Intraspecific diversity of threespine stickleback (*Gasterosteus aculeatus*) populations in eastern Canada

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ABSTRACT

The threespine stickleback (*Gasterosteus aculeatus*) is a small, mesopredatory fish that is widespread in coastal regions of the northern hemisphere. Although this species does not directly support a commercial or recreational fishery, threespine stickleback often serve as important prey for larger fish that do support important fisheries, as well as many bird species. Although studied extensively as a model organism in evolutionary biology, behavioral ecology, genomics, and numerous related subfields, this research relies heavily on populations from the Pacific coastal regions of North America and Asia, and those of northern Europe. However, based on the morphology of some western Atlantic populations, the different ecological context, and the evolutionary history of the species, not all of the knowledge gained from Pacific and European lineages is likely to be fully transferrable to the populations of North America's East Coast. Nevertheless, work in eastern Canada does suggest high levels of intraspecific phenotypic diversity and local adaptation, though much of this diversity may be under threat from changing climate, altered land use patterns, and introduced species. These factors warrant a research program focused on broad sampling of previously identified populations, identifying previously undocumented populations, determining whether there are unique genetic mechanisms underlying the unusual trait combinations present in the region, and exploring novel community interactions. Such a research program would facilitate the documentation of phenotypic change and establish baselines for future work. Because the work on nearshore marine threespine stickleback populations is sparse in the western Atlantic, I focus here on freshwater populations—with the exception of a brief discussion of the "white" stickleback populations of Nova Scotia—but this is not to suggest that nearshore marine populations are not phenotypically diverse.

Evolutionary History and Population Establishment

Anatomically modern threespine stickleback have lived in the Pacific for at least 13 million years (Bell et al. 2009), and the split between the modern Atlantic and Pacific lineages occurred over 31,000 years ago (Fang et al. 2018). However, the populations of eastern North America are considerably more recently derived. Molecular data suggest that the divergence of the western Atlantic lineage from lineages in the Barents and Norwegian Seas occurred between 7.1 and 17.1 kya, with divergence of Canadian populations from one another between 4.5 and 12.4 kya (Fang et al. 2018). When fossil data and the deglaciation of the St. Lawrence valley are considered, this window of possible establishment times is narrowed to a span of approximately 2 thousand years between 10 kya and 12 kya for southern Ontario and Quebec populations (McAllister et al. 1981; Dyke 2004). In agreement with this estimate, two fossilized stickleback dated to approximately 10 kya have been found in the Green's Creek Formation just south of the Ottawa River (Dawson 1893; McAllister et al. 1981). At that point in time, the region would have been part of the Champlain Sea, facilitating inland migration from the Atlantic, and within a few hundred years, these populations would become isolated in freshwater by isostatic uplift and draining of the Champlain Sea (McAllister et al. 1981). This approximate timeline is also supported by subfossil remains of large freshwater populations in western Greenland aged to at least 9 kya (Bennike 1997), and molecular dating of divergence times between lake populations in the same region to up to 9.9 kya (Liu et al. 2018). The existence of populations in Greenland during this period indicates that threespine stickleback were present along their hypothesized migration route from Europe in the approximate period of time that they arrived in what is now Canada.

In the roughly 10-12.4 thousand years since becoming established in eastern Canada, threespine stickleback have spread across the coastal regions of the Atlantic and Hudson Bay drainages. Populations can be found as far south along the Atlantic coast as the Chesapeake Bay in the United States, north to Baffin Island, west to the northwestern edge of Hudson Bay and inland to Nueltin Lake in Nunavut, and up the Great Lakes-St. Lawrence River Basin to Niagara Falls or Lake Ontario, as they may be absent from the lower

Niagara River (Oliver 1964; Scott and Crossman 1973; Lee et al. 1980; McKillop and McKillop 1997; Lamothe et al. 2021). Threespine stickleback are typically anadromous—spawning in freshwater and then migrating out to sea—and have established numerous freshwater populations across their eastern North American range. Since the early 1980s, they have also been introduced and become established, possibly through shipping routes or as baitfish, in all the Great Lakes upstream of their historic range (U.S. Geological Survey 2021).

The populations in the Hudson Bay region are believed to have become established more recently than those of waterbodies draining into the St. Lawrence. Prior to 8.5 kya, passage into Hudson Bay by stickleback would have been prevented by the Laurentide Ice Sheet, which had melted sufficiently by 8 kya to open the Hudson Strait, south of Baffin Island, but the shores of the bay were not accessible until 7.6 kya (Dyke 2004; Schroeder 2012). The westernmost population in the eastern North American region – that of Nueltin Lake, which spans the Manitoba-Nunavut border—is now approximately 100 m above sea level, and believed to have been isolated from anadromous populations nearly 7 ky by isostatic rebound (Dyke 2004; Schroeder 2012).

Although threespine stickleback have been established on the eastern half of North America for much less time than in Europe or the Pacific (Fang et al. 2018), freshwater populations in this region have extensively, and relatively rapidly, diversified in phenotype.

Unusual Populations and Phenotypes in Eastern North Americ

There are several phenotypes that differentiate the better-studied freshwater populations of stickleback in the Pacific and Europe from those of eastern North America. The most prominent and widespread of these differences is the unusually high frequency of completely-plated individuals in freshwater populations of eastern North America (Figures 1-3). There is also, however, a great deal of diversity between populations that is expressed as variation in color, pelvic reduction, body size, and behavior.

I. PLATE MORPHS

Threespine stickleback have lateral rows of bony defensive plates beginning just behind the head and proceeding to the end of the caudal peduncle, where the final 5-8 plates are tightly fitted together in a keel (Hagen and Gilbertson 1973). The lateral plates appear in three main morphs, each with some within-morph variation (Figure 1): the "complete" (*C*) plate morph includes the keel and an unbroken row of jointed plates continuing along the entire torso; the "low" (*L*) plate morph has no keel and typically an average of ~7 plates near the pelvic girdle; the "partial" (*P*) plate morph has a keel, but then a gap before the rest of the plates, which are generally more numerous than in the *L* morph (Hagen and Gilbertson 1972).

Although freshwater *C* morph populations do occur on occasion, in most parts of the world, a well-documented and widespread pattern of parallel evolution occurs when populations with marine ancestors become isolated in freshwater, where the *C* morph, which is nearly monomorphic in the ocean, is selected against and mostly replaced by *L* morph (Barrett et al. 2008). This shift has occurred remarkably quickly in numerous instances, even over the course of one or a few decades (Klepaker 1993; Bell et al. 2004; Gelmond et al. 2009; Lescak et al. 2015). The adaptation of low-platedness in freshwater habitats has made high-frequency *C* morph freshwater populations a relative rarity globally (Hagen and Gilbertson 1972; Klepaker 1995). In eastern Canada, however, such populations are remarkably common.

In an extensive survey conducted by Hagen and Moodie (1982), the typical pattern of plate morph frequencies in freshwater was completely inverted. This work focused on Prince Edward Island, New Brunswick, and the Gaspésie and Bas-Saint-Laurent regions of Québec, but also included sites in northern Nova Scotia, two on Lake Ontario, and one on the Vermont side of Lake Champlain. They found not a single freshwater population in which the majority of individuals exhibited the *L* morph, but in lakes alone, they identified 19 populations with a *C* morph majority (Figure 2). There are also numerous freshwater streams and rivers, and brackish habitats on both the St. Lawrence and Bay of Fundy sides of QC and NB, respectively, where high *C* morph frequencies are maintained, although it is more difficult to determine whether the freshwater stream and river localities are isolated from anadromous populations (Hagen and Moodie 1982; Delbeek and Williams 1987). Hagen and Moodie (1982), however, argue that freshwater resident and anadromous *C* morph populations can be differentiated by the size and robustness of the lateral plates, which is congruent with my own observations, but difficult to establish as general rule.

The pattern of complete platedness in Atlantic Canada freshwater populations is particularly remarkable because the extant lake populations of western Greenland—the probable sister lineage to the eastern N. American threespine sticklebacks—exhibits the same pattern of *L* morph predominance seen in most of the rest of the world (Liu et al. 2018). Hagen and Moodie (1982) proposed that *C* plate morphs may be favored by the extreme winters in the northwest Atlantic, but the data from Greenland are a compelling case to the contrary (Liu et al. 2018). This East Coast deviation from the typical patterns of plate morph parallelism in fresh water was also observed by McCairns and Bernatchez (2012). However, their study sites were confined to the St. Lawrence estuary and its tributaries, so their hypotheses for the maintenance of *C* monomorphism assumed this trend was specific to the St. Lawrence watershed, and not as widespread as others have demonstrated (McCairns and Bernatchez 2012). Parallel adaptation in freshwater habitats—including reduction of plate counts—is predicted to be constrained in the western Atlantic by founder effects that have resulted in less standing genetic variation at adaptive loci (Fang et al. 2020).

Another, even more unusual trend in plate frequencies among western Atlantic threespine stickleback populations is the number of populations, including several in the Arctic drainage basin identified from museum collections, in which the *P* morph is the most common, or even only, plate morph present (Figures 2 & 3; Coad and Power 1974; Hagen and Moodie 1982; Edge and Coad 1983). This is remarkable because the genetic basis of most variation between the three major plate morphs is a single gene, *Eda*, which generally produces *C* or *L* plated individuals when homozygous for their respective alleles, but produces partially-plated heterozygotes (Colosimo et al. 2004; Colosimo et al. 2005). The low plated allele on North America's East Coast (based on fish from a polymorphic population in Massachusetts) *is* descended from the one that appears on its West Coast (Colosimo et al. 2005), but under this single-locus model of inheritance and in the absence of implausibly strong selection, no more than 50% of individuals in any population should be partially plated, let alone 100%. Hagen and Moodie (1982) did reference six populations on the West Coast that have high frequencies of partial plate morphs. But these lakes have open connections to the ocean, meaning they are likely mixed resident-anadromous populations in which the anadromous adults were unrepresented due to the timing or location of sampling (Hagen and Gilbertson 1972; Kynard and Curry 1976; Moodie and Reimchen 1976). Some of the East Coast high-frequency *P* populations are open to migration permanently or possibly seasonally, but those of QC and NS appear isolated from anadromous populations by the absence of passable outlet streams to marine or estuarine habitats.

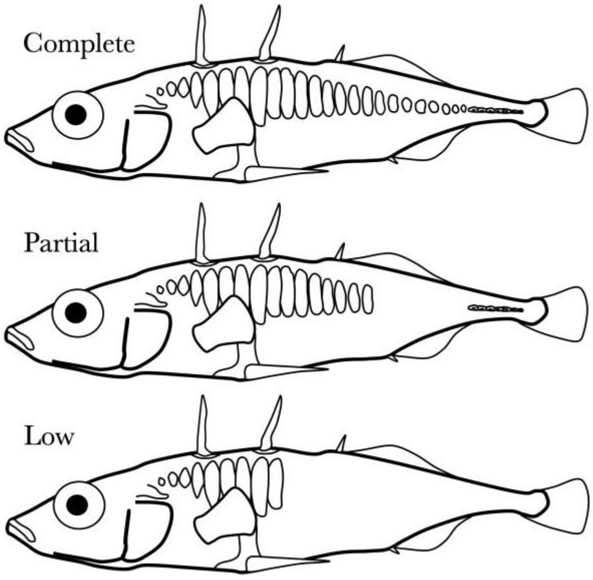

Figure 1. Threespine sticklebacks with Complete (*C*), Partial (*P*), and Low (*L*) plate morphs. Note the series of small, closely associated plates of the caudal keel in the Complete and Partial morphs.

The *L* plate morph, the most globally common plate morph for stickleback in freshwater, appears to be almost entirely absent in eastern Canadian populations outside the Arctic drainage basin. The apparent exceptions to this tendency are a single individual with the *L* plate morph found in the Matamek River, a tributary of the Gulf St. Lawrence northwest of Anticosti Island (Coad and Power 1974), and a population with all three plate morphs in Jamaica Pond in Olmstead Park near Boston, Massachusetts (Colosimo et al. 2005), which is the only known exclusively freshwater population in that state (NHESP ; Hartel et al. 2002). The Jamaica Pond population is believed to be the southernmost freshwater population on the East Coast, although it has been isolated from the Charles River estuary only since the 19th century (NHESP ; Hartel et al. 2002). The geographic distribution of plate morphs in Canada suggests either distinct Arctic Basin and Atlantic Basin lineages, or ongoing gene flow from Greenland to the Canadian Arctic. Neither of these possible histories would explain the origin of the *L* plate morph in Jamaica Pond, however.

II. PELVIC COMPLEX

In threespine sticklebacks, the pelvic complex includes the defensive spines formed by fused pelvic fin rays which are supported by an external pelvic girdle. This structure increases survival in interactions with predatory fishes (Reimchen 1991). Reduction of the pelvic complex does occur rarely in some freshwater populations, beginning with loss of the pelvic spines, and occasionally proceeding to the loss of the entire pelvic girdle that supports them (Bell 1987). However, even in the absence of native predators, reduction of the pelvis occurs only in populations with low calcium concentrations (Bell et al. 1993; but see also Reimchen et al. 2013). At least two freshwater populations in Parc National du Lac-Témiscouata (in Lacs Rond and

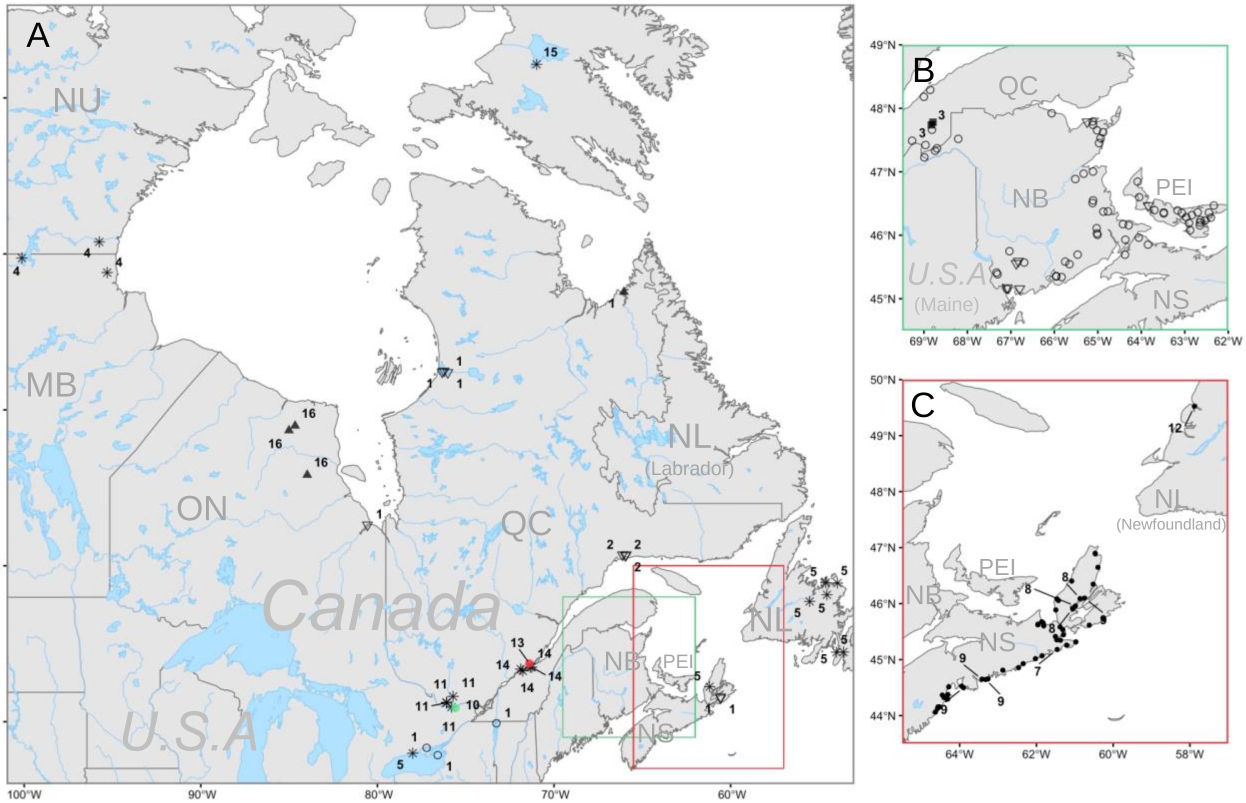

Figure 2. Some documented populations of threespine stickleback in eastern Canada. Most populations shown are from published works, with the exceptions of two iNaturalist observations (refs. 12 & 13). Many museum specimens that have not been included in published works are known, but are not indicated here due to lack of phenotypic data, and most brackish or marine populations are excluded unless morphologically unusual. Panels A and B show freshwater populations, some brackish populations in the Arctic Basin, and a small number of populations in the coastal areas of PEI, NB, and NL that may be brackish based on location, but for which not enough site information was provided to be certain. The green point in panel A indicates two fossil specimens from approximately 10 kya, and the red point indicates the barred color morph identified by O. Morissette, respectively. Panel C shows only populations of the white color morph of sticklebacks, including an observation of a possible white morph in Newfoundland. All sites in panel C are marine. Defensive trait morphs are indicated as follows in panels A and B: Open circle – *C* plate morph most common; Open triangle – *P* plate morph most common; Closed triangle – *L* plate morph most common; Square – majority *P* plate morph and reduced pelvis; Star – plate morphs not determinable from information provided or $n < 20$. For the three low plate morph populations in northern Ontario, the most common plate morph was based on mean plate counts for the populations.

References are indicated by numeric labels as follows:
1) Hagen & Moodie (1982). Can J. Zool. 60:1032-1042. 2) Coad & Power (1974). J. Fish. Res. Board Can. 31:1155-1157. 3) Edge & Coad (1983). Can. Field-Nat. 97:334-336. 4) McKillop & McKillop (1997). Can. Field-Nat. 111:662-663. 5) Garside & Hamor (1973). Can. J. Zool. 51:547-551. 6) Blouw & Hagen (1990). Biol. J. Lin. Soc. 39:195-217. 7) Jamieson et al. (1992). Can. J. Zool. 70:956-962. 8) Jamieson et al. (1992). Can. J. Zool. 70:1057-1063. 9) Haley et al. (2019) Ecol.Evol Res. 20:145-166. 10) McAllister et al. (1981). Can. J. Earth Sci. 18:1356-1364. & Dawson (1893). The Canadian ice age. 11) McAllister & Coad (1974). Fishes of Canada's National Capital Region. 12) Walsh (2021). iNaturalist. Observation 90757153. 13) Morissette (2021). iNaturalist. Observation 100646323. 14) McCairns & Bernatchez (2008). Mol. Ecol. 17:3901-3916. 15) Oliver (1964). Arctic. 17(2):69-83. 16) Schroeder (2012) MS Thesis, Univ. of Manitoba.

All unlabeled points in panel B are from reference 1, and unlabeled points in panel C are from reference 6. The sites of some populations are estimates based on place names due to absence of geographic coordinates or sufficiently detailed maps. To reduce clutter, rivers or streams with multiple sites in their respective original publications are reduced to a single point here.

Croche) have unusual reduction of the pelvic complex (Edge and Coad 1983). The geology of Parc National du Lac-Témiscouata, however, is karstic (Ministère du Développement durable de l'Environnement et des Parcs 2008), and nearby Lac Témiscouata is slightly alkaline (pH 7.8) with moderate conductivity (136 µS /cm; Organisme de bassin versant du fleuve Saint-Jean (OBVFSJ) 2016), so low calcium concentrations in these lakes is unlikely. Additionally, there appear to be no other documented instances of *C* or *P*-plated, reduced pelvis populations in the wild (but see Cresko et al. 2004 for production of such individuals from lab crosses). Thus, the patterns of selection and genetic constraints resulting in the maintenance of this combination of phenotypes in lacs Rond and Croche remains obscure and requires further investigation.

III. COLOR, SIZE, AND BEHAVIOR

Finally, there are several populations with unusual non-armor related morphological phenotypes. These

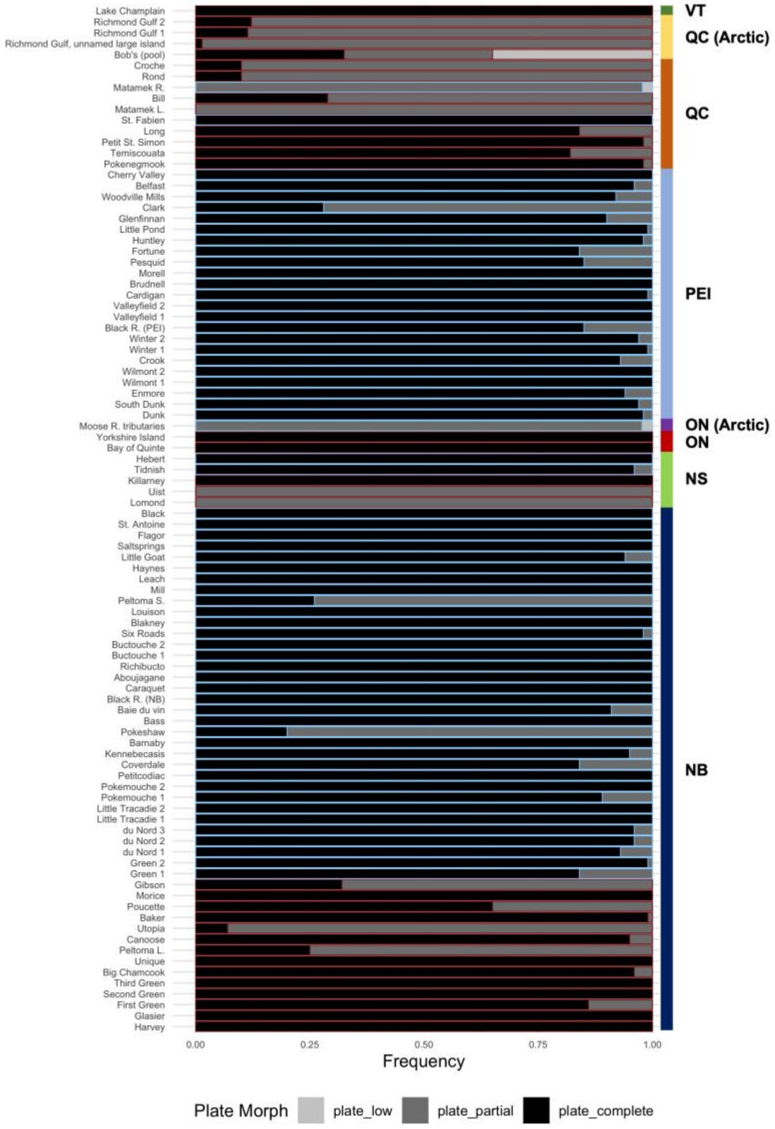

Figure 3. Plate morph frequencies in populations with $n>20$ reported by Hagen & Moodie (1982), Edge & Coad (1983), and Coad & Power (1974). These are all freshwater lake or stream/river populations, with the exception of the Richmond Gulf populations, which are brackish. This body of water is also now known as Lake Tasiujaq, but sites are referred to as "Richmond Gulf" tomaintain clarity with respect to the source publication. Bars for lake populations are outlined in red, and those for stream and river populations are outlined in blue. Although many of these sites are inaccessible to migration from the ocean, it is unfortunately not possible to determine if any are anadromous with the information provided in the source publications. Note that Lac Croche and Rond morph frequencies were pooled together when initially reported. Colors along the right axis indicate the province in which each site was located, and whether it is in the Arctic Ocean drainage basin.

include coastal marine populations of small-bodied "white" stickleback on the Atlantic coast of NS and Bras d'Or Lake, NS, which have iridescent white nuptial coloration in males, reduced parental care, and reversed sexual dimorphism of brain size compared to "common" threespine stickleback (Blouw and Hagen 1990; Jamieson et al. 1992b, a; Samuk et al. 2014; Haley et al. 2019). These populations may mate assortatively, reinforcing isolation from the common threespine stickleback populations (but see Corney 2021 for a contrary result), then lay eggs on filamentous algae, and disperse the embryos, allowing them to sink into the algae mats (Blouw and Hagen 1990; Blouw 1996; Haley et al. 2019). In spite of some gene flow between sympatric common and white stickleback populations, one estimate suggests that common and white stickleback diverged approximately 1 mya (Samuk 2016), implying that the white stickleback are descendants of a lineage that migrated from the Pacific earlier than the rest of the Atlantic lineage (Fang et al. 2018). This divergence time estimate should be understood as tentative, however, due to limitations of the estimation methods, inclusion of a small number of non-western Atlantic specimens from only Denmark, and the finding that the white and sympatric common stickleback are more closely related to each other than either is to the Danish population (Samuk 2016).

A male stickleback that is very similar in appearance to the known white stickleback populations has been recorded on iNaturalist in Neddy Harbour, NF (Figure 4B; Walsh 2021), and further investigation in this location may be worthwhile to determine if it is part of a population that is genetically and ecologically similar to the NS white sticklebacks. The Neddy Harbour individual was collected in Bonne Bay, in a habitat with abundant filamentous algae, similar to the NS populations, and was being kept in captivity when it underwent its most dramatic color change (D. McIlroy, personal communication). The captive status and timing of color change have resulted in differing opinions as to whether this individual is an example of the white stickleback morph (R. J. Scott and A. Dalziel, personal communications).

Dr. Olivier Morissette of Québec's Ministère des Forêts, de la Faune et des Parcs has also observed a population in the Rivière Nelson, a tributary of QC's St. Charles River, which has very pale coloration with bold vertical bars or alternating blotches spanning the lateral line (Figure 4A; Morissette 2021). Freshwater populations have been documented with barring on the Pacific Coast of North America, and at least three genetic regions associated with this pattern in juveniles have been identified (Greenwood et al. 2011), but it is unknown whether the coloration of the population in Rivière Nelson shares this genetic basis. Although such color patterns are sometimes reduced in adults (Kim and Velando 2015), the Rivière Nelson individuals observed are adults that have maintained prominent markings. Barred patterns in stickleback caused by melanophore concentration are associated with antipredator behavior (Kim and Velando 2015), and are favored in selection experiments with cutthroat trout (*Oncorhynchus clarki*) predators, especially in waters with low turbidity (Gygax et al. 2018). Whether barred patterns also serve an antipredatory function against East Coast native salmonid potential predators arctic char, lake trout, brook trout, Atlantic salmon, whitefish, and grayling (*Salvelinus alpinus, S. namaycush, S. fontinalis, Salmo salar*, *Coregonus spp.* & *Prosopium cylindrafomis*, and *Thymallus arcticus*, respectively) is a potentially fruitful avenue of locally relevant community eco – evo research. Notably, the Rivière Nelson stickleback are sympatric with brook trout (Morissette, personal communication).

The Lac Témiscouata population, in addition to having high *C* plate morph frequency, has very large fish (adult mean = 67.05 ± 5.87 mm; LaCasse and Aubin-Horth 2012), not far from the 75 mm threshold used to designate the Drizzle and Mayer Lake sticklebacks of Haida Gwaii as "Giant" for purpose of SARA protection (COSEWIC 2013). The Lac Témiscouata population is also strikingly similar in body shape to the Mayer Lake population (Spoljaric and Reimchen 2007), and has a superficially similar habitat (a large lake with high dissolved organic carbon concentrations and several likely predatory species). Near Lac Témiscouata, the karstic geology has also resulted in some water-filled caverns, and there are second-hand reports that they may contain small populations of stickleback with some accompanying cave adaptations, although research is needed to confirm their phenotypic differentiation from nearby populations (M. Grégoire, personal communication).

Threats to Intraspecific Diversity and Responses to Environmental Change

Intraspecific differences in phenotype between populations have environmental effects that are often as large as the impacts of different species (Des Roches et al. 2018). Morphological and consequent functional differences between populations shape communities and influence ecological function (Mimura et al. 2017), and in stickleback, trait shifts have been shown to influence the composition of their invertebrate prey communities (Schmid et al. 2019). Intraspecific diversity in such a widespread species as threespine stickleback should therefore be an important consideration for conservation practitioners and other environmental scientists whose work involves measuring, forecasting, and mitigating changes in ecological functions. The threats facing individual populations of threespine stickleback (and other native species) in eastern Canada are similar to those on the West Coast, but potentially more challenging for conservation practitioners because so much less is known about the ecology and evolution of East Coast populations. Environmental change and invasive species have the potential to be particularly destructive to both locally adapted specialist phenotypes, and whole populations that are unable to adapt to their presence.

On several occasions, threespine stickleback have been driven to extinction by the introduction of invasive species, including species of particular ecological and scientific interest. The most notable of these are the benthic-limnetic "species" pairs of British Columbia which occupy distinct planktivorous and benthivorous ecological niches and rarely hybridize. Both populations in one of these species pairs were driven to extinction in Hadley Lake, BC, by the introduced brown bullhead catfish (*Ameiurus nebulosus*), now the only species in the lake (Hatfield 2001). Most famously, the Enos Lake, BC species pair went extinct as separate populations and merged into a single, generalist hybrid population following the introduction of the signal crayfish (*Pascifasticus leniscuius*; Taylor et al. 2006). In 2002, the stickleback of Prator Lake, Alaska, a relatively rare reduced pelvis population was driven to extinction by the invasive—and voracious —northern pike *(Esox lucius*; Patankar et al. 2006). This extinction was also a near miss for the similarly weakly armored stickleback population of nearby Bear Paw Lake, which happens to be the source population of the first threespine stickleback to have its whole genome sequenced (Patankar et al. 2006; Jones et al. 2012). The preceding are only some of the most remarkable examples of local extinctions resulting from the establishment of invasive species, but many more have resulted either from introduced species or efforts to limit their spread (e.g., rotenone treatment; Haught and von Hippel 2011; Dunker et al. 2020; Massengill et al. 2020). Within Québec, pike introductions have caused the extinction of a small, isolated population of threespine stickleback in Lac Ramsay in Gatineau Park, along with the lake's populations of Allegheny pearl dace (*Margariscus margarita*) and brook stickleback (*Culea inconstans*; Vachon et al. 2005). Centrarchid basses and sunfishes have also been widely introduced and are likely to be predators of stickleback. Smallmouth bass (*Micropterus dolomieu*), in particular, have already been associated with changes in defensive traits in British Columbia (Kienzle 2018), and in Nova Scotian lakes, introduced smallmouth bass and chain pickerel (*Esox niger*) populations appear to be negatively associated with threespine stickleback presence (A. Dalziel, personal communication).

In addition to local extinctions, there is some evidence that introduced and invasive species are inducing adaptive changes in some populations in the eastern Canada. In Lacs Rond and Croche, the sites of the reduced-pelvis populations in Parc National du Lac-Témiscouata, brook trout (*Salvelinus fontinalis*) were introduced to both lakes for the purposes of sportfishing and have been present since at least 1973 (Edge and Coad 1983). Several cyprinid species—presumably introduced as live bait—are now also present (personal obs.) in addition to the native fathead minnow (*Pimephales promelas*; Edge and Coad 1983). Since the initial assessment of these populations' morphologies from samples taken in 1980-81, the frequency of pelvic reduction in Lac Rond had declined from 100% (n= 82), to 61% (n = 11) in 2010 (Edge and Coad 1983; LaCasse and Aubin-Horth 2012), and the frequency in Lac Croche had declined from 99.2% pelvic reduction (all but one individual of n = 120) to 11% (one individual of n = 9) by 2021 (Edge and Coad 1983; G. Haines 2021, unpublished data). This decline in frequency of reduced pelvises is likely a response to predation in a previously predator-free habitat. In addition to Lac Croche, I sampled Lac Rond in Sept. 2021 in conjunction with Sépaq using Gee-style minnow traps, but did not capture any sticklebacks. This should not be taken as evidence of their complete absence, however, as we conducted only a limited survey at one site on the lake.

In addition to local extinctions and adaptive changes, invasive species cause community biodiversity changes that could influence species interactions and ecological function in the long term. Further south in the Appalachians, invasive species have caused declines in endemic species and homogenization of community composition between habitats (Sleezer et al. 2021). This could pose particular problems for sticklebacks and salmonids (including whitefish), most of which have a propensity for extreme local adaptation (Dynes et al. 1999; Chaverie et al. 2016; Muir et al. 2016; Arostegui and Quinn 2019)—including a series of sympatric dwarf-normal population pairs of lake whitefish occupying the lakes of the St. John River system (Landry et al. 2007). The zebra and quagga mussels (*Dreissena polymorpha* and *D. rostriformis*) that have invaded many habitats in and around the Great Lakes have unknown effects on threespine stickleback. However, following their introduction to Lake Huron they have reduced zooplankton abundance, causing numerous population

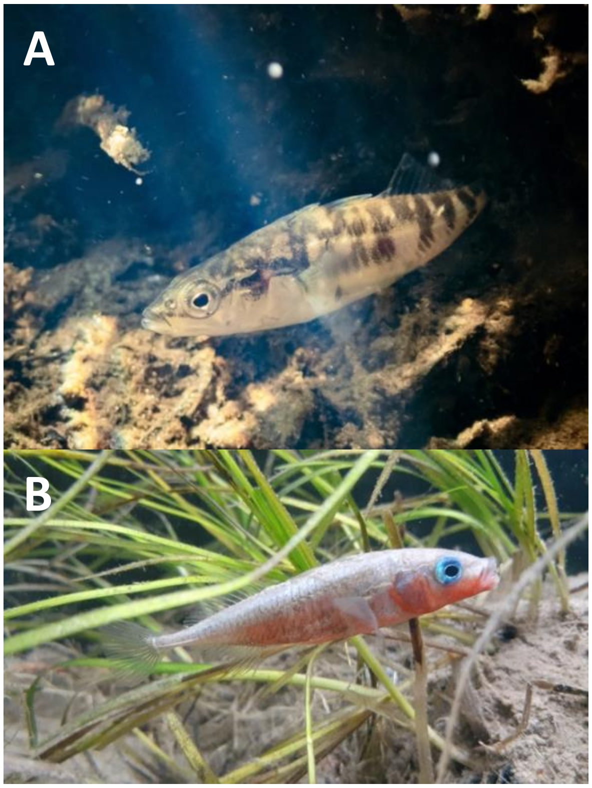

Figure 4. Coloration in understudied populations of threespine stickleback. Panel A shows an individual from a population near Québec City with pale coloration and prominent barred pattern. Image ©Olivier Morissette, used under CC-BY-NC license. Panel B shows a male individual from Bonne Bay, Newfoundland, with similar coloration to the Nova Scotia white stickleback populations. Image ©Tim Walsh, used with permission.

declines of forage fish species, including the threespine stickleback's ecologically similar confamilial species, the ninespine stickleback (*Pungitius pungitius*; Roseman and Riley 2009).

Environmental change, too, can modulate the ecological and evolutionary relationships between sticklebacks and their environments. Des Roches et al. (2019) showed that in systems where precipitation and flow rates are strongly linked, warmer, drier climate led to increases in frequencies of *L* plate morphs. In an abrupt change in the opposite direction, the stickleback population of Lake Washington, Washington experienced a rapid increase in body size and frequency of *C* plate morphs, likely because increased water clarity caused by the opening of a canal left stickleback more vulnerable to salmonid predation (Kitano et al. 2008). Use of surrounding lands has also caused local extinctions by altering habitat morphology (Wootton 2010). In cases of adaptive responses to environmental change like these, it is important to consider that morphological traits are correlated with behavior (LaCasse and Aubin-Horth 2012; De Winter et al. 2016) and function (Bergstrom 2002). Environment-induced adaptive change can therefore not just result in change to a stickleback population, but the whole way it interacts with its habitat. Such changes could have large ecological impacts in systems with few other fish species.

It is important to stress here that, while some knowledge from West Coast or European systems may be transferable, the general paucity of ecological and evolutionary work on threespine stickleback in eastern North American systems means we don't know *how much* will be transferable. Although the primary fish predators of stickleback on the West Coast and Europe are salmonids, they are not the same species as on the East Coast, where we have, eg., lake trout (*Salvelinus namaycush*) and brook trout (*S. fontinalis*). Non-salmonid predators (e.g., topminnows (*Fundulus spp.*), American eel (*Anguilla rostrata*), and centrarchid basses and sunfishes) may also be relatively more important on the East Coast than the West Coast. There is also the issue of the more extreme temperature swings on the East Coast (Kottek et al. 2006), that led Hagen and Moodie (1982) to hypothesize climate was responsible for the pattern of high *C* and *P* plate frequencies themselves, compared to most other locations (with the exception of eastern Europe and Japan), which has the potential to constrain future adaptations to new conditions. Finally, the recency of the entire radiation may mean that a reduced availability of genetic variation could limit adaptive potential to novel changes (Barrett and Schluter 2008).

Conclusions and Recommendations

Although the threespine stickleback radiation of eastern North America should be of particular interest to evolutionary biologists and ecologists, it has been neglected compared to its counterparts in Northern Europe and the Pacific. It exhibits a great deal of variation between freshwater populations in color, body size, and behavior, despite being a maximum of 12.4 thousand years old—younger than any other threespine stickleback lineage that is so geographically expansive. It is also a notable exception to the global pattern of reduced lateral plate counts in freshwater habitats. Furthermore, the extremely high frequencies of partially plated morphs in some isolated populations suggests the presence of an unknown alternative mechanism to heterozygosity at the *Eda* gene for partial-platedness. Determining the causes of this variation could not only help settle the evolutionary conundrum of plate phenotype determination within this lineage, but also help to anticipate responses to ecological change induced by species introductions or environmental change.

Efforts to build the literature on threespine sticklebacks in eastern Canada should prioritize four research directions: 1) Resampling documented populations for previously measured traits to assess whether and how they have changed in the intervening years. For many populations, this can include going back to original specimens—many of which are catalogued in Canadian museums—for phenotyping, although genotyping will be difficult for the many formalin-fixed specimens. 2) Sampling likely sites of populations that have not yet been documented. This work could focus on the inland lakes of the St. John River basin (Hagen and Moodie 1982; Edge and Coad 1983), and the group of lakes in the Moisie and Matamek River systems (Coad and Power 1974), as these have already been demonstrated to have some stickleback populations, and are likely to have more that are undocumented. This exploratory work could further be guided by iNaturalist observations and museum collections, which include documented specimens from the Saguenay River basin, Arctic basin, and inland Nunavik, about which little morphological information exists, despite their isolation from other populations. 3) Determining the genetic basis of the region's plate morphs. Determining the gene or genes responsible for the prevalence of the ***P*** morph is particularly critical. 4) Elucidating ecological and evolutionary relationships between sticklebacks and other East Coast species, particularly salmonids. Of special interest in this respect are stickleback populations of the St. John River system that are sympatric with lake whitefish population pairs, which may be sensitive to any changes that affect zooplankton communities (Landry et al. 2007). This will provide a better understanding of the threats to freshwater environments posed by invasive species and environmental changes induced by a changing climate and patterns of development and land use.

Acknowledgements

Many thanks to Andrew Hendry for his comments on this manuscript and his encouragement to expand it from a brief white paper to a review. Dr. Anne Dalziel, Dr. Olivier Morissette, and Alan García-Elfring also provided comments on this manuscript, which improved it a great deal. Thanks to Rosalie Morin-Nadeau, who arranged permits, served as my guide in Parc National du Lac-Témiscouata, and assisted with field sampling there, and to Michel Grégoire, who brought the park's unusual stickleback populations to the attention of the Hendry Lab and who has supported the lab's work in the park. From Memorial University, thanks to Drs. Duncan McIlroy and Robert J. Scott for providing information about the unusually colored stickleback on display in the Bonne Bay Aquarium and other stickleback populations in the area, and to Tim Walsh, for permission to use his photograph of this individual. For information about the specimens in the southern and western Hudson Bay region, and for providing data on the Manitoba Museum's stickleback collections from this part of the country, thanks to Dr. Randy Mooi. Sampling in Parc National du Lac-Témiscouata was conducted under QC MFFP scientific collection permit No. 20210810-007-01-S-P.

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