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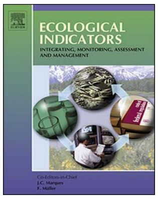

Original Articles

Are culvert assessment scores an indicator of Brook Trout *Salvelinus fontinalis* population fragmentation?

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ABSTRACT

Fragmentation is a major threat to the integrity of stream ecosystems and maintaining connectivity is a primary goal for conservation to promote natural system functioning. In human dominated systems with extensive anthropogenic fragmentation, resource managers are faced with prioritizing restoration actions to provide the most cost-effective conservation strategies. Road crossings, particularly poorly designed culverts, limit fish passage and thus population connectivity and access to seasonally important resources. Culvert assessment protocols, such as the one developed by the North Atlantic Aquatic Connectivity Collaborative (NAACC), are useful tools for managers to evaluate culvert passability and prioritize restoration actions across broad spatial extents, however the justification of such protocols requires empirical field based investigations. In this study we collected Brook Trout genetic samples from 28 headwater streams, of which 17 were separated by a culverted road crossing and 11 were included as a comparison to natural genetic structuring. The objectives of this research were to 1) determine what effect culverts had on the genetics of Brook Trout populations, 2) evaluate the ability of the NAACC culvert assessment protocol to predict genetic patterns and 3) identify the culvert characteristics that best explained genetic variability. We found significant increases in genetic differentiation at sites with culverts compared to those without, but no differences in genetic diversity metrics. Although the NAACC classifications did not predict the extent of genetic differentiation, the openness ratio of culverts (cross sectional area/length) was significantly correlated, suggesting it may need to receive a higher weighting in assessment protocols. Our results highlight the necessity to match appropriate prioritization strategies with desired management objectives in order to design the most effective conservation actions.

1. Introduction

Promoting and maintaining connectivity are highly prioritized objectives for managing ecological systems in an effort to promote natural processes. Stream systems are particularly susceptible to fragmentation due to their dendritic orientation (Fagan, 2002), and a single barrier can disrupt a diverse array of upstream and downstream processes across multiple organizational levels (Ward and Stanford, 1983). Although stream fishes were historically thought to be relatively sedentary (Gerking, 1959), evidence is accumulating that suggests larger scale movements often happen frequently enough to warrant conservation attention (Fausch et al., 2002; Gowan, 1994). Indeed, potamadromy, or seasonal migrations entirely within freshwater (Lucas and Baras, 2001), are now recognized in many different fish taxa. By preventing the dispersal of fishes through stream systems, fragmentation can limit access to seasonally important resources (Schlosser, 1991), disrupt gene flow (Hughes et al., 2009; Wofford et al., 2005), and increase risk of local extirpation (Campbell Grant, 2011; Fagan, 2002; Letcher et al., 2007; Morita and Yamamoto, 2002). Maintaining connectivity promotes population resilience by increasing diversity in accessible habitat, genetics, and life history strategies, which can allow populations to persist under future environmental stochasticity (Waldman et al., 2016). Reestablishing and promoting connectivity is one of the primary challenges in the conservation of stream ecosystems (Pringle, 2003).

Identifying causes of fragmentation and evaluating potential barriers are necessary steps towards designing effective management strategies and prioritizing restoration actions. Both direct and indirect methods are commonly used to assess fish passage across a potential barrier. Direct methods focus on actively observing the movement of individuals (e.g. over a dam or through a culvert), either by mark and recapture, telemetry, direct observations, or a combination of such

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strategies (Kemp and O'Hanley, 2010). These strategies have the advantage in that they provide counts or frequencies of passage, which can be used as targets for management goals. However, high costs and labor intensity often limit the use of direct observations across large numbers of sites or spatial extents and observing passage does not necessarily indicate the effect of individuals on recipient populations (i.e. effective dispersal). Alternatively, indirect methods can be used to predict movement probabilities based on fishes' swimming capabilities and hydraulic simulations (e.g. FishXing; Furniss et al., 2006). This technique is widely used because of its low cost and ease of large scale application, however empirical evaluations have suggested low levels of agreement between model predictions and mark and recapture results (Burford et al., 2009) and more passage has been observed than predicted based on swimming capabilities alone (Blank, 2008; Mahlum et al., 2014). In a multi-tiered evaluation study, Burford et al. (2009) documented fish passage through 91% of the culverts classified as total barriers by FishXing, suggesting exclusive use of such techniques may not correctly identify the barriers of highest restoration priority. Additional indirect methods can be used to evaluate the influence of barriers by comparing populations above and below culverts (Kemp and O'Hanley, 2010). Such techniques can be based on fish species abundance or size structure (Blank, 2008; Burford et al., 2009; Nislow et al., 2011), genetic relatedness (Knaepkens et al., 2004; Timm et al., 2016), or through genetic assignment tests (Neville and Peterson, 2014; Whiteley et al., 2014; Wood, 2014). Indirect techniques are beneficial in that they allow for a measurement of more biologically relevant effects of effective dispersal on extant populations and, in the case of population genetic data, a more temporal evaluation of movement over multiple generations.

Road crossings, particularly culverts that are not designed with fish passage as a priority, are common causes of stream fragmentation. Shallow in-pipe water depths, restricted flows which increase velocity, perched outlets, steep culvert gradients, and mismatched substrate are just a few of the potential ways that culverts restrict the movement of fish by limiting their ability to move through a culvert (Blank, 2008; Powers and Orsborn, 1985). Further, discharge can also influence individual motivation to attempt culvert passage (Goerig and Castro-Santos, 2017). Road crossings are widespread throughout much of the Eastern US, particularly in human-dominated landscapes like Connecticut, where roads intersect streams on average greater than once per stream km (USCB, 2010; USGS, 2010). Evaluating such a large number of potential barriers over broad spatial scales presents logistical challenges for prioritizing management actions. Assessments typically rely on classifying culverts into course categories or by assigning quantitative scores to each culvert based on the swimming capabilities of fish and the likelihood of passage (e.g. Clarkin et al., 2005; Milone and MacBroom, Inc., 2009; WDFW, 2000). Based on assessments across a number of culvert locations, restoration recommendations can be made in an effort to promote connectivity (Anderson et al., 2012; CRWP, 2011; Januchowski-Hartley et al., 2013; Poplar-Jeffers et al., 2009). The need for a regional synthesis of extant fragmentation concerns led to the development of a cooperative effort across much of the Northeast United States. The North Atlantic Aquatic Connectivity Collaborative (NAACC) was formed between more than 70 state, federal, and private conservation groups across 13 states in order to promote a standardized culvert scoring protocol that could be used to identify areas of conservation opportunities (NAACC, 2014). Culvert assessment protocols and prioritizations, such as the one developed by the NAACC, are designed based on a consensus of expert opinion of the factors limiting aquatic organism passage (see Section 2.2) and to encompass a broad range of taxa and ecosystem processes. Although supported by an extensive background of ecological knowledge, empirical field-based evaluations of the scoring systems to ensure they accurately predict fish passage are necessary in order to further justify and to develop the use of such protocols for restoration purposes.

Brook Trout *Salvelinus fontinalis*, typically a non-anadromous stream salmonid species, are a common focus for stream restoration actions. Historically found throughout much of the Appalachian region (MacCrimmon and Campbell, 1969), contemporary populations are largely reduced in size and distribution due to a suite of anthropogenic influences (Hudy et al., 2008). Brook Trout populations are commonly fragmented due to movement barriers or unsuitable habitat, potentially limiting dispersal between headwaters. Many populations persist, despite being fragmented into small habitats and sizes, likely due to a combination of demographic and life history strategies (Hudy et al., 2010; Kanno et al., 2011; Letcher et al., 2007). Rare dispersal events can play an important role in maintaining demographic processes, genetic diversity, and long term adaptability (Kazyak et al., 2016; Letcher et al., 2007). The prioritization of promoting connectivity in Brook Trout populations combined with their central focus of many restoration actions makes them an ideal species on which to evaluate the ability of culvert scoring metrics to predict effects of population fragmentation.

In this study we used a genetics approach to evaluate to what extent culvert classifications and scores predicted the extant genetic differentiation across, and the genetic diversity above, road crossing culverts. We aimed to address three specific research questions that would help elucidate patterns that could assist in the further development of such protocols for use in restoration prioritization strategies. First, we tested the effect of culverts on genetic differentiation between Brook Trout separated by a culvert and the genetic diversity in upstream populations by comparing them to sites without road crossings. The presence of a culvert was expected to increase genetic differentiation between reaches and decrease genetic diversity in the upstream populations due to reduced gene flow and increased genetic drift (Neville et al., 2009; Torterotot et al., 2014; Whiteley et al., 2010). Second, we assessed whether genetic differentiation and upstream diversity were related to culvert classification and passability scores based on the NAACC protocol. We predicted that higher levels of differentiation and lower levels of upstream diversity would be observed at sites with culverts that scored poorly for fish passage because of restricted gene flow to the upstream stream reach. Finally, we assessed which individual culvert features or characteristics best correlated with observed genetic patterns. Among the parameters included in the NAACC scoring protocol, we hypothesized the height of culvert outlet drop would have the greatest effect on genetic differentiation due to restricted fish passage in perched culverts.

2. Methods

2.1. Sample collection

Brook Trout were sampled from July–September of 2014 and 2015 in headwater streams across Connecticut by single-pass backpack electrofishing (Smith-Root model LR-24, Vancouver, Washington) as part of a broader landscape genetics study. Streams were typically Strahler first or second order (Strahler, 1957) with an average bankfull width of 5.23 m (range 2.59–10.06 m). Mixed aged individuals were collected over two approximately 200-m segments to avoid oversampling family groups, which are commonly clustered spatially due to limited dispersal, particularly at early life stages (Hansen et al., 1997; Hudy et al., 2010; Whiteley et al., 2012). Caudal fin clips were used for genetic samples and stored in labeled vials of molecular grade ethanol until processing. From a larger sample database consisting of 152 sample locations, 17 were selected to be included in this study based on sufficient sample sizes ($N \geq 20$) distributed above and below culverted road crossings, and another 11 were included as non-crossing sites to evaluate the genetic relatedness and patterns across space in the absence of a culvert crossing (Fig. 1; Table 1).