exposed to the same general threats, although in some cases with slightly different nuances in terms of impacts (e.g., in case of drought, the stream will dry out before lake). Lentic DU includes those in the outlet stream, while Lotic only includes those in the inlet stream; Lotic co-occur with Lentic in narrow, swampy transition zone between lake and inlet. | |
| DU 5,6 | Paxton Lake | Benthic-Limnetic sympatric pair | **Overall Threat Comments** | The threat of introduction of aquatic invasive species is high and the consequences would likely be severe (as demonstrated in other stickleback species pairs). | |
| DU 7,8 | Vananda Creek (Emily, Priest, Spectacle lakes) | Benthic-Limnetic sympatric pair | **Overall Threat Comments** | The threat of introduction of aquatic invasive species is high and the consequences would likely be severe (as demonstrated in other stickleback species pairs). | |

| | | | | |
|---|---|---|---|---|
| DU 9,10 | Little Quarry Lake | Benthic-Limnetic sympatric pair | **Overall Threat Comments** | Owing to the relatively inaccessible location on Nelson Island, BC, the threat of introduction of aquatic invasive species is medium-low, but consequences would likely be extreme (as demonstrated in other stickleback species pairs). |
| DU 11,12 | Enos Lake | Benthic-Limnetic sympatric pair | **Overall Threat Comments** | The American Signal Crayfish has already invaded Enos Lake and is associated with the breakdown of reproductive isolation between the Limnetic and Benthic Threespine Stickleback pair in Enos Lake and their collapse into a hybrid swarm. |
| Category 1 and 2 threats are summarized for each DU for which a full threats calculator was done (Appendices 1-4) if there was a score (including unknown and negligible); comments are given in Appendix 1 (Giant), 2 (Unarmoured), 3 & 4 (Misty Lake Lentic & Lotic), and 4 (Vananda Cr Benthic & Limnetic). | | | | |
| Category 1 and 2 threats and comments are given here for DUs in which a full threats calculator was not done (Paxton L, Little Quarry L, Enos L Benthic & Limnetic); full threats calculator done for Vananda Cr Benthic & Limnetic pair (Appendix 4). | | | | |

| Threat | | | Impact (calculated) | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 1 | Residential & Commercial Development | | | | | | |
| 1.1 | Housing & urban areas | Giant (DU1) | | | | | See Appendix 1 |
| | | Unarmoured (DU2) | | | | | See Appendix 2 |
| | | Misty Lake (DU3,4) | | | | | See Appendix 3 |
| | | Paxton Lake (DU5,6) | | | | | Riparian buffer zone should be maintained as part of Paxton Lake Critical Habitat Order |
| | | Vananda Creek (DU7,8) | | | | | See Appendix 4 |
| | | Little Quarry Lake (DU9,10) | | | | | |
| | | Enos Lake (DU11,12) | | | | | |
| 1.2 | Commercial & industrial areas | Giant | | | | | |
| | | Unarmoured | | | | | |
| | | Misty Lake | | | | | |
| | | Paxton Lake | | | | | Riparian buffer zone should be maintained as part of Paxton Lake Critical Habitat Order |
| | | Vananda Creek watershed | | | | | |
| | | Little Quarry Lake | | | | | |
| | | Enos Lake | | | | | |
| 1.3 | Tourism & recreation areas | Giant | | | | | |
| | | Unarmoured | | | | | |
| | | Misty Lake | | | | | |
| | | Paxton Lake | | | | | Riparian buffer zone should be maintained as part of Paxton Lake Critical Habitat Order |
| | | Vananda Creek watershed | | | | | |

| Threat | | | Impact (calculated) | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| | | Little Quarry Lake | | | | | |
| | | Enos Lake | | | | | |
| 2 | Agriculture & Aquaculture | | | | | | |
| 2.1 | Annual & perennial non-timber crops | | | | | | |
| 2.2 | Wood & pulp plantations | | | | | | |
| 2.3 | Livestock farming & ranching | | | | | | |
| 2.4 | Marine & freshwater aquaculture | | | | | | |
| 3 | Energy Production & Mining | | | | | | |
| 3.1 | Oil & gas drilling | | | | | | |
| 3.2 | Mining & quarrying | | | | | | |
| 3.3 | Renewable energy | | | | | | |
| 4 | Transportation & Service Corridors | | | | | | |
| 4.1 | Roads & railroads | | | | | | |
| 4.2 | Utility & service lines | | | | | | |
| 4.3 | Shipping lanes | | | | | | |
| 4.4 | Flight paths | | | | | | |
| 5 | Biological Resource Use | All DUs | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| 5.1 | Hunting & collecting terrestrial animals | | | | | | |
| 5.2 | Gathering terrestrial plants | | | | | | |
| 5.3 | Logging & wood harvesting | | | | | | |
| 5.4 | Fishing & harvesting aquatic resources | Giant | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| | | Unarmoured | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| | | Misty Lake | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| | | Paxton Lake | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | Scientific collections are regulated by permit and specific guidelines (includes lethal sampling and removal of live specimens). Bait ban in effect province wide. |
| | | Vananda Creek watershed | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| | | Little Quarry Lake | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | Scientific collections are regulated by permit and specific guidelines (includes lethal sampling). Bait ban in effect province wide. |

| Threat | | | Impact (calculated) | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| | | Enos Lake | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | Scientific collections are regulated by permit and specific guidelines (includes lethal sampling). Bait ban in effect province wide. |
| 6 | Human Intrusions & Disturbance | | | | | | |
| 6.1 | Recreational activities | | | | | | |
| 6.2 | War, civil unrest & military exercises | | | | | | |
| 6.3 | Work & other activities | Giant | | | | | |
| | | Unarmoured | | | | | |
| | | Misty Lake | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| | | Paxton Lake | | | | | Non-lethal mark-recapture regulated under scientific collection guidelines and permit. Transplants prohibited. |
| | | Vananda Creek watershed | | | | | |
| | | Little Quarry Lake | | | | | Non-lethal mark recapture regulated under scientific collection guidelines and permit. Transplants prohibited. |
| | | Enos Lake | | | | | Non-lethal mark recapture regulated under scientific collection guidelines and permit. Transplants prohibited. |
| 7 | Natural System Modifications | Giant | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |
| | | Unarmoured | | | | | |
| | | Misty Lake | Negligible | Pervasive (71-100%) | Negligible (<1%) | Moderate - Low | |
| | | Paxton Lake | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate - Low | |
| | | Vananda Creek watershed | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate (Possibly in the short term, < 10 yrs/3 gen) | |
| | | Little Quarry Lake | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate - Low | |
| | | Enos Lake | Very High | Pervasive (71-100%) | Extreme (71-100%) | High (Continuing) | |
| 7.1 | Fire & fire suppression | | | | | | |
| 7.2 | Dams & water management/use | Giant | | | | | |
| | | Unarmoured | | | | | |
| | | Misty Lake | | | | | |
| | | Paxton Lake | Low | Pervasive (71-100%) | Slight (1-10%) | High (Continuing) | Existing water licenses for Paxton Lake remain large relative to the volume of the lakes and size of the catchments. The impact of historical and current use of water is uncertain. |
| | | Vananda Creek watershed | Medium-Low | Pervasive (71-100%) | Moderate - Slight (1-30%) | High (Continuing) | |

| Threat | | | Impact (calculated) | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| | | Little Quarry Lake | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| | | Enos Lake | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| 7.3 | Other Ecosystem Modifications | Giant | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |
| | | Unarmoured | | | | | |
| | | Misty Lake | Negligible | Pervasive (71-100%) | Negligible (<1%) | Moderate - Low | |
| | | Paxton Lake | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate - Low | The threat of introduction of AIS is likely high. Risk assessments concluded that for most regions of BC, the probability of invasive fish species becoming established after release is high or very high, and the likely magnitude of ecological impact in small water bodies is very high. |
| | | Vananda Creek watershed | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate (Possibly in the short term, < 10 yrs/3 gen) | |
| | | Little Quarry Lake | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate - Low | With regard tor sympatric species pairs, Little Quarry Lake is particularly vulnerable to AIS that impact littoral vegetation/macrophyte beds (which are important breeding areas and important in maintenance of segregated breeding sites) as it has a substantially smaller amount. |
| | | Enos Lake | Very High | Pervasive (71-100%) | Extreme (71-100%) | High (Continuing) | The American Signal Crayfish has already invaded Enos Lake and is associated with the breakdown of reproductive isolation between the Limnetic and Benthic Threespine Stickleback pair in Enos Lake and their collapse into a hybrid swarm. |
| 8 | Invasive & Other Problematic Species & Genes | Giant | Low | | | | |
| | | Unarmoured | Very High - High | | | | |
| | | Misty Lake | Very High - High | | | | |
| | | Paxton Lake | Very High | | | | |
| | | Vananda Creek watershed | Very High | | | | |
| | | Little Quarry Lake | Very High | | | | |
| | | Enos Lake | Very High | | | | |
| 8.1 | Invasive non-native/alien species | Giant | Low | Large (31-70%) | Slight (1-10%) | High (Continuing) | See Appendix 1 |
| | | Unarmoured | Very High - High | Pervasive (71-100%) | Extreme - Serious (31-100%) | High (Continuing) | See Appendix 2 |

| Threat | | | Impact (calculated) | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| | | Misty Lake | Very High - High | Pervasive (71-100%) | Extreme - Serious (31-100%) | Moderate - Low | See Appendix 3 |
| | | Paxton Lake | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate - Low | Risk assessments concluded that for most regions of BC, the probability of predatory invasive fish species becoming established after release is high or very high, and the likely magnitude of ecological impact in small water bodies is very high. |
| | | Vananda Creek watershed | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate (Possibly in the short term, < 10 yrs/3 gen) | With regard to the sympatric species pairs, Vananda Creek system most accessible |
| | | Little Quarry Lake | Very High | Pervasive (71-100%) | Extreme (71-100%) | Moderate - Low | With regard to the sympatric species pairs, Little Quarry L is less accessible but still within a geographic area where overall threat of AIS is high |
| | | Enos Lake | Very High | Pervasive (71-100%) | Extreme (71-100%) | High (Continuing) | Although the effect of the American Signal Crayfish, which has already appeared in the lake and resulted in collapse of the Enos Lake pair into a hybrid swarm, is likely due to habitat modification (scored under 7.3), crayfish are voracious omnivores in littoral areas and they may be significant nest predators. |
| 8.2 | Problematic native species | | | | | | |
| 8.3 | Introduced genetic material | | | | | | |
| 9 | Pollution | Giant | | | | | |
| | | Unarmoured | | | | | |
| | | Misty Lake | Low | Pervasive (71-100%) | Slight (1-10%) | Moderate - Low | |
| | | Paxton Lake | | | | | |
| | | Vananda Creek watershed | Negligible | Large (31-70%) | Negligible (<1%) | High (Continuing) | |
| | | Little Quarry Lake | | | | | |
| | | Enos Lake | | | | | |
| 9.1 | Household sewage & urban waste water | Giant | | | | | |
| | | Unarmoured | | | | | |
| | | Misty Lake | Negligible | Pervasive (71-100%) | Negligible (<1%) | High (Continuing) | |
| | | Paxton Lake | | | | | |
| | | Vananda Creek watershed | Negligible | Large (31-70%) | Negligible (<1%) | High (Continuing) | |
| | | Little Quarry Lake | | | | | |
| | | Enos Lake | | | | | |

| Threat | | | Impact (calculated) | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 9.2 | Industrial & military effluents | Giant | | | | | |
| | | Unarmoured | | | | | |
| | | Misty Lake | Not Calculated (outside assessment timeframe) | Pervasive (71-100%) | Extreme - Serious (31-100%) | Low (Possibly in the long term, >10 yrs/3 gen) | Catastrophic spill from road transport. |
| | | Paxton Lake | | | | | |
| | | Vananda Creek watershed | Not Calculated (outside assessment timeframe) | Large (31-70%) | Moderate - Slight (1-30%) | Low – Insignificant /Negligible | Road runs alongside some of shore. Chances of spill low (considering type and volume of road traffic) but impact could be significant. |
| | | Little Quarry Lake | | | | | |
| | | Enos Lake | | | | | |
| 9.3 | Agricultural & forestry effluents | Giant | | | | | |
| | | Unarmoured | | | | | |
| | | Misty Lake | Low | Pervasive (71-100%) | Slight (1-10%) | Moderate - Low | Forestry sedimentation |
| | | Paxton Lake | | | | | |
| | | Vananda Creek watershed | | | | | |
| | | Little Quarry Lake | | | | | |
| | | Enos Lake | | | | | |
| 9.4 | Garbage & solid waste | | | | | | |
| 9.5 | Air-borne pollutants | | | | | | |
| 9.6 | Excess energy | | | | | | |
| 10 | Geological Events | | | | | | |
| 10.1 | Volcanoes | | | | | | |
| 10.2 | Earthquakes/tsunamis | | | | | | |
| 10.3 | Avalanches/landslides | | | | | | |
| 11 | Climate Change & Severe Weather | All DU | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |
| | | Unarmoured | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |
| | | Misty Lake | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |
| | | Paxton Lake | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |
| | | Vananda Creek watershed | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |
| | | Little Quarry Lake | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |
| | | Enos Lake | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |

| Threat | | | Impact (calculated) | Scope (next 10 Yrs) | Severity (10 Yrs or 3 Gen.) | Timing | Comments |
|---|---|---|---|---|---|---|---|
| 11.1 | Habitat shifting & alteration | | | | | | |
| 11.2 | Droughts | Giant | | | | | |
| | | Unarmoured | | | | | |
| | | Misty Lake | | | | | |
| | | Paxton Lake | | | | | All species pairs susceptible, potentially exacerbated by water extraction. Droughts impacting littoral shoreline will most directly impact Limnetic breeding fish, but will indirectly impact Benthics by reducing segregated breeding sites and increasing chance of hybridization |
| | | Vananda Creek watershed | | | | | |
| | | Little Quarry Lake | | | | | Same |
| | | Enos Lake | | | | | Same |
| 11.3 | Temperature extremes | Giant | | | | | |
| | | Unarmoured | | | | | |
| | | Misty Lake | | | | | |
| | | Paxton Lake | | | | | High adult male mortality at nest observed at Paxton Lake following extreme heat days; all pairs susceptible. Dolph Schluter reported deaths of many males in shallow water when the weather turned hot rapidly, no doubt associated with stress of reproduction; additional males observed setting up new territories in the same locations at the same time. Climate change will likely bring hot days more often, which could impact reproduction if males die while tending nests in the shallows (<= 1 m depth); likely mainly affecting Limnetics, since Benthic nests are deeper. |
| | | Vananda Creek watershed | | | | | |
| | | Little Quarry Lake | | | | | Same |
| | | Enos Lake | | | | | Same |
| 11.4 | Storms & flooding | Giant | | | | | |
| | | Unarmoured | | | | | |
| | | Misty Lake | Unknown | Pervasive (71-100%) | Unknown | High (Continuing) | |
| | | Paxton Lake | | | | | |
| | | Vananda Creek watershed | | | | | |
| | | Little Quarry Lake | | | | | |