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Banded Killifish (*Fundulus diaphanus*) and Mummichog (*Fundulus heteroclitus*) distributions in insular Newfoundland waters: implications for a Species at Risk

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Abstract

Newfoundland's Banded Killifish (*Fundulus diaphanus*) population is listed as a species of Special Concern under Canada's *Species at Risk Act* and Vulnerable under *Newfoundland and Labrador's Endangered Species Act*. Mummichog (*Fundulus heteroclitus*) is a similar looking fish species and is currently under review by Newfoundland and Labrador's Species Status Advisory Committee. Both species have limited known distributions in Newfoundland waters that overlap. They may occur sympatrically in estuaries and occasionally hybridize; thus, field identifications can be challenging. We found that dorsal fin position and caudal fin depth were the most useful morphological characters for distinguishing Banded Killifish and Mummichog in the field. We used local ecological knowledge, literature review, museum records, and field surveys to update the known distribution ranges and found both species in more locations than previously documented in Newfoundland. Thus, we extend their known ranges. Our results will be critical in future status assessments of these species in Newfoundland.

Key words: Banded Killifish; distribution range; *Fundulus*; identification; Mummichog; Newfoundland; range extension; species at risk

Introduction

Oviparous cyprinodontiform fishes, commonly known as killifishes and topminnows, occur naturally on all continents except Australia and Antarctica and are common in fresh, brackish, and occasionally coastal seawater (Scott and Scott 1988). In North America, they extend as far north as southern Canada where there are three *Fundulus* species (family Fundulidae): Blackstripe Topminnow (*Fundulus notatus*), Banded Killifish (*Fundulus diaphanus*), and Mummichog (*Fundulus heteroclitus*; Houston 1990). There are two subspecies of Banded Killifish, the eastern subspecies (*Fundulus diaphanus diaphanus*) and the western subspecies (*Fundulus diaphanus menona*), both of which are present in Canada (Scott and Crossman 1973) but only the eastern subspecies occurs in Newfoundland. There are also two subspecies of Mummichog, the southern subspecies (*Fundulus heteroclitus heteroclitus*) and the northern subspecies (*Fundulus heteroclitus macrolepidotus*), of which only the latter occurs in Canadian waters (Able and Felley 1986). In Canada, Blackstripe Topminnow and the Newfoundland population of Banded Killifish were assessed as species of Special Concern by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2012, 2014) and are listed as such under Canada's *Species At Risk Act* (SARA Registry 2019a,b). The Newfoundland population of Banded Killifish is also listed as Vulnerable under *Newfoundland and Labrador's Endangered Species Act* (Endangered Species List Regulations 2002). Throughout most of its distribution range Banded Killifish is considered Not at Risk (COSEWIC 2014; SARA Registry 2019b). The Newfoundland population's Special Concern and Vulnerable designations are due to a limited and clustered distribution in insular Newfoundland, which makes them vulnerable to catastrophic events and local disturbances (COSEWIC 2014). Also present in Newfoundland waters is Mummichog (Scott and Crossman 1973), whose status is currently under review by the Newfoundland and Labrador Species Status Advisory Committee (T. Knight pers. comm. 28 January 2016). Literature suggests Mummichog distribution is restricted to south-

western Newfoundland (Scott and Scott 1988).

Banded Killifish (Figure 1a) and Mummichog (Figure 1b) are both considered euryhaline, but Banded Killifish is more of a freshwater species, whereas Mummichog is more of a brackish-water species with only a few documented freshwater populations (Klawe 1957; Denoncourt *et al*. 1978; Scott and Scott 1988). Both species are very similar in appearance and may school together where sympatric populations occur in brackish waters (Scott and Crossman 1964). In addition, these two species have been reported to occasionally hybridize (Fritz and Garside 1974; Dawley 1992). These factors make field identification difficult, which may affect population studies and delineation of their respective distribution ranges (Fisheries and Oceans Canada 2011).

Little effort has been spent in delineating the distribution range of Banded Killifish in Newfoundland. Most early reports have been accidental discoveries by anglers and researchers studying other species (Chippett 2004). Localized surveys for Banded Killifish, conducted just prior to the 2003 COSEWIC assessment and update status report (in Terra Nova National Park [Cote *et al.* 2002]; Gros Morne National Park [Knight 2002]; Indian Bay watershed [Chippett 2004]), added only one watershed to their known distribution. Since 2006, the Mi'kmaq Alsumk Mowimsikik Koqoey Association (MAMKA) documented by-catch of Banded Killifish from the American Eel (*Anguilla rostrata*) commercial fishery in western Newfoundland (MAMKA 2006) and conducted Banded Killifish surveys (MAMKA 2011). However, except for a few earlier reports (e.g., Templeman 1951; Scott and Crossman 1964; Day 1993), there was no direct evidence of Banded Killifish at many of the locations presented in COSEWIC (2014).

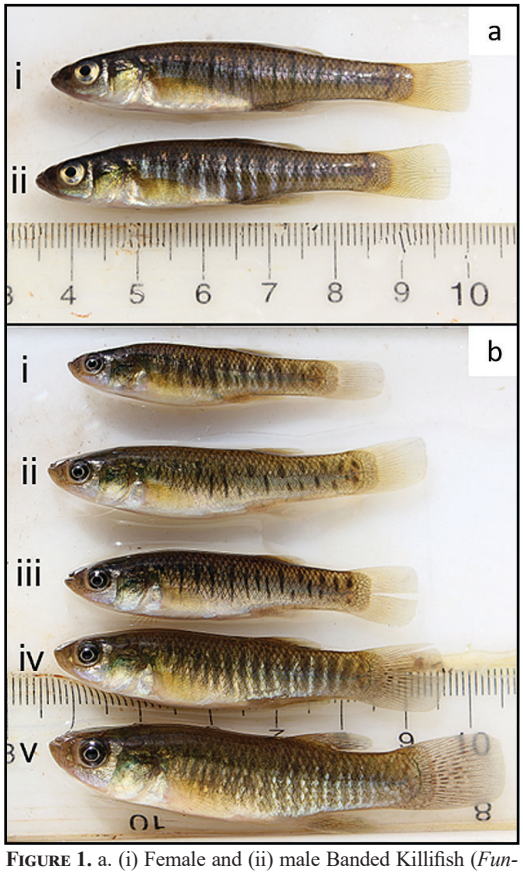

Figure 1. a. (i) Female and (ii) male Banded Killifish (*Fundulus diaphanus*); b. (i, iv, v) male and (ii, iii) female Mummichog (*Fundulus heteroclitus*), each exhibiting variations in banding patterns. Photos: Kate Dalley.

The objectives of this study were to determine the morphometric characters that allow for clear differentiation of Banded Killifish and Mummichog in the field and to update their known distribution ranges in insular Newfoundland waters. Field surveys, literature reviews, museum records, and local ecological knowledge (LEK) were used to update distribution ranges. Results from this study will provide new data that will help in the assessment on their listing status both at the federal and provincial levels.

Methods

A literature search was conducted for records of Banded Killifish and Mummichog in Newfoundland. Museums, including the Canadian Museum of Nature (CMN, Ottawa, Ontario [ON]), Royal Ontario Museum (ROM, Toronto, ON), Atlantic Reference Centre (ARC, St. Andrew's, New Brunswick [NB]), The Rooms Natural History Department (The Rooms, St. John's, Newfoundland and Labrador [NL]), Maurice Lamontagne Institute (MLI, Mont-Joli, Quebec [QC]), Ministère des forêts, de la faune et des parcs du Québec (Longueuil, QC), and Nova Scotia Museum (Halifax, Nova Scotia), were also contacted for reports of Banded Killifish. Federal fisheries officers from Fisheries and Oceans Canada, personnel from the Provincial Departments of Fisheries and Aquaculture, and Environment and Conservation, MAMKA river guardians, commercial eel harvesters, academic researchers, and local residents were contacted via telephone, e-mail, or in-person to gather local knowledge and determine potential locations of both species in Newfoundland. Pictures of the species were provided, and people contacted were asked if and where they had been observed. Maps were provided to assist the identification of drainage systems where *Fundulus* spp. were observed, where a 'drainage system' was defined as any water system with

a separate drainage to the ocean. Several commercial eel harvesters were requested to retain Banded Killifish/Mummichog specimens captured in their fishing gear and, in some instances, incidental conversations with locals during field surveys were used to gather additional information. Information gathered from the aforementioned sources was used to plan field surveys and focus effort in general areas where there were reports of these species.

A total of 102 sites were sampled for Banded Killifish and Mummichog from 2013 to 2018. Most were sampled using four Gee Minnow Traps (42 cm L × 19 cm D, 22 mm opening, 6.4 mm mesh; Fillmore, New York, USA) that had been presoaked in saltwater for 24 h to remove the surface shine. Traps were each baited with 10 Original Ritz crackers (Mondelēz International, Toronto, Ontario, Canada). Additional opportunistic collection methods included minnow traps baited with sardines, fyke nets, hoop nets, dip nets, pole seines, LR-24 Electrofisher (Smith-Root, Vancouver, Washington, USA), and from stomach contents of Brook Trout (*Salvelinus fontinalis*) caught icefishing with baited hook and line, while other specimens were provided by local residents. Catches from each survey were identified, counted, and standard length (SL) measured. Samples of *Fundulus* spp. (typically 4–6 individuals) were preserved in 70% ethanol from each location for later analysis of morphological and meristic characters for species identification and museum archival. Most Banded Killifish and Mummichog specimens retained in this study were deposited at The Rooms, Provincial Museum of Natural History Annex (St. John's, NL), whereas those sent to the ARC (St. Andrew's, NB) for identification were deposited there (Table S1). Sampling data from this study has been submitted to the Ocean Biogeographic Information System (OBIS) for public archive. Reports of each species were verified using physical specimens or detailed photographs (Figure S1).

In the laboratory, meristic characters (Scott and Crossman 1964) and one morphological character (Scott and Scott 1988) were used to verify field identifications. Meristic characters (Table 1) included the number of dorsal fin rays, the number of gill rakers on the first gill arch, and the number of scale rows, and the stepped forward location (SFL) morphometric were used to identify the species (Table 1; Figure 2). Smaller juveniles were difficult to identify, and several other individuals showed a mix of characteristics suggesting the possibility of hybridization and were sent to L. van Guelpen (ARC) for species identification.

After initial identification of specimens collected from 2013 to 2016, additional morphological measurements were recorded as potential characters to differentiate species for individuals ≥27.5 mm SL in the field (Figure 2). Morphological characters identified from the literature to differentiate these species included the dorsal fin index (DFI; Scott and Crossman 1964; Table 1; Figure 2) and the ratio of the caudal peduncle depth (CD) relative to the distance from the dorsal origin to posterior end of vertebrae (DO–EV); the inverse of the ratio used by Fritz and Garside (1974) and Hernández Chávez and Turgeon (2007).

Data for Banded Killifish, Mummichog, and possible hybrids were plotted using SigmaPlot version 13.0 (Systat 2014) to determine the amount of over-

TABLE 1. Definitions of meristic and morphometric characters used to identify Banded Killifish (*Fundulus diaphanus*) and Mummichog (*Fundulus heteroclitus*) in Newfoundland. See Figure 2 for illustrations of the morphometric measurements.

Character type	Character	Description
Meristic	Number of dorsal fin rays (DFR)	Number of fin rays on the dorsal fin
Meristic	Number of gill rakers (GR)	Number of gill rakers on the first gill arch
Meristic	Number of scale rows (SR)	Number of scales in the longitudinal row from just posterior to the operculum to the end of the caudal peduncle
Morphometric	Caudal depth (CD)	Vertical distance from the dorsal to the ventral part of the caudal peduncle
Morphometric	Dorsal origin to posterior end of vertebrae (DO–EV)	Distance from the anterior origin of the dorsal fin to the posterior end of the vertebral column
Morphometric	Dorsal origin to tip of snout (DO–ST)	Distance from the anterior origin of the dorsal fin to the tip of the snout
Morphometric	Standard length (SL)	Distance from the tip of the snout to the posterior end of the vertebral column
Morphometric	Dorsal fin index (DFI)	Measure the DO–EV distance and subtract it from the DO-ST distance (see Scott and Crossman 1964)
Morphometric	Stepped forward location (SFL)	Measure the DO–EV distance and step that distance anteriorly from the anterior origin of the dorsal fin; the location on the head at which this measurement lands determines the species; if the location lands near the eye it was identified as Banded Killifish, whereas if it landed on the operculum it was identified as Mummichog (Scott and Scott 1988: 612)

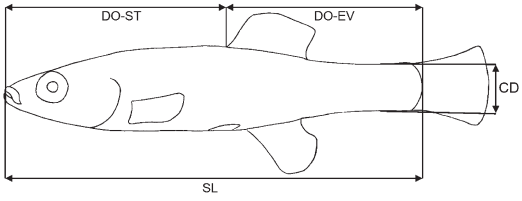

Figure 2. Morphological measurements recorded from killifish (*Fundulus* spp.) in insular Newfoundland. Abbreviations: CD = caudal depth; DO–EV = origin of dorsal fin to end of vertebrae; DO–ST = origin of dorsal fin to snout tip; SL = standard length.

lap of each character. Characteristics with <5% overlap were identified as the most useful for differentiating Banded Killifish and Mummichog.

Results

In general, Banded Killifish (Figure 1a) has a more slender and compressed body form with a more tapered mouth compared to Mummichog (Figure 1b). Mummichog has a stout, robust body form and deeper caudal peduncle compared to Banded Killifish. Females of both species exhibit thin vertical black bands along their sides, but on Mummichog, these bands, posterior to the anal fin, are usually shortened and do not span the width of the body (Figure 1). Males of both species do not have black bands but instead have alternating dark olive and lighter white/silver/blue bands during the breeding season that are closer together compared to bands of females (Figure 1).

From our measurements and analyses (Table S2; Figure S2), we developed the following identification key from the most useful meristic and morphometric characters. Individuals that exhibited characters that when keyed out did not clearly identify as either Banded Killifish or Mummichog were considered potential hybrids.

Number of gill rakers on the first gill arch, 4–7 (usually five); number of scale rows, 42–55; stepped forward location reaches just anterior to the eye to between the eye and operculum (usually middle of the eye); ratio of caudal peduncle depth relative to the distance between the origin of the dorsal fin and the end of the vertebrae, 0.15–0.25 .... Banded Killifish, *Fundulus diaphanus*

Number of gill rakers on the first gill arch 6–10 (usually eight); number of scale rows, 32–39; stepped forward location reaches posterior to the eye to posterior to the operculum (usually posterior operculum); ratio of caudal depth relative to the distance between the origin of the dorsal fin and the end of the vertebrae, 0.25–0.40 .... Mummichog, *Fundulus heteroclitus*

Two adult specimens from Little Paradise Park (>50 mm SL) and eight juvenile specimens (<20 mm SL) from Saltwater Pond sent to the ARC for identification could only be identified as *Fundulus* sp. (Table S2; Figure S2). The juveniles from Saltwater Pond appeared to be Mummichog but the adults from Little Paradise Park exhibited a mix of characters from both species (L. van Guelpen pers. comm. 13 June 2014; Figure S2).

We confirmed the presence of Banded Killifish at 45 sites within 35 drainage systems (Figure 3) and Mummichog at 30 sites within 24 drainage systems (Figure 4) in insular Newfoundland (Tables 2 and S1). From the 102 sites surveyed during this study, 30 and 18 were new (i.e., previously undocumented or unconfirmed from LEK and grey literature, such as internal reports; Table S1) for Banded Killifish and Mummichog, respectively. Locations were considered unconfirmed when grey literature and LEK lacked sufficient physical evidence for accurate species identification. Banded Killifish and Mummichog were not detected at 70 and 82 sites, respectively (Figures S3 and S4). Potential locations of Banded Killifish and Mummichog occur where unconfirmed reports were not investigated (Figures S3 and S4). Banded Killifish and Mummichog were detected sympatrically at two unnamed ponds that connect directly to estuaries at high tide: one near Little Paradise Park, St. Andrew's and the other near Stephenville Crossing (Table S1). When present, catch numbers from minnow traps ranged up to 102 individuals with catch per unit effort (CPUE) from 0.04 to 16.92 fish/h for Banded Killifish and up to 159 individuals with CPUE from 0.04 to 12.52 fish/h for Mummichog.

We found two unreported museum records for Banded Killifish (Burin and Gravels Pond; Table S1) and one unreported record for Mummichog (Terrenceville; Table S1). We also detected two erroneous reports of Banded Killifish from Star Lake and York Harbour, Newfoundland. The Star Lake record was reported by Chippett (2004) based on a personal communication, but we found that the original source indicated Banded Killifish was actually observed in Stag Lake (B. Dennis pers. comm. 9 January 2015). We sampled Stag Lake but could not confirm the presence of Banded Killifish. Fisheries and Oceans Canada (2011) and COSEWIC (2014) reported Banded Killifish from York Harbour, however, no specimens were retained from the original sampling, only photographs. Morphological features measured from these photos (Figure S1d–f) indicated Mummichog, not Banded Killifish. In addition, our surveys at this site yielded only Mummichog.

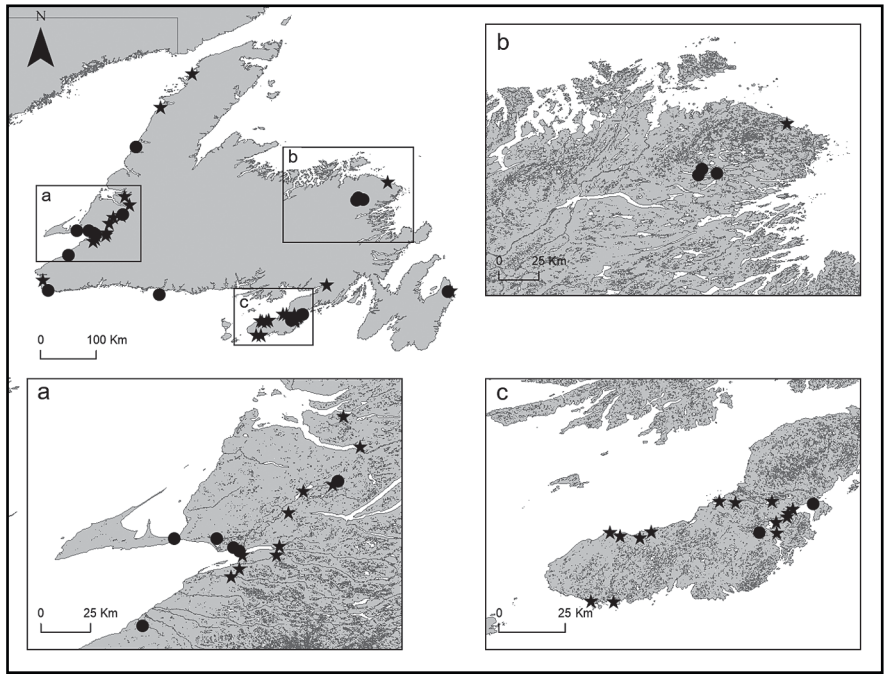

FIGURE 3. Confirmed locations of Banded Killifish (*Fundulus diaphanus*) in insular Newfoundland. ★ = locations confirmed through direct sample collection or samples provided by residents during this study. ● = locations confirmed by museum and literature records, or unpublished data with substantial evidence of species identification (e.g., high quality photographs).

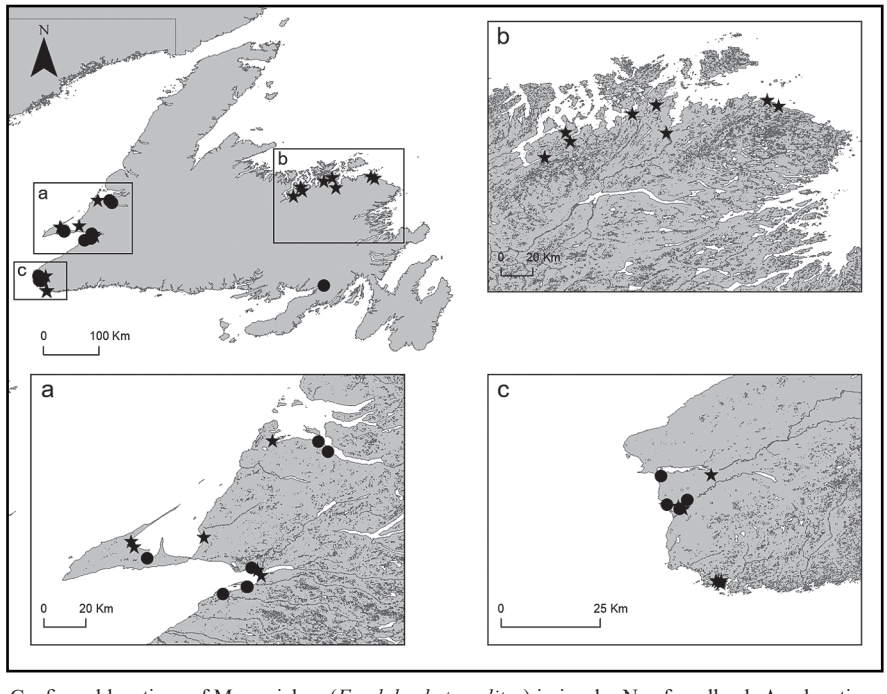

FIGURE 4. Confirmed locations of Mummichog (*Fundulus heteroclitus*) in insular Newfoundland. ★ = locations confirmed through direct sample collection or samples provided by residents during this study. ● = locations confirmed by museum and literature records, or unpublished data with substantial evidence of species identification (e.g., high quality photographs).

Table 2. Sources of site confirmations of Banded Killifish (*Fundulus diaphanus*) and Mummichog (*Fundulus heteroclitus*) in insular Newfoundland.

Species	Literature* only	Literature* with corresponding museum records	Museum records only	LEK with photos	Surveys confirming LEK	Surveys confirming grey literature	Specimens provided by locals	Surveys alone	Totals
Banded Killifish	7	4	2†	2‡	10	0	2	18	45
Mummichog§	3	5	3	0	7	6	1	5	30

*Literature sources included: Johansen (1926); Templeman (1951); Scott and Crossman (1964); van Vliet (1970); Dickinson and Threlfall (1975); Gibson *et al*. (1984); Day (1993); Chippett (2004); Mann and Nambudiri (2005); Mitchell and Purchase (2014).
†One site (Burin) could not be counted in total as site co-ordinates were not provided with sample.
‡See Figure S1a–c used to confirm Banded Killifish reports from LEK (local ecological knowledge).
§Site confirmations based on LEK and grey literature (internal documents) were for reports believed to be Banded Killifish.

Discussion

As previously identified by Scott and Crossman (1964) and Hernández Chávez and Turgeon (2007), the number of scale rows along the lateral line was the most useful meristic character to differentiate these species, because there is no overlap. We found the stepped forward position and the ratio of caudal depth to the distance between the origin of the dorsal fin and the end of the vertebrae to be the most effective morphometric characters for differentiating these species in the field. These characters, previously identified by Scott and Scott (1988) and Fritz and Garside (1974), respectively, only require three measurements in the field, minimizing handling stress and even allow identifications from high quality lateral view photographs.

Several of our specimens could only be identified as *Fundulus* sp.; two individuals from Little Paradise Park (Little Codroy River estuary) were considered potential hybrids, as the species were sympatric in a pond that connects to the estuary near the collection site. Hybridization has been documented at two locations in Nova Scotia (Fritz and Garside 1974) but is probably more widespread (Hernández Chávez and Turgeon 2007). However, hybridization has yet to be confirmed in Newfoundland.

Several previously reported locations of Banded Killifish are likely in error due to misidentification. Similar to the erroneous York Harbour record, specimens were not retained from locations in West Bay of the Port au Port Peninsula (MAMKA 2011). We surveyed most of these West Bay sites and several were surveyed earlier by Johansen (1926) and van Vliet (1970) but only Mummichog were detected.

Our results greatly expanded the known number of locations for Banded Killifish and Mummichog in insular Newfoundland and extended their known ranges. We confirmed Banded Killifish at nearly four times as many sites in more than three times as many drainage systems as previously reported in COSEWIC (2014). Two new locations were considerably further north (77 and 146 km respectively), than previously reported (COSEWIC 2014) and we documented them in one additional drainage system on the northeast coast where only one was previously reported (Chippett 2004). We also corrected two Banded Killifish locations previously misreported by Chippett (2004) and Fisheries and Oceans Canada (2011) due to a miscommunication of a location and a misidentification of Mummichog, respectively. Mummichog was previously reported at ten locations from eight drainage systems along the southwest coast of insular Newfoundland (Johansen 1926; Scott and Crossman 1964; van Vliet 1970; Dickinson and Threlfall 1975; Scott and Scott 1988). We confirmed them at three times as many sites and drainage systems, including eight sites along the northeast coast where they have never been previously reported. The previously reported limited distributions of both Banded Killifish and Mummichog was most likely due to a lack of survey data; the new locations we identified highlight a general lack of basic aquatic biodiversity data for insular Newfoundland.

Banded Killifish can colonize new territory and expand their range when conditions are suitable. A population of Banded Killifish was reportedly introduced into a pond in the city of St. John's (east coast) in 1999 (Mitchell and Purchase 2014). In 2014, we detected a downstream expansion of this population into a lake but did not detect an expansion upstream likely because of a ~4 m high waterfall, which may present a significant barrier (Gibson *et al*. 1984). The expansion of this population over the last 20 years suggests resilience and the potential for population restocking (Mitchell and Purchase 2014) elsewhere if deemed necessary. Expansion of at-risk populations of Banded Killifish in the United States (Illinois) have also been reported (Mankowski 2012). Populations of the eastern and the western subspecies have been rapidly expanding in Lake Michigan since 2001 and the Mississippi

River since 2009 (Willink *et al.* 2018). Recent changes in environmental conditions within the Great Lakes due to introduced species and climate change may have benefited the eastern subspecies in this area, while the western subspecies may have been introduced into the Mississippi River (Willink *et al.* 2018).

In light of the expanded distribution we found, the status of Newfoundland's Banded Killifish population should be reassessed by COSEWIC. This population was initially designated as Vulnerable (renamed Special Concern since 2000; COSEWIC 2003, 2014) in 1989 (Houston 1989, 1990), based on only two known widely separated localities reported by Scott and Crossman (1964) and Gibson *et al*. (1984). The Special Concern status was maintained in the 2003 and 2014 assessments despite the addition of four locations (COSEWIC 2003) and five locations (COSEWIC 2014), two of which (Star Lake and York Harbour) we have found to be in error.

To date, targetted surveys for Banded Killifish have been extremely limited. Including the present study, fewer than 200 (<1%) of Newfoundland's ponds, lakes, brooks, and barachois have been surveyed specifically for Banded Killifish (see Gibson *et al*. 1984; Cote *et al.* 2002; Knight 2002; Chippett 2004; MAMKA 2006, 2011). Given the vast number of water bodies in insular Newfoundland, future sampling must be prioritized and optimized. Representative water bodies from each drainage system with suitable environmental conditions for Banded Killifish should be considered first. We recommend sampling: 1) areas with suitable habitat including shallow quiet waters of ponds and lakes with a sand, gravel, or detritus-covered bottom and patches of submerged aquatic plants (Scott and Crossman 1973); 2) water bodies in the lowest parts of each drainage system without steep gradients that may create barriers to upstream migration (Gibson *et al*. 1984); 3) using minnow traps baited with Ritz crackers, the most efficient sampling method for Banded Killifish and Mummichog >30 mm (SL) during our study; 4) when the water temperature is ≥17°C (Chippett 2004), typically between July and mid-September; and 5) during sunny days, when Banded Killifish were more readily observed during our study. Additionally, detection may depend on size of the water body surveyed. To increase chances of detecting Banded Killifish in larger water bodies, sampling effort should be increased proportionally to water body size. Future surveys should also explore the distribution of Banded Killifish on the northeast coast, as it is unclear how the species arrived there and how widespread it is in this region. To confirm the presence of hybrids in Newfoundland, morphometric and genetic data (both mitochondrial and nuclear sequence polymorphism) from specimens would have to be examined, as suggested by Hernández Chávez and Turgeon (2007).

Author Contributions

Writing – Original Draft: P.S.S.; Writing – Review & Editing: D.R.O., K.L.D., and P.S.S.; Conceptualization: P.S.S.; Investigation: K.L.D. and P.S.S.; Methodology: K.L.D. and P.S.S.; Formal Analysis: P.S.S.; Data Curation: P.S.S.; Project Administration: P.S.S.; Resources: P.S.S. and D.R.O; Supervision: P.S.S.; Validation: P.S.S. and K.L.D.; Funding Acquisition: P.S.S.

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SUPPLEMENTARY MATERIAL:

TABLE S1. List of museum collection records of Banded Killifish (*Fundulus diaphanus*), Mummichog (*Fundulus heteroclitus*), and potential hybrids (*Fundulus* sp.) from insular Newfoundland.

TABLE S2. Meristics and morphometrics of Banded Killifish (*Fundulus diaphanus*), Mummichog (*Fundulus heteroclitus*), and potential hybrids (*Fundulus* sp.), collected throughout insular Newfoundland (2013–2016).

FIGURE S1. Photographs used to verify presence of Banded Killifish (*Fundulus diaphanus*) and Mummichog (*Fundulus heteroclitus*) in insular Newfoundland where physical specimens could not be acquired.

FIGURE S2. Frequency distributions comparing meristic and morphological characters of Banded Killifish (*Fundulus diaphanus*), Mummichog (*Fundulus heteroclitus*), and potential hybrids (*Fundulus* sp.) collected in insular Newfoundland (2013–2016).

FIGURE S3. Locations where Banded Killifish (*Fundulus diaphanus*) were not detected in insular Newfoundland.

FIGURE S4. Locations where Mummichog (*Fundulus heteroclitus*) were not detected in insular Newfoundland.