Natural History Note

Evolution of Threespine Stickleback (*Gasterosteus aculeatus*) Defensive Traits following the Stocking of Brook Trout (*Salvelinus fontinalis*)

Grant E. Haines,[1,*] Sarah Sanderson,[2] Rosalie Morin-Nadeau,[3] and Andrew P. Hendry[2]

1. Department of Aquaculture and Fish Biology, Hólar University, Hólar, Iceland; 2. Department of Biology, McGill University, Montreal, Québec, Canada; 3. Sépaq, Parc national du Lac-Témiscouata, Témiscouata, Québec, Canada

Submitted October 1, 2024; Accepted August 6, 2025; Electronically published February 3, 2026

Online enhancements: supplemental PDF.

abstract: In recent decades, numerous observations have been made of evolution induced by anthropogenic change in natural populations. Evolution in response to harvest, climate change, pollution, landscape change, and introduced invasive species are common. Here, we provide evidence for evolution in a new context by documenting a large increase in the frequency of defensive pelvic spines in two unusual threespine stickleback (*Gasterosteus aculeatus*) populations previously shown to mostly lack such structures. These populations, in Parc national du Lac-Témiscouata, Québec, Canada, were historically free of predatory fish and consisted nearly entirely of pelvic-spineless stickleback. This phenotypic change coincided with the stocking of the lakes with brook trout (*Salvelinus fontinalis*), a stickleback predator, and the introductions of other species used by anglers as live bait. The rapid evolutionary change toward a more defensive morph in the populations should prompt increased caution regarding the effects of management practices on native species.

Keywords: contemporary evolution, local adaptation, fisheries, stocking, stickleback, brook trout.

Introduction

In threespine stickleback fish (*Gasterosteus aculeatus*), a model species in ecology and evolutionary biology, pelvic spines typically reduce mortality caused by gape-limited predators, including salmonids (Hoogland et al. 1957; Reimchen 1991; Reimchen and Nosil 2002). Populations with frequent pelvic reduction, including spine loss, are unusual globally and extremely rare in the Atlantic drainage of North America, where they are known to occur in only two locations: Narrows Pond, Newfoundland (Scott et al. 2023), and two of more than a dozen permanent lakes (contrasted with the numerous but temporary beaver ponds) in Parc national du Lac Témiscouata (PNLT)—a provincial park in the Bas-Saint-Laurent region of Québec (fig. S1; figs. S1, S2 are available online). In 1980–1981, the vast majority of sticklebacks in Lac Croche and Lac Rond in PNLT lacked pelvic spines (Edge and Coad 1983; Bell 1987). The PNLT lakes and Narrows Pond both have environmental conditions that differ considerably from other regions where reduced-pelvis phenotypes are present, which typically have low calcium concentrations and low levels of predation (Bell et al. 1993; Klepaker et al. 2013). The PNLT lakes and Narrows Pond both have calcium-rich geology, and Narrows Pond also has abundant native brook trout (*Salvelinus fontinalis*), a gape-limited predator (Ministère du Développement durable de l'Environnement et des Parcs 2008; Scott et al. 2023). Additionally, Edge and Coad (1983) found that a large majority of the PNLT sticklebacks have the "partial" lateral plate morph, which is extremely unusual in combination with pelvic spine loss (Klepaker et al. 2013).

Adaptations of native species to selection imposed by introduced populations are widespread in freshwater systems (Taylor et al. 2006; Fisk et al. 2007; Kitano et al. 2008; Sharpe et al. 2012) and can have extensive impacts on ecosystem integrity and functioning (Townsend 1996; Cucherousset and Olden 2011). In lakes and streams, such introduced populations often arrive by inadvertent transport (Lawrie 1970; Bussman and Burkhardt-Holm 2020), through escape/release from aquaria or aquaculture facilities (Courtenay 1990), as baitfish used by anglers (McEachran

* Corresponding author; email: grant.haines@mail.mcgill.ca.

ORCIDs: Haines, https://orcid.org/0000-0001-9085-0022; Sanderson, https://orcid.org/0000-0003-1525-2326; Morin-Nadeau, https://orcid.org/0009-0006-1635-6695; Hendry, https://orcid.org/0000-0002-4807-6667.

 Published by The University of Chicago Press for The American Society of Naturalists. https://doi.org/10.1086/739174

et al. 2022), or through stocking programs (McDowall 1994; Sandlund et al. 2019). Native "nongame" species that are not widely valued as targets for fishing have historically been ignored, considered a nuisance by anglers, or even deliberately exterminated by fisheries managers (Rypel et al. 2021). As a consequence, some strategies commonly employed for the management of "game" species are detrimental to native populations of species with less direct economic value. Within the brook trout's range in eastern North America, for example, introductions into previously trout-free habitats have even been encouraged for conservation (Habera and Moore 2005) or increased production (Kirn 2018; Clancy et al. 2020). Through permitting stocking to such habitats, inappropriate management practices pose a threat to locally adapted and sometimes highly specialized populations.

Between 1973 and 1991, the two PNLT lakes previously documented to contain nearly spine-free stickleback populations were stocked with brook trout (fig. S2). Within a few years, the native sticklebacks had become important prey items of the introduced trout (Edge and Coad 1983) and have remained so well beyond the cessation of stocking (Burman-Plourde 2014; Paquet-Walsh 2014; Lavigne 2015). With new samples collected from 2021 to 2023, we show that in the years since the stocking, the frequency of complete pelvises with defensive spines has increased dramatically in these previously spineless populations. We also document two additional threespine stickleback populations within the park's Sutherland Creek watershed that have likely adapted in response to brook trout.

Methods

The Study Organism

The threespine stickleback populations in Lac Rond and Lac Croche, the lakes we will focus on within PNLT, have been cited as part of the justification for the park's creation (Ministère du Développement durable de l'Environnement et des Parcs 2008) because of their unusual phenotypes. Although the species is fairly common in eastern Canada and the northeastern United States, these particular populations are noteworthy because they exhibited reduced pelvises with missing spines when surveyed in 1980–1981 by Edge and Coad (1983). Mutations resulting in pelvic reduction typically occur de novo in freshwater populations rather than being transported from the marine environment via anadromy (Xie et al. 2019), so it is probable that pelvic reduction is derived from mutations that occurred within this system.

In addition to having two pelvic spines, stickleback typically have a series of bony lateral plates that serve as a defense against predation. These series of plates are commonly assigned to three different morphs: complete, partial, and low. The complete morph is characterized by a continuous series of plates that extend from the operculum to the caudal peduncle (fig. 1). The low morph lacks the caudal keel and most of the plate series. In the intermediate partial morph, the series of plates is broken, leaving a gap between the trunk and the caudal keel. Populations in which partial plate morphs are in the majority are extremely unusual because these individuals are typically produced by heterozygosity at the *EDA* (ectodysplasin) gene, along with minor alleles at other loci related to *EDA*, whereas homozygotes are typically complete or low morphs (Colosimo et al. 2004; Colosimo et al. 2005; Laurentino et al. 2022). However, in Lac Croche and Lac Rond, partially plated individuals were in the majority (>90%), without any low-plated individuals and few completely plated individuals, when surveyed in 1980–1981 (Edge and Coad 1983). This unusual frequency of partial morphs is therefore another characteristic of interest in the Lac Rond and Lac Croche populations (Klepaker et al. 2013). Because some individuals have asymmetric lateral plate series in PNLT lakes, we also recorded a fourth intermediate "asymmetric complete" category for individuals with a complete plate series on one side and a partial one on the other.

Site Description and History

PNLT is located in the Bas-Saint-Laurent region of eastern Québec, lying within the northernmost portion of the St. John River watershed. Most PNLT lakes are situated in the Cabano-Squatec syncline, a largely calcareous basin to the north and east of the western portion of Lac Témiscouata, which contains the entire Sutherland Creek watershed (fig. S1; table S1, available online; Lespérance and Greiner 1969; Tessier 2008). Here, we focus in particular on two lakes within the watershed, Lac Rond and Lac Croche, from which collections of threespine stickleback were made in 1980 and 1981 by Edge and Coad (1983), who identified only three fish species in these lakes: threespine stickleback, brook trout (a recent introduction; see below), and fathead minnows (*Pimephales promelas*; Lac Rond only). The two focal lakes are separated by a series of waterfalls from migrations upstream from the much more speciose Lac Témiscouata (fig. S1). Because Lac Croche sits higher in the basin and is separated from Lac Rond by another small lake (Lac à Foin) followed by a small cascade, downstream migration of fish from Lac Croche to Lac Rond is likely, but the reverse is much less so.

Although all three fish species identified by Edge and Coad (1983) are within their native ranges in PNLT, brook trout had been absent until their introduction was made possible by the construction of logging roads in what would become the park (Tessier 2008). The two focal lakes, Lac Rond and Lac Croche, were first stocked with brook trout

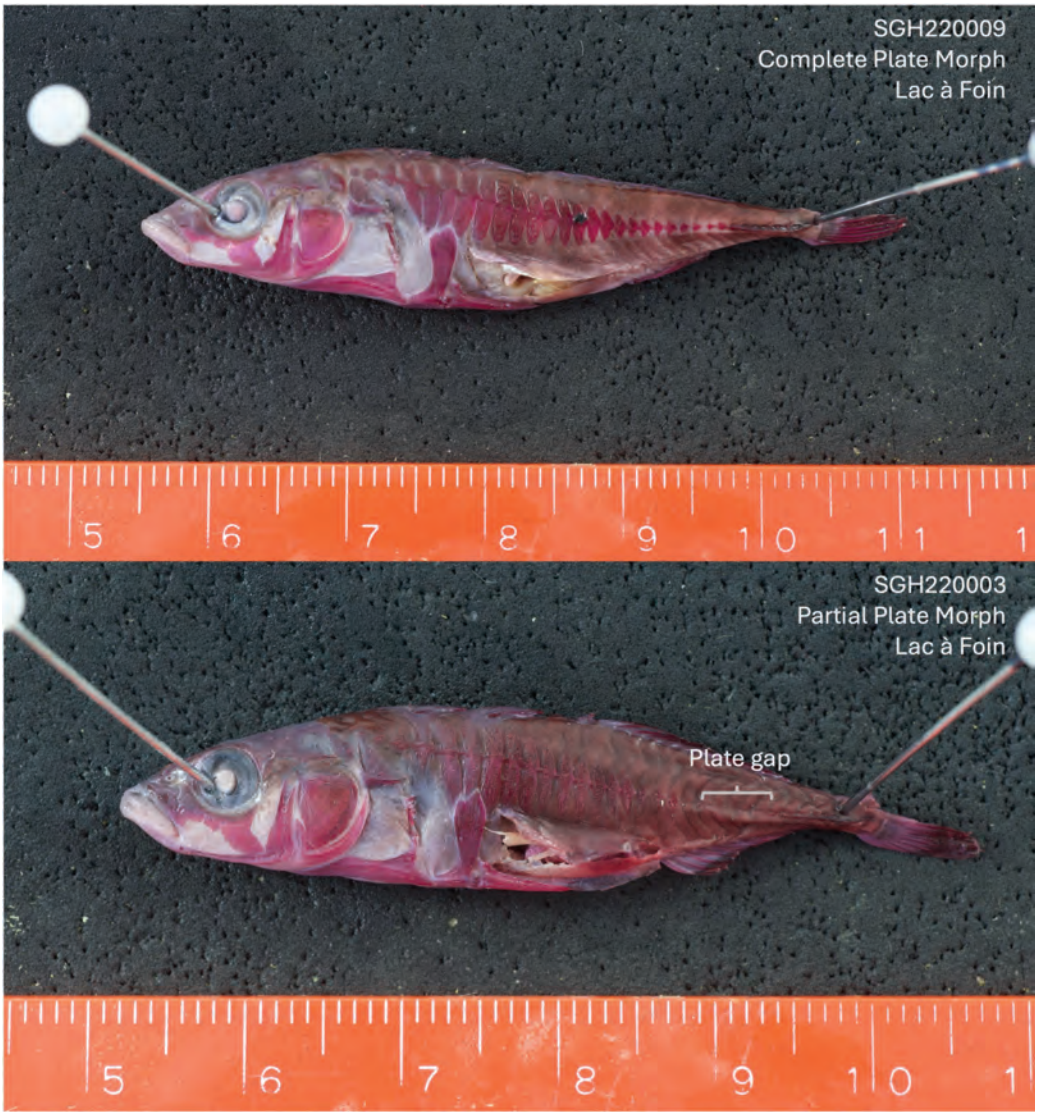

Figure 1: Complete (C) and partial (P) plate morphs. Shown are two threespine stickleback specimens that have been stained with alizarin to highlight bony structures. The gap between the continuous lateral plates and the caudal keel is highlighted on the fish with the partial plate morph. Rulers on the photographs indicate length in centimeters. Both specimens have complete pelvises. Photograph by Sarah Song.

in 1973 and 1974, respectively. Lac à Foin (fig. S1) was also stocked with smaller numbers of brook trout in 1967 and 1968 (fig. S2). Prior to the 1967 stocking of Lac à Foin, no brook trout were present within the Sutherland Creek watershed (Tessier 2008). Emigration from Lac à Foin to both Lac Croche and Lac Rond is possible, and judging by the Edge and Coad (1983) sample size of 75 individuals, trout populations were well established by the time of sampling in 1980 and 1981. Despite the stocking program ending in 1991, thriving populations of the introduced trout persisted more than two decades later, with individuals commonly reaching sizes of more than 30 cm (Paquet-Walsh 2014; Lavigne 2015). Stickleback are major prey items of the brook trout in these lakes and make up a larger proportion of the diets of larger fish (Burman-Plourde 2014; Lavigne 2015). At least two other fish species have become established in Lac Rond and Lac Croche: northern redbelly dace (*Chrosomus eos*) and creek chub (*Semotilus atromaculatus*). Both of these species had become common by 2014 (Paquet-Walsh 2014; Lavigne 2015), likely before the park's establishment in 2009, as both species were frequently used as live bait by recreational anglers in Québec

(Scott and Crossman 1973). Neither creek chubs nor dace are expected to commonly prey on stickleback, although both might act as competitors, and some evidence suggests that large chubs may consume stickleback.

Field and Laboratory Methods

Between 2021 and 2023 we captured stickleback using unbaited Gee-style minnow traps set at numerous sites throughout PNLT (table S1), focusing on Lac Rond and Lac Croche but also including other potential stickleback habitats in the park. We measured basic water-quality variables using a YSI sonde (ProQuatro multiparameter meter; Yellow Springs Inc., Yellow Springs, Ohio) at many of the sites where minnow traps were set. Captured stickleback were euthanized using a clove oil solution, then preserved in 95% ethanol.

For two reasons, pelvic spine counts (zero, one, or two) were used for analysis rather than the pelvic scores of Bell et al. (1993), which are more common in recent work. First, the pelvic score was introduced and became a standard way to describe pelvic reduction only after Edge and Coad (1983) was published, and the descriptions of pelvic phenotypes they used can only be directly mapped onto pelvic scores in some cases. Use of pelvic scores would therefore have prevented us from conducting statistical analyses comparing older data to modern specimens. Second, the number and length of pelvic spines is presumably the target of selection imposed by gape-limited predators. As a consequence, reduction of the pelvis beyond the loss of spines is not directly relevant to the vulnerability to predation by trout.

We recorded lateral plate morphs described above as follows: complete (C)—a full series with no gaps greater than the width of two plates; asymmetric complete (C•)—a complete series on one side but gaps at least three plates wide on the other; partial (P)—a series of plates with anterior plates and a caudal keel but a gap at least three plates wide between them; or low (L)—anterior plates only.

Finally, we compared the current stickleback defensive trait phenotypes in Lac Rond and Lac Croche to those recorded in 1980–1981 by Edge and Coad (1983) and in 2010 by LaCasse and Aubin-Horth (2012). We conducted Wilcoxon rank-sum tests to compare pelvic spine phenotypes between the 1980–1981 samples and the 2021–2023 samples to determine whether the populations changed morphologically in the decades following brook trout stocking. In the LaCasse and Aubin-Horth (2012) dataset, one-spined and two-spined individuals were not recorded as separate categories, so these data are excluded from our statistical analyses and are instead evaluated qualitatively. The plate morphs of the 1980–1981 specimens were not described in sufficient detail by Edge and Coad (1983) to allow comparisons using the Wilcoxon rank-sum test.

To assess whether the available sample sizes were sufficient to detect change, we also performed one-sided exact binomial tests of spine and plate morphs. These tests used the proportions of spineless and partially plated fish in the 1980–1981 samples as population proportions, and because the plate morphs of the populations were not reported separately by Edge and Coad (1983), we used their pooled minimum P morph frequency of 90% for both lakes. LaCasse and Aubin-Horth (2012) also used plate number to differentiate plate morphs—with <10 plates as low, 10–30 plates as partial, and >30 plates as complete morphs—which would have resulted in similar classification outcomes as our method if our C and C• fish were grouped together. Statistical tests were performed as implemented in the stats R package (ver. 4.5.1; R Core Team 2025).

Results

Pelvic Spines

Frequencies of the reduced-pelvis phenotypes decreased significantly between the early 1980s and the early 2020s in both of our focal lakes (table 1; fig. 2; Croche: $W = 96$, $P < .001$; Rond: $W = 129$, $P < .001$). Although substantial phenotypic change has occurred in the Lac Rond and Lac Croche stickleback populations, a small number of stickleback at these sites still had reduced pelvises in the early 2020s—as was common historically (table 1). Exact binomial tests showed that the 2021–2023 sample sizes were sufficient to detect differences from 1980–1981 population proportions in frequencies of fish lacking pelvic spines for both lakes (Croche: $n = 71$, $P < .001$, 95% confidence interval [CI] $= 0$ to 0.106; Rond: $n = 9$, $P < .001$, 95% CI $= 0$ to 0.655). A majority of the specimens collected from Lac Rond by LaCasse and Aubin-Horth (2012) in 2010 were spineless, consistent with increasingly frequent complete pelvises.

In our sampling efforts, we captured threespine stickleback at five sites: Lac Croche, Lac Rond, Lac à Fion, Lac Témiscouata, and a large shallow pond downstream of Lac Rond informally referred to as Lac Sutherland (fig. S1). No earlier stickleback samples from Lac à Foin (the site of the earliest stocking) have been reported, but all 19 individuals collected between 2021 and 2023 have two pelvic spines. One of the seven stickleback collected from Lac Témiscouata had no pelvic spines. One of three specimens collected in Lac Sutherland had a reduced pelvis with one spine. We did not directly measure aqueous calcium concentrations, but low aqueous calcium concentrations are associated with low or very high pH (Weyhenmeyer et al. 2019), and at all sites with stickleback we recorded pH values between 7.07 and 8.20 (table S1). This is further evidence that

Table 1: Counts of specimens from Lac Rond and Lac Croche by defensive trait phenotypes

	Plate morphs				Spine counts			
Lake, year	P	C•	C	Total	0	1	2	Total
Lac Rond:								
1980–1981	...	...	...	...	79	3	...	82
2010[a]	28	...	...	28	17	n.r.	11[a]	28
2021–2023	7	...	1	8	3	1	5	9
Lac Croche:								
1980–1981	...	...	...	...	36	1	1	38
2021–2023	25	6	39	70	3	2	66	71

Note: Plate morphs are as follows: P = partial; C• = complete but asymmetric; C = complete. Spine counts refer to pelvic spines only. n.r. = not reported in source publication.

[a] See the results section for an explanation of differences in data classification between the current study and LaCasse and Aubin-Horth (2012). Individuals not reported as spineless are reported here as having two spines.

adaptation to low calcium is not likely to be responsible for historical pelvic reduction in these populations.

Plate Morphs

Edge and Coad (1983) did not report plate morph data separately for each lake but found that >90% of stickleback in Lacs Croche and Rond had the partial plate morph. Assuming that their 1980–1981 sample was representative of both populations, the available data from these lakes suggests that the frequency of C (complete) or C• (complete asymmetric) fish has remained low in Lac Rond but increased substantially in Lac Croche since 1980–1981 (P plate morph: $n = 70$, $P < .001$, 95% CI = 0 to 0.462; fig. 2). The proportion of partially plated fish in our Lac Rond sample was not statistically different from the frequency present in the earlier sample, and the exact binomial test results suggest that a much larger sample would

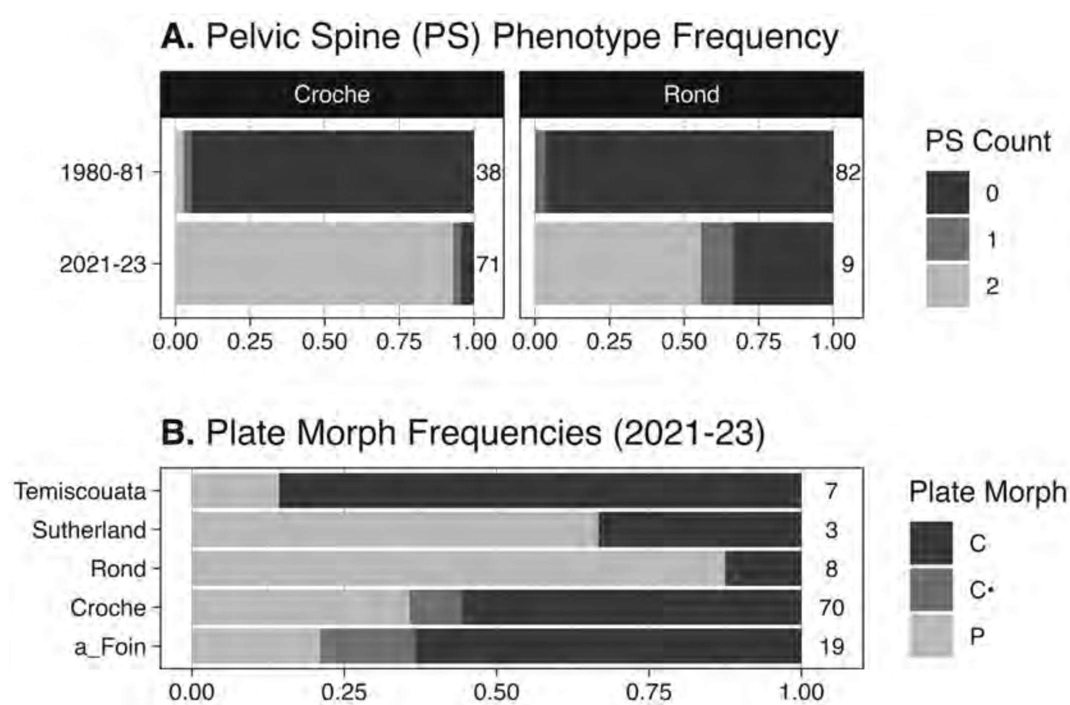

Figure 2: Defensive trait phenotypes. *A*, Pelvic spine counts in the Lac Croche and Lac Rond threespine stickleback samples collected in 1980–1981 (Edge and Coad 1983) and those collected for the present study between 2021 and 2023. *B*, Frequencies of plate morphs in Parc national du Lac Témiscouata lakes with threespine stickleback. "C" indicates a complete plate morph, "C•" indicates an asymmetric complete morph, and "P" indicates a partial plate morph. In both panels, the numbers to the right of the bars indicate sample sizes.

have been required to detect any differences ($n = 8$, $P = .570$, 95% CI = 0 to 0.994), if present.

LaCasse and Aubin-Horth (2012) found that 100% of Lac Rond sticklebacks had the partial plate morph in their specimens collected in 2010 (but see differences in classification methods above), whereas we found that seven of eight fish in our Lac Rond sample had the P morph, with one C morph. Although both the P and C plate morphs have been found at all sites with threespine stickleback, no low-plated individuals have ever been found in any sampling of these lakes.

In their descriptions and illustrations of pelvic reduction, Edge and Coad (1983) and Bell (1987) noted substantial asymmetry in pelvic reduction but did not remark on asymmetry of the lateral plates. We found nine C• morph individuals in our data, along with plate asymmetry within the other morph categories. Furthermore, although they were not measured for this study, lateral plates closer to the keel in all upstream populations appeared reduced in size compared with the plates of fish from Lac Témiscouata, where defensive traits were more robust, a point also remarked on by Edge and Coad (1983).

Discussion

In PNLT, deliberate stocking of brook trout began in the 1960s and appears to have prompted the rapid evolution of elevated pelvic spine frequencies in at least two—and possibly several more—threespine stickleback populations in a previously isolated catchment. Prior to brook trout stocking, these stickleback populations were extremely unusual for both their historical pelvic reduction and their high frequencies of partial plate morphs, which have also declined in one population.

The Cabano-Squatec syncline includes at least four populations of threespine stickleback, a model species in ecology and evolutionary biology. In the two populations that have been studied previously, frequencies of pelvic spine loss have declined rapidly since shortly after the introduction of brook trout, resulting in stickleback with more robust defenses against predation. In another lake (Lac à Foin), which in 1967 became the first to be stocked with brook trout in this watershed, stickleback possess complete pelvises including both spines, but no historical documentation exists of pelvic phenotypes before the introduction of trout for this lake. It is likely that the historical population in Lac à Foin also had reduced pelvises, but this cannot be confirmed with the evidence available at present. Additionally, the C lateral plate morph appears to have increased in frequency in Lac Croche. The L plate morph is absent from these lakes, which is consistent with the broad geographical pattern of phenotypes in eastern North America (Haines 2022). However, the absence of the L plate morph in populations adapted to freshwater is unusual globally due to selection against complete plating in low salinity (Marchinko and Schluter 2007) and is difficult to reconcile with the presence of the P morph, given the known genetic influences on this trait (Colosimo et al. 2004; Colosimo et al. 2005; Laurentino et al. 2022).

Historically, these lakes have had species-poor fish communities, including only threespine stickleback and fathead minnows. Reduced pelvises are typically only found in habitats where they are not selected against by gape-limited predators, particularly salmonids (Bell et al. 1993). It is therefore probable that the shift away from reduced-pelvis phenotypes was an adaptation to predation by the stocked brook trout populations and that low levels of reduced-pelvis phenotypes in Lac Témiscouata are likely maintained by asymmetric gene flow (as suggested by a single specimen in our sample).

Only one other reduced-pelvis population, Narrows Pond in Newfoundland, is known within the threespine stickleback's range in eastern North America (Scott et al. 2023). The more extensive reduction of the pelvis for stickleback in Narrows Pond, which results in complete absence of the pelvic girdle in the majority of individuals, likely points to different genetic bases for pelvic reduction in these two locations (Edge and Coad 1983; Scott et al. 2023). The fragility and resulting high mutation rate of the main genetic region responsible for pelvic reduction in stickleback means that for this trait, separate de novo mutations in the PNLT lakes and Narrows Pond are more probable than a mutation derived from a common marine ancestor (Schluter and Conte 2009; Xie et al. 2019).

The establishment of self-sustaining brook trout populations in at least Lac Rond and Lac Croche, as well as subsequent responses of stickleback antipredator morphology, illustrates the evolutionary hazards of stocking programs to native fish communities. This supports previous work showing stocking-induced changes to native fish communities, including local extirpations of some species (Patankar et al. 2006), extirpation of native locally adapted morphs of the stocked species (Wilson 2021), and shifts in prey community composition (Parker et al. 2001) that can also induce an eco-evolutionary feedback on the stocked population (Combrink et al. 2023). Awareness of the importance of local adaptation in populations of native species has begun to be reflected in policies around stocking (Cowx et al. 2012; Maine 12 M.R.S. §12461 2013; United States of America 16 USC §90(c)(3) 2014). Still, although stocking regulations increasingly restrict the introduction of nonnative species functionally if not explicitly (Pennsylvania 58 Pa. §71a.5 2024; Vermont 10 V.S.A. §4605 2024; DEC 2025), they rarely protect native fish populations from stocking of regionally "native" species that were previously absent from specific waters. Stocking programs similar to those once carried out in PNLT are no longer possible in Québec, as its stocking guidelines provide a rare warning about the hazards to native populations, largely due to the extirpations of some populations in Gatineau Park (Chapleau et al. 1997; Ministère des Ressources Naturelles et de la Faune 2008) However, the dramatic changes in response to stocking that we have documented in these unique threespine stickleback populations should prompt freshwater fisheries managers and regulators in other jurisdictions to evaluate the rationale, necessity, and possible consequences of existing stocking programs and new programs under consideration, even for species native to the region.

Acknowledgments

Many thanks to Michel Grégoire for comments on the manuscript, as well as for his advice and assistance in planning this project. Thanks also to Joanne Marchesseault for informing us of previous work on brook trout within the park, including the surveys of brook trout in Lac Rond and Lac Croche. Sarah Song stained and photographed the specimens shown in figure 1 and assisted in data collection.

Statement of Authorship

G.E.H.: conceptualization, experimental design, data collection, data analysis, data validation, data visualization, writing—original draft, writing—review and editing; S.S.: experimental design, data collection, writing—review and editing; R.M.-N.: experimental design, data collection, provided resources; A.P.H.: funding acquisition, experimental design, data analysis, supervision, writing—review and editing.

Data and Code Availability

Code and data associated with this study can be found in the Dryad Digital Repository (https://doi.org/10.5061/dryad.xwdbrv1r5; Haines 2025).

Literature Cited

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., 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.

Burman-Plourde, C. 2014. Régime alimentaire de la population d'omble de fontaine (*Salvelinus fontinalis*), du lac Croche, au Parc national du Lac-Témiscouata. Y. Lemay, ed. Université du Québec à Rimouski, Rimouski.

Bussman, K., and P. Burkhardt-Holm. 2020. Round gobies in the third dimension—use of vertical walls as habitat enables vector contact in a bottom-dwelling invasive fish. Aquatic Invasions 15:683–699.

Chapleau, F., C. S. Findlay, and E. Szenasy. 1997. Impact of piscivorous fish introductions on fish species richness of small lakes in Gatineau Park, Québec. Écoscience 4:259–268.

Clancy, M., M. Cohen, J. Daley, L. Durfey, R. Fiorentino, M. Flaherty, F. G. Henson, et al. 2020. New York State trout stream management plan. New York State Department of Environmental Conservation.

Colosimo, P. F., K. E. Hoseman, S. Balabhadra, G. Villarreal Jr., M. Dickson, J. Grimwood, J. Schmutz, et al. 2005. Widespread parallel evolution in sticklebacks by repeated fixation of ectodysplasin alleles. Science 307:1928–1933.

Colosimo, P. F., C. L. Peichel, K. Nereng, B. K. Blackman, M. D. Shapiro, D. Schluter, and D. M. Kingsley. 2004. The genetic architecture of parallel armor plate reduction in threespine sticklebacks. PLoS Biology 2:0635.

Combrink, L. L., W. C. Rosenthal, L. J. Boyle, J. A. Rick, E. G. Mandeville, A. C. Krist, A. W. Walters, et al. 2023. Parallel shifts in trout feeding morphology suggest rapid adaptation to alpine lake environments. Evolution 77:1522–1538.

Courtenay, W. R., Jr. 1990. The introduced fish problem and the aquarium fish industry. Journal of the World Aquaculture Society 21:145–159.

Cowx, I. G., A. D. Nunn, J. P. Harvey, and R. A. A. Noble. 2012. Guidelines for stocking of fish within designated natural heritage sites. Scottish Natural Heritage Commissioned Report No. 513.

Cucherousset, J., and J. D. Olden. 2011. Ecological impacts of nonnative freshwater fishes. Fisheries 36:215–230.

DEC (Department of Environmental Conversation). 2025. NY Fish Stocking Permit. https://dec.ny.gov/regulatory/permits-licenses/fish-wildlife-plant/special-licenses/fish-stocking-permits. Accessed February 18.

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.

Fisk, D. L., L. C. Latta IV, R. A. Knapp, and M. E. Pfrender. 2007. Rapid evolution in response to introduced predators I: rates and patterns of morphological and life-history trait divergence. BMC Evolutionary Biology 7:22.

Habera, J., and S. Moore. 2005. Managing Southern Appalachian brook trout. Fisheries 30:10–20.

Haines, G. E. 2022. Intraspecific diversity of threespine stickleback (*Gasterosteus aculeatus*) populations in eastern Canada. Environmental Biology of Fishes 106:1177–1194.

Haines, G. E., S. Sanderson, R. Morin-Nadeau, and A. Hendry. 2025. Data from: Evolution of threespine stickleback (*Gasterosteus aculeatus*) defensive traits following the stocking of brook trout (*Salvelinus fontinalis*). American Naturalist, Dryad Digital Repository, https://doi.org/10.5061/dryad.xwdbrv1r5.

Hoogland, R., D. Morris, and N. Tinbergen. 1957. The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as means of defense against predators (*Perca* and *Esox*). Behaviour 10:205–236.

Kirn, R. 2018. The Vermont management plan for brook, brown, and rainbow trout. Vermont Fish and Wildlife Department, Montpelier.

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

Klepaker, T., K. Østbye, and M. A. Bell. 2013. Regressive evolution of the pelvic complex in stickleback fishes: a study of convergent evolution. Evolutionary Ecology Research 15:413–435.

LaCasse, J., and N. Aubin-Horth. 2012. A test of the coupling of predator defense morphology and behavior variation in two threespine stickleback populations. Current Zoology 58:53–65.

Laurentino, T. G., N. Boileau, F. Ronco, and D. Berner. 2022. The ectodysplasin-A receptor is a candidate gene for lateral plate number variation in stickleback fish. G3 12:jkac077.

Lavigne, K. 2015. Diagnose et caractérisation de la population d'omble de fontaine (*Salvelinus fontinalis*) du lac Rond, situé dans le Parc national du Lac-Témiscouata. Y. Lemay, ed. Université du Québec à Rimouski, Rimouski.

Lawrie, A. H. 1970. The sea lamprey in the Great Lakes. Transactions of the American Fisheries Society 99:766–775.

Lespérance, P. J., and H. R. Greiner. 1969. Squatec-Cabano area: Rimouski, Riviére-du-Loup and Témiscouata Counties. Geological Reports, Quebec Department of Natural Resources.

Maine 12 M.R.S. §12461. State heritage fish waters, last revised 2013. Accessed August 27, 2025.

Marchinko, K. B., and D. Schluter. 2007. Parallel evolution by correlated response: lateral plate reduction in threespine stickleback. Evolution 61:1084–1090.

McDowall, R. M. 1994. The origins of New Zealand's Chinook salmon, *Oncorhynchus tshawytscha*. Marine Fisheries Review 56:1–7.

McEachran, M. C., A. H. Mohr, T. Lindsay, D. C. Fulton, and N. B. D. Phelps. 2022. Patterns of live baitfish use and release among recreational anglers in a regulated landscape. North American Journal of Fisheries Management 42:295–306.

Ministère des Ressources Naturelles et de la Faune. 2008. Lignes directrices sur les ensemencements de poissons. Québec.

Ministère du Développement durable de l'Environnement et des Parcs. 2008. Parc national du Lac-Témiscouata: Plan directeur provisoire. Bibliothèque et Archives nationales du Québec, Québec.

Paquet-Walsh, A. 2014. Diagnose et caractérisation de la population d'ombles de fontaine (*Salvelinus fontinalis*), du lac Croche, au Parc national du Lac-Témiscouata. Y. Lemay, ed. Université du Québec à Rimouski, Rimouski.

Parker, B. R., D. W. Schindler, D. B. Donald, and R. S. Anderson. 2001. The effects of stocking and removal of a nonnative salmonid on the plankton of an alpine lake. Ecosystems 4:334–345.

Patankar, R., F. A. von Hippel, and M. A. Bell. 2006. Extinction of a weakly armoured threespine stickleback (*Gasterosteus aculeatus*) population in Prator Lake, Alaska. Ecology of Freshwater Fish 15:482–487.

Pennsylvania 58 Pa. §71a.5. Notice of Stocking to the Commission, last revised 2024. Accessed August 27, 2025.

R Core Team. 2025. R: a language and environment for statistical computing. Foundation for Statistical Computing, Vienna.

Reimchen, T. E. 1991. Trout foraging failures and the evolution of body size in stickleback. Copeia 1991:1098–1104.

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

Rypel, A. L., P. Saffarinia, C. C. Vaughn, L. Nesper, K. O'Reilly, C. A. Parisek, M. L. Miller, et al. 2021. Goodbye to "rough fish": paradigm shift in the conservation of native fishes. Fisheries 46:605–616.

Sandlund, O. T., H. H. Berntsen, P. Fiske, J. Kuusela, R. Muladal, E. Niemelä, I. Uglem, et al. 2019. Pink salmon in Norway: the reluctant invader. Biological Invasions 21:1033–1054.

Schluter, D., and G. L. Conte. 2009. Genetics and ecological speciation. Proceedings of the National Academy of Sciences of the USA 106:9955–9962.

Scott, R. J., G. E. Haines, and C. A. Trask. 2023. Armour reduction and pelvic girdle loss in a population of threespine stickleback (*Gasterosteus aculeatus*) from western Newfoundland, Canada. Environmental Biology of Fishes 106:685–695.

Scott, W. B., and E. J. Crossman. 1973. Freshwater fishes of Canada. Fisheries Research Board of Canada, Ottawa.

Sharpe, D. M. T., S. B. Wandera, and L. J. Chapman. 2012. Life history change in response to fishing and an introduced predator in the East African cyprinid *Rastrineobola argentea*. Evolutionary Applications 5:677–693.

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.

Tessier, I. 2008. États des Connaissances: Parc national du Lac-Témiscouata. J. Boisclair, ed. Développement durable, Environnement et Parcs, Québec.

Townsend, C. R. 1996. Invasion biology and ecological impacts of brown trout *Salmo trutta* in New Zealand. Biological Conservation 78:13–22.

United States of America 16 USC §90(c)(3). North Cascades National Park Service Complex Fish Stocking Act, last revised 2014. Accessed August 27, 2025.

Vermont 10 V.S.A. §4605. Placing fish in waters; fish importation permits, last revised 2024. Accessed August 27, 2025.

Weyhenmeyer, G. A., J. Hartmann, D. O. Hessen, J. Kopáček, J. Hejzlar, S. Jacquet, S. K. Hamilton, et al. 2019. Widespread diminishing anthropogenic effects on calcium in freshwaters. Scientific Reports 9:10450.

Wilson, P. N. 2021. Extirpation of a native population following a stocking program: genetic population structure and demographics of kokanee (*Oncorhynchus nerka*) in a large impounded watershed. University of British Columbia.

Xie, K. T., G. Wang, A. C. Thompson, J. I. Wucherpfennig, T. E. Reimchen, A. D. C. MacColl, D. Schluter, et al. 2019. DNA fragility in the parallel evolution of pelvic reduction in stickleback fish. Science 363:81–84.

Natural History Editor: Leticia Avilés