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Wild Brook Trout (Salvelinus fontinalis) Demographics and Movement in the Presence of Undersized Road Crossings in Headwater Streams in Central New Hampshire

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Wild Brook Trout (*Salvelinus fontinalis*) Demographics and Movement in the Presence of Undersized Road Crossings in Headwater Streams in Central New Hampshire

By

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Title: Impacts of Historic Barriers on Wild Brook Trout (*Salvelinus fontinalis*) in Central New Hampshire

Abstract approved:

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Brigid C. O'Donnell, Ph.D.

Populations of wild Brook Trout (*Salvelinus fontinalis*) continue to decline across their historic range, making relatively healthy populations and intact habitats within northern New England increasingly important for conservation. The Beebe River watershed, located in central New Hampshire, is home to intact headwater populations of wild Brook Trout despite movement barriers and riparian manipulation affecting tributaries to the mainstem river. The region has also experienced two centuries of widespread timber harvest and a century of stream acidification, creating further ecological stressors. We focused on three headwater tributaries with 1) impassable road crossing and reduced canopy cover, 2) passable road crossing and reduced canopy cover, and 3) no impediments to movement and unaltered canopy. We documented Brook Trout abundance, density, age structure, condition, biomass, growth, net movement, cumulative movement, home range, and recruitment with the goal of better understanding potential habitat influences on fish across tributaries and among geomorphic threshold regions. Our primary sampling methods included depletion electrofishing, PIT tag mark-recapture techniques, and detailed habitat assessments and temperature monitoring. We hypothesized that undersized crossings and no-low canopy reaches would create physical and thermal barriers for fish. In particular, we predicted that fish in streams with these barriers would exhibit lower density, fewer age classes and lower growth rates while seasonal and annual movement would increase compared to fish in an unimpacted stream. Overall, tributary populations were comprised of young fish that exhibited little movement. We failed to support many of our hypothesis metrics due to underestimating the indirect influences of no-low canopy reaches. Although we documented a crossing barrier inhibiting upstream movement, fish with unrestricted access to the no-low canopy primarily grew more and moved less, while density remained stable interannually. In contrast, fish in the most impacted stream and the unimpacted stream exhibited increased movement and significant declines in interannual density. This project was a unique opportunity to compile a detailed description of the spatial and temporal differences in

Brook trout populations for two seasons prior to multiple crossing replacements and habitat enhancement. Our research helps fisheries managers to better understand the benefit of watershed-wide restoration to inform the protection of wild Brook Trout populations.

Master of Science thesis of Tyson R. Morrill presented on July 22, 2019

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I understand that my thesis will become part of the permanent collection of Plymouth State University, Lamson Library. My signature below authorizes release of my thesis to any reader upon request.

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This study was part of a larger project intended to improve water quality, connectivity, aquatic habitat and improve degraded infrastructure across the majority of a watershed. I am thankful to become involved in such an expansive project, driven by diverse partners with a moral desire to ensure our natural resources persist for future generations. I thank everyone who's primary goal was large-scale restoration, but accepted our ongoing research during project planning and ongoing construction, including The Conservation Fund, NH Fish and Game Department, the Towns of Campton and Sandwich, NH, Trout Unlimited: Pemigewasset Chapter and New England Culvert Project, Tin Mountain Conservation Center, Squam Lake Conservation Society, NH Department of Environmental Services, USDA Forest Service, US Natural Resources Conservation Service and local contractors. Our work could not have been possible without the trust of the landowner, Nancy Bell and her vision of preserving vulnerable natural resources. I would like to thank Ben Nugent for his tireless support intellectually and in the field. His passion for fisheries is inspiring to myself and anyone he interacts with. Our project could not have been accomplished to the scale I envisioned without the dozens of Trout Unlimited volunteers. Their long days of shuttling buckets of fish and passion for wild trout keep the spirt alive. I am thankful for financial support by Plymouth State University through the Joe and Gail White Graduate Fellowship, Student Research Advisory Council (SRAC) and tuition assistance, The Conservation Fund and Trout Unlimited: Pemigewasset Chapter. Furthermore, I am appreciative for Plymouth State University by providing the opportunity to teach. It defined my unrecognized desire to advocate for the natural world by educating others. I would like to thank Dr. Len Reitsma for his critical yet thoughtful feedback on my writing and project as a whole. His expertise as a naturalist is a critical skill that I strive to gain as a future scientist. I sincerely appreciate the devotion and persistence of Dr. Brigid O'Donnell and Dr. Amy Villamagna over the last three years, beginning with taking the risk of accepting a skunk trapper to establish a fish project. Their grounded inspiration helped me grow as a researcher, even as I tested their patience. I will forever attribute my future career success to the opportunity they provided me. Finally I would like to thank my family and friends. They understood how difficult it was for someone who lives for the outdoors to spend days on end behind a computer, but they knew how important this degree was for me. And to my parents- I promise I will move out soon, again.

CONTRIBUTION OF AUTHORS

Dr. Brigid O'Donnell assisted in project study design, led acquisition and use of sampling and monitoring equipment, provided intellectual expertise, thesis revisions, and overall guidance.

Dr. Amy Villamagna assisted in project study design, led acquisition and use of sampling and monitoring equipment, provided intellectual expertise, thesis revisions, and overall guidance.

Ben Nugent assisted in project study design, provided sampling and monitoring equipment, co-led field sampling, provided specialized intellectual expertise, and was the primary NH Fish & Game Department liaison.

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Chapter 1

Introduction

Brook Trout (*Salvelinus fontinalis*) are a cold water dwelling char species native to eastern North America and a game fish. Their habitat requirements are relatively narrow, requiring pristine, highly oxygenated water, defining their role as an indicator species (Raleigh 1982). The Brook Trout's range includes cold, well-oxygenated streams, rivers, lakes, ponds and coastal estuaries in their northern range across southern Quebec, but are restricted to headwater streams in the southern Appalachians (DeWeber and Wagner 2015, Kanno et al. 2015, Snook et al. 2015). Home to the most intact remaining populations (Hudy et al. 2005, 2008), the northeastern United States was the first landscape in the New World to experience wide-spread European settlement, establishing its recognition as 'New England'. New England has undergone an historic transformation (Foster et al. 2008). Beginning in the 1770s, old-growth forests were cleared for agriculture and the remaining forest was extensively cut for an array of wood products (Foster 1992). In 1839, New England and New York accounted for 41% of the nation's timber harvest, solidifying its recognition as the hub for natural resource exportation. As regional resources quickly became depleted and settlement began to expand westward, timber harvest decreased by almost half 20 years later (Williams 1982). The cumulative effects of widespread land-use change and human expansion caused a 59% decline in Brook Trout across the United States and extirpation from 21% of subwatersheds. New Hampshire (NH) maintains the second most abundant watersheds with the presence of Brook Trout, but also some of the least studied populations (Hudy et al. 2005, 2008). The state has a complex history of land-use (Justice et al. 2002) and fisheries management (NH Fish and Game Department 1939), creating profound challenges surrounding the species (Thompson et al. 2013). As managers look to restore and protect remaining populations, further understanding of influential factors must be addressed at the landscape scale (Fausch et al. 2002, Petty et al. 2005). By recognizing historic and ongoing influences on Brook Trout populations while documenting current population trends, we can better manage Brook Trout in a region encompassing some of their most intact, contiguous range.

Reference research forests

Two regional research forests have contributed to a detailed archive documenting the lasting effects of historic land-use practices and ongoing ecological trends in New England, both recognized as Long-Term Ecological Research sites by the National Science Foundation. Research originating from the Harvard Forest succeeded by the Hubbard Brook Experimental Forest, helps paint a combined timeline of regional landscape ecology over the past 300-350 years. Understanding historical ecology allows managers to better analyze current habitat conditions, improving the understanding required for research and decision making.

Harvard Forest

Located in northern Massachusetts (MA), the Harvard Forest was established in 1907 as a field laboratory for students, a research center in forestry, soils, wildlife biology, geography and botany and for demonstrations of sustained forestry (Harvard College 2018). Dr. David Foster has extensively studied historic land-use and resulting ecosystem processes within the Harvard Forest, which are indicative of regionally widespread landscape influences. He designates historic landscape impacts into three ‘use’ categories: forest that was cut for wood products followed by burning or livestock grazing, intensively grazed pasture that was grassy and mostly treeless, and land that was cleared and plowed for cultivation. Land-use history was found to almost always have a significant influence on soil properties and accompanying vegetation (Compton and Boone 2000). All three impacts created a primarily homogenized layer of topsoil 15-25cm deep (Foster 1992). In addition, the drainage of wetlands and creation of lakes and reservoirs has permanently altered abiotic and biotic environments. Many of today’s forests have been abandoned for only 120-150 years since last impacted by settlement. Given the tree species present in the region, forest regeneration has not reached its maturity due to the longevity of many of these species (Foster et al. 1998). The current composition and distribution of regional forests has been largely shaped by the location of settlements, further influenced by the intensity of agricultural and forestry practices (Gerhardt and Foster 2002). Compton and Boone (2000) further studied the long-term effects of deforestation, cultivation and subsequent reforestation on forest soil composition within the forest. They found that historic cultivation increased soil nitrogen (n) and phosphorus (p) concentrations, which persisted for a long period after agricultural sites were abandoned. In the same study areas, carbon was 13%-16% lower within the topsoil in sites last cultivated 90-120 years prior.

Hubbard Brook Experimental Forest

Located in central NH, the Hubbard Brook Experimental Forest (HBEF) was established in 1955 by the USDA Forest Service and is one of the longest running and most comprehensive ecosystem studies in the world (USDA Forest Service 2018a). The study began with an initial goal of documenting the relationship between forest cover and water quality/supply by documentation of post-colonial forest recovery (Likens et al. 1996) before transitioning to ongoing landscape remediation (Bernhardt et al. 2016). Additional long-term monitoring results present a host of anthropogenic influences, ranging from clear cutting to greenhouse gas emissions which are further reviewed by Likens (2004). HBEF’s most profound finding occurred in 1968 with the first detection of acid rain in North America (Likens et al. 1972). Forest soils showed accumulating nitrogen through 1977 before acid rain caused n fixation, resulting in continuously decreasing levels (Yanai et al. 2013). While studying the influence of stream-side timber harvest on Brook Trout, Nislow and Lowe (2003) found logging history (years since harvest) is negatively correlated with the stability of substrate embeddedness, which can be washed into streams and negatively affect fish and macroinvertebrates. The soil pH is relatively low because sediments have been affected long-term by acid rain. As a result, they found that the lowered pH input was negatively correlated with overall Brook Trout abundance in headwater streams adjacent to timber harvests. Prior to the Clean Water Act and establishment of HBEF, Hubbard Brook's pH was 6.6 in 1927 (NH Fish and Game 1939) but has increased to a median pH of 6.8 across 16 first-order streams by 2000 (Nislow and Lowe 2003). During the initial pH sampling, NH Fish and Game (1939) recognized Hubbard Brook as a 'fish market stream' with high fishing pressure. Exploitation of wild Brook Trout and the degradation of their habitat has been well documented in the region, but intact populations still remain throughout the Hubbard Brook watershed (Nislow and Lowe 2003). Results from the effects of logging in HBEF was further compiled into analysis of 15 watershed-scale experiments across the Appalachian Highlands region, analyzed by Swank et al. (1988). They found stream-side clear cutting increased streamflow and evapotranspiration and these increases varied by landscape orientation and tree species composition.

Although land clearing and subsequent soil disturbance was evident across MA and NH, a notable contrast is present in the use of the cleared land between these two states. Hamburg (1984) describes lower densities of domestic animals in NH than MA, believed to have peaked in NH in 1845 at 0.3 animals/ha. Central NH was comprised of ≤10-15% cultivated land which received animal manure as fertilizer (Compton and Boone 2000). The small amount of fertilized, cultivated fields in NH is presently supported by the lack of elevated n in cultivated soils.

Each study forest contributes factual data to historical landscape ecology across the region, but at the state-level, n soil content in NH must be further reviewed. Prevailing winds cause pollutant deposition across New England and eastern Canada, distributed by precipitation or suspended clouds and fog (Driscoll et al. 2001). HBEF is more frequently exposed to both methods of deposition, increased by the condensation of suspended moisture within the higher elevation watershed (Yanai et al. 2013). Hamburg (1984) describes increased soil acidification across lower elevation farmland historically used for agriculture, increased in lower stages of the water cycle. Variation can be explained by differences in ecosystem processes present between study areas across the state. As more acid rain is distributed along the landscape, n fixation results in decreased levels of soil acidification. Although varying ecological changes occurred throughout the region, diverse abiotic impacts on regional ecology remain at small scales (Thompson et al. 2013).

Legacy effects on Brook Trout

The diversity in historic land-use varies across the range of Brook Trout, but many lasting impacts remain. The first colonial settlements occurred in New England, creating a more complex and extended range of manipulated landscapes (Foster et al. 2008). After extensive fisheries and stream ecology research, multiple factors have been identified to continuously affect Brook Trout distribution, population demographics, movement and population-level and individual-level condition. These include habitat fragmentation attributed to dams and road crossings, habitat degradation caused by timber harvests and altered water chemistry and complicated fisheries management practices.

I. Dams

Dams have been shown to negatively affect movement and suitable habitat of cold water stream fishes like Steelhead Trout (Rainbow Trout, *Oncorhynchus mykiss*) (Winans et al. 2018), Brown Trout (*Salmo trutta*) (Törnblom et al. 2017) and White-spotted char (*Salvelinus leucomaenis*) (Morita and Yamamoto 2002). Regulated dam discharge can provide cold water refuge downstream, creating a thermal regime suitable for dependent fish species like Brook Trout and Slimy Sculpin (*Cottus cognatus*) (Kelly et al. 2017a), but the effects of dams on fish populations is generally negative. Additionally, authors found that Brook Trout below the dam had a greater length-at-age and increased metabolism, highlighting the need to monitor multiple indicators of fish health while observing river regulation by dam managers (Kelly et al. 2017b). In contrast, others argue the benefit of cool water release is only present when reservoirs have stratified and flow is released from low in the waterbody (Lessard and Hayes 2003, Maheu et al. 2016). Smaller dams have been found to increase seasonal water temperature, significantly altering daily mean temperatures up and downstream (Maheu et al. 2016), further impacting Brook Trout, which are vulnerable to warmwater (Baird and Krueger 2003, Lessard and Hayes 2003). Furthermore, dams can block or restrict Brook Trout movement to suitable habitat, including long distance, migratory life history strategies (Ecret and Mihuc 2013, Kelson et al. 2015, Kusnierz et al. 2014).

In New England, research has documented the negative effects of dams on a broad array of fishes. Dam removal has primarily focused on restoring movement and habitat for individuals with anadromous and catadromous life histories in MA (Magilligan et al. 2016b, Snook et al. 2015) and Maine (ME) (Gardner et al. 2013). Removals with a goal of restoring Brook Trout habitat continues to occur across the region, however, little research has been published. Notable removals to benefit Brook Trout occurred on Swett Brook in ME, Nissittissit River in MA, Wells River in Vermont (VT) and on McQuesten Brook in NH. Approximately 10% of the nation's dam removals have occurred in New England, which is low given the region possesses the largest quantity of remaining dams. By 2013, one third of regional dam removal occurred in NH (n=26). Resulting dam removals have primarily occurred in upper riverine catchments, capitalizing on improving water quality and ongoing forest revegetation (Magilligan et al. 2016b). In MA, restored connectivity has been found to improve sediment flux, increased fish access to upstream habitat and improved flood resiliency (Magilligan et al. 2016b, Snook et al. 2015). But Fitzpatrick and Neeson (2018) found a combined 'mixed removal strategy' of replacing dams and road crossings within a system was the most beneficial return-on-investment practice. This practice is most implemented in the northeastern US with the highest human population densities and abundant road networks (Kemp and O'Hanley 2010).

The region still retains the highest density of dams across the country. The National Dam Inventory (NID) estimates approximately 400 dams have remained across New England for the past 200 years, primarily constructed for mills, small water supplies and larger hydroelectric facilities (Graf 1999). Magilligan et al. (2016a) found the NID significantly underestimated the quantity of dams across New England due to many undocumented lowhead structures scattered across small streams. NH is home to the most remaining dams (n=5,076) in the region and has 29-40% more than other leading states, such as Connecticut (CT) and MA. Although dams remain a problem to Brook Trout, one of the most common anthropogenic barriers in the region continues to be road crossings (Kemp and O'Hanley 2010).

II. Road crossings

Road crossings have been found to negatively affect numerous fish species by reducing or blocking upstream passage, driven by outlet drop and increased stream velocity (Diebel et al. 2015). The influence of crossings on movement are predominantly found with undersized culverts and slab crossings (Warren and Pardew 1998). These crossings can alter natural flow and thermal regimes (Wheeler et al. 2005) and increase downstream sediment input (LaChance et al. 2008), which can reduce reproduction and survival in fragmented populations (Gibson et al. 2005, Jones et al. 2004). Habitat alterations also directly affect fish health, behavior, and tend to decrease species richness (Maitland et al. 2015).

Headwater streams maintain the most robust populations of wild Brook Trout throughout their historic range (Hudy et al., 2005; 2008) where isolation is frequent due to behavioral traits and habitat conditions (Castric et al. 2001, Hebert et al. 2000, Kanno et al. 2011, Kelson et al. 2015, Whiteley et al. 2012). Road culverts can further reduce the genetic diversity upstream among already vulnerable Brook Trout populations (Nathan et al. 2018, Torterotot et al. 2014, Wood et al. 2018).

Throughout the northeastern U.S.A., state agencies have recognized the benefit of understanding quantity and quality of road crossings in place, creating the North Atlantic Aquatic Connectivity Collaborative (NAACC). Regional values, finances and historic land-use influence have caused many agencies to create state-specific road crossing assessments. NH's agencies measure two main parameters, aquatic organism passage (AOP) and hydraulic compatibility (HC) with the lowest combined scores prioritized for restoration (NH Department of Environmental Services 2018). Over half of the state's road crossings have reduced AOP, and more crossings are impassable than fully passable (Fig. 1.1) (NH Department of Environmental Services n.d.). Our analysis of surrounding states concluded that most crossings have reduced AOP and the fewest crossings with full AOP are found in VT (Table 1.1) (NAACC 2019).

III. Timber harvest

VanDusen et al. (2005) found that widespread, historic logging in northern Michigan had a greater impact on coldwater fish and macroinvertebrate communities than previously assumed. Because small, headwater streams receive the majority of energy from adjacent terrestrial ecosystems (Fisher and Likens 1973), timber harvest becomes a major source of disturbance to these habitats (Lowe et al. 2004) by reducing organic material input and increased sediment loading. Instream wood is a critical attribute of aquatic habitat, providing cover, food and other functions benefiting fish (Morris et al. 2012). In North Carolina, higher Brook, Brown and Rainbow Trout densities and biomass have been found in reaches with higher LWD compared to reaches with little or no LWD (Flebbe and Dolloff 1995). Pools critical to trout habitat (Rosenfeld, 2014) formed by large woody debris were three-fold more abundant in old-growth sites compared to clear-cut and second-growth sites (Bilby and Ward 1991).

Clear cutting or logging along riparian areas can result in higher stream siltation, negatively impacting Brook Trout populations by decreasing critical spawning habitat (Hayes et al. 1998, Marschall and Crowder 1996). Kreutzweiser and Capell (2001) found that logging activity in Quebec, Canada significantly increased sediment influx along road crossings built for equipment transport. Argent and Flebbe (1999) quantified egg survival within redds influenced by sediment deposition in a laboratory setting and found the survival of incubating Brook Trout eggs decreased as the amount of sediment coverage increased. The influx of sediments can also overtake fine organic matter naturally occurring in streams. This organic matter is a quality food source for filtering and gathering organisms, which are among the most common and abundant food items for juvenile fish (Cummins and Wilzbach 2005).

Although many studies have observed both positive and the negative influences of timber harvest on trout, one study area surfaces as the most relevant to NH's land-use history and subsequent reforestation. In the HBEF, Brook Trout populations, macroinvertebrate communities and aquatic habitat were quantified to understand the influence of logging on headwater streams. Streams flowing through recently logged stands had higher macroinvertebrate abundance but decreased diversity, which reversed with regeneration (Nislow and Lowe 2006). Years post-logging was negatively correlated with substrate embeddedness and Brook Trout density and biomass, suggesting the influence of sediment input declines with the time elapsed since last disturbance (Lowe and Bolger 2002, Nislow and Lowe 2003).

Approximately 80% of NH forests had been cleared by the mid-1880s (Langley-Turnbaugh and Keirstead 2005). Harvested timber was floated downstream along routes ranging from tributaries to entire catchments, scouring waterways. Williams (1982) reviewed the clearing of US forests during the early 1800s and compared New England's log transport practices as analogous to grazing cattle across open plains. Since that time, NH is recognized for its dramatic forest recovery and is now the second most forested state in the nation (Fig. 1.2) (Hudy et al. 2005, 2008). Recent residential development has increased deforestation across New England during the last 30 years, totaling a loss of almost half a million hectares (Olofsson et al. 2016). Most remaining forests have yet to reach maturity (Foster et al. 1998), subjecting many streams to a young canopy. This results in decreased LWD input, a critical attribute to sustain Brook Trout populations (Nislow and Lowe 2003). While the combination of waterway scouring and lack of LWD input are regional concerns, they are subjected to historic land-use practices at local scales (Thompson et al. 2013).

Current timber harvest regulations in NH do little to protect smaller waterways, which have been found to negatively affect Brook Trout by increasing sedimentation (Nislow and Lowe 2003), stream temperature (Wilkerson et al. 2006) and altering water chemistry (Lawrence and Driscoll 1988). The Basal Area Law (RSA 227-J:9) provides a 31m total buffer on perennial streams and 46m on ≥4th order streams. RSA 227-J:10 prohibits slash from being left in a perennial waterway or within 8 m of a ≥4th order stream. Because NH's timberland is 76% privately owned, increased riparian buffers must be developed by regulating state agencies or implemented by conservation-minded landowners (Smith and Anderson 2014).

IV. Water chemistry

Prior to the US Environmental Protection Agency's (EPA) Clean Air Act, widespread fossil fuel emissions became condensed through sulfuric and nitric acids in precipitation and through dry deposition, causing acidification of lakes and streams (Driscoll et al. 2001). The long-term effects of episodic acidification has been found to negatively influence fish communities in small streams across the northeastern US (Baker et al. 1996). Acidification can also limit the distribution and richness of native fishes due to varying species tolerance (Baldigo and Lawrence 2001, Baldigo et al. 2016, Johnson et al. 1987) and in some cases, leave streams fishless (Baker et al. 1996).

Northern New England boasts the most remaining intact Brook Trout populations, but has also been exposed to some of the most severe deforestation and acid deposition in the US (Hudy et al. 2005, 2008; Warren et al. 2017). Brook Trout are comparatively intolerant of acidic conditions compared to many other coexisting species (Baker and Christensen 1991), but they are still negatively affected by stream acidity. In northern New York (NY) and VT, Warren et al. (2010) found pH to be one of three primary habitat factors accounting for the variability in biomass within headwater streams. Findings by Baker et al. (1996) were similar across the northeast, documenting lower Brook Trout density and biomass in streams with lower pH and higher aluminum (Al). NH's geological features create conditions that cause waterbodies to become especially vulnerable to the effects of acid rain (Driscoll et al. 2001, NH Department of Environmental Services 2015a). Stream acidification is further amplified by soil disturbance created during timber harvest. Logging disturbance in NH has resulted in downstream acidification five times the area that was actually harvested, creating detrimental watershed-wide impacts on Brook Trout (Lawrence and Driscoll 1988).

The mobilization and transport of Al has become an increased focus of research in forested watersheds due to its toxicity on aquatic biota (McHale et al. 2007). Elevated acidity has been found to mobilize inorganic l within soil runoff (Lawrence and Driscoll 1988), increasing Brook Trout mortality in impacted systems (Baker and Christensen 1991). Baldigo et al. (2005) placed caged Brook Trout in reaches downstream of active logging to further understand the effect of landscape management practices on aquatic systems. Trout survival seven days post-harvest was 0% and 15% two years post-logging below clear-cut regions, induced by mobilized Al in the soil that dispersed into the stream.

Baldigo and Lawrence (2001) analyzed water quality, stream habitat and fish communities across 16 reaches of a NY river basin to understand how acid rain influenced water chemistry, fish abundance and distribution. No fish were found in the most contaminated headwater reaches and Brook Trout, subject to very low pH and high Al concentrations, were the sole species documented from the headwaters to lower reaches. Additional fish species became present downstream as pH increased and Al decreased. Johnson et al. (1987) and Gagen et al. (1993) exposed small stream cohabitants to a gradient of acid and Al concentrations. Slimy Sculpin and Blacknose Dace (*Rhinichthys atratulus*) almost always experienced higher mortality than juvenile Brook Trout in lab and stream settings. We found Brook Trout to be abundant throughout tributaries of the Beebe River watershed; however, Blacknose and Longnose Dace (*Rhinichthys cataractae*) are only found in lower reaches, and Slimy Sculpin are completely absent. Although pH and Al were not measured during our study, we suspect species distribution among and across tributaries has been influenced by historic and/or current water chemistry, further compounded by long-term logging (Lawrence and Driscoll 1988).

Pollutant-driven soil and water chemistry has changed over time across New England, but the degree varies. Haines and Baker (1986) reviewed early (1930s) and recent (1974-1983) fish abundance, richness and pH across 11 eastern states to understand the effects of decreased waterbody pH. They found dramatic declines in many populations, correlated with decreased pH which was most profound in Brook Trout across the Adirondack region of NY. It is believed that very few NH lakes have experienced fish population declines caused by acidification, but detrimental declines have been documented in riverine habitats across nearby states (Haines and Baker 1986). Results from Haines and Baker (1986) were further quantified in the Adirondacks and VT by Warren et al. (2010) who recognized stream acidification as a remaining, primary impact on headwater streams. Baldigo and Lawrence (2001) suggest the restricted distributions of fish species found in NY's Nerversink River may not be unique, but also a factor in similar acidified systems. To sustain intact populations, New England's remaining stream fish populations must be resilient to the effects of acidification and/or have access to diverse stream habitat with well-buffered refugia.

Many aquatic ecosystems in NH still remain acidified as a result of the legacies of historic acid deposition (Warren et al. 2017). In southern NH and northern MA, sediment accumulation in three ponds has declined since widespread deforestation, but none have returned to low, pre-disturbance amounts (Francis and Foster 2000). Rainfall pH has significantly increased (become less acidic) across NH since 1972, but pH of remote ponds has remained relatively stable (NH Department of Environmental Services 2015a,b). The historic and continued effects of combined logging and acid deposition produce stream conditions toxic to juvenile Brook Trout (Baldigo et al. 2005). Although legacy effects continue to negatively influence the species, managers have begun to alleviate and even remove lasting anthropogenic disturbances.

V. Fisheries management

Recognizing historic, detrimental impacts to the regional landscape has resulted in improved management practices, but residual and new threats to Brook Trout persist. Rainbow Trout (Cunjak and Green 1983, Marschall and Crowder 1996), Brown Trout (Hoxmeier and Dieterman 2013) and Atlantic Salmon (*Salmo salar*) (Mookerji et al. 2004) are known to outcompete Brook Trout for suitable habitat and pose a predation threat on young individuals. Hoover (1939) studied wild Brook Trout population dynamics in NH's White Mountains, intending to identify sources for ongoing hatchery production. He identified strains recognized as primitive, meaning they had not received stocked fish introduction or fishing pressure in over ten years while referencing that regional stocking had been occurring since 1937. Results concluded that previously observed "fingerling" trout were actually three or four years old, remaining small due to spring-fed streams that limited growth. Stocking in the region is documented as early as 1867 when 20,000 Atlantic Salmon eggs from New Brunswick were deposited in the upper Pemigewasset River (NH Fish and Game Department 1939).

The legacy of NH's fisheries management was built on hatcheries, which continues today with nearly a million fish stocked annually (NH Fish and Game Department 2018). Few studies have utilized genetic analysis to understand the interactions between stocked and wild Brook Trout in the northeast (Kelson et al. 2015, White et al. 2018). Although little hatchery introgression has been quantified, the ongoing repercussions of widespread, extensive stocking in NH still remains unknown. Vague historic stocking records and complacent practices leave to question the purity of the entire state's wild fish lineage. In the Beebe River watershed, state biologist's initial stocking quota has been surpassed in 74 of the last 79 years and consistently for the last 50 years (Fig. 1.3). Until our research, 99 years have passed since the watershed's ecological attributes described in the Report were measured to a similar scale (Table 1.2). The lead survey biologist Earl E. Hoover introduced the State's 1939 report by stating, "It is believed that the Department's activities in the transplantation of fishes may do more harm than good". We suspect today's 'stable' populations remain exposed to the compounding impacts of introduced hatchery fish, resulting in wild Brook Trout populations' decreased abundance, condition and distribution. Although it is challenging to quantify fish species presence prior to colonization, Brook Trout remain the species at greatest risk to the introduction of other fish across New England (Whittier and Kincaid 1999).

Beebe River watershed

The Beebe River watershed (HUC 12) is located in central New Hampshire and flows into the Pemigewasset River watershed (HUC 10), the primary headwater source to the Merrimack watershed (HUC 8). The Merrimack drains 44.5% of NH land area and 19.5% in MA before entering the Atlantic Ocean. The mainstem of the Beebe River flows 26.9 km southwest through the towns of Campton and Sandwich, NH before reaching the Pemigewasset River, decreasing 526m in elevation along the way. The 8,168.8 ha drainage basin is fed by 106 ha of high elevation ponds and 23 ha of wetlands before entering a distinct bowl where spring-fed, headwater streams become the primary water source (Fig. 1.4). The Beebe watershed is bordered in the north by 303,859 ha of conserved land in the White Mountain National Forest (USDA Forest Service 2018b) and on the south by the Squam Range including over 10,000 ha of conserved land (Lakes Region Conservation Trust 2018).

The Beebe River watershed lies within the White Mountains/Blue Mountains ecotone and borders the Upper Montane/Alpine Zone, Sebago-Ossipee Hills and Plains and White Mountain Foothills ecotones (Fig. 1.5) (Anderson et al. 2013), creating a unique ecosystem mosaic (Duveneck et al. 2015, Sperduto and Nichols 2012). The close proximity of diverse habitats (cliff or talus slope, northern hardwood-conifer) coupled with >85% forest cover creates a region boasting the highest nationally observed species richness in birds with diverse life history strategies, including forest-interior nesters, ground nesters, neotropical migrants and short-distance migrants (NH Fish and Game Department 2015a, Radeloff et al. 2007). Northern hardwoods-conifer forest is found at higher elevations and hemlock-hardwood-pine forest are adjacent to the mainstem Beebe River (NH Fish and Game Department 2015b). Geologic features are diverse across the watershed including cliff outcroppings with primarily granite and pelitic schist (Bennett et al. 2006).

History of the Beebe Watershed

Less than four miles from the Beebe River watershed outlet, Thomas Baker led an attack on the Pemigewasset band of Abenaki in 1712. Sixty-six years later, 400 residents had settled within the Beebe River watershed in the newly chartered town of Campton (Campton Historical Society n.d.). Sandwich Notch Road was the first critical route through the nearby mountainous region, constructed in the watershed's headwaters in 1801. The road carved a path into the wilderness where 30-40 families settled at its height, supported by two sawmills and two schoolhouses. Farmers and tradesmen used the route as a shortcut to transport goods from the Pemigewasset River Valley to the seacoast, primarily during winter. Its unique position passes through two notches and three watersheds (Bearcamp, Beebe River and Mad River) that support the Saco and Merrimack River systems (Sandwich Historical Society n.d.).

From 1810-1840, local towns experienced a sheep farming boom in response to demand from southern NH textile mills, drawing people away. A sharp population decline followed and Campton's population was reduced by 22% from 1840-1880, reaching a 33% reduction by 1900 (Campton Historical Society n.d.). The same trend followed in Sandwich and by 1860 only eight families remained along the Notch Road (Sandwich Historical Society n.d.).

Indications of logging within the watershed was first documented on a map produced in 1860 when a completed road paralleled the Beebe River toward Sandwich and a sawmill was constructed on the upper River (Old Maps of New Hampshire 2018). The turn of the century marked a change in regional land-use as the demand for forest products increased. The peak of logging occurred in 1895 when ten active railroads transported timber through the nearby White Mountains (WhiteMountainHistory.org 2017).

Not long after, a local forester began photographing the denuded northern New England landscape, primarily the White Mountains. Coupled with tales of the destruction, Philip Wheelock Ayers shared his influential photos across the nation with hopes of change. Support came from a NH-born Massachusetts congressman, leading to his sponsorship and the subsequent passing of the Weeks Act of 1911, which bears his name (Plymouth State University 2018, USDA Forest Service 2011). Today, a 180 ha State Park pays homage to his introduction of the Act which resulted in protection of over 8,093,713 ha of National Forests (Forest History Society 2018, NH Department of Natural and Cultural Resources 2018). Three years later, the US Forest Service began purchasing land along the northern perimeter of the Beebe River watershed (Forest History Society 2018), resulting in permanent conservation of over 322,000 hectares of abutting wilderness on the watershed's northern border (USDA Forest Service 2011).

The ~42 km Beebe River Railroad was constructed from 1917-1921 and leads from a critical railroad junction and mill in Campton before paralleling the Beebe River further into the watershed's headwaters (Upper Pemigewasset River Historical Society and Russack 2017). The establishment of this access route initiated extensive logging in the region, which continues a century later. Toward the end of WWI, virgin Red Spruce (*Picea rubens*) from the Beebe River area contributed one quarter of the timber used in construction of US military aircraft. It is reported that 70-80 men with 20 horses hauled 743,224 m^2 of spruce in one year (Gove 2006). All the spruce was removed by 1920, just seven years after starting, but the railroad remained as one of three active routes through the White Mountains (Upper Pemigewasset River Historical Society and Russack 2017). In 1923, slash from decades of timber cutting was left along the railroad and is thought to have ignited from coal embers spread by a train engine. The fire burned 1,416 ha of the forest in the Beebe headwaters before it was contained (Gove 2006). Much of the regional softwood had been harvested and other large expanses burned, while patches of hardwood forests remained (Campton Historical Society n.d.).

In 1925, the Draper Company purchased the Campton sawmill and surrounding timberland, transitioning their forestry practices to focus on construction of hardwood bobbins for textile looms. The mill has been recognized as creating the most bobbins in the world during peak production, reaching ~100,000 per day (Campton Historical Society n.d.). A 1939 watershed report documented sewage discharge into the

Pemigewasset River near the Beebe River confluence, originating from mill houses serving workers of the Draper Lumber Co. The trend of watershed pollution at the time was evident across the entire state. Authors further described angler opinions of the state's large rivers, "Unfortunately the larger rivers are not favorable waters for the New Hampshire angler. This anomaly may be attributed to the angler's dislike of fishing waters which are polluted…" (NH Fish and Game Department 1939). As coal-fired trains became inefficient, 1,200 tons of steel railroad tracks were removed in 1942 to aid in the war effort (WhiteMountainHistory.org 2017). Land-use in the previous 40-50 years had cleared the region of usable timber, and companies struggled to remain in business. Draper Co. sold the Campton mill and subsequent owners ceased operation in 1980 (Campton Historical Society n.d.).

In 1977, the Draper Co. (later, the Rockwell International Timber Corp.) sold holdings to the Yorkshire Timber Co. Several thousand acres were sold off to various owners including 1,960 ha to the US Forest Service, further expanding the White Mountain National Forest. As timberland began to regenerate, Yorkshire Timber Co. managed for forest products before selling holdings to Yankee Forest LLC in 1998. At the turn of the century, the shift of timberland ownership across the Beebe region is similar to most northeastern trends. Large, single owner parcels were broken up, creating increased ownership of smaller tracts of land (Hagan et al. 2005). The remaining Yankee Forest LLC parcels were further subdivided when 350 ha in the southern portion of the watershed was sold to Spencer Brook Forest, LLC. In 2014, Yankee Forest LLC sold their remaining 1,965 ha to the current owner, The Conservation Fund (Redstart Forestry 2017, Sandwich, NH 2017, Town of Campton, New Hampshire 2018).

NH Fish and Game biologists conducted expansive sampling of rivers and lakes in the early 1900s, including the Beebe River from 1916-1918 and Upper Hall Pond in the watershed's headwaters. Sampling included site-specific assessments of abiotic conditions (air/water temperature, pH, flow, wetted width), habitat (condition of pools and amount of cover, canopy cover, substrate type), prey (macroinvertebrate abundance) and fishing pressure. Based on ecological conditions, human influence, fish assemblage and demographics, stocking practices were further evaluated. If results concluded that stocking was beneficial, recommendations included fish species and size class, frequency of stocking and quantity of fish per river mile. Notable conditions of the Beebe River include the recognition as being heavily fished, suggesting ≥75% of fish were removed by recreational and commercial anglers annually. Four tributaries received medium to low pressure, suggesting ≥25 to 50% of fish were removed annually. Based on survey results, biologists in 1939 recommended approximately 195 Brook Trout should be stocked per river mile across the watershed, totaling 2,000 fish annually. Species introduced to the Beebe River and its tributaries have been derived from seven hatcheries and several unknown sources. Stocking records indicate 60,190 Rainbow Trout were introduced from 1939-1991 (primarily before 1955), 4,600 Brown Trout in 1990 and 325,800 Atlantic Salmon from 1992-2002. The predominant species introduced continues to be hatchery Brook Trout including 529,406 fish in the past 79 years with a median of 6,475 per year (Fig. 1.3) (NH Fish and Game Department 2018).

Long-term data collected within two headwater waterbodies document combined changes in water chemistry and fish assemblages in Kiah and Upper Hall Ponds. These ponds are the most susceptible to acid rain because of their high elevation locations, small input drainage area, shallow soils and elevated precipitation rates (NH Department of Environmental Services 2015a). Introduction of hatchery fish was first recommended in Upper Hall Pond during 1916 and again in 1938, while recognized as a 'proven EBT [Brook Trout] pond' (NH Fish and Game Department 1939). The acidity of the ponds increased from the early 1900s until 1974-1987 when decreases were observed (Table 1.3). The most pronounced recorded acidification occurred in Upper Hall Pond during 1984, reaching the critical pH threshold for Brook Trout (4.7-5.2) (Baker and Christensen 1991). Continued sampling in Upper Hall Pond showed a stable pH from 1987-1990, comparable to similar sites state-wide (NH Department of Environmental Services 2015b). No wild Brook Trout were documented in either pond during 2015 and as of 2018, only hatchery Brook Trout inhabit both ponds (Table 1.3) (NH Fish and Game Department n.d.). Historic stocking was not well documented and misinformed rationale was common across the region. Haines and Baker (1986) suggest hatchery-maintained fisheries may be less susceptible to the detrimental effects of acidification, and all records indicate that these ponds have followed suit. They further advise caution in attempts to interpret water chemistry as an impact on the state's fish populations because they have become further complicated by extensive fishery management like chemical reclamation and hatchery introductions (Haines and Baker 1986).

Historic landscape features remain in the area surrounding our focal study streams, including numerous stone walls blanketing the valley created for sheep pasture. Evidence of industry was found within a tributary and along the mainstem with the presence of mill foundations. Among select tributaries, railroad tracks and bunks to support a trestle remain within the wetted width, disrupting the natural stream sinuosity. Widespread decayed stumps, discarded saw blades and steel cables used to haul timber indicate long-term logging has occurred. In steeper tributary headwaters, few old growth trees remain primarily across inaccessible riparian areas.

Present day Beebe River Watershed

NH's forests have regenerated to 77.8% cover by 2002 (Fig. 1.2) (Justice et al. 2002) and 88.9% cover ten years later (Nowak and Greenfield 2012), but the signs of historic forestry practices still remain. In the Beebe River watershed, streams have been impacted by continuous, and sometimes poor, forest management practices for over a century. Forests intersecting Brook Trout streams show signs (i.e. stand age and community composition) of recent timber harvest. Forest stands adjacent to GR3, a tributary flowing from WMNF into the Beebe River, indicate aggressive cutting within the past 10 years and subsequent regeneration. Stands adjacent to GR4, another tributary flowing from WMNF into the Beebe River, show signs of clearcutting but some remaining overstory is estimated to be 80-100 years old. The average coarse woody material (CWM) of stands surrounding GR4 are highest at 41.15 stems/ha compared to stands around GR3 (26.53 stems/ha) and ECR1 (17.3 stems/ha), a north facing tributary to Beebe River. Stands around ECR1 have not been logged in ~30 years and even longer in steeper terrain, but evidence of historic clearcutting remains. The forest adjacent to ECR1 has the most standing dead trees (SDT) at 7.61 trees/ha when compared to average SDT of stands adjacent to GR3 (5.98 trees/ha) and GR4 (5.71 trees/ha) (Redstart Forestry 2017). Depletion of in-stream wood caused by historic logging practices is regionally documented (Kratzer and Warren 2013, Nislow and Lowe 2006) and further quantified through detailed habitat assessments as part of ongoing research in the Beebe River watershed.

Headwater stream systems are inherently resource limited, increasing the importance of connectivity for salmonids (Meyer et al. 2007). Ecological influences like water temperature (Baird and Krueger 2003, Bowlby and Roff 1986), food availability (Petty et al. 2014, Sweka and Hartman, 2008) and suitable habitat (Flebbe 1991, Flebbe and Dolloff 1995) can impact a myriad of demographic traits including carrying capacity, abundance and condition. Anthropogenic conditions that can limit Brook Trout growth, survival, and distribution often go unnoticed until populations become extirpated (Schofield et al. 1993). Life history traits and suitable breeding habitat can seasonally influence Brook Trout movement (Castonguay et al. 1982; Snook et al. 2015). Within these systems, Brook Trout populations have been shown to experience restricted or isolated movement and decreased genetic diversity (Castric et al. 2001, Kanno et al. 2011, Kelson et al. 2015). Additionally, road crossings have been recognized to restrict or act as barriers to upstream fish movement, further impacting upstream populations (Diebel et al. 2015, Perkin and Gido 2012, Warren and Pardew 1998). The probability of extirpation increases when inherently isolated headwater populations become further restricted (Letcher et al. 2007).

The objective of this study was to document baseline population attributes prior to watershed-wide restoration in the Beebe River watershed. We documented wild Brook Trout population attributes two-fold: abundance, density, age structure, condition, biomass and growth, and net movement, cumulative movement, home range and immigration in the presence of anthropogenic barriers and reduced riparian cover. Study areas focused on three headwater tributaries with 1) impassable road crossing and reduced canopy cover, 2) passable road crossing and reduced canopy cover, and 3) no impediments to movement and unaltered canopy. We hypothesized that Brook Trout subjected to a permanent barrier (road crossing) and reduced canopy cover (powerline easements) would exhibit lower density, fewer age classes and lower growth rates than fish populations while fish in an unimpacted stream exhibit more robust, seasonally and interannual stable populations. Additionally, we hypothesized that Brook Trout subjected to a barrier/reduced canopy cover would exhibit increased seasonal and annual movement while fish in an unimpacted stream remain largely sedentary in better suited habitat conditions.

Our work is a critical step contributing to an understudied process (Wood et al. 2018) by measuring how stream restoration can benefit Brook Trout while educating regional managers to further promote conservation of the species. We fear the detrimental effects of land-use change, pollution and hatchery fish introduction have altered native fish assemblages across the watershed, most profoundly observed within the watershed's headwaters (Table 1.3). Similar trends among Brook Trout have been documented elsewhere in NH (Hall et al. 1980, Nislow and Lowe 2003, Warren et al. 2008), in relative northern landscapes across Vermont (Kratzer and Warren 2013), New York (Baldigo et al. 2016, Schofield et al. 1993) and across their native range (Hudy et al. 2005, 2008). We hope our research provides results that can initiate conversations among managers by recognizing the value of remaining wild Brook Trout populations in the Beebe River watershed while further evaluating ongoing management strategies with a goal of restoring native fish assemblages.

FIGURES

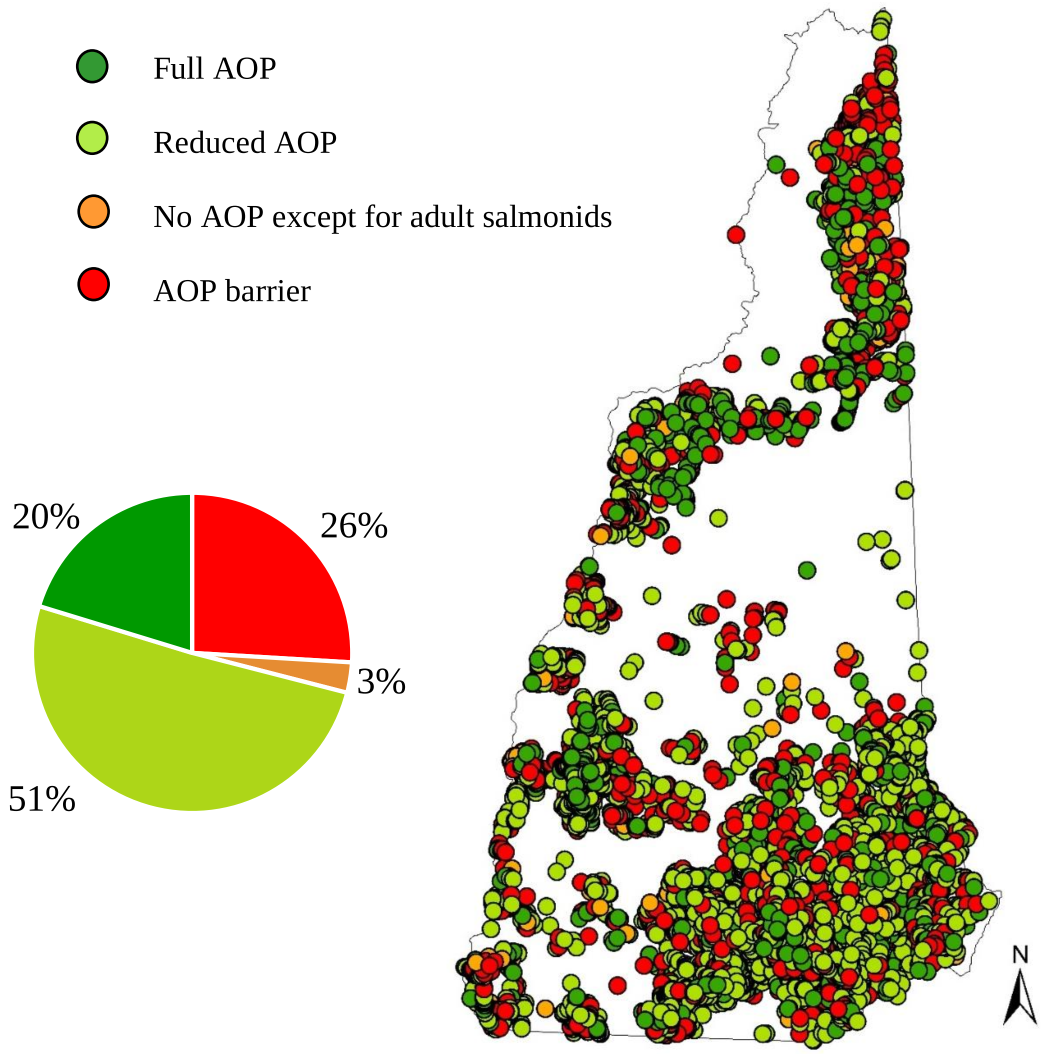

Fig. 1.1. Aquatic Organisim Passage (AOP) of road crossings in NH including percent of each crossing passage type present (NH Department of Environmental Services, n.d.).

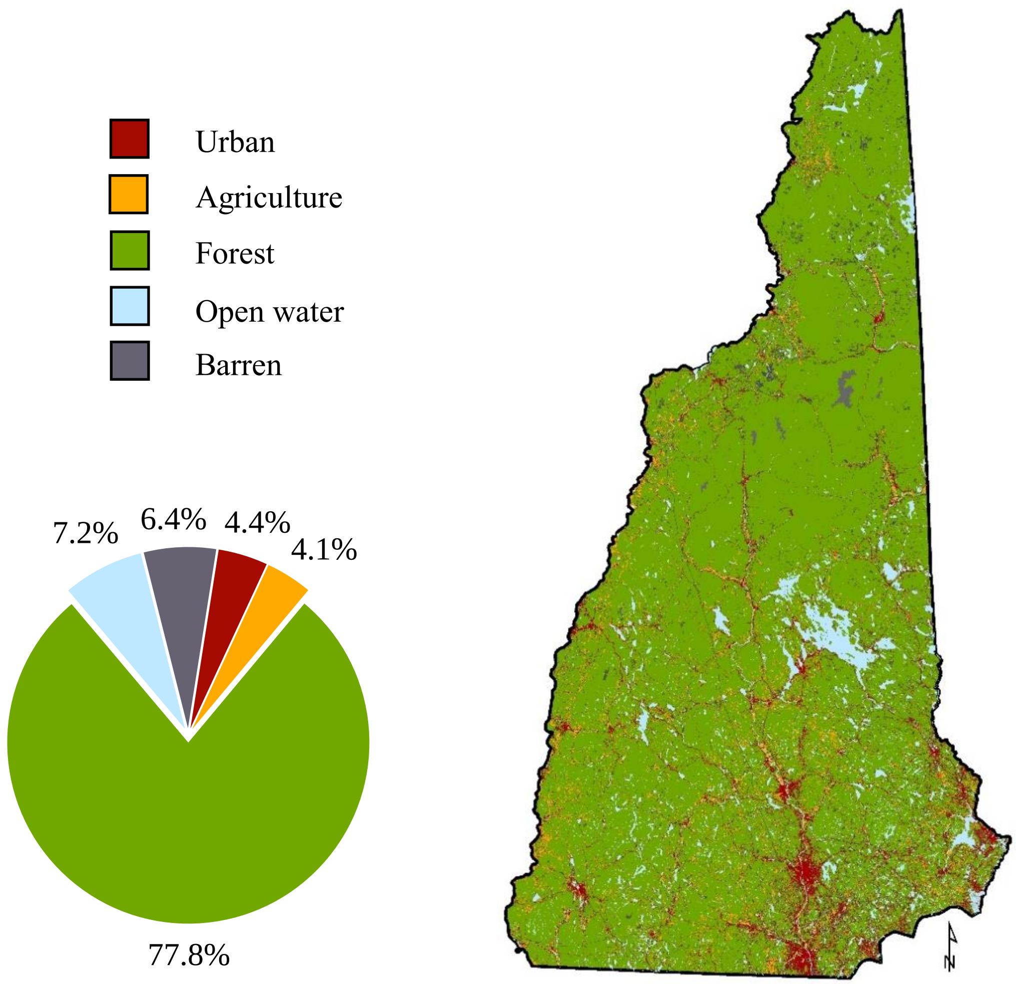

Fig. 1.2. Land cover types in the state of New Hampshire including percent of each type present (Justice et al., 2002; UNH CSRC, 2002).

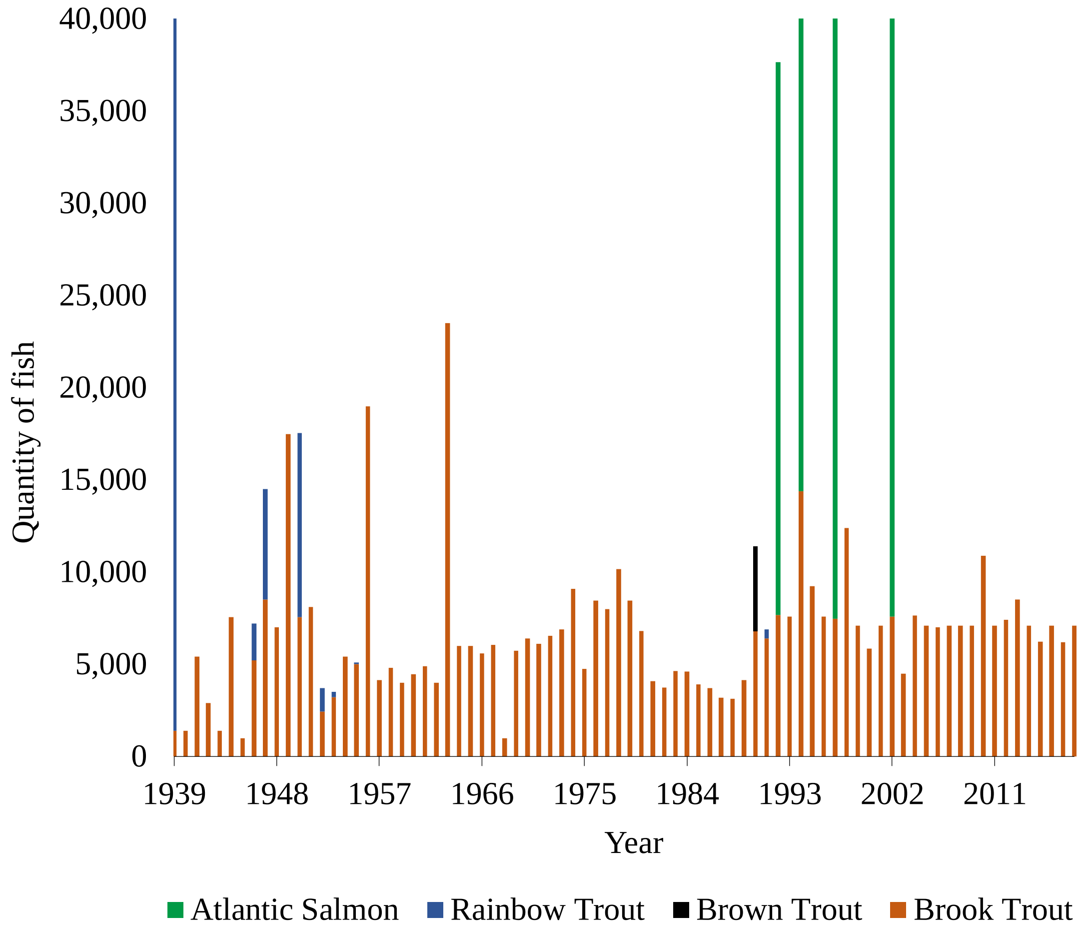

Fig. 1.3. Quantity of fish species stocked in the Beebe River by NH Fish and Game from 1939-2018. Records extending above the displayed quantity of fish include Atlantic Salmon in 1994 (n=80,800), 1997 (n=140,000) and 2002 (n=75,000).

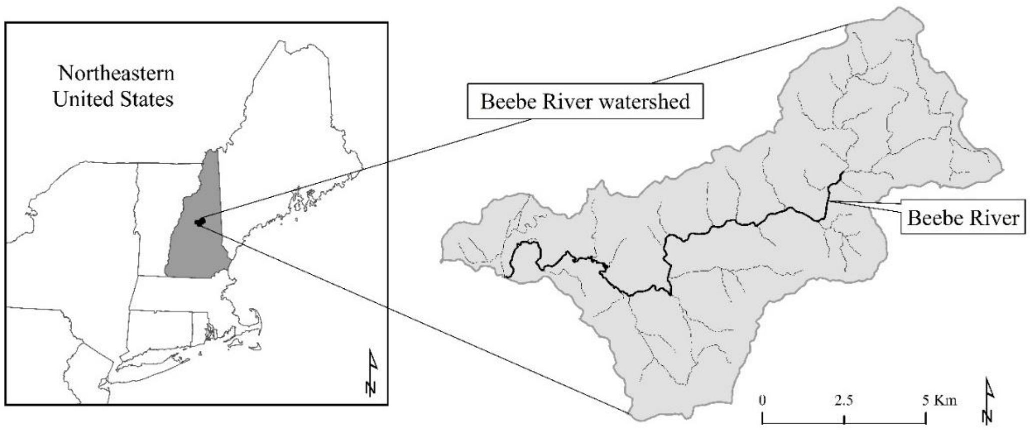

Fig 1.4. The Beebe River watershed is located in Campton and Sandwich, NH, U.S.A., a subwatershed of the Merrimack River within the Northeastern United States.

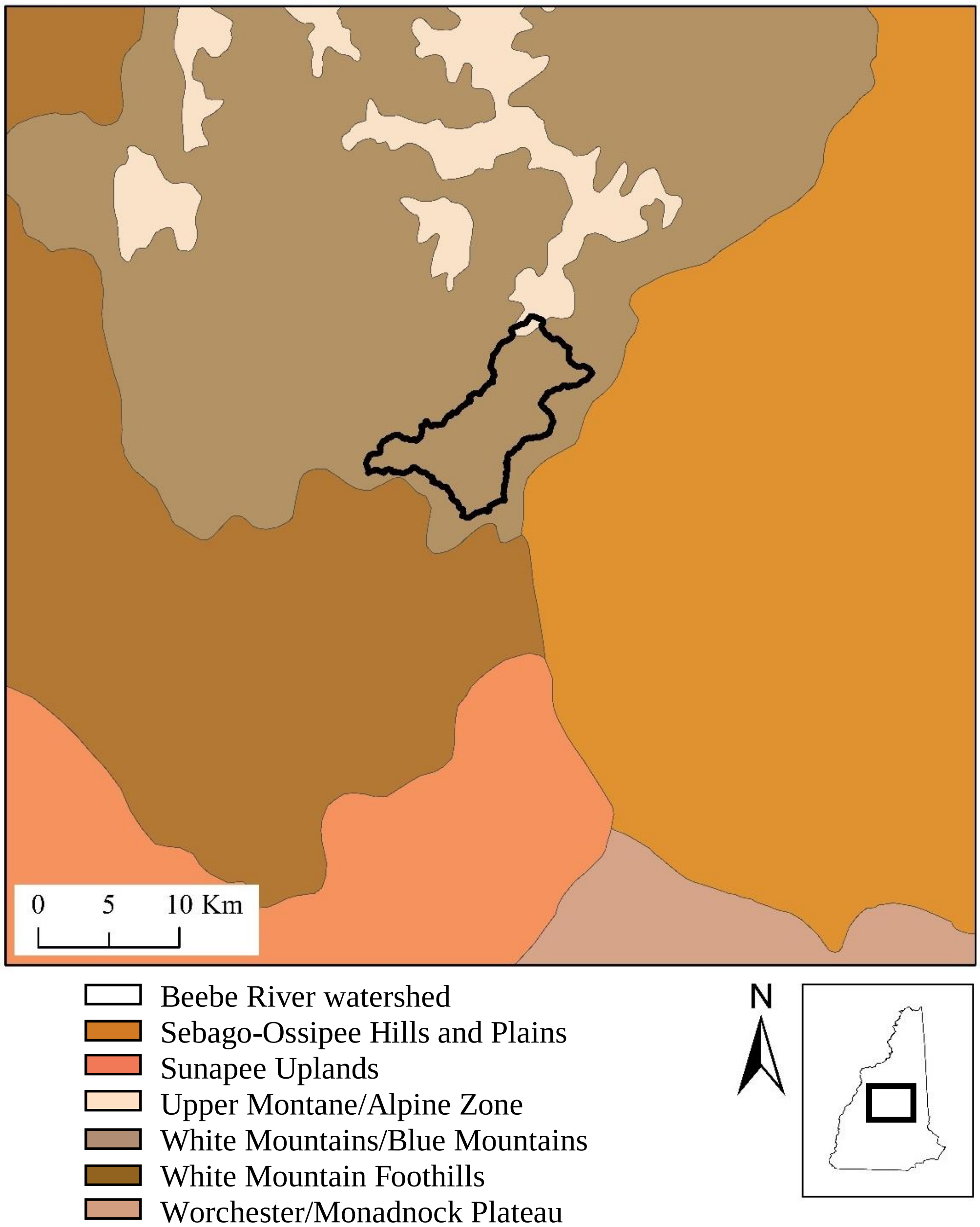

Fig. 1.5. The location of the Beebe River watershed in relation to proximity of ecotones found within New Hampshire (U.S. EPA ORD 2012).

TABLES

Table 1.1. Capability of aquatic organism passage (AOP) through road-stream crossings (# / %) across New Hampshire (NH), Maine (ME), Vermont (VT) and Massachusetts (MA). ME's AOP protocol from 2007-2015 followed reduced detail in road crossing assessments, resulting in the 2016-2018 dataset to be compiled into limited categories that reduced AOP determinations. MA's earlier assessment protocol occurred during initiation of a new protocol, so only data from earlier assessments were used.

	NH	ME	VT	MA
AOP barrier	1,583 / 26	4,592 / 34	2,805 / 35	867 / 14
No AOP except for adult salmonids	187 / 3		235 / 3	128 / 2
Reduced AOP	3,094 / 51	6,210 / 46	4,667 / 58	3,773 / 61
Full AOP	1,237 / 20	2,752 / 20	357 / 4	1,418 / 23
Sampling period	2006-2018	2007-2018	2002-2018	2005-2016

Table 1.2. Habitat attributes measured in the middle region of the Beebe River and stocking data throughout the Beebe River in 1918 (NH Fish and Game, 1939) and 2017 (NH Fish and Game Department 2018).

					Stocking recommended		
Sampling date	Air temp. (°C)	Water temp. (°C)	Mean width (m)	Mean depth (cm)	Species	Age	Quantity
July 1918	25.56	18.89	3.05	7.62	Brook Trout	1 YO	2,000
					Stocking completed (2017)		
Sampling date	Air temp. (°C)	Mean water temp. (°C)	Mean width (m)	Mean depth (cm)	Species	Age	Quantity
July 2017	20.7	18.45	10.37	21.48	Brook Trout	1 YO	6,100
					Brook Trout	2 YO	100

Table 1.3. Compiled water chemistry and fish species presence trends in two headwater ponds of the Beebe River watershed. Data from 1916 and 1938 are sourced from NH Fish and Game Department (1939), 1951-1983 data from Singer and Boylen (1984), 1984-1990 data from [2]NH Department of Environmental Services (2015), 2015 data from NH Fish and Game Department (n.d.).

Location	Year sampled	pH	Fish species present	Reclaimed?
Kiah Pond outlet Brook (unnamed)	1916	6.4	Recommended: 300 EBT Fingerlings to be stocked annually	-
Kiah Pond	1951	5.7	EBT, BT (hatchery)	No
Kiah Pond	1974-1983	5.9	EBT, BBH	No
Kiah Pond	2015	-	EBT (hatchery)	-
Upper Hall Pond	8/22/1938	6.2, 6.0, 6.0	"Proven EBT pond", Recommended: 2,000 ≥15cm EBT stocked annually	-
Upper Hall Pond	1960	5.6	EBT (hatchery), LT, LND	No
Upper Hall Pond	1974-1983	5.8	EBT	No
Upper Hall Pond	1984	5.2, 5.3	-	-
Upper Hall Pond	1987	6.0	-	-
Upper Hall Pond	1988	5.9	-	-
Upper Hall Pond	1990	5.9	-	-
Upper Hall Pond	2015	-	EBT (hatchery)	-

Chapter 2

Headwater Brook Trout Demographics in the Presence of Undersized Road Crossings and Managed Riparian Areas in Central, NH

Introduction

Brook Trout (*Salvelinus fontinalis*) are native to the eastern United States (U.S.A.) and Canada with populations primarily found in small, headwater streams, except in the northern part of their range where populations persist in larger lakes and rivers (Kanno et al. 2015). Brook Trout are sensitive to environmental changes and the cumulative effects of urbanization have limited their occurrence and distribution (Hudy et al. 2005). These effects compound to influence stream conditions, most notably their preferred temperature range of 10-16°C and maximum tolerance of 20°C (Cherry et al. 1976, Coutant 1977, Schofield et al. 1993, Xu et al. 2010, Yoder 2012). Although Brook Trout are not considered threatened across the eastern U.S.A., stream and lake populations have been dramatically reduced throughout their historic range. The majority of large, riverine habitats no longer support self-sustaining populations, reducing intact populations primarily to headwater streams across 27% of their range (Hudy et al. 2005). Populations restricted to headwater habitats lack the connectivity required to reestablish populations elsewhere, making them prone to extirpation by increased human impacts or stochastic events (Hudy et al. 2008). Moreover, 39% of historic populations are considered reduced and 29% of populations at the subwatersheds level have been extirpated (Thieling 2006). Hudy et al. (2005) identified agriculture and urbanization as the top two ongoing threats to stream populations of Brook Trout across their range.

Throughout the northeast U.S.A., fragmentation is the main impact on headwater Brook Trout populations. Gibson et al. (2005) surveyed recently constructed road crossings along the Trans Labrador Highway and found that poorly constructed stream crossings can destroy aquatic habitat, fragment and isolate fish populations and increase vulnerability to disturbance events, compounding the chances of extirpation. Within the same ecotone, Maitland et al. (2015) found that habitat quality in northern boreal forests was negatively affected by sediment mobilization and deposition related to road crossings. The stream crossing structure most detrimental to fisheries has been recognized to be culverts (Warren and Pardew 1998). Furthermore, Park et al. (2008) documented the cumulative effects of culverts at the watershed scale, recognizing acute streambed scouring at the outlet which increases the potential to cause a hanging culvert. Populations restricted to headwater habitats lack the connectivity required to reestablish populations elsewhere, making them prone to extirpation by increased human impacts or natural, stochastic events (Hudy et al. 2008). Harvey and Railsback (2012) used inSTREAM to explore the effects of barriers on a virtual stream-dwelling trout population. They found different life history characteristics of tributary subpopulations isolated by barriers, including shorter lengths at given ages and a lower survival rate beyond Age-3 resulting in earlier spawning in younger fish. Overall, degraded habitat can negatively impact fish health and behavior, further influencing population demographics.

Historic forestry practices have compounded multiple anthropogenic stresses to stream ecosystems across New England (Kratzer and Warren 2013, Nislow and Lowe 2006). Since the 1770s, regional landscapes have transitioned from primarily deforested, farmed and subsequently reforested (Foster, 1992). During forest recovery, records describe wild Brook Trout populations and distribution as ‘abundant’ (Hoover 1939, Lord 1933, Vermont Fish and Wildlife Department 2017). New England’s present day forests primarily consist of secondary growth that have yet to reach their average lifespan (Foster et al. 2008), but are experiencing increased deforestation driven by human expansion in specific areas (Olofsson et al. 2016). Consequently, many streams lack input of old, large woody debris characteristic of streams draining old-growth forests (Neumann and Wildman 2002) that has been found to directly and indirectly benefit populations (Flebbe 1991, Kratzer and Warren 2013, Morris et al. 2012, Neumann and Wildman 2002). Even with these landscape impediments, New Hampshire (NH) maintains the second highest number of watersheds containing Brook Trout across their historic range (Hudy et al. 2005).

Recognizing ongoing threats of resource limitation and fragmentation, NH has initiated assessments of existing road crossings with survey parameters now including aquatic organism passage (NH Department of Environmental Services et al. 2018). These assessments have sparked a trend in watershed-wide aquatic restoration by crossing replacement and instream wood additions, most notably at Nash Stream (Whitaker unpublished), within the Beebe River watershed (NH Fish and Game Department, 2015a) and ongoing in the Androscoggin River watershed (Ben Nugent, personal communication). Furthermore, fragmentation impacts are expected to persist and increase into the future, strengthening the importance of protecting primary, long-term habitat like headwater streams (Hunnington et al. 2009; Kanno et al. 2015, Merriam et al. 2017).

Understanding pre-restoration population trends is a critical step to measure the effects of restored connectivity across historically impacted headwater streams. The objective of this study was to document wild Brook Trout demographics in the presence of seasonal and permanent barriers and related habitat degradation, serving as a baseline assessment in the Beebe River watershed (Campton/Sandwich, NH, U.S.A.). We focused on three headwater tributaries with 1) impassable road crossing and reduced canopy cover, 2) passable road crossing and reduced canopy cover, and 3) no impediments to movement and unaltered canopy. We documented Brook Trout abundance, density, age structure, condition, biomass, growth and habitat. Potential abiotic influences are further characterized at the geomorphic threshold region-scale, which share similar habitat characteristics per region across tributaries (Church 2002). We hypothesized that Brook Trout subjected to a permanent barrier (road crossing) and reduced canopy cover (powerline easements) would exhibit lower density, less complex age structure and lower growth rates than fish populations in an unimpacted stream.

Methods

Study area

The Merrimack River watershed (HUC 8) is located in central New England, U.S.A. and drains 44.5% of land area in New Hampshire and 19.5% in Massachusetts before entering the Atlantic Ocean. The Pemigewasset watershed (HUC 10) is the primary headwater source to the Merrimack R. watershed, deriving flow from the White Mountains of NH. The Beebe River watershed (HUC 12) is an eastern subwatershed to the Pemigewasset that flows 26.9 km through the towns of Campton and Sandwich, NH. This 8,168.8 ha drainage basin is fed by 106 ha of high elevation ponds and 23 ha of wetlands before entering a distinct bowl where spring-fed, headwater streams become the primary water source (Fig. 1.4). The watershed is bordered in the north by 303,859 ha of conserved land in the White Mountain National Forest (USDA Forest Service 2018b) and on the south by the Squam Range including over 10,000 ha of conserved land (Lakes Region Conservation Trust 2018). The mainstem of the Beebe River (further referred to as ‘mainstem’) flows southwest before reaching the Pemigewasset River, decreasing 526 m in elevation. Northern hardwoods-conifer forest dominates at higher elevations and Hemlock-hardwood-pine forest is adjacent to the mainstem (NH Fish and Game Department 2015b). Geologic features are diverse across the watershed including cliff or talus outcroppings with primarily granite and pelitic schist (Bennett et al. 2006).

A private dirt road (denoted as GR) parallels the mainstem to the north, traversing five south-facing, perennial, first-order streams (GR1-GR5)(Fig. 2.1). Five stream-road crossings range from complete to no aquatic organism passage (AOP) (NH Department of Environmental Services et al. 2018). Paralleling the Grade Road to the north, vegetation management occurs approximately every five to seven years along a ~50m wide powerline easement, resulting in little to no canopy cover. The easement crosses tributaries GR1-GR5 less than 200m upstream from their confluence with the mainstem and two streams in the northeast portion of the watershed. Fish species documented within the Beebe River watershed include wild and hatchery Brook Trout (*Salvelinus fontinalis*), Blacknose Dace (*Rhinichthys atratulus*), Longnose Dace (*Rhinichthys cataractae*), Common Shiner (*Luxilus cornutus*), White Sucker (*Catostomus commersonii*), Atlantic Salmon (*Salmo salar*) and Slimy Sculpin (*Cottus cognatus*) (Nugent 2014, Page et al. 2013). Lower portions of headwater tributaries contain wild and hatchery Brook Trout, Longnose Dace, Blacknose Dace and rarely, White Sucker. Brook Trout are the sole species documented from the lower to upper portions of the headwater tributaries in this study area. Study reaches account for a subset of each tributary occupied by Brook Trout, including ~27% of GR3, ~32% of GR4 and ~41% of ECR1 (NH Fish and Game Department- 2015 sampling).

Our study focused on 200m reaches immediately upstream of the mainstem confluence in three headwater tributaries: GR3, GR4 and ECR1. We also sampled 100m portions of the mainstem, 50m downstream and 50m upstream of each study tributary confluence. These reaches include three main geomorphic thresholds typically found in first order streams: floodplain valley, upland valley and upland (Church 2002). GR3 flowed beneath the Grade Road through two 91cm diameter corrugated steel culverts and GR4 flowed beneath a degraded and undersized wooden bridge. GR3 and GR4 study areas also include a powerline easement with reduced canopy cover and a portion of intact forest downstream of the Grade Road and upstream of the powerlines. Each 200m study reach was flagged in 2m increments including main channel portions and braided flows/side channels.

Habitat

I. Lotic and terrestrial attributes

We conducted a stream habitat analysis following a modified version of NH Fish and Game's *Rapid Habitat Assessment* (Decker, 2000) and an additional, in-depth wood survey following *TWF Monitoring Program: Large Woody Debris Survey* (Schuett-Hames et al. 1999). Assessments occurred from late July to mid-August 2017 in study tributaries during low flow (determined by USGS station #01016500, Fig. 2.2) within the main channel of each tributary, excluding seasonally intermittent braided flows/side channels. Surveys were modified to quantify habitat that becomes seasonally available to aquatic organisms during increased discharge, potentially influencing food webs (Schneider and Winemiller 2008) and stream geomorphology (Fisher and Likens 1973). We measured 'rafted organic material' (ROM) suspended within bankfull and excluded organic substrate, further separated by submerged (wet) or above water level (dry). When multiple pieces of wood were aligned in close proximity, the formation was considered a 'jam' measured by the approximate volume (m^3). Following modified methods by Lemmon (1956), we measured canopy cover in the approximate center of wetted width in 10 m intervals by orienting a spherical densitometer (Forestry Suppliers, Inc., Jackson, MS, U.S.A.) upstream during late June and early July 2017. Canopy cover measurements for forested reaches and the managed powerline easement (no-low riparian cover) were calculated as mean ±SE. Channel slope was determined using a clinometer, measured from a 2m increment to where line of sight was impeded by landscape slope change. No landscape alterations occurred along study reaches between 2016 and 2017, so we assigned 2017 riparian canopy cover values to the same transects in 2016. Analysis between sampling events were referenced in association with seasons: Spring=May-June, Summer=June-July and Fall=July-September and results from an individual sampling event were referenced as the sampling month.

We analyzed habitat reaches to categorize geomorphic threshold regions (further denoted as regions) designed to understand how expected variability influenced Brook Trout demographics. Regions were determined post-habitat and fish sampling by recognizing regional changes in channel slope, riparian landscape topography and in-stream ecological features (Church 2002). Additionally, the same regions exhibited similar habitat across study streams.

II. Temperature

Temperature loggers (ONSET HOBO U-22, Bourne, MA, U.S.A.) were deployed at fourteen locations in 2016 and 17 locations in 2017 (Fig. 2.3) and recorded instantaneous air and water temperature at 15-minute intervals and daily maximum temperature. Loggers were deployed in May and retrieved in October each year. Two loggers were not recovered in 2017 presumably due to a 100-year flood event in early July. Short-term fluctuations in temperatures were further explained by rain events, recorded at a nearby weather station. We used a one-way analysis of variance (ANOVA) to test for differences in monthly water temperature variation among tributaries to determine tributary-specific, seasonal fluctuations. For all statistically significant ANOVA results, we used a Bonferroni post-hoc test to determine where significance was derived. We analyzed hydrologic data from USGS monitoring stations closest to the study area to compare current (2016-2017) conditions to typical historic conditions (Table A2.1). A two-sample T-test was used to test differences historic and current hydrological data during both spring and fall.

Fish sampling

We sampled Brook Trout in July and October 2016 and May, June, July and September 2017 and during three dewatering events during restoration (September 2017). Smith-Root model LR-24 DC (Vancouver, WA, U.S.A.) backpack electrofishing units were utilized at 600-800 volts. We used two-pass depletion methods described by Zippin (1958) and blocked fish movement with nets every 10m. Mainstem segments were sampled from 50m downstream to 50m upstream of each confluence with a single pass using 2-4 synchronous backpack electrofishing units. Mainstem sections below all three tributaries were periodically not sampled due to elevated flow (May 2017 and in June 2017, below GR4 and ECR1). Fish sampling during crossing dewatering events occurred between natural breaks or with block nets deployed downstream and upstream of stream channels directly impacted by construction.

The location of all captured Brook Trout was recorded to the nearest 2m and all fish were measured for total length (TL) in mm and weighed to the nearest gram before being released at the location of capture. Brook Trout ≥60 mm TL and ≥2g were tagged with a 12 mm, half duplex (HDX) PIT tag (Biomark, Andover, MA, U.S.A.). Tags were implanted in the ventral region of the body cavity (Gries and Letcher 2002), and each fish was marked with an adipose and/or ventral fin clip(s) immediately preserved in 95% ethanol for future genetic analysis. Scales for aging fish were sampled from all tagged fish above the lateral line, posterior to the dorsal fin (Schneider et al. 2000a). Only the quantity of fish captured per tributary was recorded in October 2016 and no fish were tagged. In May 2016, handheld nets with 3/16" mesh size limited the capture success of small young-of-year/Age-0 fish (denoted as YOY). Age-1+ minimum TL and mass -SE all met the minimum sizes required for tagging, therefore untagged captures were only analyzed for Age-1+ fish. Additionally, we elected to remove untagged fish captured during May due to the extended time since the most recent tagging event (July).

Fish population metrics

The mainstem was not sampled during habitat analysis, therefore the association between fish location and habitat type only occurred in tributaries. The longest duration between tagging events occurred from July 2016 to May 2017. We elected to remove untagged fish captured during May from these analyses because we focused on seasonal movement within and into study reaches. Analysis for each sampling event was referenced as the sampling month. We determined 'seasons' within each year because our sampling events were chosen to document variability based on Brook Trout natural history traits and fluctuating habitat conditions, like spawning and peak water temperature. Analysis between sampling events are referenced by season, including Spring=May-June, Summer=June-July and Fall=July-October (2016) and July-September (2017). Interannual analysis occurred from July 2016 to July 2017. Capture probability and biomass estimates were calculated using Trout Count Lite (Carle and Strub 1978 modified by Kratzer). Probability could not be calculated during October 2016 because scales were not sampled and age determination was needed to run probability analysis.

I. Abundance and density

Beebe A two-sample T-test was used to compare age-specific abundance within each tributary between 2016 and 2017. A one-way ANOVA was used to test variation in abundance across tributaries within each sampling event. Abundance estimates are calculated by capture probability using Trout Count Lite (Carle and Strub 1978 modified by Kratzer) and standardized by sample area. Capture probability could not be calculated during October 2016 because the lack of data collection did not allow analysis to occur. Scales (for age determination) were not sampled, and untagged captures were not measured (TL, mass, location), which was needed to run probability analysis. Finally, sampling precluded calculating capture probability and biomass estimates for YOY during May 2017.

Beebe We calculated tributary-specific Brook Trout density for all sampling events. Wetted width calculations were based on July 2017 measurements, but widths were variable among seasons and across years, causing some variability in expected Brook Trout density.

Regional The density of Beebe River tributary populations were compared to statewide populations at the scale of fish/acre. We expanded the comparison of Beebe River tributary populations to the 'Wild Trout' protection standards designated by nearby states by analyzing results at the river mile scale. Maine's (ME) lotic standard is ≥1,350 fish/mile (ME Department of Inland Fisheries and Wildlife 2009) and although Vermont (VT) recognizes standards at the same scale, their classification could not be compared to our populations because it includes a minimum fish TL at the fish/mile scale.

II. Age structure

Beebe Aging of scale samples involved dry plating scales beneath a light microscope. Determination followed a modified protocol described by Schneider et al. (2000a) with further instruction by NH Fish and Game fisheries biologists. Length-frequency histograms were available during age determination and the confidence of assigned ages followed rankings provided by Idaho Department of Fish and Game (2015). If confidence was Rank-3 (low) or Rank-4 (unreadable), ages were reanalyzed after the completion of remaining samples and the previously assigned ages were not referenced during secondary analysis. All samples read a second and third time were found to have matching ages compared to earlier analysis. Age verification by a second reviewer was not completed; therefore, the results should be considered preliminary at this stage until a third party can conduct QA/QC.

The body size and capture probability of YOY were variable across tributaries and among seasons. This made it difficult to confidently differentiate untagged YOY captures, due to a) not meeting minimum TL requirements for tag implantation during a previous capture, b) missed capture during sampling due to small size, or c) both. Therefore, the analysis of YOY was limited compared to adults (Age-1+) throughout the study.

III. Condition

Size at age We determined condition using a multispectral approach by analyzing snapshots of seasonal size of age classes and population trends across our study streams. TL was used to compare fish size among years and across tributaries while mass was used to compare fish size across seasons and tributaries. Fish TL is a more constricted growth metric, better used to observe trends formed by interannual conditions while mass can fluctuate seasonally based on food source abundance and abiotic influences. Captures from July of 2016 and 2017 were used to analyze interannual condition using two-sample T-tests. Captures from all sampling events were used to analyze seasonal changes in condition, focusing on Age-1 Brook Trout. We tested variation in Age-1 median body mass among tributaries for each sampling event using one-way ANOVAs. We calculated sex-based mass per event, analyzed as median mass ±SE.

Population condition We compared Beebe River tributary Brook Trout populations to statewide populations to further understand how study populations rank among populations found in similar conditions across the state. Statewide populations were sampled using 2-5 pass depletion electrofishing from 1995-2018 with the intent of targeting wild Brook Trout or Atlantic Salmon (NH Fish and Game Department unpublished). We further restricted our analysis to >10 wild Brook Trout of all age classes captured in study areas >200m^2. We used data from July 2016 Beebe tributary sampling for comparison but some statewide populations were sampled multiple times with the most recent event displayed. Study stream populations were further transformed to analyze populations at the state-wide scale of pounds per fish (lbs./fish).

Body condition Population-level condition (TL-mass relationship) from each tributary, each July, was plotted against the American Fisheries Society (AFS) standard for lotic, wild Brook Trout populations (Schneider et al. 2000b).

IV. Biomass

Beebe Population biomass was estimated using Trout Count Lite and was analyzed at kg/study area to remain consistent with our metrics. A two-sample T-test was used to compare biomass between July 2016 and 2017 for each tributary. We tested variation in biomass among tributaries for each sampling event using a one-way ANOVA. We tested interannual variability in biomass for each tributary using two-sample T-tests.

Regional Understanding regional populations can provide managers with a more informed understanding of population persistence, often prompting protection, habitat enhancement, restoration, changes in angling regulations or influencing surrounding land-use practices. NH and VT utilize biomass thresholds to enhance protection of wild Brook Trout, including NH's standard of ≥13 lbs./acre (NH Fish and Game Department 2018) and VT's standard ranging from >20-30 lbs./acre (Vermont Fish and Wildlife Department 2018). The wetted width of each study transect represented a proportion of state standard area recognized, so results were increased to meet measurement guidelines (acre or mile). Additionally, we compared the biomass of 225 statewide populations to our study population results, analyzed at lbs./acre to align with state-level management methods.

V. Growth

First, we measured Brook Trout mass using a digital scale accurate to ≥1g, resulting in an unknown mass for fish weighing <1g. We reassigned unknown mass to 0.75g for a total of 49 fish (GR3: 2016 n=4, 2017 n=2, GR4: 2016 n=5, 2017 n=2 and ECR1: 2016 n=36). Body mass change and TL change (as a proxy for daily growth) were calculated between recapture events within a season using tributary-specific time intervals calculated from the equation modified from Jensen (1990) and Petty et al. (2014):

$$G = \left[\frac{\Delta TL}{TL_{t1}}\right] / \text{\# of days}$$

where G is the mean daily growth, ΔTL is the change in TL between capture events, TL_{t1} is TL at first of two capture events and # of days is the number of days between capture events. When we reference percent TL change, G is multiplied by 100. This equation is used for body mass change as well, but with mass substituting TL.

These calculations standardize the growth of individual fish recaptured after the number of days since last capture. YOY were excluded from the dataset used in length and mass changes because growth until minimum tagging size varied. We tested for variation in percent body mass change among Age-1 fish and percent TL change among Age-1+ fish. Both were tested among tributaries after each recapture event within the same season using one-way ANOVAs. We tested the variation in both body mass and TL change interannually among the same tributaries using two-sample T-tests. Among tributaries, a Bonferroni correction post-hoc was used to determine where significance was derived. All recapture events within a season were combined per tributary to compare growth within the same age class. We calculated sex-based seasonal growth, analyzed as median mass ±SE. Statistical analyses and figures were constructed using Microsoft Excel: Version 2016 and mapping, including spatial analyses, was completed using ArcMap 10.6.

Results

Habitat

I. Lotic and terrestrial attributes

Mean canopy cover measurements indicate 15% canopy cover in powerline easements and 88% elsewhere along forested stream reaches. ECR1 contained 4-6x more instream wood (15,740.98 m^3) than GR3 and GR4 (3,866.96m^3 and 2,459.85m^3, respectively). An additional contributing value to instream wood volume includes the presence and size of log jams. The size of jams did not significantly differ among streams ($F= 0.42$, $df= 17$, $p= 0.6622$), but ECR1 had 3x more jams than other streams. The larger quantity of jams in ECR1 create the greatest quantity of pools formed by organic material among tributaries, documented in ECR1 (13.79%).

Across New England, 2016 was widely recognized as a drought year, including central NH. Discharge, groundwater and precipitation were not recorded within the study area but records are available from within 7km of the Beebe River watershed outlet (Table A2.1). Cumulative monthly precipitation in spring and summer of 2016 was significantly less (18.6%) than the same period in 2017 ($t= -2.398$, $df= 9$, $p= 0.02$)(Fig. 2.2). Habitat attributes of the three study tributaries are reported in Table 2.1.

<u>GR3:</u> The most downstream ~50m of GR3 consists of wide, shallow glides and riffles separated by low cascades with a 5-6% channel slope. During 2016 spring flooding, the main channel relocated and began flowing over the forest floor before carving a new channel during the summer/fall. This highly variable reach is braided and comprised of small gravel and sediment substrate deposited as the channel slope abruptly decreases into the Beebe River floodplain valley. The transition to upland valley has a channel slope of approximately 8%, primarily comprised of pools until the impassable road

crossing. Aggregation of bed material from the scour pool created a 14% channel slope below a cobble and gravel hydraulic control, resulting in a seasonal barrier along the downstream edge of the pool that stretched 10m downstream. The scour pool was approximately 21.6m^2 and almost 1m deep with the banks lacking vegetation, exposing eroding gravel. Above the crossing, the landscape transitions to upland within the ~50m no-low riparian canopy easement as channel slope ranges from 9-12% and is comprised of step pools separated by cascades.

GR4: The floodplain valley zone extends upstream ~120 m to just below the road crossing. This zone is primarily comprised of shallow pools and riffles and the channel slope remains below 8%, as low as 1-2% in extended reaches. Although the road crossing is fully passable to fish, it remains geomorphically undersized. Restricted flow concentrates velocity to create a ~21.4m^2 scour pool beneath the crossing. This pool was the largest and second deepest in the stream, while the bridge retained the largest jam within the study area. Aggregated bed material stretching 10 m immediately downstream created an 11% channel slope. Transitioning upstream to upland valley, slope increased to 8-9% with longer riffles and cascades between pools through the ~50m no-low riparian canopy easement. The channel slope increased to 11-17% on the upper edge of the easement through wooded reaches. Alternating cascade/pool steps increased in frequency and depth while decreasing in length. Large boulders created a waterfall within this area, measuring 1m long x 1.1m wide x 1.2m tall.

ECR1: The study reach flows northwest entirely through closed canopy forest. The floodplain valley zone extends ~35m upstream with channel slope ranging from 3-8%. This zone is primarily comprised of glides bordered by vertically cut banks up to 1 m tall. Limited steps of up to 12% slope occur throughout, created by cobble aggregate or instream wood. Channel avulsion present in lower reaches causes habitat depths to decrease while braided channels frequently redistribute small gravel across wide areas. Upstream, the upland floodplain is comprised of glides and pools created by an abundance of instream wood. This creates alternating pool/small cascades with 9-12% channel slope. An abrupt transition to upland occurs ~125m upstream from the confluence where the tributary cuts through a steep, defined valley. The intact riparian area experienced limited timber harvest allowing mature conifers to dominate (Redstart Forestry, 2017). Frequent jams (12 per 200m) held by boulders create step pools throughout the upper reach with a channel slope ranging from 11-24%.

Substrate size and channel slope decreased from upland to floodplain valley zones in all streams. Steeper channel slope in the headwaters increases flow velocity, collecting bed material as flow moves downslope (Church 2002). As channel slope and velocity decreased downstream, the hydraulically suspended bed material becomes deposited within the floodplain valley zone. Mean bankfull width was most variable within the lower (floodplain valley) and upper (upland) regions of GR3 and GR4, but became more restricted in the upland valley zone. In ECR1, the mid region of the study area (upland valley) had the most variable mean bankfull width, approximately double the variation compared to any regions of GR3 and GR4.

The greatest variation in channel slope and bankfull width occurred in ECR1, created by a distinct transition between upland and upland floodplain landscapes. ECR1 was also the steepest and most narrow stream with a single 21m reach measuring 24% channel slope. The lowest mean channel slope across all habitats among all streams occurs in GR4. This low slope occurs in the floodplain valley, which is also the longest threshold zone among all tributaries. The greatest wetted stream area found among tributaries was in this zone of GR4, caused by a more widely distributed flow regime for a longer reach than other zones. The dominant habitats present in all upland zones were pools and cascades, or a combination of both, while all upland valley zones primarily consisted of pools.

II. Temperature

Below average precipitation resulted in reduced input to the system, compounding the effects of no-low canopy cover in multiple reaches. Shallow mainstem and powerline reaches increased the thermal impact on stream habitat, resulting in temperatures exceeding the thermal maximum for Brook Trout (Table A2.2). In the mainstem, the mean daily maximum temperature of all seven mainstem loggers reached ≥20°C for a combined average of 60 days throughout 2016. The duration was only 33 days in 2017. The daily maximum water temperature within the GR4 powerline easement (no-low canopy) reached ≥20°C during two continuous periods, totaling nine days. Warmed water from the no-low canopy cover segment continued 98m downstream through a 74m closed canopy reach for an additional two days. This resulted in a significantly warmer median temperature in GR4 during June, July and August 2016 (p value range: <0.001-0.016).

Across all tributaries from June to October, ECR1 maintained the coolest mean monthly temperature while GR4 remained warmest from June to September. Combined 2016 tributary/mainstem results show GR4 and its associated mainstem segment were consistently warmest, except during October when tributary temperatures were warmer in GR3.

All tributaries remained below 18°C during 2017 and only August showed a significant difference in mean monthly temperature (F= 3.269, df= 90, p= 0.043), noted by a 0.7°C (±2.08) increase in GR4 over GR3 and ECR1. During June, July and August 2017, mean monthly tributary temperatures were all significantly lower than the same period of 2016 (p value range: <0.001-0.016). Later in the season, the annual trend was reversed: October tributary temperatures were significantly warmer in 2017 than 2016 (p values <0.001). When observing mean tributary temperatures across 2017, GR3 remained the coolest while GR4 was the warmest. GR3 and its associated mainstem segment remained the coolest locations from June-October 2017 (Fig. 2.3).

Fish population metrics

Throughout the study, 41% (n=157) tagged fish were captured at least once. The overwinter recapture rate of 12% (n=21) is defined by fish tagged in July 2016 and recaptured in May 2017 and consisted of 211-215 days between tagging events. There were 157 (41%) tagged fish recaptured at least once within tributaries during a given season, creating the dataset of fish used in demographic and movement analysis. This included 79% of the total Brook Trout captured in ECR1, 71% in GR3 and 60% in GR4 throughout the study, including replicate recaptures. During 2017, the highest percent of fish recaptured during every sampling event was observed in ECR1 (16%). Furthermore, the fewest recaptures were observed in GR3 (6%); in contrast, the highest proportion of fish recaptured two times occurred in this stream (40%).

I. Abundance and density

Beebe A total of 940 wild Brook Trout were captured across our study sites during six sampling events. Mainstem captures included 54 wild and four Age-2 hatchery fish, of which only 48 were tagged and three recaptured (two in the mainstem and one in GR3). We excluded hatchery fish from further analysis. Capture probability calculated with Trout Count Lite ranged from 76.32%-100% except for May 2017. Total monthly precipitation was almost 2x higher than average that May (Fig. 2.2), which resulted in a decrease in capture probability to 26.88%, driven by the lowest captures of the study (Table A2.3). Throughout the study, 41% (n=157) tagged fish were captured at least once. The overwinter recapture rate of 12% (n=21) is defined by fish tagged in July 2016 and recaptured in May 2017 and consisted of 211-215 days between tagging events. Fish exclusively tagged during 2017 were recaptured at the rate of 49% in GR3 (n=23), 68% in GR4 (n=26) and 75% in ECR1 (n=38). Tag retention was high with only eight fish noted for tag loss (i.e. observed fin clip, but no tag). Regardless of habitat types present, Brook Trout were most commonly captured in pools than any other habitat (Fig. 2.4).

Significantly more Brook Trout were captured in ECR1 than GR3 and GR4 during July 2016 sampling ($F= 48.35$, $df= 326$, $p<0.001$) (Fig. 2.5). Fish abundance significantly decreased in ECR1 and GR3 between July 2016 and July 2017 (GR3 $t= 5.63$, $df= 155$, $p<0.001$; ECR1 $t= 9.52$, $df= 213$, $p<0.001$). Abundance trends shifted among tributaries throughout 2017. Significantly more fish were captured in GR3 during June ($F= 11.93$, $df= 113$, $p<0.001$) followed by the most fish captured in ECR1 during July and September (July $F= 8.16$, $df= 174$, $p<0.001$; September $F= 6.18$, $df= 159$, $p=0.003$).

Beebe Brook Trout density was significantly greater in ECR1 during July 2016, but there were no significant differences in density among tributaries during the remainder of the study ($F= 14.92$, $df= 90$, $p<0.01$) (Fig. 2.6). The highest total densities observed within a sampling event at the tributary scale occurred in July 2016 for GR3 (0.3 fish/m^2) and ECR1 (0.51 fish/m^2). The following July, density was significantly lower in both tributaries (GR3 t= 3.22, df= 45, p<0.01, ECR1 t=5.8, df= 71, p<0.01). Brook Trout total density in GR4 was the highest in September 2017 (0.11 fish/ m^2), also the same period with the lowest total density observed in GR3 (0.11 fish/m^2). The total density of individuals for populations in all tributaries exhibited similar seasonal trends across 2017. All populations increased from June to July (13-132%) followed by a decline from July to September in populations within GR3 (34%) and ECR1 (14%) while the density in GR4 continued to increase (36%).

Regional The density of all three study populations rank in the highest 2% (n=275) of statewide Brook Trout populations, led by fish in ECR1. Three statewide populations have a higher density than the lowest ranked study stream, found on opposite ends of the state but all in second order streams. Nash Stream and Mill Brook are located in the northern part of the state and Witches Spring Brook is located just north of the MA border.

II. Age structure

Age-1 fish were the most abundant age class among ages captured during every sampling event throughout our study, including 91% of 2016 recaptures and 44% of 2017 recaptures. YOY were the most abundant age class captured overall, even with the age class not recorded during May 2017 sampling. YOY represented 51% of 2016 captures and 41% of 2017 captures. Age-1 fish contributed 91% to the total October recaptures, and the same significant trend of recaptures was observed the following fall (F= 3.74, df= 50, p= 0.031). Significantly more YOY were captured in ECR1 than the other tributaries in 2016 (F= 43.33, df= 166, p<0.001). The largest shift in abundance throughout the study occurred within the dominant YOY age class in ECR1, significantly decreasing from 74% to 44% from July of 2016 to 2017 (t= 12.58, df= 153, p<0.001) (Fig. 2.5). With the massive decline in YOY, only one YOY tagged in 2016 was recaptured in 2017 which was tagged/recaptured in ECR1. After an almost four-fold interannual decline of YOY within ECR1, there was no significant difference in abundance across tributaries during the following July (F= 1.77, df= 79, p= 0.177). YOY were the most abundant age class in 10 of 12 events across all tributaries during July 2016 and June, July and September 2017. The dominant age class per tributary was most consistent in GR4, led by YOY and then Age-1 fish during all events. YOY were the most dominant age class in GR3 during all events, except July 2016. There were almost three times more Age-1 fish present than all other age classes combined, followed by Age-2 fish. This pattern was followed into 2017 with Age-2 fish observed as the second most abundant age through all events. After the interannual decline in YOY in ECR1, older adults were the most abundant during June followed by YOY then Age-1 fish, respectively.

III. Condition

Size at age Overlapping lengths among age classes were observed across all tributaries between Age-1 and 2 fish and in tributaries GR3 and ECR1 between age-2 and

3 fish (Fig. 2.7, Fig. A2.1). The largest TL variation within a single age class occurred among fish in GR3 during July across both seasons: Age-1 fish at 50mm and Age-2 fish 44mm, respectively (Table 2.2).

Age-1 fish were the most consistently represented age class among tributaries (Table 2.2), therefore analysis of Age-1 body mass was completed across the duration of the study. Furthermore, we utilized Age-1+ TL results to compare variation interannually. A significant difference in body mass was detected among tributaries during four of the six sampling events, including October 2016 (F=3.85, df=65, p=0.02), June 2017 (F=3.56, df=25, p=0.04) and September 2017 (F=3.62, df=45, p=0.03). Brook Trout body mass was significantly less within GR3 during May 2017 (F=6.11, df=35, p<0.01) (Fig. 2.8). No significant difference in Age-1+ TL was detected among tributaries during July 2016 (F=2.07, df=139, p=0.19) and July 2017 (F=1.07, df=73, p=0.35). Fish in GR3 had the shortest TL in 4 of 5 events among Age-1, 2 and 3 fish during October 2016 and all of 2017. The smallest mass per age class was commonly tied among tributaries, but Age-1 fish in GR3 weighed the least during July 2016 and all of 2017. During the same time, Age-2 and Age-3 fish in GR3 weighed the least during 3 of 5 events.

Contrasting condition metrics were observed when mass and TL were analyzed during the same sampling events. Age-1 fish in GR4 had the least median TL in July 2016 followed by fish in GR3 during July 2017. In contrast, Age-1 fish in GR4 had the least median mass and were longest during October 2016 plus all four 2017 events. Fish in ECR1 had the greatest mass and length during 2016 while fish in GR3 had the least through both metrics during 2017.

Although 94% of all fish captured were within tributaries, fish captured within the mainstem Beebe River frequently exhibited larger body sizes when compared to captures in tributaries. YOY and Age-1 mainstem captures had a greater median TL and median mass than tributary fish in every sampling event. Mainstem Age-2 fish were larger than tributary fish in 10 of 14 independent date/location events, three occurring in July. Mainstem fish only contributed to <10% of total fish captured in age classes YOY-Age-2, but 24% of Age-3 fish were captured in the mainstem. Additionally, six of nine Age-4 fish were captured in the mainstem and what we suspect to be an Age-5 fish (female) was captured below the GR3 confluence.

Population condition The condition of Brook Trout among GR4 (0.01 lbs/fish) and ECR1(0.008 lbs/fish) rank in the lowest 90% compared to similar statewide populations (Table A2.4). Fish in GR3 rank in the 65% of statewide populations at 0.023 lbs/fish. Similar to condition, average lbs./fish was highest in GR3 (0.023 lbs./fish) followed by GR4 (0.01 lbs./fish) and ECR1 (0.008 lbs./fish), analyzed during July of 2016. Fish in GR3 ranked above 36% of all statewide populations, followed by 5% and 3% in GR4 and ECR1 (Fig. A2.2). None of our study populations surpassed the statewide average of 0.034 lbs./fish, but populations within and surrounding the Beebe River watershed either meet or surpassed two-thirds of study populations.

Body condition The condition of study populations primarily met the AFS national standard during July of both 2016 and 2017 (Fig. A2.3). Condition improved across years among fish in GR3 and GR4 and declined in fish within ECR1. Across all tributaries, there was a slight trend of younger fish below the AFS condition standard while older fish surpassed it, more pronounced during 2016.

IV. Biomass

Beebe Interannual Brook Trout biomass differed dramatically across tributaries, including among YOY and adult fish biomass (Fig. 2.9). GR3 supported 0.072kg of Brook Trout biomass during July 2016 and 0.008kg during July 2017. Comparing the same sampling events, GR4 decreased from 0.01kg to 0.003kg and ECR1 from 0.056kg to 0.046kg. When biomass was combined spatially, it did not significantly differ temporally from 2016-2017 ($t= 1.0$, $df= 3$, $p= 0.195$) or spatially across tributaries in 2017 ($F= 4.04$, $df= 6$, $p= 0.08$). Biomass from June to September decreased in GR3 by 21% and increased in GR4 and ECR1 by 78% and 118%, respectively.

Regional We compared out study populations to enhanced protection standards in other New England states. This required the biomass of our study populations to be transformed to meet a range of management criteria. Across NH, Brook Trout biomass is variable among southwestern populations while many northern populations remain below the biomass documented in our study streams (Fig. A2.4). Additionally, 34-36% of statewide populations exhibit a greater biomass than GR3 and ECR1 and over 90% of populations surpass the biomass found in GR4 (Table A2.4). The mean statewide biomass is above the standard for NH's 'Wild Trout' designation (16.779 lbs./acre), including 88 of 225 populations surpassing the threshold (Table A2.5). Population biomass in GR3 and ECR1 met NH and/or VT 'Wild Trout' protection standards during at least one sampling event, but no study populations met ME's fish/mile standards. Regional populations are relative to biomass observed in GR4 and few surpass all focal populations (Fig. A2.5a). Similar to biomass, most populations within and surrounding the Beebe River watershed fall below the mean lb./fish values documented in our study populations (Fig. A2.5b).

IV. Growth

Fish in the most impacted study reach (GR3) gained significantly less median body mass than fish in GR4 and ECR1 during 2016 ($F= 5.12$, $df= 55$, $p=0.009$). During 2017, no significant differences in body mass change were detected across tributaries. The greatest mass increase during spring occurred in ECR1 at 25% ±3.76, during summer in GR3 at 8.82% ±1.77, and growth was similar during fall among fish in GR3 and ECR1 at 4.0% ±1.12 and 4.0% ±0.88, respectively. No significant differences in body mass change were detected within age classes 1, 2 and 3+ fish across tributaries. The most growth per tributary within the same age classes was detected among fish in GR3; YOY shared between GR3 and ECR1 at 4% ±0.0 and 4% ±2.29, respectively. The most growth among the remaining age classes all occurred in GR3: Age-1 at 8% ±1.16, Age-2 at 8.82% ±15.98 and Age-3+ at 14.82% ±4.27. The least growth was observed in Age-1 fish at 3.64% ±0.94 captured in GR4 while Age-2 and Age-3+ fish had the least growth in

ECR1, ranging from 6% ±1.71 to 0% ±4.0, respectively.

Total length growth of Age-1+ Brook Trout among tributaries significantly varied during fall 2016, with the most growth among fish in ECR1 (F=4.59, df=55, p=0.01) (Fig. 2.10). Additionally, median growth rates were more similar during spring and fall of 2017 than summer, when growth decreased. When comparing TL growth among tributaries per age class, no significant differences were detected in age classes 1, 2 and 3+. The most growth per tributary within the same age classes was detected among Age-1 and Age-2 fish in ECR1 at 1.56% and 3.84% respectively, and Age-3+ fish in GR3 at 1.21%.

The greatest median TL and body mass growth among all age classes occurred in fish residing in GR3; Age-3+ fish (n=4) with the greatest mass increase and Age-1 fish (n=9) with the greatest TL increase. Seasonally, the greatest median mass and TL growth occurred in ECR1: spring 2017 at 39.29% mass increase and fall 2017 at 3.84% TL increase. Overall, little variation was observed within age-classes across tributaries but the degree of variation increased as fish matured.

Discussion

Density and Age Structure Variability

We failed to support our hypothesis that Brook Trout density would be lowest in the most impacted tributary (GR3)h. Density during July of both years was lowest in GR4, but interannual density significantly decreased among fish in GR3 and ECR1. Trends in density also shifted throughout 2017. Brook Trout density was significantly higher in GR3 compared to other tributaries during June, followed an increase across all tributaries through July. By September, density declined in GR3 and ECR1 while in contrary, fish density in GR4 remained predominantly stable before increasing during fall. Although we saw changes within and among tributary-level densities, populations remain very productive compared to others in NH. Led by Brook Trout in ECR1, July 2017 density within our study streams rank in the highest 2% of statewide populations (N=275). Second, we failed to support our hypothesis that the most impacted tributary (GR3) would have less age classes. Population age structures were similar across all tributaries, consistently dominated by younger Brook Trout. Sixty-eight percent of all captures in GR3 were comprised of YOY and Age-1 fish, while populations in GR4 and ECR1 were comprised of 13% and 14% more of the same age classes. The youngest population was consistently observed in GR4, dominated by YOY and then Age-1 fish during all events. Populations in the most impacted tributary (GR3) and the unimpacted tributary (ECR1) were comprised of more Age-3+ fish than GR4, but interannual density among populations with older fish were less stable. Overall, Brook Trout density and dominant age classes were most variable in the unimpacted tributary and tributary with an impassable crossing and no-low a crossing barrier, while the density and age structure of fish with unrestricted access to the no-low canopy reach remained stable.

Following tagging methods described by Gries and Letcher (2002), we can exclude tagging-induced mortality as a measurable variable in population reductions. It must be recognized that recapture probability increases with fish length as described by McFadden (1961), but the decrease in YOY abundance between 2016 and 2017 far surpasses tagging-induced mortality and percent expected recapture based on fish sizes. Significantly less spring/summer precipitation during 2016 compared to the previous year caused environmental stress, influencing the variability in Brook Trout abundance between seasons. Induced by drought, these include microhabitat isolation by reduced access to reaches providing increased, seasonally critical, terrestrial food, and thus increased intraspecific competition. System-wide warmer water temperatures during 2016 likely increased mortality, resulting in population reductions the following season (Amundsen et al. 2007; Hutchings, 1994, 2006).

Size at Age, Population Condition and Growth Variability

Brook Trout in the most impacted tributary (GR3) had the smallest body size, but had an increased population-level condition. During all tag/recapture events, fish in GR3 had the shortest TL in 4 of 5 events among Age-1, 2 and 3 fish. Although the smallest mass per age class was commonly tied among tributaries, Age-1 fish in GR3 weighed the least during all tag/recapture events. Furthermore, both Age-2 and Age-3 fish in GR3 weighed the least during 3 of 5 events. Brook Trout in GR4 were consistently the longest per age class, and YOY exhibited the most overall growth. During 2016, fish in GR4 gained significantly more body mass but fish in ECR1 gained significantly more length, while no significant size differences were observed during 2017. Fish body condition was below the AFS standard in younger fish during 2016, but showed interannual improvement within each population that had access to stream reaches subject to no-little canopy. Adults had the least TL and mass growth for the greatest number of sampling events among tributaries GR3 and GR4. Sotiropoulos et al. (2006) concluded the most informative growth analysis occurred within Age-1 fish due to the highest proportional abundance. This was also evident throughout our study; Age-1 were captured during every sampling event, across all tributaries. We hypothesized that fish in the most impacted stream (GR3) would grow less, which was supported during 2016, but we failed to support our hypothesis during 2017. Age-1 fish grew the least (TL) across 2016, but grew the most (TL) the following year, also observed for growth in mass. Age-1 fish in GR4 grew the most (mass) during the fall of 2016 and across all three 2017 seasons. In contrast, Age-1 fish in the same stream exhibited the least TL growth among three of four recapture events. Although GR3 and GR4 are both impacted systems, there were large differences in population attributes between the two. We attribute contrasting body size, growth and population-level condition to a combination of factors created by heterogenous stand composition (no-low canopy): increased primary productivity influencing invertebrate food webs and optimal Brook Trout thermal regimes. The extent to which these factors become influential is further determined by access to the no-low canopy, restricted in GR3 and unimpacted in GR4.

The most diverse macroinvertebrate community assemblages are found in streams possessing reaches of younger forest (Sweeny 1993, Wilson et al. 2014) because light is likely to be the primary abiotic constraint on photosynthesis (Hill et al. 1995). We suspect lower macroinvertebrate abundance and growth within the forested reaches further limited the growth and abundance of observed headwater trout populations. After spring benthic invertebrate hatches end, Brook Trout diet is restricted to primarily terrestrial invertebrates (Hubert and Rhodes 1989, Sotiropoulos et al. 2006). Summer terrestrial invertebrate biomass is greater in early successional habitat than late successional habitat (Wilson et al. 2014), relative to riparian forest structure throughout the no-low canopy cover reaches in our study streams. We conducted preliminary macroinvertebrate sampling to further document baseline conditions to be analyzed by post-restoration research. Qualitative observations showed smaller size macroinvertebrates and decreased species richness within intact, forested stream reaches while greater sized individuals among more diverse assemblages (e.g. Corydalidae and Tipulidae) were captured within the no-low canopy reach. We observed more abundant and more diverse macroinvertebrate communities in no-low canopy areas, consistent with results observed by Sweeny (1993) and Wilson et al. (2014).

The no-low canopy reaches may create seasonally beneficial habitat by improving primary productivity benefitting lower trophic level food webs, however, these same areas may become less preferred or inhabitable under high temperature and/or low flow conditions. Bowlby and Roff (1986) acknowledge maximum summer water temperatures as the variable most important to trout due to direct physiological impacts, food availability, stream morphology and velocity. When temperatures become consistently warmer in the no-low canopy cover reaches, metabolic costs can increase, decreasing fish growth cumulatively as temperatures increase (Baldwin 1953, Chadwick and McCormick 2017, Xu et al. 2010). However, if temperatures are below the critical threshold, limited riparian canopy cover can promote increased productivity via algal growth (Hill et al. 1995), macroinvertebrates (Sweeny 1993) and terrestrial invertebrates (Wilson et al. 2014), benefitting Brook Trout.

Although our study focused on tributary reaches, analysis of both main findings indicate a greater need for research regarding mainstem and tributary subpopulations frequently documented across the region. Only 6% of the total captures occurred in the mainstem, but captures exhibited a greater median size and complex age structure than fish captured in tributaries, similarly documented by Huntsman and Petty (2014) and Petty et al. (2014) in West Virginia. Very few YOY were captured in the mainstem, augmented by older, larger adult fish which may reflect optimal mainstem habitat utilized by older fish while YOY are excluded to less-optimal habitat (Huntsman and Petty 2014, Petty et al. 2014). YOY and Age-1 fish in the mainstem exhibited a greater TL and body mass than tributary fish during every sampling event, while Age-2 fish were larger in 10 of 14 independent date/location events. We completed preliminary mainstem sampling every 100m along cross-sectional transects, including additional seeps and intermittent springs. Four supplemental inputs ranged from 13.9-18.3°C during peak summer temperatures, all cooler than study tributaries at the time of sampling. Overall, we suspect larger, more dominant fish may colonize mainstem seeps and upwelling sites during peak summer temperatures while utilizing reaches with increased productivity (Baird and Krueger 2003, Huntsman and Petty 2014, Petty et al. 2014).

Our first two major findings lead to further questions regarding young populations that fluctuate across time and space, explained by system-wide resource limitation. Brook Trout productivity can be limited by lower pH, habitat availability or other factors, all variable at the regional and local scale (Warren et al. 2010). Hoover (1939) first documented resource limitation and deprived habitat condition in NH across headwater streams, observing reduced Brook Trout growth and size potential. More recently in Massachusetts, Letcher et al. (2007) found preliminary evidence of long-term resource limitation within southern New England headwater streams, resulting in individuals maturing earlier in life. Additionally, organisms evolve to achieve the optimal life history, like sacrificing survival and fertility in later life stages for the benefit of early reproduction and survival (Partridge and Bartron 1993).

Our exploratory analysis may document early maturation in response to resource limitation, infrequently quantified in headwater Brook Trout populations. Mature gonad development was observed during spawning in 88% of Age-1 fish (further described in Appendix, Brook Trout maturity), contrasting literature recognizing Brook Trout sexual maturity beginning at Age-2 or 3 (Letcher et al. 2007, Öhlund et al. 2008, Werner 2004). Few studies have documented this trend of early maturation in Brook Trout. In Pennsylvania, both Wydoski and Cooper (1966) and Detar (2007) observed maturity at Age-1 and 1-2 years old, respectively. In Wisconsin, Brasch et al. (1966) observed maturity in 95% of YOY males and 83% of Age-1 females. Hutchins (1994) found small body size can increase physiological costs in females but increase physical costs in males, and the few reported studies primarily observe early maturation in males. Although we did not find a significant difference in the abundance among sexes, Age-1 females accounted for 13% more than males while analyzing Age-1 and Age-2 classes. Hutchings (2006) conducted a five year mark-recapture in Newfoundland, Canada to further understand survival consequences of sex-biased growth. He discovered that growth rate is independent of body size, and the rate of growth can have a positive influence on survival. We observed females consistently weighing more at Age-1 and males at Age-2. Overall, we cannot make assumptions of survival in an open system, but we can observe growth and abundance among populations per tributary across two seasons. Though our methods did not measure fecundity, our preliminary results suggest the possibility of long-term resource limitation within headwater systems that may apply selection pressure for individuals who mature earlier in life.

While we document the important benefits of habitat features commonly recognized as critical to species viability, we suspect plentiful instream wood and a high pool ratio have become less important among our study streams than previously regarded. The importance of instream wood has been documented along the Brook Trout’s southern range (Flebbe 1991, Flebbe and Dolloff 1995), western range (Morris et al. 2012) and northeastern range (Kratzer and Warren 2013, Neumann and Wildman 2002). The importance of pool habitat has been documented by Rosenfeld (2014) among a modeled

Cutthroat Trout population and Johnson et al. (2016) among Brook Trout in New York. Habitat preference across our study populations modeled range-wide findings, with the most Brook Trout captured in pool habitat, regardless of the availability. Hutchings (2006) reported that growth rate can positively influence survival, which we observed among fish with access to the no-low canopy. The highest growth rate across most sampling events and the most stable interannual density occurred in the tributary with the least instream wood and the least quantity of pools (GR4). Unrestricted access to the no-low canopy allows fish to navigate freely into and out of heterogenous habitat infrequent among resource-limited headwater systems common across New England (Nislow and Lowe 2006). Overall, our focal Brook Trout populations and their subsequent habitat preferences do not conform to the range-wide understanding of habitat features critical to population viability.

Wild Brook Trout populations in Beebe River tributaries remain young with widely fluctuating seasonal and interannual density, population-level condition and growth. We observed similar trends among populations in GR3 and ECR1, which range from the most impacted reach to an unimpacted reach, respectively. GR3 had an impassable barrier, but we suspect downstream access to heterogenous habitat (no-low canopy) provided beneficial habitat in resource-limited headwater streams, yet populations experienced the lowest growth and exhibited a significant interannual decline in density. ECR1 had no human impacts, plentiful instream wood and the most pool habitat, but population density still significantly declined interannually. Fish in GR4 remained the most stable. Brook Trout consistently grew the most, consisted of the fewest age classes and interannual density declined <10%. We attribute the most beneficial growth characteristics to unrestricted access to no-low canopy among headwater streams where water typically remains cool, longitudinal heterogeneity lacks and populations densities are greatly fluctuating.

FIGURES

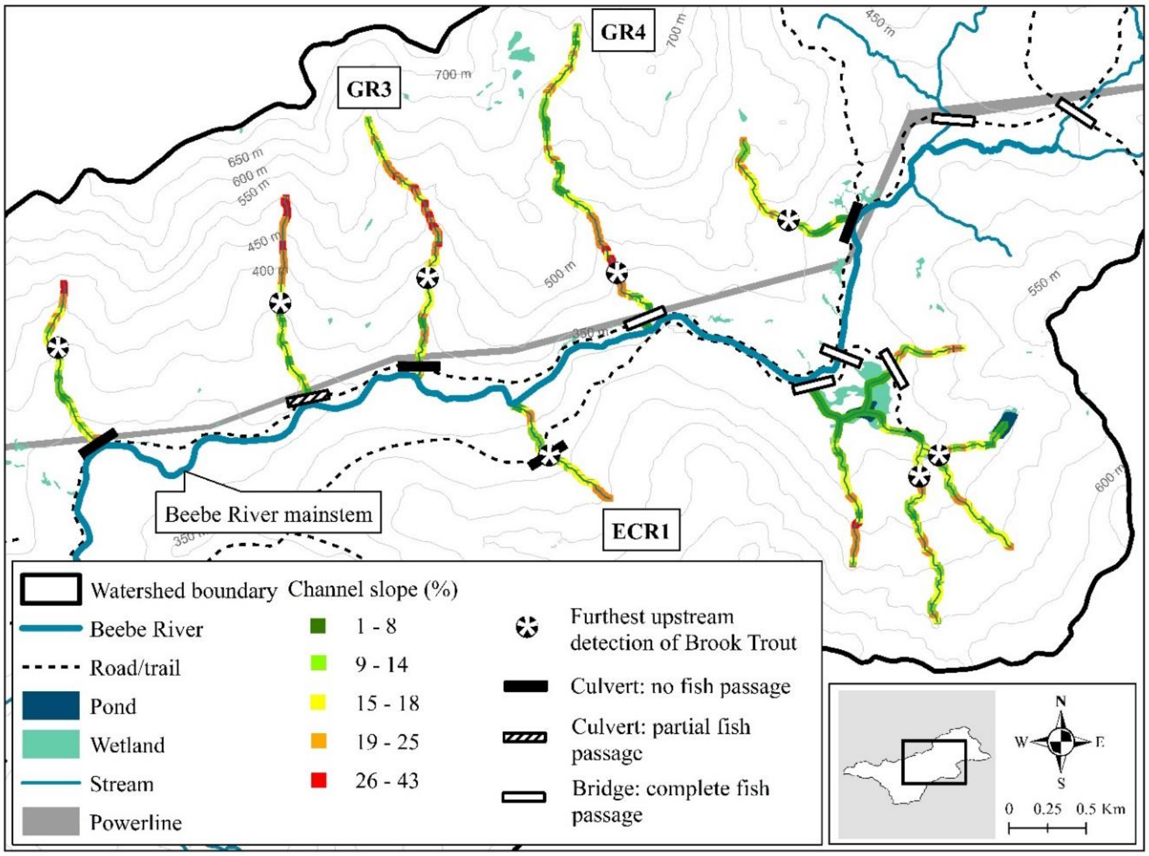

Fig. 2.1. Map of tributaries in the Beebe River watershed including furthest upstream detection of Brook Trout determined by NH Fish and Game electrofishing surveys (2015), hydrological and landscape features. Study tributaries are represented by bold labels noted in the headwaters.

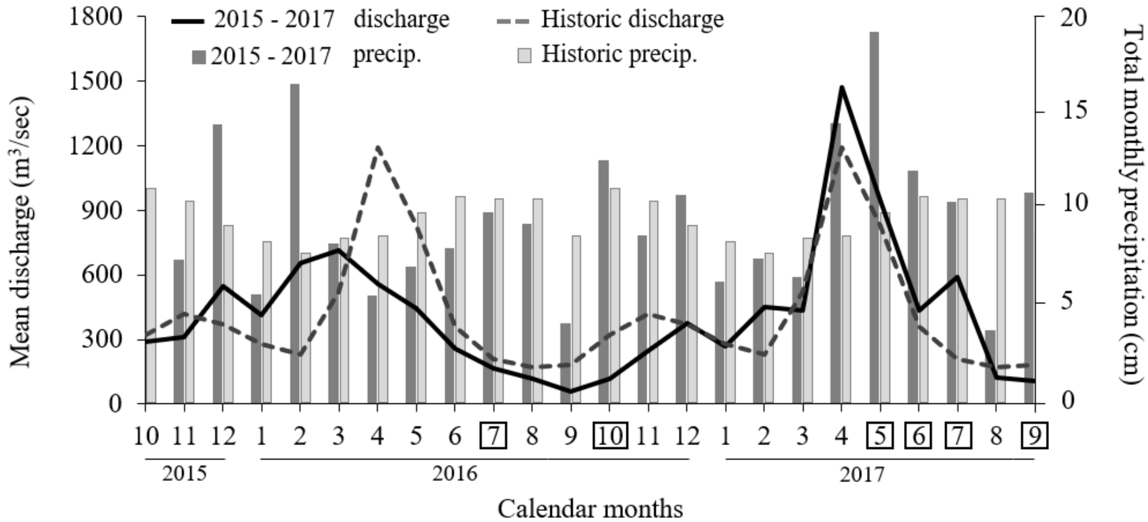

Fig. 2.2. Discharge and precipitation rates displaying 2015-2017 rates and historic trends, including discharge from 1903-2017 and precipitation from 1981-2010 recorded 6.53km-6.98km SSW of watershed outlet. Lines indicate mean monthly discharge with black solid representing current rates and gray dotted representing historic trends. Bars indicate total monthly precipitation with dark gray representing current rates and light gray representing historic trends. Boxed calendar month indicates a fish sampling event.

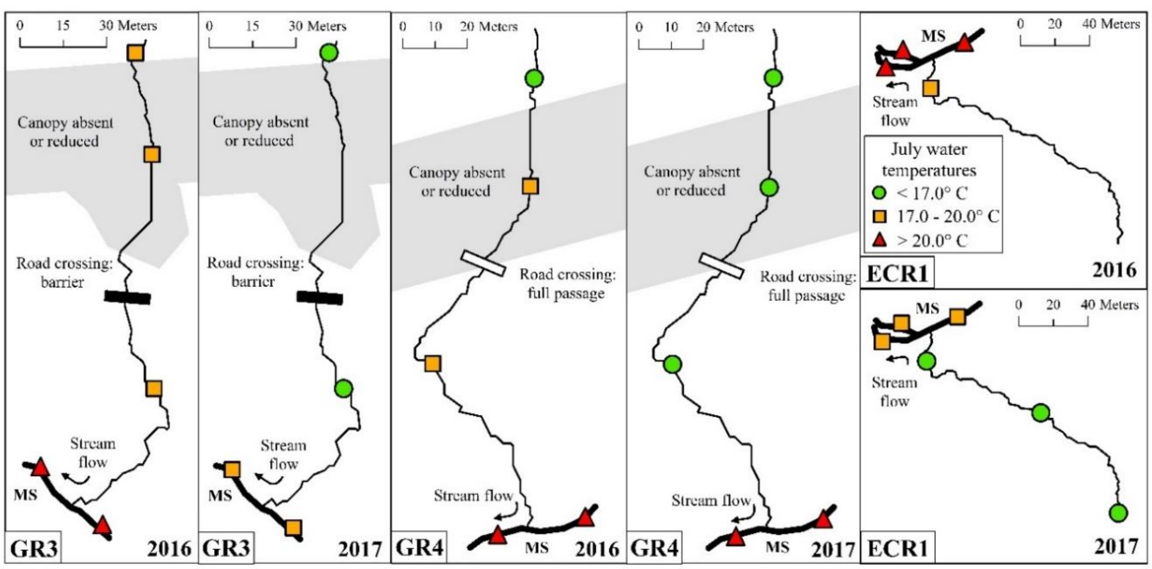

Fig. 2.3. Monthly mean of daily maximum stream temperatures (°C) in July 2016 and 2017 within study reaches of focal tributaries in the Beebe River watershed. Mainstem reaches are indicated by bold lines and bold "MS" labels.

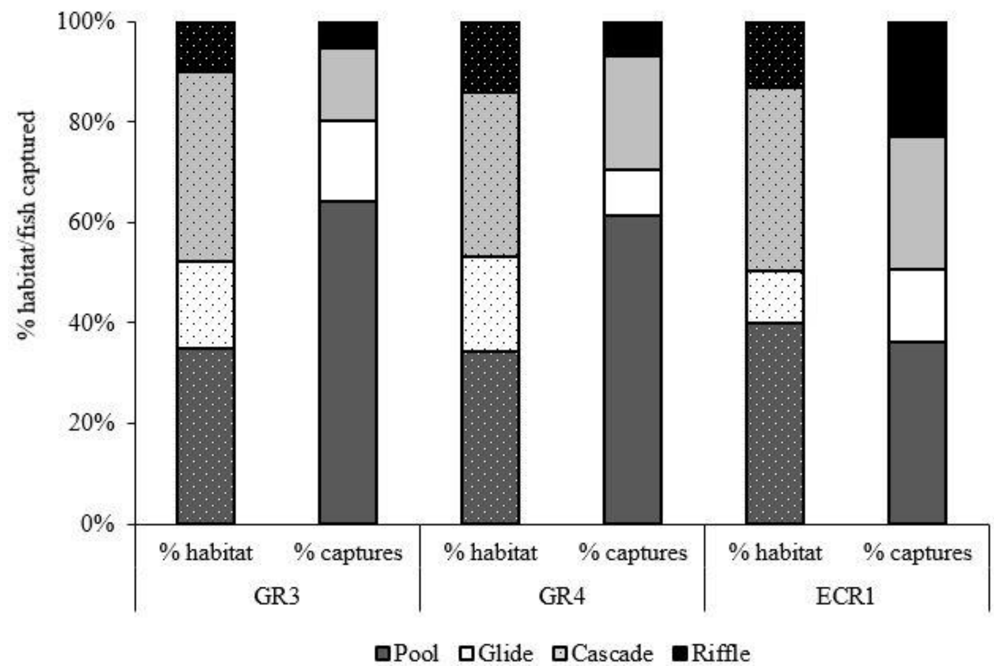

Fig. 2.4. Habitat types and associated Brook Trout presence within three tributaries of the Beebe River watershed. Fish and habitat sampling occurred during July 2017.

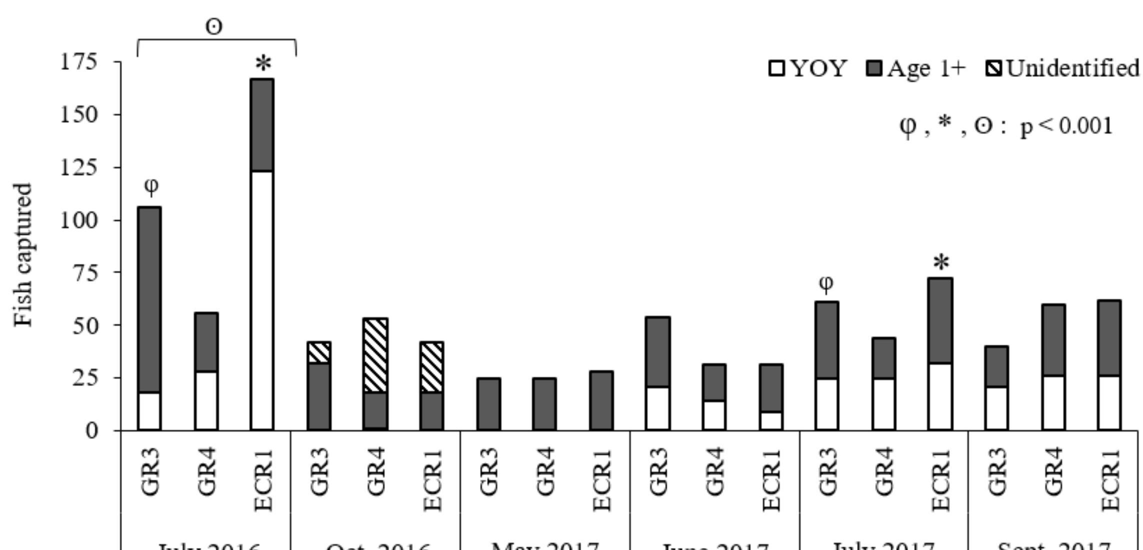

Fig 2.5. Brook Trout captured by electrofishing from focal Beebe River tributaries during 2016 and 2017. October 2016 represents only recaptured PIT tagged individuals (gray bars) including count of untagged fish (dashed bars) due to limited sampling activities. May 2017 represents only ≥Age-1 fish due to low detectability of YOY. Corresponding symbols above data indicate statistical significance.

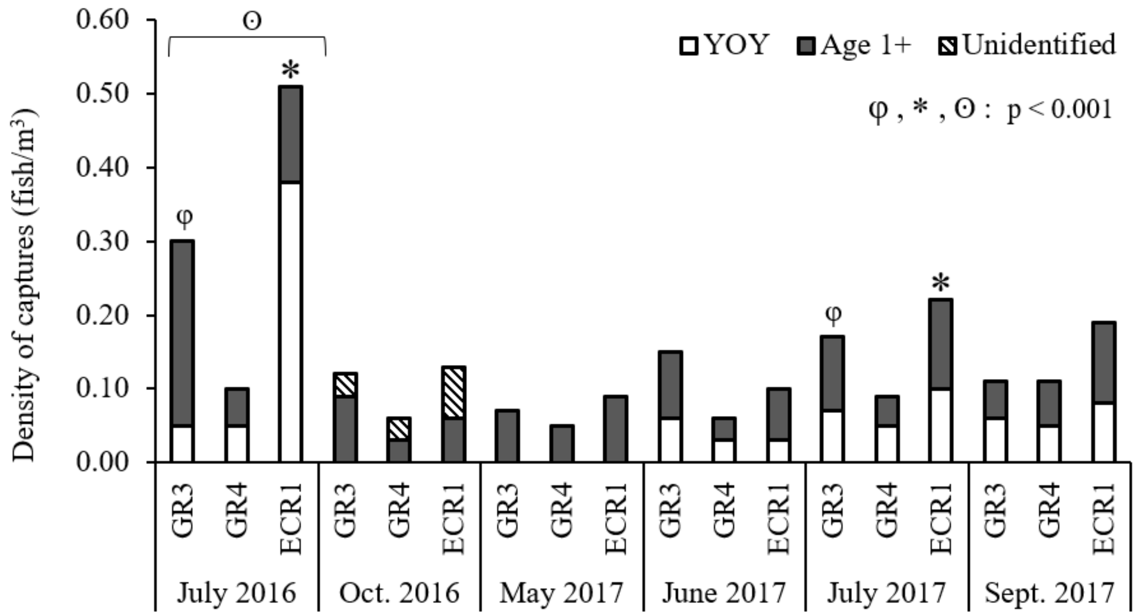

Fig. 2.6. Density of Brook Trout captured (fish/m^3) by electrofishing from focal Beebe River tributaries during 2016 and 2017. October 2016 represents only recaptured PIT tagged individuals (gray bars) including a count of untagged fish (dashed bars) due to limited sampling activities. May 2017 represents only ≥Age-1 fish due to low detectability of YOY. Corresponding letters above data indicate statistical significance.

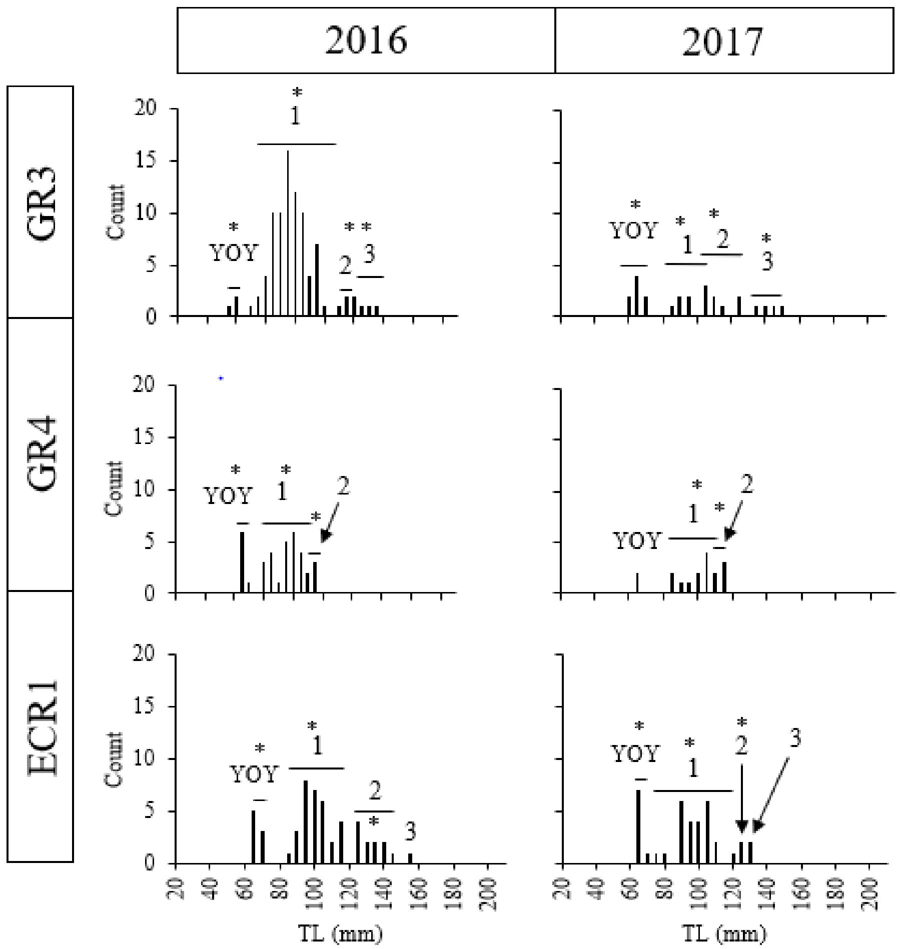

Fig. 2.7. Length frequency and age classes of wild Brook Trout captured in focal tributaries within the Beebe River watershed. Sampling occurred during July and asterisks above each age signify median total length (TL).

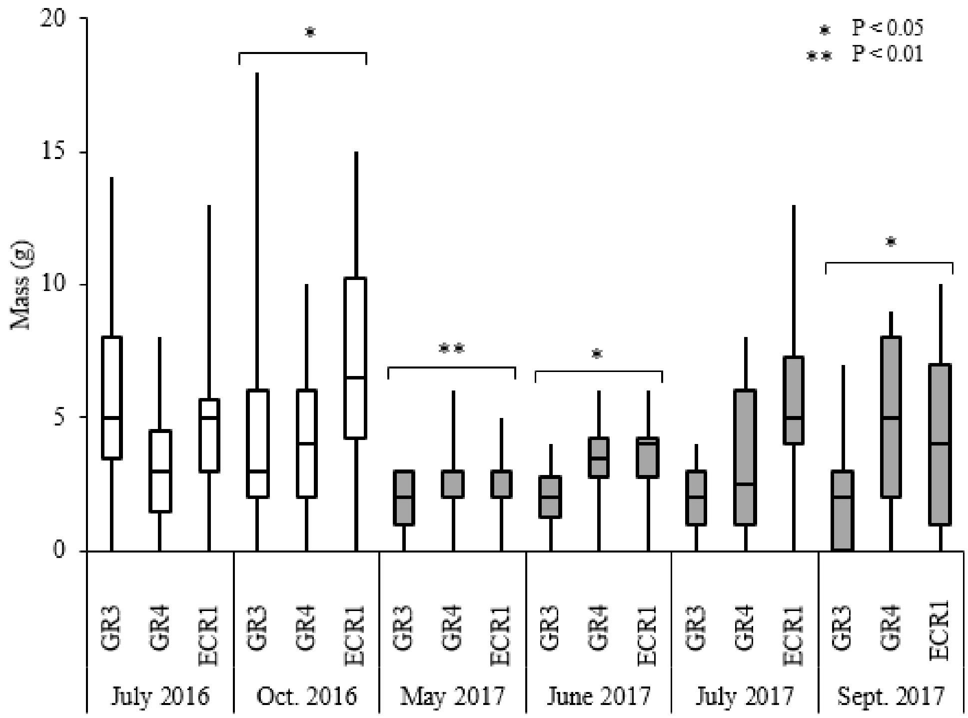

Fig. 2.8. Box-whisker plot representing monthly body mass of wild Age-1 Brook Trout across focal tributaries within the Beebe River watershed. Corresponding symbols above data signify statistical significance.

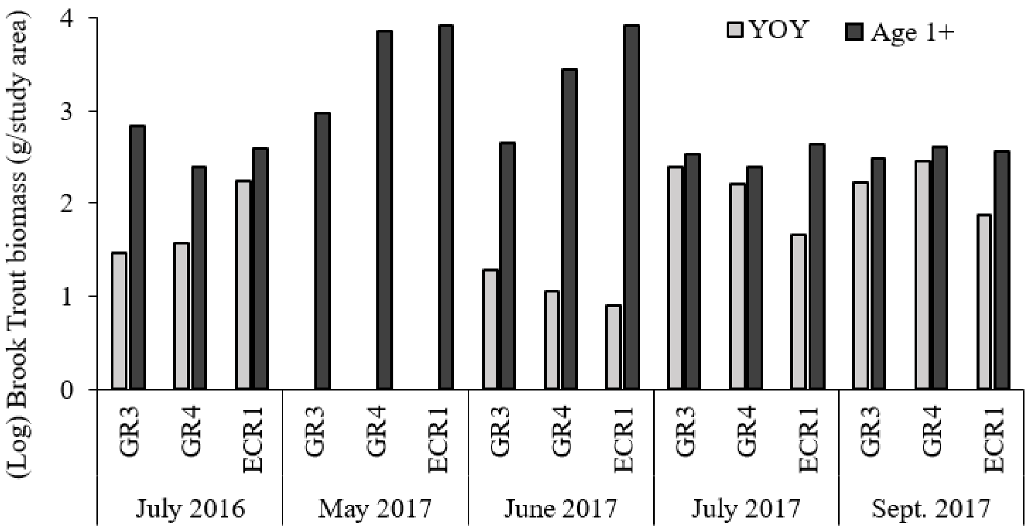

Fig. 2.9. Log-transformed YOY and Adult (Age-1+) Brook Trout biomass per tributary area (m^2) within each sampling period in focal tributaries of the Beebe River watershed. During May, sampling techniques limited the capture success of YOY resulting in removal from analysis.

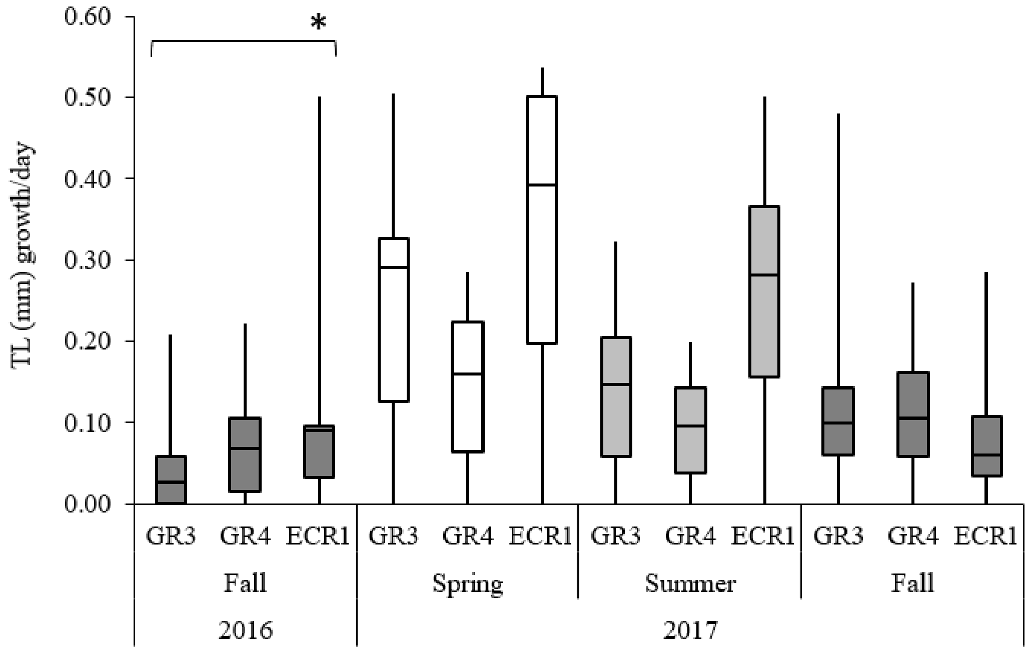

Fig. 2.10. Box-whisker plot representing Adult Brook Trout TL (mm) growth within three focal tributaries of the Beebe River watershed. Spring growth occurred from May-June, summer growth occurred from June-July and fall growth occurred from July-September. Asterisk indicates significantly more growth by fish in ECR1 during the fall of 2016.

TABLES

Table 2.1. Habitat attributes collected during seasonal low flow in July and August 2017 within focal study tributaries of the Beebe River watershed.

Habitat attribute		GR3 mean / range	GR4 mean / range	ECR1 mean / range
Bankfull width (m)		4.58 / ±5.59	4.48 / ±4.42	3.85 / ±7.86
Slope (%)		9 / ±9	7 / ±16	10 / ±21
Canopy cover (%)		69 / ±100	71 / ±100	89 / ±27
Organic material (m^3)	Within bankfull	3,767.36	2,248.10	11,749.63
	Dry	3,043.34	2,274.18	15,144.20
	Wet	830.09	185.09	600.54
Pool-forming organic material (%)		8	7.14	13.79
Substrate type (dom / subdom)		boulder / cobble	boulder / cobble	bounder / s. gravel
Riparian vegetation type (dom / subdom)		E. hemlock / N. hardwood	N. hardwood / E. hemlock	E. hemlock / N. hardwood
Habitat type (dom / subdom)		pool (48%) / riffle (18%)	riffle (41%) / cascade (29%)	cascade (60%) / pool (16%)

Table 2.2. Descriptive statistics of wild Brook Trout age classes captured during July 2016, 2017 within the Beebe River and tributaries, including count (n), percent (%), density (D), mean total length in mm ±SE, TL range, mean mass in grams ±SE and mass range. Mainstem density could not be calculated because braided wetted area measurements were not surveyed.

					GR3					
	n / % / D		mean TL ±SE, median		TL range		mean mass ±SE, median		mass range	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
YOY	18 / 16.98 / 0.05	34 / 60.71 / 0.1	52.78 ±1.21, 54	57 ±1.01, 56	43 - 60	44 - 69	1.33 ±0.15, 1	2 ±0.1, 2	0.75 - 2	0.75 - 3
Age-1	79 / 74.53 / 0.23	5 / 8.93 / 0.01	94.72 ±1.2, 95	91.17 ±2.89, 90.5	68 - 118	81 - 102	8.68 ±0.33, 8	8.17 ±0.6, 8.5	3 - 17	6 - 10
Age-2	4 / 3.77 / 0.01	14 / 25 / 0.04	132.25 ±1.44, 133.5	114.14 ±3.04, 110	128 - 134	98 - 142	22.25 ±0.85, 22.5	16.71 ±1.51, 15.5	20 - 24	11 - 31
Age-3	5 / 4.72 / 0.01	3 / 5.36 / 0.01	144 ±3.11, 144	140.25 ±3.64, 141.5	137 - 154	131 - 147	29.2 ±2.22, 28	29.5 ±2.02, 30.5	27 - 36	24 - 33
Total	106 / 100 / 0.3	56 / 100 / 0.16	91.34 ±2.28, 93.5	79.77 ±3.93, 63	43 - 154	44 - 131	8.92 ±0.64, 8	7.91 ±1.17, 2	0.75 - 36	0.75 - 33
					GR4					
	n / % / D		mean TL ±SE, median		TL range		mean mass ±SE, median		mass range	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
YOY	23 / 45.1 / 0.04	25 / 56.82 / 0.05	53.86 ±1.34, 52	53.12 ±1.03, 54	43 - 69	42 - 62	1.31 ±0.11, 1	1.66 ±0.1, 2	0.75 - 3	0.75 - 2
Age-1	24 / 47.06 / 0.04	10 / 22.73 / 0.02	92.61 ±1.75, 93	96.9 ±2.61, 99	79 - 106	84 - 107	8.22 ±0.51, 8	9.5 ±1.01, 8.5	5 - 13	6 - 14
Age-2	5 / 9.8 / 0.01	9 / 20.45 / 0.02	113.5 ±1.29, 114	118 ±3.83, 115	109 - 115	105 - 139	14.6 ±0.68, 15	17.11 ±1.61, 14	13 - 16	12 - 25
Total	51 / 100 / 0.09	44 / 100 / 0.08	75.02 ±3.1, 74	76.34 ±4.34, 59.5	43 - 115	42 - 139	14.6 ±0.68, 15	6.6 ±1.02, 2	0.75 - 16	0.75 - 25
					ECR1					
	n / % / D		mean TL ±SE, median		TL range		mean mass ±SE, median		mass range	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
YOY	86 / 66.15 / 0.26	31 / 48 / 0.1	50.53 ±0.71, 52	58.06 ±0.89, 59	34 - 70	50 - 69	1.37 ±0.06, 1	1.48 ±0.1, 1	0.75 - 4	1 - 3
Age-1	31 / 23.85 / 0.1	24 / 37.5 / 0.07	98.74 ±1.41, 98	93.45 ±1.82, 94	81 - 115	77 - 109	9.9 ±0.51, 10	8.23 ±0.5, 8	5 - 10	3 - 12
Age-2	12 / 9.23 / 0.04	10 / 15.63 / 0.03	133.75 ±3.45, 130.5	121.5 ±3.47, 121	122 - 166	105 - 143	23.36 ±2.34, 21.5	18.3 ±1.57, 18	16.3 - 46.6	12 - 22
Age-3	1 / 0.77 / <0.01	2 / 3.13 / 0.01	152.00	129.5 ±0.5, 129.5	152	129 - 130	36	22.5 ±0.5, 22.5	36	22 - 23
Total	130 / 100 / 0.4	64 / 100 / 0.2	66.07 ±2.22, 54	81.39 ±3.25, 76.5	34 - 152	50 - 130	4.77 ±0.55, 2	6.83 ±0.86, 4	0.75 - 46.6	1 - 23
					Beebe River Mainstem					
	n / %		mean TL ±SE, median		TL range		mean mass ±SE, median		mass range	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
YOY	8 / 28.57	3 / 12	59 ±2.26, 59	70.67 ±5.9, 75	50 - 70	59 - 78	1.9 ±0.21, 1.9	5 ±0.58, 5	1.1 - 2.8	4 - 6
Age-1	10 / 35.71	4 / 16	106 ±1.84, 106	106.25 ±3.9, 105	94 - 113	99 - 116	11.6 ±0.68, 11.8	15.25 ±0.85, 15.5	7.9 - 15	13 - 17
Age-2	10 / 35.71	3 / 12	132.7 ±4.75, 131.5	115 ±8.02, 122	110 - 166	99 - 124	23.23 ±2.97, 21.65	17.33 ±4.98, 19	13 - 46.6	8 - 25
Age-3		8 / 32		135.75 ±3.41, 134		124 - 147		27.5 ±2.59, 28.5		16 - 38
Age-4		6 / 24		155.17 ±2.63, 155		148 - 166		38.17 ±2.56, 36.5		33 - 49
Age-5		1 / 4		196		196		69		69
Total	28 / 100	25 / 100	101.11 ±5.98, 107.5	127.8 ±6.25, 131	50 - 166	59 - 196	13 ±1.95, 12	25.84 ±3.03, 25.84	1.1 - 46.6	4 - 69

Chapter 3

Headwater Brook Trout Movement in the Presence of Undersized Road Crossings and Managed Riparian Areas in Central, NH

Introduction

Throughout the northeast US, fragmentation is the main impact on headwater Brook Trout populations. Gibson et al. (2005) surveyed recently constructed road crossings along the Trans Labrador Highway and found that poorly constructed stream crossings can destroy aquatic habitat, fragment and isolate fish populations and increase vulnerability to disturbance events, compounding the chances of extirpation. Within the same ecotone, Maitland et al. (2015) found that habitat quality in northern boreal forests was negatively affected by sediment mobilization and deposition related to road crossings. Degraded habitat can negatively impact fish health and behavior due to changes to habitat. In particular, Warren and Pardew (1998) found culverts to create more barriers to fish passage than any other stream crossing structures. Undersized culverts alter stream flow, which was found to directly relate with the level of fish passage. Park et al. (2008) documented the cumulative effects of culvert-induced fragmentation at the watershed scale, recognizing acute streambed scouring at the outlet that increased the potential to cause a hanging culvert outlet. The hanging outlet is the most important factor in trout passage success, because outlet drop can restrict of block upstream fish movement (Burford et al. 2009). Harvey and Railsback (2012) used inSTREAM to explore the effects of barriers on a virtual stream-dwelling trout population. They found different life history characteristics of tributary subpopulations isolated by barriers, including shorter lengths at given ages and a lower survival rate beyond Age-3 resulting in earlier spawning in younger fish. Stochastic events can reduce trout survival and growth, but barriers further eliminate the possibility of reoccupying tributaries after such events.

Life history, reproduction and habitat conditions have been shown to seasonally influence Brook Trout movement (Castonguay et al. 1982, Snook et al. 2015). Movement can be further restricted by natural landscape attributes often present in headwater stream systems (Castric et al. 2001; Kanno et al. 2011, 2015) and by anthropogenic barriers. Road crossings have been recognized to restrict or act as barriers to upstream fish movement, further impacting populations (Diebel et al. 2015, Perkin and Gido 2012, Warren and Pardew 1998). The probability of extirpation increases when inherently isolated headwater populations become further restricted (Letcher et al. 2007). Pepino et al. (2012) discusses two main impacts to fish created by road crossings: (i) restricted passage of individuals (fragmentation), and (ii) reduction in habitat quality downstream by increases in sediment load (habitat degradation).

Few studies have focused on movement in systems dominated by wild Brook Trout, commonly streams and headwater systems. Wild Brook Trout movement has been analyzed in streams co-occupied by introduced Brown Trout (Hoxmeier and Dieterman 2013) and Rainbow Trout (Baird et al. 2006), native salmonids like Atlantic Salmon (Johnson 2008) and in Brook Trout populations with anadromous life histories (Castonguay et al. 1982, Snook et al. 2015). Of the few studies, researchers have primarily utilized radio telemetry monitoring, which allows movement records over larger areas and infrequently subjects fish to direct human interactions through multiple electrofishing events. This method is frequently subject to a smaller sample size, short tag life and a larger minimum fish size required for tagging.

Gerking (1959) first introduced the ‘restricted movement paradigm’ of adult stream fishes, concluding that movement of resident (non-anadromous) fish is generally restricted within a meso-habitat type (e.g. a pool) or stream reach (<10 m). Gowan et al. (1994) challenged the restricted movement paradigm, arguing that long-range movement is more detectable when fish sampling covers longer stream reaches. Our goal was not to document long-range movement, but to document movement patterns in relation to human influenced landscapes. In doing so, we recognized subsets of fish that seasonally used habitat within the study reaches and others that remained primarily sedentary. The benefit of tributary systems for seasonal refuge does not exclusively lie within the tributary itself. Cool water input can provide supplemental heterogenous habitat in main rivers near confluences. Baird and Krueger (2003) found Brook Trout to congregate within the tributary-input cool water plume in the Moose River in… during high summer temperatures. Similar results by Petty et al. (2012) found that when mainstem temperatures increased, Brook Trout selected mainstem microhabitats that provided thermal refugia instead of moving into tributaries. They also documented mainstem fish moving considerably more (50 m/day) than tributary fish (2 m/day) within the same West Virginia system. When suitable habitat is present, fish may remain stationary. If access to seasonally vital habitat is restricted, further strain is placed upon resource limited populations.

Recognizing ongoing threats of resource limitation and fragmentation, NH has initiated assessments of existing road crossings with survey parameters now including aquatic organism passage (NH Department of Environmental Services et al. 2018). These assessments have sparked a trend in watershed-wide aquatic restoration by crossing replacement and instream wood additions, most notably at Nash Stream (Whitaker unpublished), within the Beebe River watershed (NH Fish and Game Department 2015a), and ongoing in the Androscoggin River watershed (Ben Nugent personal communication). Furthermore, fragmentation impacts are expected to persist and increase into the future, strengthening the importance of protecting primary, long-term habitat like headwater streams (Hunnington et al. 2009, Kanno et al. 2015, Merriam et al. 2017).

Understanding pre-restoration population trends is a critical step to measure the effects of restored connectivity across historically impacted headwater streams. Measuring fish movement is an important application to further understand how habitat availability, intra-species interactions and anthropogenic influences can impact populations (Davis et al. 2015, Ecret and Mihuc 2013, Snook et al. 2015). The objective of this study was to document wild Brook Trout movement in the presence of seasonal and permanent barriers and related habitat degradation, serving as a baseline assessment in the Beebe River watershed (Campton/Sandwich, NH, U.S.A.). We focused on three headwater tributaries with 1) impassable road crossing and reduced canopy cover, 2) passable road crossing and reduced canopy cover, and 3) no impediments to movement and unaltered canopy. To address these objectives, we assessed Brook Trout net movement, cumulative movement, home range, immigration and habitat. Potential abiotic influences are further characterized at the geomorphic threshold region-scale, which share similar habitat characteristics per region across tributaries (Church 2002). We hypothesized that Brook Trout subjected to a permanent barrier (road crossing) and reduced canopy cover (powerline easements) would exhibit greater seasonal and annual movement to find suitable habitat when further access is restricted, while fish in an unimpacted stream remain largely sedentary in better suited habitat conditions.

Methods

Additional descriptions of our study area, landscape features and fish assemblages are detailed in Ch. 2 Methods: *Study Area*.

Habitat

We analyzed habitat reaches to categorize geomorphic threshold regions (further denoted as regions) designed to understand how expected variability influenced Brook Trout movement across regions and time. Regions were determined post-habitat and fish sampling by recognizing regional changes in channel slope, riparian landscape topography and in-stream ecological features (Church 2002). Additionally, the same regions exhibited similar habitat across study streams. The three regions observed among study reaches are further denoted by their acronyms, floodplain valley: FV, upland valley: UV, and upland: U. Additional descriptions of habitat, landscape and temperature measurements completed are detailed in Ch. 2 Methods: *Habitat*. Techniques completed to sample Brook Trout are further described in Ch. 2 Methods: *Fish sampling* and study-wide analysis of Brook Trout are further described in Ch. 2: Methods: *Fish Population Metrics*.

Movement

I. Net movement

We measured net movement, defined as the distance between two (re)capture events, during either consecutive (re)captures or between two (re)capture events with one uncaptured sampling event in between, to understand how far fish seasonally moved across tributaries, explaining habitat preference among seasonally variable features. The net movement metrics were analyzed by the distance moved and the direction moved (upstream, sedentary or downstream). We standardized movement by time as meters per day (m/day). This metric was the only calculation completed from 2016 results while 2017 calculations included additional movement parameters. We examined if net movement among tributaries varied by age class and with combined ages using a one-way ANOVA. We tested the variation in net movement per age, across tributaries using a two-sample T-test. We used a one-way ANOVA to compare Adult median net movement across tributaries during all of 2017. Upstream, sedentary and downstream movement was quantified by percent of the population exhibiting each metric.

II. Cumulative movement

We analyzed cumulative movement as the directionless, absolute sum of all movement distances within a year (2017) to understand how much fish moved within a year (2017) seeking seasonally available food sources, suitable habitat for refuge or for spawning. We analyzed movement results from fish captured ≥3 times during 2017, standardized by time as meters per day (m/day). We used a one-way ANOVA to test if there were differences in cumulative movement across tributaries among YOY and Adult fish. A two-sample T-test was used to test differences among YOY and Adult fish movement with all tributaries combined.

III. Home range

We measured home range, the distance between the farthest upstream and downstream locations of a tag/recapture event during 2016 and 2017 (Young 1996). to understand the size of the area where fish spend their lives and where these areas are located within each study reach relative to road crossings and no-low canopy reaches. Calculations were limited to fish tagged and recaptured during 2017. We used a one-way ANOVA to test for differences in home range size among tributaries. Habitat conditions are consistently different for each region, so we further tested the quantities of home ranges per threshold region within and across tributaries using one-way ANOVAs. A post-hoc Bonferroni correction was used to determine the dominant region within GR3 and to determine the dominant region when testing across tributaries. Analysis of home range size per region is reported as median ±SE. Additional categories were created when home ranges overlapped defined regions. These data were only analyzed when quantifying the abundance and size of home ranges per tributary, and also tested using a one-way ANOVA.

IV. Immigration

We were interested in further analyzing untagged Adults recruited into study reaches, whether by seasonal habitat present or intra-species interactions. We analyzed abundance to quantify immigration of untagged Brook Trout captured during June, July and September 2017. We tested variation among untagged Age-1+ (Adult) captures spatially (among tributaries) and temporarily (among months) using a one-way ANOVA.

Temporal variability was further tested using a post-hoc Bonferroni correction. We also used a one-way ANOVA to test the differences in habitat use by untagged Adults, quantified by region. Statistical analyses and figures were constructed using Microsoft Excel version 2016 and mapping, including spatial analyses, was completed using ArcMap 10.6.

Results

Habitat in each study tributary are further described in Ch. 2 Results: *Habitat*, I. Lotic and terrestrial attributes, and II. Temperature. Study-wide Brook Trout captures and annual recaptures used in movement analysis are further described in Ch. 2. Results: *Fish population metrics*.

Movement

I. Net movement

Distance Median net movement in GR3 continued to increase throughout the season from 0 m/day in early summer, 0.06 m/day mid-summer and 0.1 m/day late summer. The opposite pattern occurred in ECR1 as movement was highest in early summer at 0.21 m/day, significantly decreasing to 0.19 m/day in mid-summer ($t= 1.699$, $df= 29$, $p= 0.007$) and 0.1 m/day in late summer, a significant decrease compared to early summer movement ($t= -2.418$, $df= 25$, $p= 0.023$). In GR4, movement was similar in early summer (0.11 m/day) to late summer (0.1 m/day) with no median net movement mid-summer. We captured 11 fish in the scour pool below the GR3 crossing barrier during July and 12 during October 2016. Seven of the 12 October recaptures were again recaptured in the same pool at some point the following year. There was no significant difference in age-specific net movement across tributaries for YOY, Age-1, 2 or in Age 3+ fish, throughout both years (p-values: 0.055, 0.453, 0.982 and 0.928, respectively). When all age classes among all net movement parameters were combined, there was no significant difference among tributaries ($F=2.22$, $df= 224$, $p=0.11$) but the movement was greatest among fish in GR4 and least among fish in GR3. Age-1+ fish were captured during all sampling events, therefore we analyzed Adult net movement seasonally during 2017. There were no significant differences among tributaries during spring, summer or fall (p-values: 0.55, 0.72 and 0.66, respectively), but the most net movement was exhibited by fish in ECR1 during spring, GR3 during summer and GR4 during fall.

Direction Directional net movement was seasonally variable, but no movement (sedentary) was also notable because far more fish remained in the same location than expected. The percent of fish remaining sedentary increased throughout the season in ECR1, while almost half the population in GR4 exhibited no movement by summer. Upstream fish movement continued to increase throughout the season in GR3 while fish in GR4 exhibited a dramatic decrease in upstream movement by summer. Fish in ECR1 exhibited the opposite pattern of fish in GR4; more fish moved upstream during summer than during spring and fall (Table 3.1).

II. Cumulative movement

YOY cumulative movement did not significantly differ from Age-1+ fish when all data were combined (t= 0.112, df= 15, p= 0.912). There was no significant difference in cumulative movement of YOY across tributaries (F= 2.782, df= 12, p=0.11) or for Adults (F= 0.431, df= 84, p= 0.651); however, Adult median cumulative movement in ECR1 was more than double the median movement by fish in GR3 and GR4. In contrast, we found the greatest individual cumulative movement in GR4 (9.43 m/day) followed by GR3 (7.09 m/day) and ECR1 (3.51m/day) (Fig. 3.2). Each age class had at least one individual that was recaptured in its previous tag/recapture location.

III. Home range

Home ranges were small across tributaries, including 92% of home ranges ≤50m in GR4, 80% in ECR1 and 73% in GR3. Home range size did not significantly differ among tributaries during 2017 when all age classes were combined (F=0.96, df= 2, 98, p=0.39). Median home range of Adults was the largest in ECR1 (11m) followed by GR4 (10m) and GR3 (7m) with no significant difference among tributaries detected (F= 0.702, df= 130, p= 0.497). Median home range of all recaptures was similar, with the same size in ECR1 but 8m in GR4 and GR3 (Fig. 3.3).

The fewest overlapping home ranges in GR3 and GR4 were found in the lower reaches of each tributary (Fig. 3.4). Within ECR1, the fewest home ranges were found at the upper and lower extents of our study area. The greatest quantity of overlapping home ranges across all tributaries occurred within the GR3 scour pool located directly downstream of an impassable culvert, as it represents the upper most extent of fish captured below the crossing. Restricted movement created by road crossings skew home range calculations, which Gerking (1953) notes as an important factor to recognize during movement analysis.

We further analyzed home ranges at the geomorphic region scale to understand which regions with similar habitat were most utilized. The proportion of each region per tributary were variable, but significantly fewer home ranges occurred in the FV region when all tributaries were combined (F=5.23, df=119, p<0.01). The smallest median home range across regions occurred in the FV at 4m (±1.2m), UV at 7m (±3.8m) and 13m (±4.3) in the U region. In GR3, 91% (n= 40) of home ranges were found in the UV and U regions (Fig. 17). Almost half within the UV occurred in the scour pool below undersized culverts (n= 9). Significantly fewer home ranges were present in the GR4 UV than FV and U regions (F= 4.35, df=34, p= 0.021), similar to the pattern detected among untagged Adult captures. The UV region of ECR1 contained 66% (n= 27) of home ranges compared to 17% (n= 7) in both FV and U regions, but no significant difference among regions was detected (F= 1.27, df= 40, p= 0.29). Not all home ranges were present entirely within a region (n=25). ECR1 contained the greatest overlap of home ranges across regions, highest between the UV and U regions (n= 10). Across all tributaries, no significant difference in the quantity of overlapping home ranges (i.e., home range density) was detected for each region (p= 0.57-0.85). We could not determine the region where four untagged Adult fish were captured because they resided in intermittent side channels not assessed during habitat analysis.

IV. Immigration

Adult capture probability was 100% during June, July and September 2017 (Trout Count Lite: Carle and Strub 1978 modified by Kratzer). All Adults captured were PIT tagged, therefore, we considered untagged Adult captures as immigrants recruited into our study reaches from June-September 2017 (Gowan et al. 1994) (Table 3.2). The most immigrants were captured in GR4 (n= 22) followed by 15 in ECR1 and 14 in GR3. The size (median TL) of Age-1 and Age-2 immigrants was larger during each successive sampling month. Six of 14 immigrants captured in GR3 occurred downstream of the GR3 crossing barrier. Four fish were captured upstream of the barrier within the no-low canopy reach, and the remaining others were captured in close proximity. The greatest abundance of each immigrant age class were captured in GR4, with the most captured within the U region. Half were captured within the no-low canopy cover reach (n=11) and two fish were captured in the pool beneath the bridge crossing. It is notable that zero Brook Trout were captured in the mainstem above or below the GR4 confluence throughout the study duration. Over half the mobile Adults captured in ECR1 occurred in the UV region (53%). Movement into this region requires fish to travel either >35m upstream from the mainstem or >75m downstream from outside our study area. We suspect immigration into our study reaches reflects seasonal preference of habitat and food sources.

Discussion

Movement among road crossing passability gradients

The GR3 road crossing in is impassable, the crossing in GR4 is fully passable, and both tributaries flow through a no-low canopy areas. The designation of fragmentation across Beebe River tributary populations was determined by stream crossing assessments completed by NH Fish and Game Department staff in 2016 (NH Department of Environmental Services et al. 2018), which we further quantified by mark-recapture movement analysis.

Our hypothesis that Brook Trout in the most impacted stream would exhibit greater seasonal and interannual movement was supported. The hydrologically undersized culvert in GR3 created the deepest pool across our study reaches. Stream banks surrounding the pool were absent of vegetation, likely caused by concentrated discharge from the undersized culvert (Jones et al. 2004). Additionally, the lack of instream wood and few substrate boulders further suggest that habitat selection was not a motivating factor of fish movement into the pool. Although fish could move downstream through the crossing, we suspect most untagged Adults captured in the scour pool moved upstream into and within GR3, induced by mainstem warming. The quantity of fish captured in the scour pool increased monthly during 2017 while mainstem temperatures warmed. Overall, the mainstem's mean daily temperature reached ≥20°C for 60 days during 2016 and 33 days during 2017, and GR3 remained below that threshold for the entire study. Refuge from lethal mainstem temperatures was restricted to ~110m below the crossing, and further movement to reach cooler water was blocked by the impassable barrier.

Restricted flow beneath the undersized GR4 crossing created the largest pool within the stream's study area. The bridge retained the largest log jam study-wide and suspended abundant dry and wet ROM, while the least volume of wood was documented in GR4. Two notable metrics of Brook Trout movement were documented in the habitat indirectly created by the undersized crossing. The largest quantity of fish captured within one habitat type in GR4 occurred within the pool, leading to the some of the most overlapping home ranges in the same location. Hartman and Nel Logan (2010) found that Brook Trout strongly selected for pools with LWD present, while further attributing LWD to the accumulation of organic material supporting increased macroinvertebrate communities. Morris et al. (2012) concluded similar findings, also relating the use of log jams as refuge from increased flow events. Restricted flow common with an undersized crossing may have contributed to preferred habitat, indirectly influencing Brook Trout movement beneath the bridge.

Movement in laboratory and wild Brook Trout populations has been studied to understand the species' physical ability to navigate past variable natural and anthropogenic features. In a laboratory setting, Kondratieff and Myrick (2006) documented the jumping height of Brook Trout driven by habitat attributes, like waterfall height, plunge pool depth and fish size. Adams et al. (2000) observed a 6% natural stream gradient as reaches likely influencing Brook Trout mobility, but documented movement past subsections of 13% slope. Adams et al. (2000) documented Brook Trout moving 14.5m upstream past a 22% slope, but our results indicate Brook Trout can navigate steeper, longer stream reaches. Seven Adult Brook Trout moved upstream through a 21m long, ~24% slope reach comprised of alternating pool-cascades in ECR1. Our research documented novel movement patterns among a subset of fish, traversing a high-gradient reach commonly thought to be a barrier to upstream movement.

Movement among no-low canopy areas

Our study streams were open systems, so we could not conclude movement into the no-low canopy was directly associated with the habitat conditions present. Instead, we analyzed Brook Trout movement across tributaries to understand how movement occurred when no-low canopy was present, and by the degree of access to it. Although no significant difference in net movement occurred among tributaries during 2017, noticeable trends were observed. Adult fish in GR3 moved the most during the greatest number of seasons throughout the study, but the most interannual net movement was variable across tributaries. Annually, the most cumulative Adult movement and the largest home range occurred in ECR1. Because Brook Trout movement among tributaries was variable across space and time, we failed to support our hypothesis that Brook Trout move more seasonally and interannually in the most impacted stream with no-low canopy cover (GR3). Many of the largest movement metrics were exhibited among fish in the most impacted tributary (GR3) and the tributary with no human impacts (ECR1). Fish in GR3 had the largest home range and cumulative movement was two-fold higher in ECR1. Furthermore, Adult net movement in GR4 was minimal during spring and fall, and fish remained sedentary during summer. Adults in GR4 also had the smallest home range and least cumulative movement. We suspect the no-low canopy provided more indirect habitat benefits than forested reaches, instead of acting as a thermal barrier. Additionally, open access to the no-low canopy allowed unrestricted movement to and from this habitat, which may reduce movement necessary to remain in seasonally optimal conditions.

Overall, the majority of fish moved very small distances and subsequently had relatively small home ranges. The smallest median home range occurred in the most impacted tributary (GR3), corroborated by Gerking's (1953) findings that home range can be influenced by barriers, limiting the extent of movement. We suspect that, given the overlapping home ranges, habitat variability within tributaries may be influencing fish move movement. Recognizing where fish move to and where their home ranges lie within tributary-specific regions enables us to observe where optimal habitat occurs. Similar habitat attributes were distributed across similar areas within tributaries, driven by geomorphic factors. The longest home ranges were exhibited in the FV of all three tributaries. The most home ranges were located in regions dominated by pools and pools/cascades across UV and U regions, but some of the fewest home ranges were documented in regions dominated by pools as well. Therefore, we suspect the distribution of home ranges across regions is not driven solely by the presence of pool habitat, but additional critical habitat preferences like increased seasonal food availability in the no-low canopy reaches, also documented in NH by Nislow and Lowe (2003, 2006) and Wilson et al. (2014). Future Brook Trout sampling should place further emphasis on regions, not only focusing on comparison of anthropogenic habitat influences.

We suspected the no-low canopy became a thermal barrier seasonally, and fish exposed to it would move further to find suitable habitat, which was not supported. From spring to fall, mean monthly temperatures remained within or occasionally surpassed the optimal range across the no-low canopy reaches, while forested reaches frequently remained below the optimal range. The only time a tributary surpassed Brook Trout's maximum thermal tolerance occurred during two continuous events for nine days during 2016. While we acknowledge GR4 surpassed 20°C for a short time, overall analysis of tributary temperatures leads us to exclude thermal stress as a potential motivator of intra-tributary movement. Furthermore, we expected to observe the greatest net movement during the period leading up to peak seasonal water temperatures, because fish would begin to seek thermal refuge from the warming mainstem and no-low canopy (Petty et al.

2012). In contrast, fish with open access to and from the no-low canopy reach exhibited the least movement when temperatures became warmest. We suspect the no-low canopy reaches contained optimal bioenergetic conditions in headwater tributaries that commonly remain below optimal conditions.

Additionally, we suspect the no-low canopy reaches within GR3 and GR4 provide seasonally beneficial food sources compared to intact canopy reaches. Hill et al. (1995) found that in most shaded streams, light is likely to be the primary abiotic constraint on photosynthesis, further influencing primary productivity and macroinvertebrate populations. Our qualitative aquatic invertebrate sampling documented fewer and smaller species among reaches with a forested canopy, while a larger, more diverse species were captured within the no-low canopy cover reach. Brook Trout seasonally target habitats where the availability of terrestrial food increases, utilizing critical terrestrial invertebrate forage during the summer (Hubert and Rhodes 1989), comprising 51-63% of annual energy consumption (Sweka and Hartman 2008). Terrestrial invertebrate biomass is greater in early successional habitat than late successional habitat (Wilson et al. 2014), which has also been found to support higher Brook Trout density (Nislow and Lowe 2003, 2006). More complete analysis of aquatic invertebrates observed, no-low canopy-specific habitat conditions and reference to similar results are further described in Ch. 2 Discussion: *Size at Age, Population Condition and Growth Variability*. Overall, we observed less movement across multiple parameters in the tributary with unrestricted access to the no-low canopy. Our review of habitat results and exploratory macroinvertebrate sampling supports baseline movement results, while further introducing the explanation of improved habitat among typically heterogenous, resource limited streams.

Movement into tributary study areas

Previous discussion of recaptured Brook Trout provides analysis of movement in association with no-low canopy reaches, but the untagged Adults (immigrants) captured further supports rationale for the observed trends. The presence of immigrants provides additional results indicating why we failed to support our hypothesis that Brook Trout move more seasonally and interannually in the most impacted stream (GR3).

There was no significant difference in the quantity of immigrants captured among tributaries, but 50% more fish were captured in GR4 than GR3 and ECR1. Almost half of the immigrants captured in GR3 occurred below the crossing barrier, indicating the road crossing was a likely barrier to upstream migration of immigrants as well as recaptures. Four of ten immigrants captured in GR3 were located in the no-low canopy, and half of the immigrants captured throughout GR4 were located in the same no-low canopy habitat. More smaller fish moved into study reaches during June, and fewer, larger fish of the same age classes moved into study reaches during September. We observed a negative trend in YOY biomass as the quantity of immigrants increased during 2017, and we suspect the immigrants may exhibit piscivory feeding habits. Multiple factors likely influenced seasonal fish movement into study reaches, including seeking thermal refuge, a desire to reach natal habitat and seasonal food availability.

Warm summer temperatures in lower tributary reaches and the mainstem may trigger seasonal movement into tributaries. In a similar mainstem-tributary system, Petty et al. (2012) observed Brook Trout moving to coldwater seeps, groundwater upwelling and tributary confluences when mainstem temperatures warmed. Fish born in tributaries might exhibit homing movement behavior, increasing their desire to return to tributary habitat. Our results may be explained by complex, intra-tributary subpopulation dynamics, with Brook Trout born in tributaries producing offspring primarily born in tributaries as well, similarly observed by Kanno et al. (2014). Terrestrial and aquatic invertebrate abundance variation among a gradient of habitat conditions are further described in our previous analysis (Ch. 2 Discussion: *Size at Age, Population Condition and Growth Variability*). Finstad et al. (2006) studied the allopatric relationship among Arctic Charr (*Salvelinus alpinus*), a close relative to Brook Trout. They discovered when food limitations create a bottleneck, some adult fish adapt to alternate life history strategies, like cannibalism. Previous discussion introduced food limitations among headwater streams, which may induce this life history characteristic (Hill et al. 1995). Furthermore, Bowlby and Roff (1986) detected the negative influence of piscivorous Brook Trout, Rainbow Trout and Brown Trout on Brook Trout biomass, similar to trends we observed among YOY and immigrant adult Brook Trout. Among the factors we introduce to possibly explain observed immigrant movement, the most supported explanation is a desire to reach no-low canopy reaches. The capture location and quantity of immigrants among tributary study reaches provides further rationale to why we failed to support our hypothesis that fish would move less in the most impacted tributary.

Our initial research questions and subsequent hypothesis were derived from influences documented among similar systems, range-wide. Brook Trout in our study streams exhibited small movement overall, similar to tributary-resident Brook Trout subpopulations described by Ecret and Mihuc (2013) and Petty et al. (2012). Relative to conditions among our study streams, road crossing barriers can create compounding, long-term influences on Brook Trout populations, including decreased species richness (Perkin and Gido 2012), decreased population-wide density (Pepino et al. 2012) and increased chances of population extinction (Letcher et al. 2007). Furthermore, thermal habitat degradation can restrict populations to headwaters or reduce Brook Trout distribution (Hudy et al. 2005, 2008). Although undersized crossings and increased thermal influences have been found to negatively affect Brook Trout populations, the indirect habitat associated with these variables provided different influences when the most intact tributaries are not the most ideal for wild Brook Trout. Stream temperatures were warmer and riparian canopy was younger in no-low canopy reaches, creating habitat that met conditions commonly recognized as beneficial. Fish with open access to the no-low canopy exhibited less movement during peak summer temperatures, indicating sufficient refuge was available. They also moved less across a year, and their home ranges were the smallest, indicating minimal movement was necessary to reach suitable habitat and food. In conclusion, it is important to review complete systems before associating singular negative influences on fish populations (Bond and Lake 2003, Fausch et al. 2002). Brook Trout are susceptible to a multitude of environmental conditions responsible for their reduced abundance and distribution range-wide. Recognizing complicated interactions among fish, the environment, and socio-cultural interactions may allow us to improve management and restoration in the future.

FIGURES

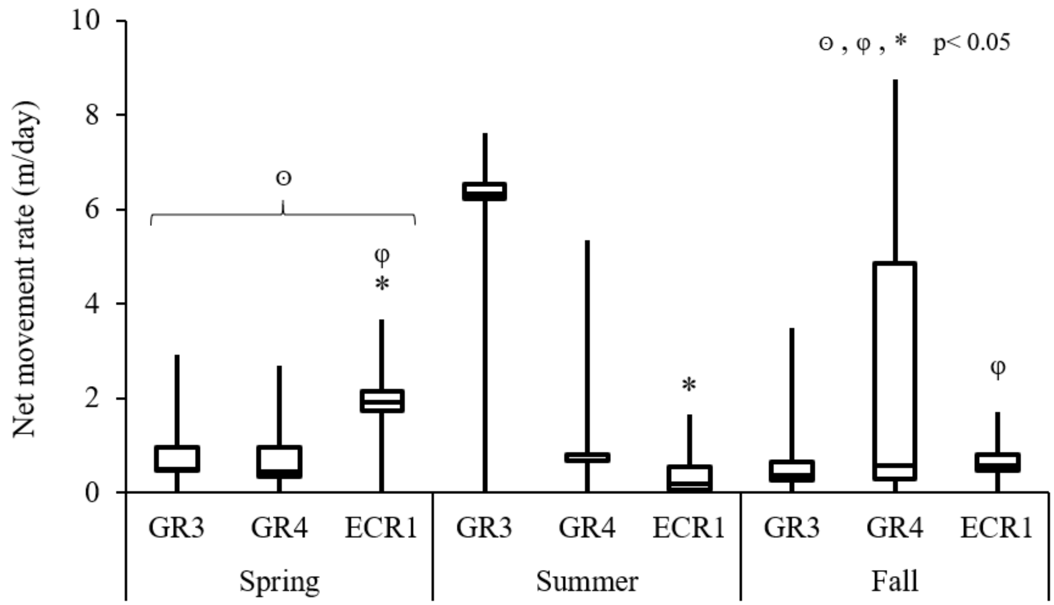

Fig. 3.1. Box-whisker representing net movement of tagged Brook Trout per tributary between sampling events in 2017 within focal tributaries of the Beebe River watershed. Significant differences in movement among sampling events is further indicated by symbols above box plots.

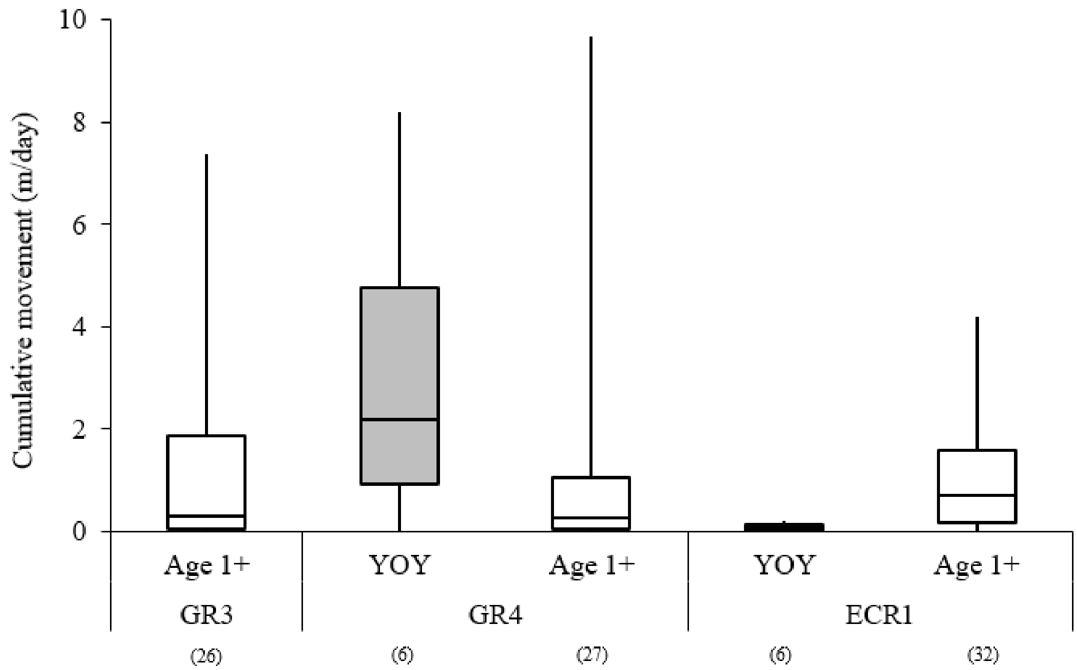

Fig. 3.2. Box-whisker plot of 2017 cumulative movement of Brook Trout in focal tributaries within the Beebe River watershed in 2017. Sample sizes per age class are indicated in parenthesis below tributary names, and one YOY captured in GR3 was removed from the figure.

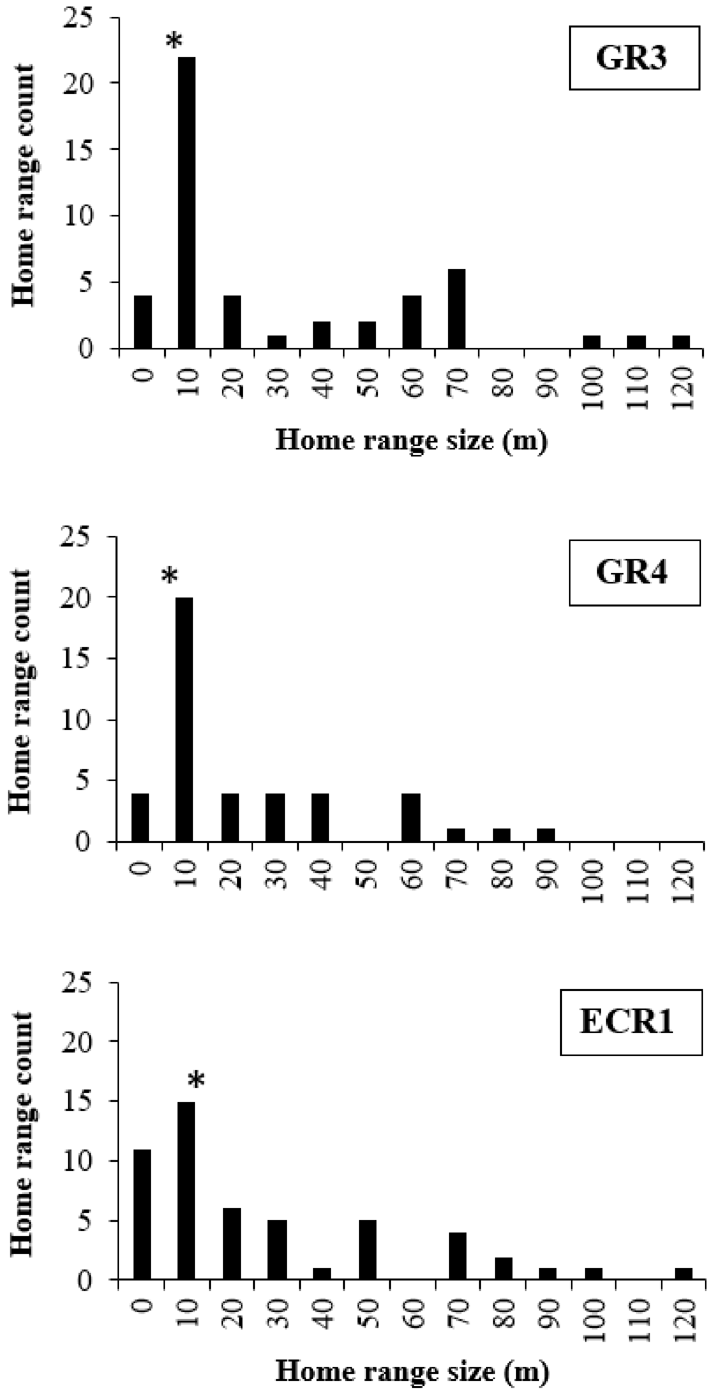

Fig. 3.3. Study-wide home ranges sizes within focal tributaries of the Beebe River. Asterisks indicate median home range size per tributary, GR3: 8m, GR4: 8m, and ECR1: 11m.

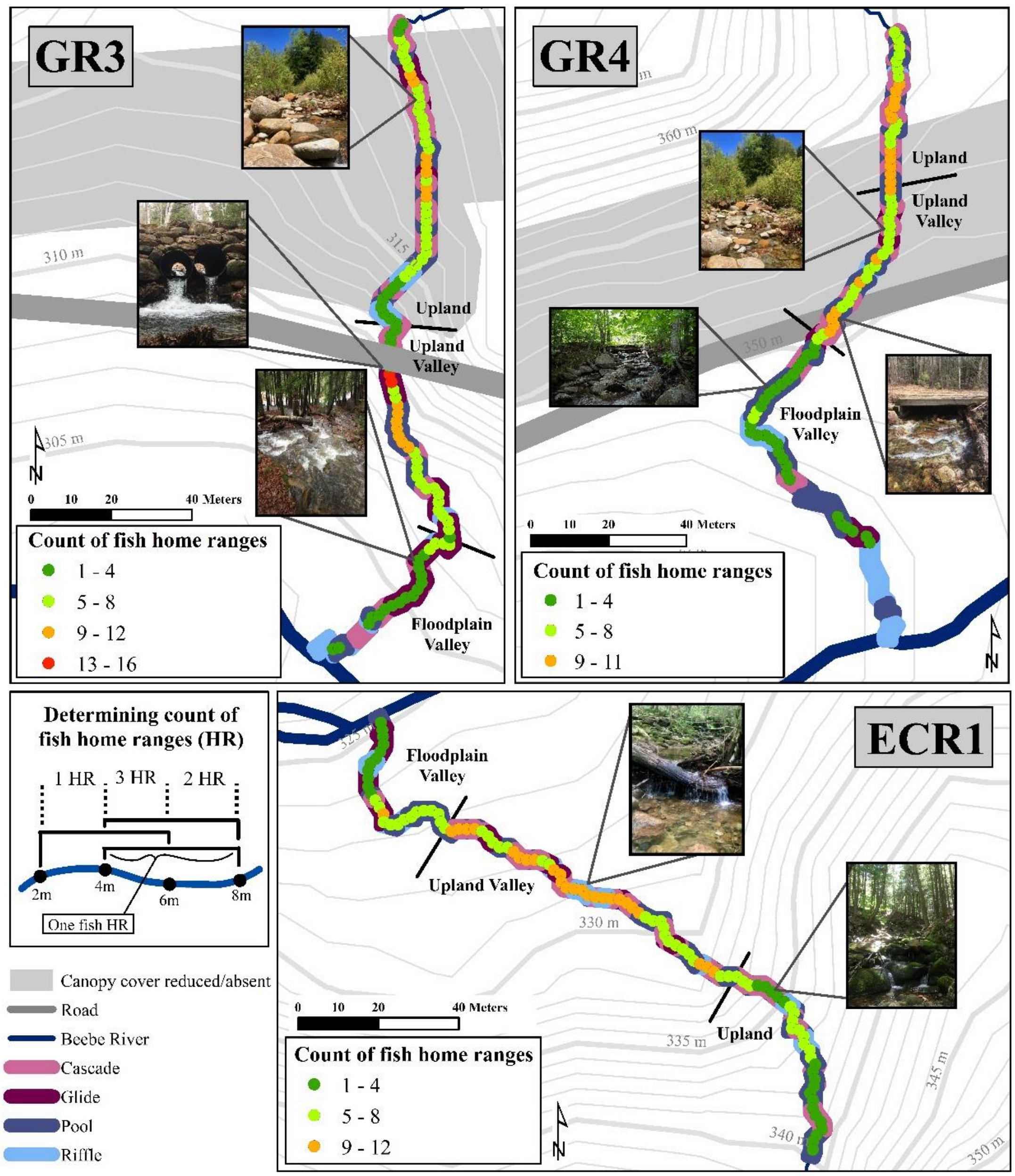

Fig. 3.4. Brook Trout home range density and habitat types in focal tributaries within the Beebe River watershed. Home range is determined from the range between furthest upstream and downstream mark and/or recapture locations of individual fish, within 2m increments of each 200m study reach (graphic above figure key). Home ranges were determined from two sampling events during 2016 and four during 2017.

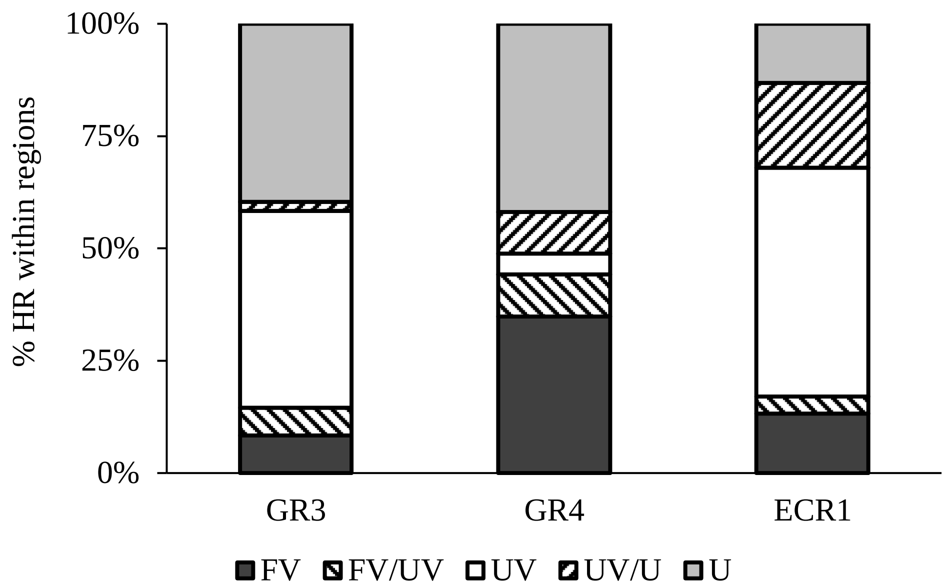

Fig. 3.5. Distribution of Brook Trout home range (HR) study-wide within geomorphic threshold regions in focal tributaries of the Beebe River watershed. Region abbreviations and grayscale/patterns displayed: FV (floodplain valley) as solid dark gray, UV (upland valley) as solid white and U (upland) as solid light gray. When home ranges overlapped multiple regions, new overlapping regions were included: FV/UV as lines with -slopes and UV/U as lines with +slopes. One fish home range extending from FV to U region in GR3 was excluded from analysis due to movement attributes not meeting home range qualifications.

TABLES

Table 3.1. Combined ages of Brook Trout directional net movement during spring, summer 2017 and fall 2016/2017 across Beebe River focal tributaries. Results include the count of recaptures between seasonal events during each year, percent of fish that moved upstream (US), percent of fish recaptured in the same location (sedentary) and percent of fish that moved downstream (DS).

	Spring				Summer				Fall			
	Count	% US	% sedentary	% DS	Count	% US	% sedentary	% DS	Count	% US	% sedentary	% DS
GR3	19	42%	26%	32%	19	53%	21%	26%	28	64%	18%	18%
GR4	8	50%	38%	13%	11	18%	45%	36%	21	57%	14%	29%
ECR1	19	21%	16%	63%	24	79%	21%	0%	45	58%	31%	11%

Chapter 4

Management Implications and Future Directions

Management in NH

The management strategy for NH's wild trout focuses on protecting naturally reproducing trout populations from pressures related to increases in fishing exploitation and urbanization. To ensure protection of wild trout, the NH Fish and Game Department established waters designated at 'Wild Trout Waters'. Our analysis of wild Brook Trout populations in the Beebe River watershed and statewide provides results that can help refine management, more specifically, the designation of Wild Trout Waters. Among multiple objectives, the designation of these protected populations includes sustaining populations with densities ≥13 lbs/acre (NH Fish and Game Department 2018).

Isolating only biomass to designate increased protection ignores other trends critical to long-term population resiliency. Fish in GR3 and ECR1 met the standard for enhanced protection, but exhibited a significant interannual decline in density. Fish in GR4 consistently gained the most mass, maintained productive recruitment through YOY-dominant age structure and interannual biomass remained stable. NH's Wild Trout protection and the broad standards utilized to meet this designation misrepresent populations that meet the most important qualifications for sustained viability under NH's greatest wild Brook Trout protection.

We further compared study populations to statewide Brook Trout populations found in similar systems that were sampled using ≥2 pass depletion electrofishing. The biomass of populations in GR3 and ECR1 surpass 2/3 of statewide populations. While comparing mean lbs/fish, over 1/3 of statewide populations fell below the mean lbs/fish documented in the population within GR3. By NH's Wild Trout Waters designation standards, the average statewide population meets requirements for enhanced protection. Only 13 lotic and three lentic systems are rewarded protection under the current designation, yet 88 lotic populations currently meet the standard (NH Fish and Game Department, 2018). Of the statewide subset we analyzed, 88 of 225 populations surpass the biomass threshold for enhanced protection. Two management tools could be implemented to address the variation in population protection and biomass present among many populations. The criteria to designate streams for enhanced protection should be revisited to assess how accurately the criteria meets long-term population management goals, including analyzing interannual trends and seasonal recruitment. Second, rationale for hatchery stocking among wild populations that meet the criteria for enhanced protection could be reassessed based on scientific rationale, not driven by historic stocking practices within each system. In particular, the Beebe River has been stocked for 80 years with fish derived from eight different hatcheries. We aim to provide results that initiate discussion related to ongoing management, but recognize additional detailed, long-term sampling will provide more support to our initial findings.

NH resides in the heart of the United States' most intact Brook Trout range, yet lacks sufficient peer-reviewed studies or an established species-focused management plan (currently being completed). State-level population and overall species monitoring is focused on management, often lacking a clearly established study design, consistently standardized data collection and critically vetted data analysis to document results that can be transferrable for regional or range-wide management (Hudy et al. 2005, 2008). In the future, managers should account for interannual population trends and seasonal environmental changes when making decisions on how wild, self-sustaining populations are managed (Warren et al. 2010).

Management Across New England

Regional datasets are commonly utilized to further understand wild Brook Trout demographics at the state-level, but misinterpretation can arise from referencing only singular population attributes. We found that analyzing density or biomass alone to understand population health was a poor indication of overall condition and resiliency across headwater streams. We compared the seasonal biomass of study populations to regional state-level management criteria standards to understand the status of study populations related to population protection across the northeast (ME Department of Inland Fisheries and Wildlife 2009, NH Fish and Game Department 2018, VT Fish and Wildlife Department 2018). The value of wild populations is recognized by a multitude of enhanced protection techniques, like restricted fishing methods (artificial bait, barbless), catch and release angling, no hatchery fish stocking, etc. Management guidelines are implemented based on factors like fishing pressure and high quality habitat, measured by population standards at the landscape scale. Populations in GR3 and ECR1 met at a standard recognizing enhanced protection in at least one state during at least one sampling event (Table A2.5). Results from a composite of metrics suggest this designation may not reflect true population condition and viability. By recognizing additional metrics, more responsible and educated decisions can me made regarding ongoing and future regulations influencing wild Brook Trout populations across New England and beyond.

National Management

We quantified individual level conditions at the tributary scale, and compared them to the American Fisheries Society (AFS) national standard. Overall, our study populations primarily meet the national standard (Fig. A2.3). AFS lotic Brook Trout standards are derived from populations inhabiting the midwestern U.S.A., generalizing wild Brook Trout attributes from Michigan to across the nation (Schneider et al. 2000b). Hudy et al. (2005, 2008) recognized the most subwatersheds supporting wild Brook Trout in New Hampshire, Maine, Vermont and northern New York, far from where the national standard is derived. We propose that national standards for wild Brook Trout condition should be reassigned to populations at the regional scale, better reflecting populations with a variety of regional impacts.

Habitat Management

Headwater tributaries are important drivers of aquatic habitat and biological diversity, including their influence on mainstem rivers. When habitat within tributary streams become degraded, it can have profound and lasting impacts on main river habitat and biodiversity (Kiffney et al. 2006). Fausch et al. (2002) describes the need for fisheries managers to have a continuous view of rivers, recognizing the connectivity necessary to meet the needs of different life stages of fishes. At the watershed scale, Petty et al. (2005) further indicates the importance of appreciating spatial and temporal variations in Brook Trout health, survival and reproduction that can further influence population distribution. Furthermore, Meyer et al. (2007) recognized the biological integrity of entire river networks which may be dependent on impacts occurring in the headwater streams.

Anthropogenic barriers can further isolate populations from reaching suitable refuge and spawning habitat critical to their persistence. When considering restoration through barrier removal, a watershed approach should be implemented to best understand sites with the most ideal financial and ecological benefit (Diebel et al. 2014). Road crossings described throughout the study were replaced with large bridges in late 2017, restoring system-wide connectivity to 9.7km of streams and over 24km of aquatic habitat throughout the watershed. Ongoing management should focus on limiting riparian mowing that occurs within the powerline easements, allowing vegetative buffers along streams but continuing to promote a young, heterogenous forest. Wilkerson et al. (2006) conducted research on timber harvests along small, headwater streams in Maine. They found that when no riparian buffers remained, stream temperature greatly increased post-harvest. Because we observed temperatures within the easement seasonally reaching ≥20°C, continued reduction of riparian cover could be detrimental to resident populations of Brook Trout, despite the potential seasonal pulse of prey from young riparian vegetation (Sweeny 1993, Wilson et al. 2014). As Fausch et al. (2002) recommends, we should focus on management at the riverscape level to achieve the best fisheries management possible. Employing conservative management strategies across the Beebe River watershed is necessary to sustain headwater populations of Brook Trout while compromising with stakeholders to ensure productive working relationships.

Future Directions

In-stream wood additions occurred among headwater tributaries throughout the Beebe River watershed from 2015-2018. Approximately 3,720 linear meters of streams have received the treatment, and revegetation of recently armored and degraded stream banks is planned for 2019. These treatments have focused on adding stream-side wood by “chop and drop”, while not creating openings in the canopy (R. Fortin- personal communication). Our results indicate light exposure within headwater streams may be become beneficial to Brook Trout by indirectly influencing populations through increased productivity, promoting macroinvertebrate abundance and diversity. We propose that further analysis of increased light should be completed among headwater streams, replicating natural canopy openings that historically occurred across New England.

For future stream crossing restoration projects, it is important to review complete systems before associating singular negative influences on fish populations (Bond and Lake 2003, Fausch et al. 2002). Meyer et al. (2007) concluded that the individual and cumulative impacts of headwater streams are important contributors to the biological integrity of entire river networks, especially within headwater tributaries to a watershed, like the Beebe. Although it is known road crossings have negative impacts on wild Brook Trout populations, it is important to understand other site features present or lacking (Burford et al. 2009, Poplar-Jeffers et al. 2009, Torterotot et al. 2014). The presence of permanent and seasonal barriers, reduced instream wood and regional stream acidification all have been found to compound stress on wild Brook Trout in NH (Nislow and Lowe 2003, 2006) and in nearby VT (Warren et al. 2010). If tributaries had remained fragmented and degraded, populations could continue to become vulnerable to extirpation or dramatic population declines from extreme weather events like flooding (Roghair et al. 2012) or drought (Hakala and Hartman 2004). Fisheries managers should address multiple factors while assessing continued wild Brook Trout declines, requiring expanded and detailed watershed-wide assessments prior to future restoration projects across New England.

Bernhardt et al. (2007) compared the success of aquatic restoration across database records and telephone interviews, finding only 11% of projects conducted research before and after restoration while including additional reference sites. Our project established pre- and post-restoration monitoring, inclusion of reference sample sites and a clear project goal of restoring fish passage, resulting in an important contribution to lacking research in the field of river restoration science. Studies have specifically focused on measuring Brook Trout demographics (Hutchings 1994, Utz and Hartman 2009), habitat relationships (Bowlby and Roff 1986, Kratzer and Warren 2013) and the influence of road crossings (LaChance et al. 2008, Pepino et al. 2012) in systems they dominate, but few have combined a myriad of important metrics into a fine-scale, multi-stream study. Our research combined multiple parameters that provide critical results to begin filling gaps in Brook Trout research, both range-wide and regionally. Ongoing and future restoration projects should better emphasize study designs with multiple replicates, reference sites, and document conditions and fish assemblages before and after restoration to better refine methods used during aquatic restoration.

Our study was a critical component in the long-term monitoring of watershed restoration, leading to research that further quantifies the benefits of restored connectivity across headwater streams. Moving forward, we recommend supporting monthly mark-recapture events with stationary PIT tag antennas. By encompassing tributary study reaches, researchers can further quantify survival and seasonal immigration/emigration missed between sampling events (Gowan et al. 1994). Crossing replacement and construction can create increased sediment input and turbidity (Kreutzweiser and Capell 2001, Pepino et al. 2012), which has been found to negatively impact Brook Trout spawning habitat and egg survival (Argent and Flebbe 1999). Increased fine sediment input has also been found to alter macroinvertebrate assemblages (Nislow and Lowe 2006) but impacts decrease as time progresses (Lowe and Bolger 2002). We sampled macroinvertebrates during the study, and future analysis will likely help explain observed variability within and among tributaries. We suggest increased seasonal habitat assessments focusing on substrate changes below replaced crossings and associated disturbed areas to understand potential negative impacts of restored connectivity.

In conclusion, our study provided results that benefit the understanding of wild Brook Trout demographics and movement at the state, regional, and national scale while contributing to the effectiveness of aquatic restoration. We aim to discuss alterations and improvements to ongoing management, but recognize current and future research may refine and further explain supporting trends. Recognizing influences that threaten current populations can promote advancement in stewardship, ensuring we do not repeat mistakes threatening our shared resources. In the future, we hope biologists, environmental groups, outside stakeholders and sportsmen will continue to work together to improve strategies to enhance and protect wild Brook Trout populations not just in NH, but range-wide as well.

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APPENDICES

Brook Trout maturity

Methods

We were able to follow changes in size of recaptured fish throughout the season while further differentiating sex, and ultimately sexual maturity. To determine sex during spawning (September 2017), we lightly pressed laterally along the vent to visually document the presence of milt or roe excretion or the clear presence of an ovipositor. A fish was considered sexually mature if it was old enough to determine its sex. Furthermore, analysis of males and females was focused on Age-1 and Age-2 fish (94% to the dataset). We tracked the recaptured PIT tag number through previous recapture events, comprising of all primary sampling events and during crossing dewatering. We utilized five recapture events to analyze sex-based abundance and body mass change across 2017. Additionally, we tested the difference in abundance among males and females across tributaries across 2017 using a two-sample T-test.

Results

We observed gonads in 88% of Age-1 fish captured during spawning and 92% of Age-2+ fish (Table A1). Mature fish were comprised of >50% Age-1 individuals. Age-1 females contributed 13% more than males to the combined mature Age-1 and Age-2 demographics. Across all sampling events and among all tributaries, sexually mature fish captured in GR4 weighed the greatest during 12 of 14 events. The median mass of each sex and age across 2017 was greatest among fish captured in GR4, while fish in GR3 had the least mass among Age-1 males and females and Age-2 males. Large variation between the greatest and least mass per sex was observed, primarily among Age-2 males. Fish in GR4 weighed 23.5 g. (±5.57), almost double the mass of Age-2 males in GR3 (12 g. ±0.63). For all males, the least seasonal body mass increase occurred among Age-2 fish in GR3 (18.18%) while the greatest occurred among Age-1 fish in ECR1 (157.14%). For females, we observed the least increase among Age-2 fish in GR3 (36.36%) and the greatest mass within the same tributary, but among Age-1 fish (133.33%). Overall, females weighed 1 g. (±0.46) more than males at Age-1, and males weighed 5.5 g. (±4.37) more than females at Age-2.

The greatest abundance of each sex was variable among tributaries and there was no significant difference among male and female abundance within either Age-1 or Age-2 fish (p=0.1 and 0.16, respectively). From May-June, mature fish body mass increased 12.5% and 5.36% in immature fish (Fig. A1). From June-July, mature fish mass increased 8.82% while immature fish mass decreased 20.59%. For the remainder of the season, immature mass increased 0.78% from July-August followed by 12.5% into September.

Mature fish mass continued to increase 3.13% from July-August then no change occurred during the remainder of August, followed by a 12.5% decline into September.

Manual of Beebe River Study Sampling Protocol

Present version is protocol initiated during 2016 & 2017 field seasons

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Transect design

Primary study tributaries include GR3, GR4 and ECR1 including 100m of MS per confluence

Tributaries

- Tributary transects begin at confluence with mainstem at 0m and end 200m upstream
- Start and end of each transect is indicated by three strands of flagging with orange painted rebar at the 200m increment
- Each 10m segment is marked by two strands of pink flagging wrapped around a tree on at least one streambank. The location is written on flagging multiple times to be visible from throughout the stream and from shore (check and/or replace annually)
- Pink stake flags placed outside bankfull every 2m with increment written on each (check and/or replace before each sampling event)
- When channel braiding occurs, use discretion to continue main channel marking or to begin marking as a side channel. Discuss options with supervisor(s)
- If braiding occurs, place flags on each side of where the braid/main channel split
- <u>Side channel</u>: when large braiding occurs or seep becomes presumably navigable by fish
- Begin side channel designation while moving upstream from confluence of tributary/MS
- Delineate side channel perpendicular to where it enters the main channel
- Mark flags for side channel as SC-A- #.#
- Regardless of side channel quantity, indicate each alphabetically in case others form throughout the season
- It is extremely beneficial to sketch the layout of each tributary including side channels indicated by where they begin/end in relation to main channel and length of each side channel (Fig. A2, A3, A4)

Mainstem (MS)

- MS transects are 100m long, each including 50m downstream of the tributary confluence and 50m upstream of the tributary confluence
- Beginning and end of MS transect is marked by orange painted rebar on tributary side of the MS
- Recommended: pink flagging wrapped around each end of the transect PLUS multiple strands of flagging hanging to be visible while walking in the MS
- Check MS hanging flagging prior to each fish sampling event

Abiotic habitat

Temperature

- Loggers used were ONSET HOBO U-22 (Bourne, MA, U.S.A.)
- Loggers should be set at 0:30 minute intervals with delayed recording ~2-3 hours after deployment to ensure acclimation with site conditions prior to recording
- Ensure sufficient logger battery life when starting the logger
- When deploying/saving data per logger, use deploy/retrieve date, stream name and an acronym to delineate location within the tributary/MS
- Water: attach logger inside PVC capsule, secured by tightening two nuts onto each other before placing inside cinderblock (inset right)

- Using polyester rope: tie one end to eyelet of eye bolt and wrap multiple times through cinderblock before tying the unit to the block
- Ensure logger is placed in a location deep within a channel so if flow becomes reduced, the logger will remain submerged
- Bury cinderblock with LARGE boulders so extreme flows do not dislodge the block
- Air: feed long zip ties through drilled holes in the PVC capsule to attach logger inside (inset right)

- Tie logger securely to a tree, >10m from water
- Temperature loggers were deployed at locations indicated by ‘W#’ for water and ‘A#’ for air (Fig. 4.1, 4.2, 4.3)
- Indicate the date/time of deployment for each logger and recording interval to determine when the logger’s battery will need to be charged (Appendix, Temperature logger datasheet)

SC/Flow

- Sensors are screwed into housings permanently attached to boulders and coupled with a metal yard stick fixed to nearby boulder (inset right)
- At first opportunity each spring, data should be downloaded from each sensor and the recording interval set to shorter time
- Winter: 30 minutes, summer: 5 or 15 minutes
- Each time data is downloaded and during additional visits, record water depth from the yard stick
- Prior to data download, clean the end of sensors with Q-tips to remove sediment/fungus accumulation
- When sensors are removed and redeployed, ensure the boulder the capsules are attached to is placed in the same location with the same water depth
- Locations are displayed on tributary maps indicated by ‘SC/flow sensors’ (Fig. A2, A3, A4)
- When redeploying sensors, note the date sensor memory will be filled to ensure download is completed before that time

Biotic habitat

Habitat inventory

- Staff completing habitat inventory MUST be either trained by NH Fish and Game biologist(s) or shadowed by staff who have previously completed the inventory to ensure parallel data collection
- Assessments should be completed during seasonal low-flow (late July to early August)
- Following NH Fish and Game’s Rapid Habitat Assessment (Decker 2000)(Appendix, Instream Habitat Standard Operating Procedure)
- Slope/gradient: cut two limbs into 1m lengths, stand in center of stream at a designated increment (flagged) and while looking upstream to a partner. The partner’s location should be at a point where either there is an abrupt slope change or you cannot see around a corner. Set the clinometer on top of the stick and look upstream, placing the indicator line at the top of the upstream stick to determine slope/gradient. On the datasheet, indicate the slope from start to finish measurement based on associated habitat type delineated by flag increments
- Wood survey following TWF Monitoring Program: Large Woody Debris Survey (Schuett-Hames et al. 1999)(Appendix, Wood survey)

- For each day of inventory, record depth of water on yard stick (corresponding with SC/Flow sensors) and qualitative notes of precipitation within the previous 48 hours
- Modifications to Rapid Habitat Assessment are further explained in Appendix, Habitat sampling protocol modifications and authorship
- Necessary supporting files include Habitat assessment datasheet (Appendix) and Wood survey datasheet (Appendix)
- Supplemental sampling information is provided in the Appendix, including the Large woody debris survey criteria & code sheet (Table A2), Wood survey directional and orientation schematics (Fig. A5) and Wood survey declass class criteria (Table A3)

Canopy cover

- Data collection should occur 2-3 times annually post-leaf out (late June-July)
- Beginning at transect location 0.0m, measure cover every 10 meters from center of wetted width, facing upstream
- Measure stream temperature at each sampling location (every 10m) using the Morrill Multi-Reader (Appendix, Morrill Multi-Reader)
- Record water depth on SC/flow yard stick during each sampling event
- Spherical densiometer (inset right): hold at waist height with forearm extended perpendicular to your body and calculate % cover per square following the cover designation 0 (0%), 1 (25%), 2 (50%), 3 (75%), 4 (100%)
- Calculate the sum of all squares per location, multiply by 1.05 for total percent canopy cover
- Datasheets are located in Appendix, Canopy cover datasheets

Macroinvertebrate sampling

Pre-sampling

- Determine pools and riffles throughout each transect and uniquely label each habitat type streamside with ribbon (i.e. P-01 = Pool #1)
- Quantify pools and riffles located within three sub-sections: downstream of powerlines (DS PL), within powerlines (PL), upstream side of powerlines (US PL)
- Use a random number generator for each section of each tributary to select which sites will be sampled

- Label each habitat location selected (through random generator) on strips of cardstock paper using an alcohol resistant permanent marker before placing into tubes
- Fill 50mL Falcon tubes ¾ full of ethyl alcohol (can range from 75-90%)

Sampling

- Using Surber sampler (net), select a location within the selected habitat that has sufficient flow to carry suspended specimens into the net
- Within the designated square frame, kick substrate for 30 seconds and scrub rocks/dig in substrate for 30 seconds
- To easily retrieve specimens from net, turn it upside down over cylindrical screen (resting on bucket) and splash or squirt water over the net
- Only search/remove specimens for designated periods of time depending on staff availability:
    - One person: 5 minutes
    - Two people: 2 minutes 30 seconds
    - Three people: ~1 minute 45 seconds
- Turn the net inside out and rinse in the stream between each sampling event
- Keep vials vertical so macros remain in alcohol without getting stuck to sides of vial outside the alcohol solution
- Store vials in coolers with ice packs during sampling
- After returning to lab:
    - Put vials in freezer
    - Mark group of vials with sampling date and document in a second location

Fish sampling

Electrofishing

All captured fish are identified by species, measured (TL in mm and mass in g.), and 2m location recorded.

Tributaries

- After testing the unit's settings, clear all effort (seconds) on each backpack unit prior to sampling
- Place block nets at start and end of each 10m sub-section
- Begin sampling at 0m, working upstream to 200m
- One person completes a first pass of the 10m sub-section, followed by a second pass after the first pass is completed (Zippin 1958)
- Fish must be removed and placed into buckets for each 2m sub-section they were captured in

- Label a slip of paper with the 2m section AND pass number (i.e. 0.2 / Run 1), then place label in bucket
- When sampling is complete, record effort (seconds) from each backpack unit for first and second pass, separately

Mainstem (MS)

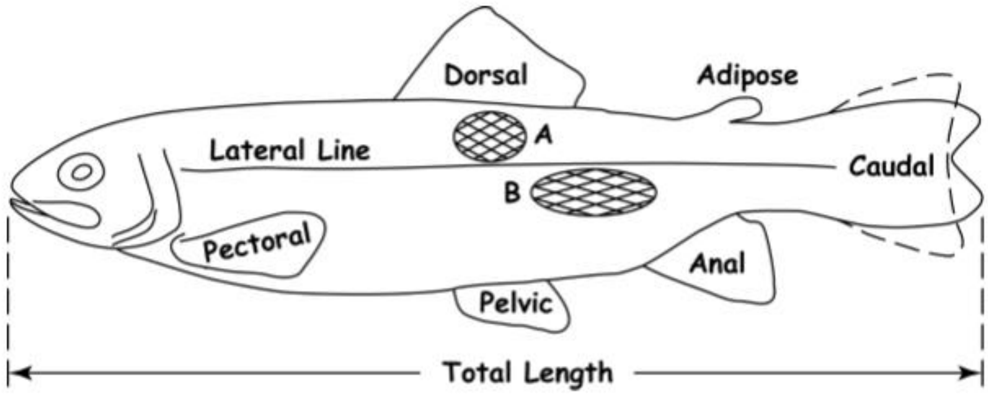
- After testing the unit's settings, clear all effort (seconds) on each backpack unit prior to sampling
- Staff includes 3-5 electrofishing units, 3-5 people netting and multiple other staff to collect and shuttle bucks to/from shore
- Begin sampling 50m downstream of tributary confluence, indicated by rebar and flagging above bankfull on the tributary side of MS
- All fish captured from 50m downstream to perpendicular of confluence are documented with a location of 'MS 0-50'
- 50m upstream of tributary confluence is indicated by rebar and flagging above seasonal flooding zone on tributary side of MS
- All fish captured from perpendicular of confluence to 50m upstream are documented with a location of 'MS 50-100'
- One-pass electrofishing is completed for entire MS section
- When sampling is complete, record effort (seconds) from each backpack unit and sum all effort for MS sampling

* For each stream and/or MS sampled, record descriptive notes of things like problems that occurred, oddities, staff involved, weather conditions during sampling and within days prior, dry side channels not sampled, fin order clipped per tributary, etc.

Data collection

- Following attached datasheet (Appendix, Electrofishing sampling datasheet) complete designated metrics including more descriptive parameters:
    - PIT tag: APP/OBS = applied/observed
    - Fin clip: LV=left ventral, RV=right ventral, AD=adipose
- If a ventral fin has grown from an initial clipping event, re-clip respective fin and indicate on data sheet as observed AND applied
- May sampling excludes YOY captures based on capture probability caused by the size of YOY and size of webbing in handheld sampling nets

- Scale sample ALL TAGGED FISH following the schematic below, sampled at location 'B' (Schneider et al. 2000)



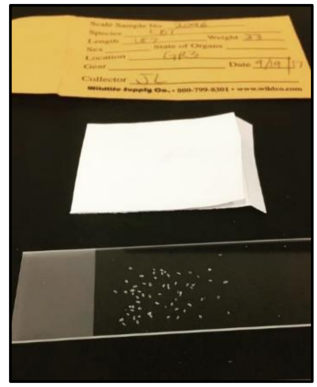
- Scrape mucous from the sampling location. Next, remove scales with a dull knife blade then wipe in paper slip within envelope, followed by labelling relevant documentation. Wipe the knife blade clean between samples to prevent cross contamination
- When clipping fins for biological sampling, dip scissors in the alcohol within the vial you are placing the sample before clipping fin(s) from other fish to prevent cross-contamination

PIT tagging

- Only tag fish ≥ 60mm AND 2g using an 8mm PIT tag
- Wave portable PIT tag reading wand over the tag prior to insertion into fish and record full tag number on datasheet
- Ensure no other tags are within approximately 0.5m of the tag you are reading! Check data sheet before implanting a new tag to ensure the new tag number is recorded on the reader (different than the previous tag number)
- Make a small incision anterior to the vent so tag must be slightly forced into body cavity, inserting tag posterior of incision (inset right)

Scale sample analysis

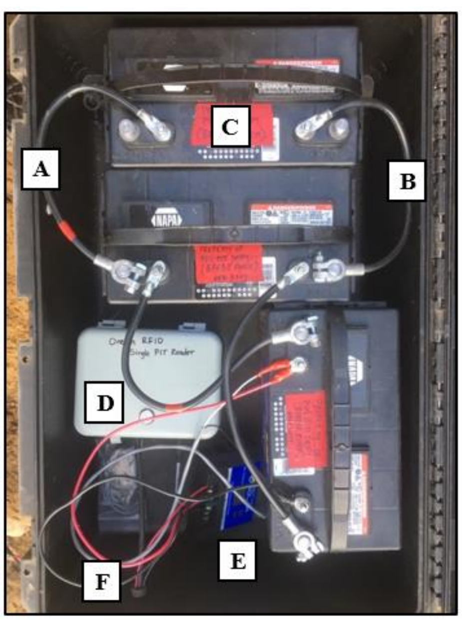
- Place black paper on the table surface before you begin prepping samples
- With scrap paper from scale envelope facing you, scrape the adhered scales away from you toward the fold
- Gently tap scrap paper onto microscope slide

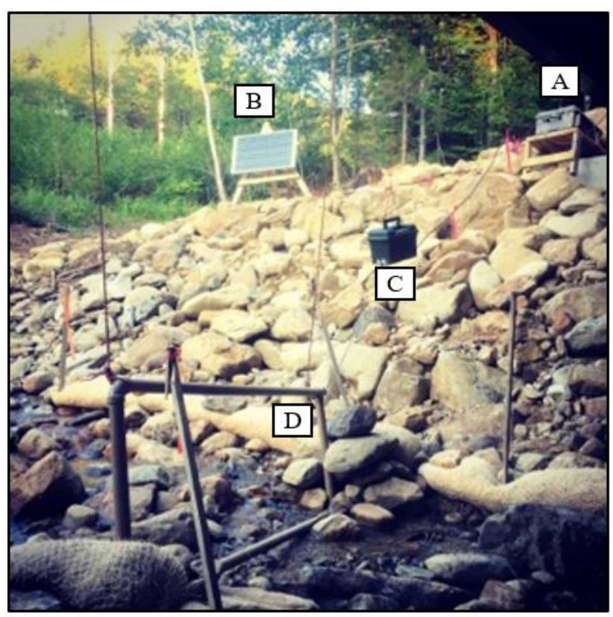


- Using knife and/or other sharp object, flatten and separate scales stuck together while moving scales from edges of the slide toward the center (inset right)
- Using pipette, place one drop of water on center of slide
- Place a second slide on top, ensuring water permeates through scales to surround them all
- Tape together one end of slides with ¾-full width masking tape and the other end with a thin strip
- Write relevant identifying information in pencil on the wide end of tape
- Wipe knife clean and work space black paper before plating the next sample

  Information regarding best microscope settings for scale aging:
  - Olympus BX53 Microscope
  - 10x magnification
  - On computer screen: using QCapture Suite PLUS program
  - 'Basic' tab:
    - Exposure: 93.3 ms
    - Gain: 1.2
    - Offset: -484
  - 'Advanced' tab: check 'preview' box for grayscale**PIT tag antennas**

Consult individuals trained use of antennas prior to attempts of setup, alteration and/or deployment. Directions listed below are to be used as a guide, not primary wiring and/or setup methods.





- Connecting batteries: in-line positive to positive (inset left, A), negative to negative using terminal connectors (inset left, B) to a deep cycle, 12 volt marine battery (inset left, C)
- From battery to HDX Single Duplex Reader (computer)(inset left, D): connect positive and negative terminals from a single battery to the connectors supplied with the Reader
- From solar panel arrestor to one marine battery: two strands of 14 gauge custom terminal connector, one positive and one negative (inset left, E)
- All wires are fed through waterproof outlet of sealed Pelican case (inset left, F), located above the stream (inset right, A)
- Entire system is powered by a solar panel, oriented south (inset right, B)
- Suspend Tuning box in an ammunition case above seasonal high-water level (inset right, C)
- From solar panel(s) to solar panel arrestor: two strands of 14 gauge wire, one positive and one negative
- From Reader to Tuning Box: one TwinAx cable
- From tuning box to antenna: two wires included in a loop formed through the antenna
- Seal where wires exit the antenna and enters/exits the ammunition box with silicone caulking
- Anchor antenna to stream bed with boulders so it remains stable but can break free during high flow events
- Anchor antenna either to a bridge (two vertical polyester ropes)(inset right, D) or from the antenna corner to a tree along the bank (using polyester rope, tied with slack horizontally)

Temperature logger datasheet

Temperature Probe Data Collection			
Site ID:	Date:	Time:	Crew:
Serial #:	Air: Water:	Status: deploy / remove	
Lat:	Long:	Condition: good / repair / replace	
Pic #:	Location description:		

Temperature Probe Data Collection			
Site ID:	Date:	Time:	Crew:
Serial #:	Air: Water:	Status: deploy / remove	
Lat:	Long:	Condition: good / repair / replace	
Pic #:	Location description:		

Temperature Probe Data Collection			
Site ID:	Date:	Time:	Crew:
Serial #:	Air: Water:	Status: deploy / remove	
Lat:	Long:	Condition: good / repair / replace	
Pic #:	Location description:		

Temperature Probe Data Collection			
Site ID:	Date:	Time:	Crew:
Serial #:	Air: Water:	Status: deploy / remove	
Lat:	Long:	Condition: good / repair / replace	
Pic #:	Location description:		

Instream Habitat Standard Operating Procedure
Rapid Habitat Assessment

Objective: To quantify the existing instream habitat, including instream wood, to determine if and approximately how much and type(s) of wood should be added to the subject stream for the benefit of aquatic species, specifically brook trout, and the retention of instream nutrients. This assessment methodology is specifically to determine the typical habitat conditions in and near a stream, but is also being used in research to determine the effect of habitat (including instream wood) on wild brook trout populations.

Standard Operating Procedure:

This is the basic habitat inventory for determining the quality and quantity of fish habitat, and to ascertain basic riparian and hydrologic condition.

Equipment needed:
- copy of survey protocol (waterproof paper)
- datasheets (waterproof paper)
- clipboard
- pencils
- stadia rod (or depth measuring tool)
- hand level or clinometer

Aquatic Habitat Units

This method classifies stream habitat into several categories such as pool, riffle, run/glide. Channel shape and scour patterns are key parameters for determining the boundaries of the habitat unit. The starting and ending points of a habitat unit are recognized by the breaks in slope (highest point) along the channel bottom. A new habitat unit is recognized whenever the length of a habitat exceeds its wetted channel width, or if it is a significant single habitat type (you are in a riffle habitat unit and come across a classic pool that is slightly shorter than its width).

POOL (P): Aquatic habitat in a stream in which the water surface slope is essentially 0% (flat) and that is typically (but not always) deeper and wider than aquatic habitats immediately above and below it.

RIFFLE (R): Shallow reaches of a stream (1-4% gradient, sometimes even steeper) characterized by small hydraulic jumps over rough bed material, causing ripples, waves, and eddies.

GLIDE (G): A transitional zone between pools and riffles, a run/glide has swift uniform (laminar) flow **without** surface agitation or waves. Maximum depth is 5% or less of the average stream width. We will consider runs and glides synonymous. Do not confuse glides with tails of pools.

CASCADE (C): An area of high turbulence and coarse substrate with a gradient at least 4%, and typically much steeper. Cascades are often very similar to waterfalls, except that cascades do not have water that drops absolutely vertically (the water still flows over substrate).

Field Measurement Procedures
Instructions for Habitat Survey Datasheet

Habitat Unit Type: Record the habitat unit type (P, R, G, C). Side channels should be surveyed as their own part of the stream.

Stream meter at downstream end of habitat unit: Record this value to the nearest 0.1 meter for those streams that are marked out by stream meter.

Habitat Unit Length: Measure to the nearest 0.1 meter

Typical Wetted Width: Measure the wetted width at 3 locations for each habitat unit to the nearest 0.1 feet. The exact locations that are measured can be chosen based on a visual assessment of the widths along the habitat unit and should include the variability of the widths of the unit.

Typical Bankfull Width: Measure the typical wetted width for each riffle habitat (or representative riffles) to the nearest 0.1 feet. The exact locations that are measured can be chosen based on a visual assessment of the widths along the habitat unit and should include the variability of the widths of the unit.

Typical and Maximum Depth: Measure typical and maximum water depth to the nearest 0.1 foot for each habitat unit. The exact location that is measured for the typical depth can be chosen based on a visual assessment of the depths along the habitat unit. The maximum depth should be measured at the deepest location.

Substrate: Enter the dominant and subdominant substrate type at each habitat unit. Use the substrate codes listed below:

SA = fines/silt/sand: < ¼ inch in diameter
BO = boulder: > 12 inches
G1 = small gravel: ¼ to 3.0 inches

BR = bedrock: large solid mass
G2 = large gravel: 3.1 to 6.0 inches
OR = wood and/or herbaceous
CO = cobble: 6.1 to 12 inches

Gradient: Measure gradient over a series of habitat units and specifically record the distance over which you measured the gradient.

Riparian Vegetation Characteristics: Determine the dominant COMMUNITY TYPE of the vegetation within the ~100 ft. riparian zone for each habitat unit on left and right banks looking upstream. In ***Comments,*** record the typical diameter at breast height (DBH) and range of DBH.

NH = Northern hardwoods (Sugar maple, Red maple, Beech, Yellow birch)
PB = Paper birch
QA = Aspen
SF = Spruce/fir
OP = Oak/Pine
EH = Eastern hemlock
WT = Wetland/Alder
OF = Old fields
AG = Agriculture
RL = Rock ledges, orchards, or “other”

Wood survey

These methods are to record the what type, where, size and what does the wood do to the channel/habitat.

1. **Wood type**: L=log; WJ=wood jam; LWJ = both a log and wood jam together.
2. **Habitat Unit Type**: P = Pool, R = Riffle, G = Glide (Run), C = Cascade
3. **Orientation**: Record the orientation of the log according to the diagram below:
4. **Log length**: record the TOTAL length of the log including any branches.
5. **Length in Water**: record the length of the log that is in the water.
6. **Length in bankfull**: record the log length that is within the bankfull channel. Note this could be somewhat of an estimate based on available bankfull indicators at the site.
7. **Log diameter**: record the diameter about the center of the log.
8. **Root diameter**: record the mean of the length of the longest axis and the length of the axis parallel to it to the nearest 0.1 feet.
9. **Wood jam length**: record the length from upstream to downstream.
10. **Wood jam width**: record the length from river right to river left (i.e., perpendicular to the flow).
11. **Pool forming**?: Record "us" for upstream, "ds" for downstream, "us/ds" for both upstream and downstream, and "n" for no pool formed by this wood. The pool must be immediately adjacent or under the subject wood.
12. **Retains ROM**?: "Rafted Organic Material" such as sticks and leaves. Record, "Y" for yes if there is some, "S" for significant amounts of ROM (subjective) or "N" for no.
13. **Retain LWM**?: record the number of pieces of Large Woody Material that is retained by the subject wood. The LWM does not necessarily have to be touching the subject wood.
14. **Sediment storage**?: record whether or not the wood is retaining sediment on the upstream side or lateral to the wood. Sediment accumulations downstream of the subject wood are not applicable.
15. **Stability**: R: Root system >1 foot of projecting roots; B: buried on either end of >50% of the log; P: pinned or pegged; U: unstable.
16. **Wood type** (C: coniferous; D: deciduous, or U: unknown). If you know the species, you can use YB for yellow birch, RM for red maple, SM for sugar maple, BF for balsam fir, AS for aspen, and other abbreviations as are needed – include a key to the abbreviations in the comments.
17. **Zone**: Record up to 4 categories (1, 2, 3, 4: see diagram).
18. **Decay Class**: Record one value from table.

    Note: if your study includes "Stream meter", record the stream meter for the most downstream end of the subject wood.

Habitat sampling protocol modifications and authorship

Instream Habitat Standard Operating Procedure: Rapid Habitat Assessment
I. Scott Decker ~2000, Program Supervisor, New Hampshire Fish & Game Department
II. Modified by John Magee (2017), Fish Habitat Biologist, New Hampshire Fish & Game Department
III. Further modified by Tyson R. Morrill (2017), MS Biology candidate, Plymouth State University

Modifications by T. Morrill:
- Multiple subsections and measurements were removed from protocol to align with sampling goals
- Addition of rafted organic material (ROM) measurement to include approximate length, width and height
- Additional classification of ROM as 'wet' or 'dry' determined by if the ROM is ≥ 50% residing in water (wet) or ≤ 50% residing in water (dry)
- Sub-habitat delineation: if two different habitats are located parallel within the stream, note the same start locations for each habitat and different approximate widths then make a comment noting different measuring style. The resulting combined width of both habitats will be used to calculate wetted with.
- Habitat unit length: to 0.1 meter
- Stream meter downstream end of habitat unit: 0.1 meter

Wood Survey
I. Schuett-Hames, D., A.E. Pleus, J. War, M. Fox and J. Light. 1999. TFW Monitoring Program method manual for the large woody debris survey. Prepared for the Washington State Dept. of Natural Resources under the Timber, Fish and Wildlife Agreement. TFW-AM9-99-004. DNR #106.
II. Modified by John Magee, Fish Habitat Biologist, New Hampshire Fish & Game Department

Modifications by J. Magee:
- When wood was added or if it is naturally recruited, the length of the wood in the water and in bankfull was measured whether or not the wood forms a pool or retains sediment and % in bankfull channel

Habitat assessment datasheet

	Instream Wood survey datasheet for PIT Tag research - Beebe River Watershed												Page #	
Project:					Survey Comments									
Stream:														
Date:														
Observer:														
Recorder:														
Measurer(s):										Flow sensor- Depth:				ft
Date of last precipitation:												Time:		

Hab. Type & hab. Number	Stream meter (ds/ start of habitat)	Habitat Length (to 0.01 m)	Habitat Width (to 0.01 m)	Habitat Average Widths (to 0.01 m)	Bank Full Width (to 0.01 m)	Typical Depth (to 0.01m)	Max Depth (to 0.01m)	Substrate (Dom)	Substrate (Subdom)	Gradient (%)	Riparian Veg. (Dom)	Riparian Veg. (Subdom)	ROM wet or dry AND length, width, height (cm)	Comments

Wood survey datasheet

	Instream Wood survey datasheet for PIT Tag research - Beebe River Watershed																			
Site			date			Staff											Page#			
Type (log (s), jam)	Hab. TYPE P,R,G,C,F and Hab. #	Stream meter (transect flag to flag)	ADDED OR NATURAL	Orientation	Log length (m)	length in water (m)	length in bankfull (m)	Log diameter (m) @ mid point	Wood jam length (m)(us to ds)	Wood jam width (m) (RR to RL)	Wood jam height (m)(in center	Pool forming? If yes, indicate us and/or ds	Retains ROM? N=none, P=present, S=significant	ROM wet or dry AND length, width, height (cm)	Log/jam sediment storage? (Y/N)	Log/jam retain LWM? (#), then measure ea.	Stability (see code sheet)	Wood type (see code sheet)	Zone(s) (see code sheet)	Decay class (see code sheet)

Morrill Multi-Reader

Supplies required:

- (1) Residential indoor/outdoor thermometer
- (1) 6 foot x 2 inch PVC pipe
- (2) 2 inch standard end caps
- Permanent marker
- Electrical tape
- (1) 3" carriage bolt
- (2) nuts fitting carriage bolt
- Cordless drill w/ drill bit fitting diameter of carriage bolt

I) Cut 2" diameter PVC to length at approximately 2m, secured with dry-fit end caps.

II) Write IN and OUT on the thermometer display switch so temperatures do not get switched in the field (Inset left). IN is temperature measured from the display unit itself (air) and OUT is measured from the end of the wire, attached to base of PVC pipe (water).

III) To attach thermometer display to the depth measurement staff, drill a hole through the staff at the diameter of the carriage bolt. The head of a carriage bolt must fit in the nail hook slot on the back of the display so it does not pull through the staff when pressure is placed o un the unit.

IV) When head of bolt is secure, pull bolt through PVC so a washer is snug against the back of thermometer unit. Tighten one nut down against other side of PVC and when snug, tighten the second nut against the first nut so they do not loosen. Extra thermometer cord is taped above typical submersion level.

V) Tape water measurement end of the thermostat cord to the base of the staff ABOVE the end cap (inset right). This prevents the tape and thermometer end from rubbing against rocks and becoming cut or broken.

VI) To mark depth on PVC staff, tape a piece of string along the staff then trace with permanent marker. Mark the depth in increments in cm using a meter tape and permanent marker. Helpful tip: mark increments of 10cm on one side of main line with larger hash marks, increments of 5cm with smaller hash marks on opposite side, and increments of 1cm with the smallest marks. This helps view the depth easier by having room to write in larger font.

Additional notes:
Chemical-based insect repellent will cause marker to wipe off the staff, so keep extra marker handy when in the field. Most cheap indoor/outdoor thermometers are meant to remain indoors, meaning they are not waterproof. It is recommended to have an extra thermometer and batteries available to easily replace in the case of moisture exposure. Taking temperature measurements on different days in the same stretch of stream can show fluctuations of temperature based on seasonal conditions and precipitation. This is prevented by having additional equipment available in case of equipment failure.

Canopy cover datasheets

Stream: Date: Staff:	
Precip. last 48 hrs:	Stream depth at yard stick:

Hold densiometer perpendicular to body at waist level with elbow touching body to ensure measurements are one forearm length from your body. Measure every 10m from center wetted width facing upstream. Calculate amount of canopy cover per cell using a rating of 1 (25%), 2 (50%), 3 (75%), 4 (100%) vegetation presence. Complete sum of entire grid, multiply by 1.05 = percent canopy cover. Record stream temp. at each canopy measurement and stream depth each day sampling occurs.

0.0

Temp.:_______ Sum of boxes: _______

10.0

Temp.:_______ Sum of boxes: _______

20.0

Temp.:_______ Sum of boxes: _______

30.0

Temp.:_______ Sum of boxes: _______

Stream: _____________ Date: ____________________ Staff: ___________________________

40.0

Temp.:________ Sum of boxes: ________

50.0

Temp.:________ Sum of boxes: ________

60.0

Temp.:________ Sum of boxes: ________

70.0

Temp.:________ Sum of boxes: ________

80.0

Temp.:________ Sum of boxes: ________

90.0

Temp.:________ Sum of boxes: ________

Stream: ____________ Date: __________________ Staff: ________________________

100.0

Temp.:_______ Sum of boxes: _______

110.0

Temp.:_______ Sum of boxes: _______

120.0

Temp.:_______ Sum of boxes: _______

130.0

Temp.:_______ Sum of boxes: _______

140.0

Temp.:_______ Sum of boxes: _______

150.0

Temp.:_______ Sum of boxes: _______

Stream: ____________ Date: __________________ Staff: ________________________

160.0

Temp.:_______ Sum of boxes: _______

170.0

Temp.:_______ Sum of boxes: _______

180.0

Temp.:_______ Sum of boxes: _______

190.0

Temp.:_______ Sum of boxes: _______

200.0

Temp.:_______ Sum of boxes: _______

0.0:	110.0:
10.0:	120.0:
20.0:	130.0:
30.0:	140.0:
40.0:	150.0:
50.0:	160.0:
60.0:	170.0:
70.0:	180.0:
80.0:	190.0:
90.0:	200.0:
100.0:	Average:

Electrofishing sampling datasheet

Beebe Tributaries - PIT Study Fish Datasheet

Date and Time:

Steam name:

Crew:

Efishing settings:
Volts:
Frequency:
Duty Cycle:

Page ____ of ______

	Run / Section	Species	Total Length (mm)	Weight (g)	PIT tag Observed = "Obs" or Applied = "App"	PIT tag #	Fin Clip APP	Fin Clip OBS	Scales taken?	Comments/Genetics #
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										

APPENDIX FIGURES

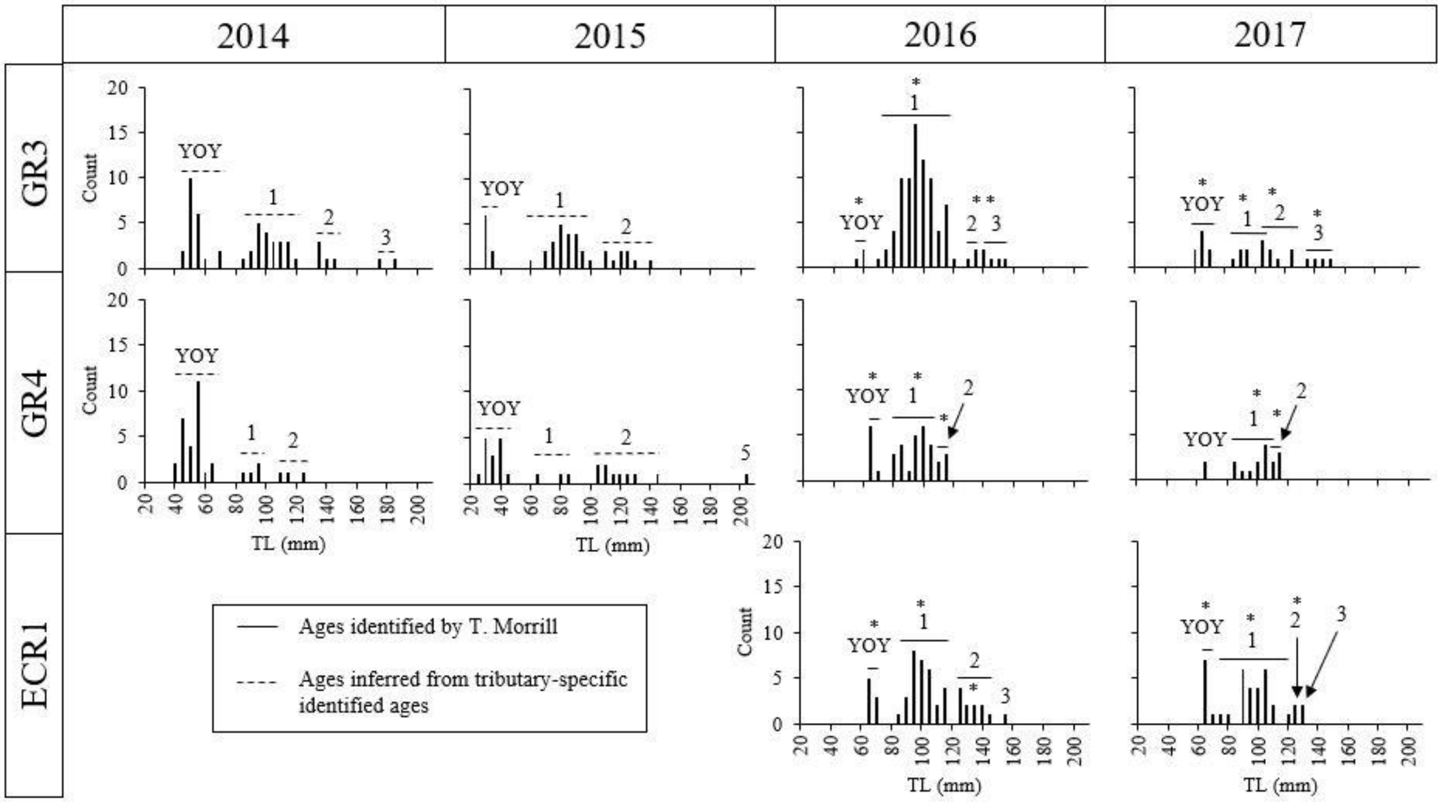


Fig. A2.1. Total length (TL) frequency and age classes of wild Brook Trout captured in focal tributaries of the Beebe River watershed from 2014-2017. Asterisks above age in 2016 and 2017 signify median TL. Counts reflect captures during July sampling for all years except 2015, which occurred in June. During 2014/2015, sampling was completed within future focal 200 m study reaches and in July 2016/2017 within complete reaches. Age was determined by scale annuli analysis for 2016 and 2017 and were inferred for 2014 and 2015 using stream-specific TL-age classes.

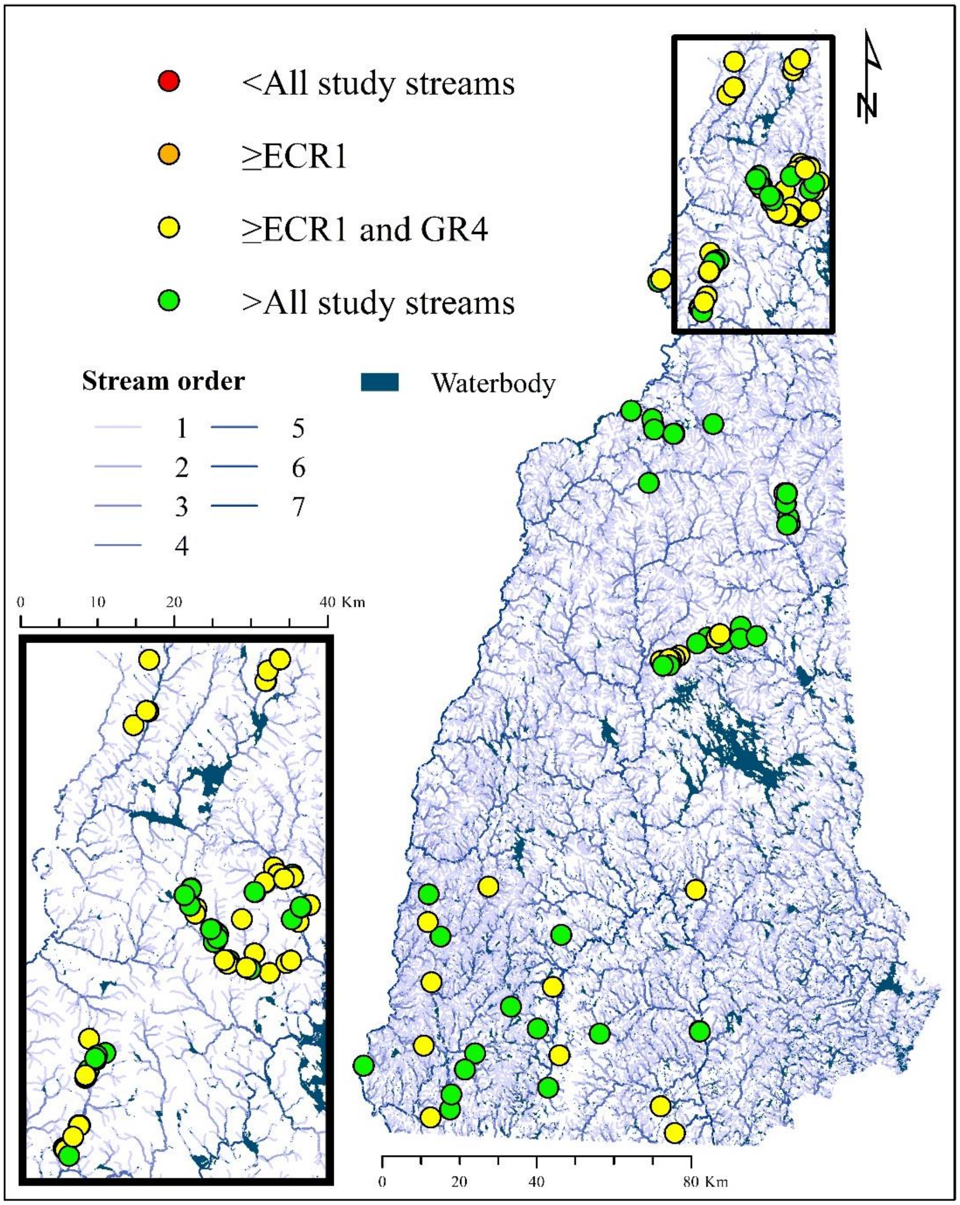


Fig. A2.2. Statewide wild Brook Trout population condition (lbs./fish) compared to populations in Beebe River watershed focal tributaries (NH Fish & Game Department, unpublished).

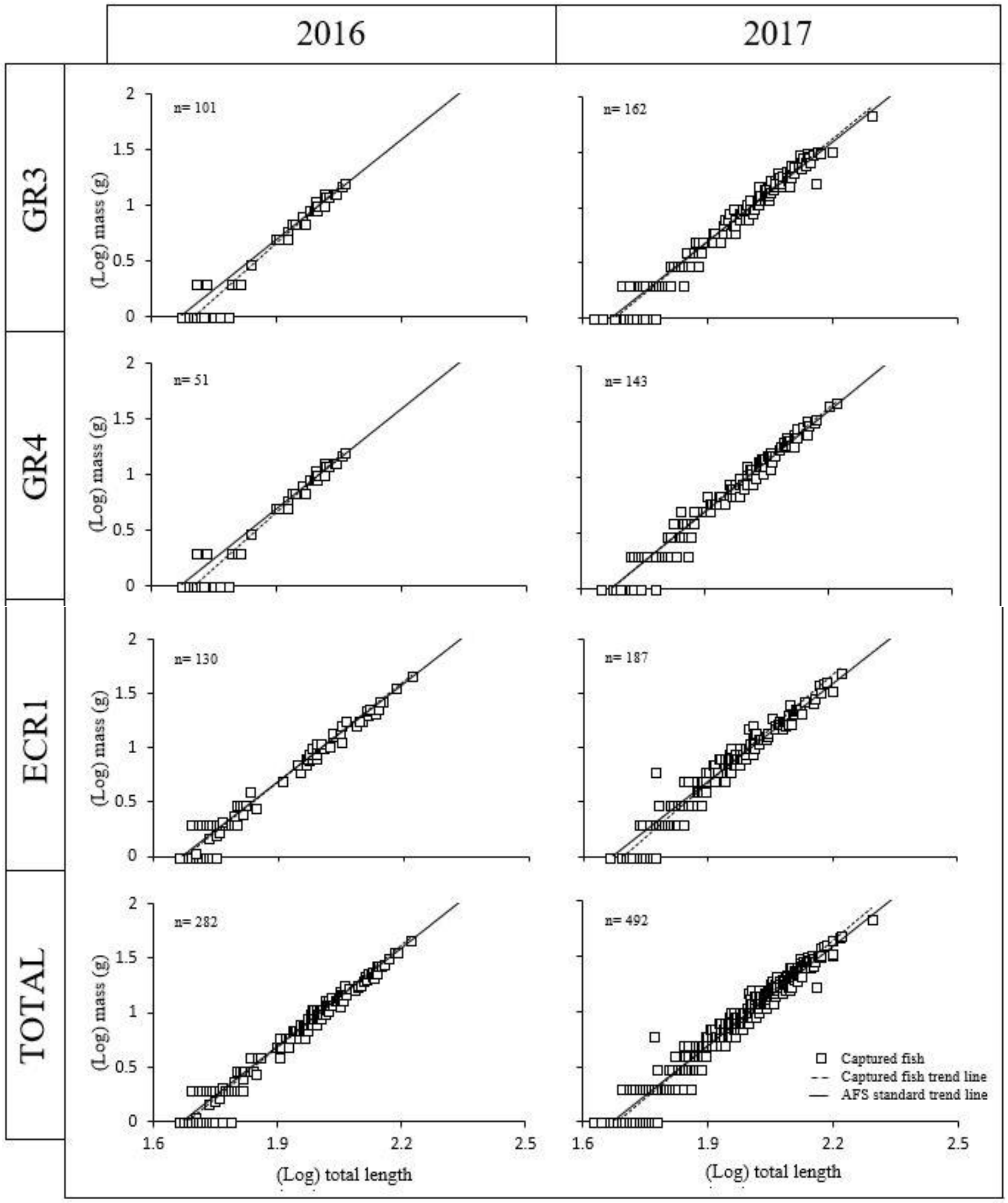


Fig A2.3. Log-transformed total length and mass relationship (individual condition) of wild Brook Trout captured in July 2016 and combined May, June, July, September 2017 within focal tributaries in the Beebe River watershed compared to AFS standard relationship (y = 2.9863x – 4.9743). All fish with mass recorded as >1g (n=88) were removed from dataset prior to analysis.

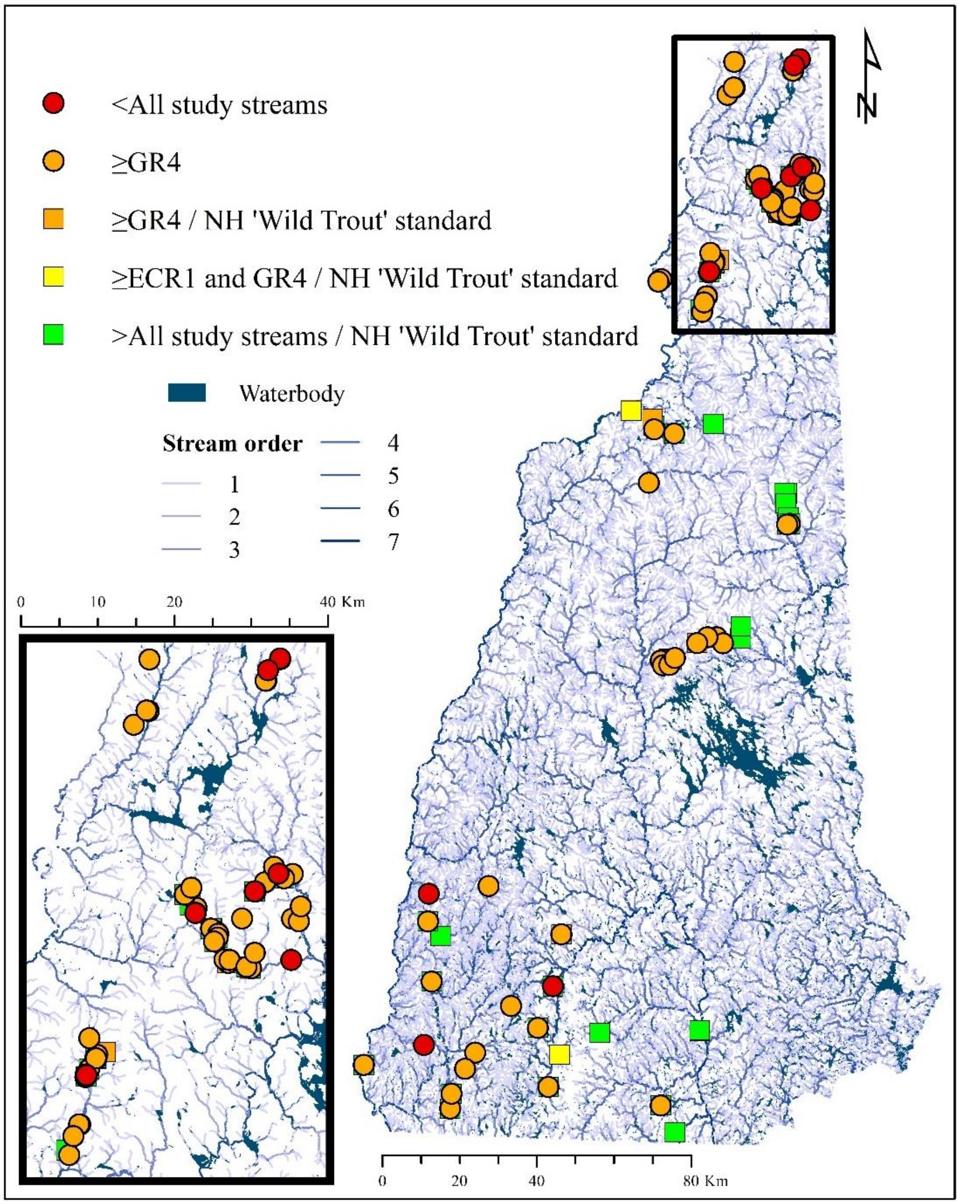


Fig. A2.4. Statewide Brook Trout biomass (lbs./acre) compared to populations in Beebe River focal tributaries while recognizing populations meeting the 13 lb./acre standard for NH 'Wild Trout' management (NH Fish & Game Department, unpublished).

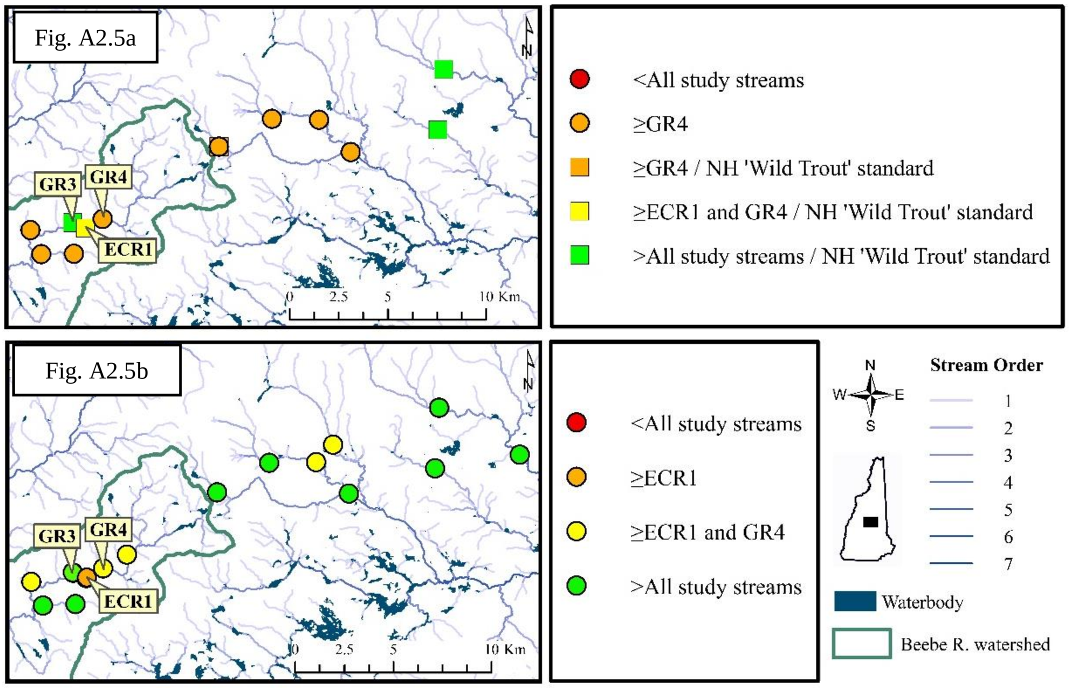


Fig. A2.5. Regional wild Brook Trout biomass (lbs./acre)(Fig. A2.5a) and population condition (lbs./fish) (Fig. A2.5b) compared to populations in Beebe River focal tributaries while recognizing populations meeting the 13 lb./acre standard for NH ‘Wild Trout’ management (NH Fish & Game Department unpublished).

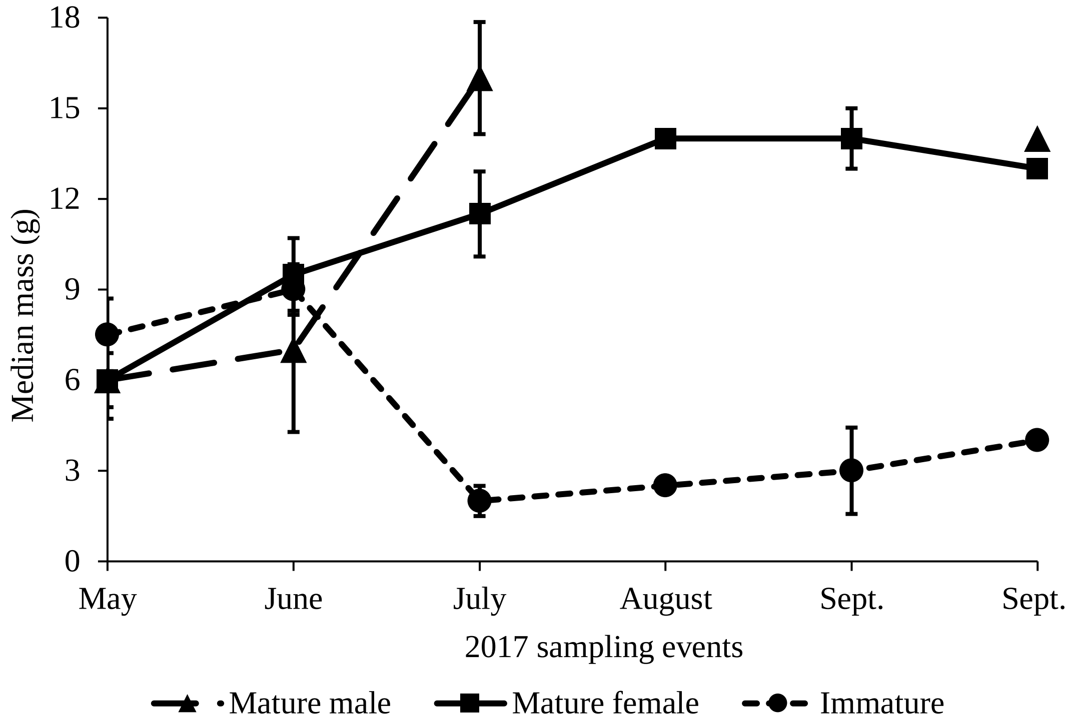


Fig. A1. Plots of median mass ±SE over time of sexually mature (adult) and immature (juvenile) Brook Trout across 2017 captured within focal tributaries of the Beebe River watershed. Sample sizes include total number of each subgroup including replicates: adult males (n=48), adult females (n=101) and juveniles (n=28). Multiple sampling events during September include results from recaptured fish during crossing dewatering and study area-wide sampling.

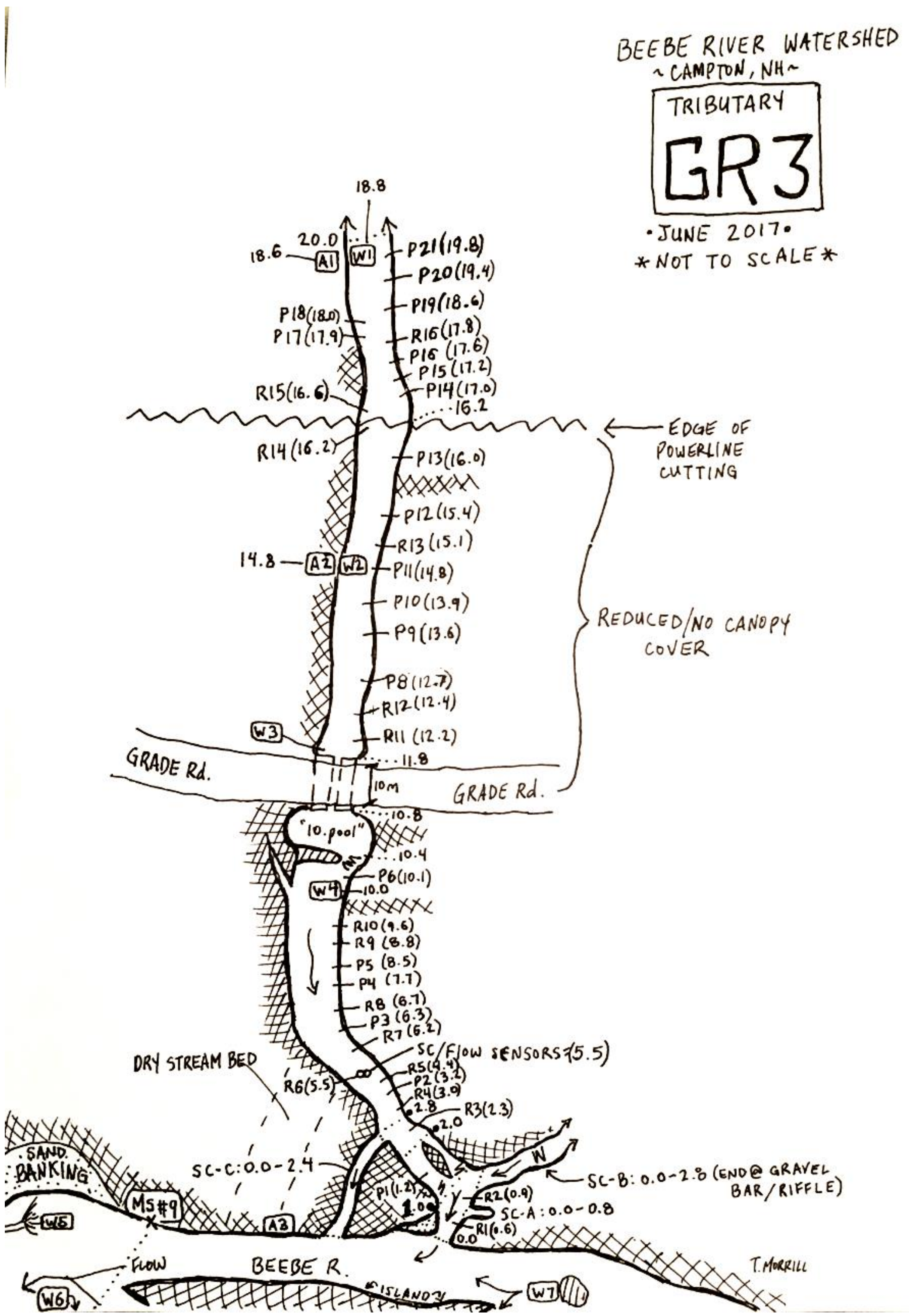


Fig. A2. Map of stream layout including sites and transects for electrofishing, macroinvertebrate sampling and temperature loggers within tributary GR3.

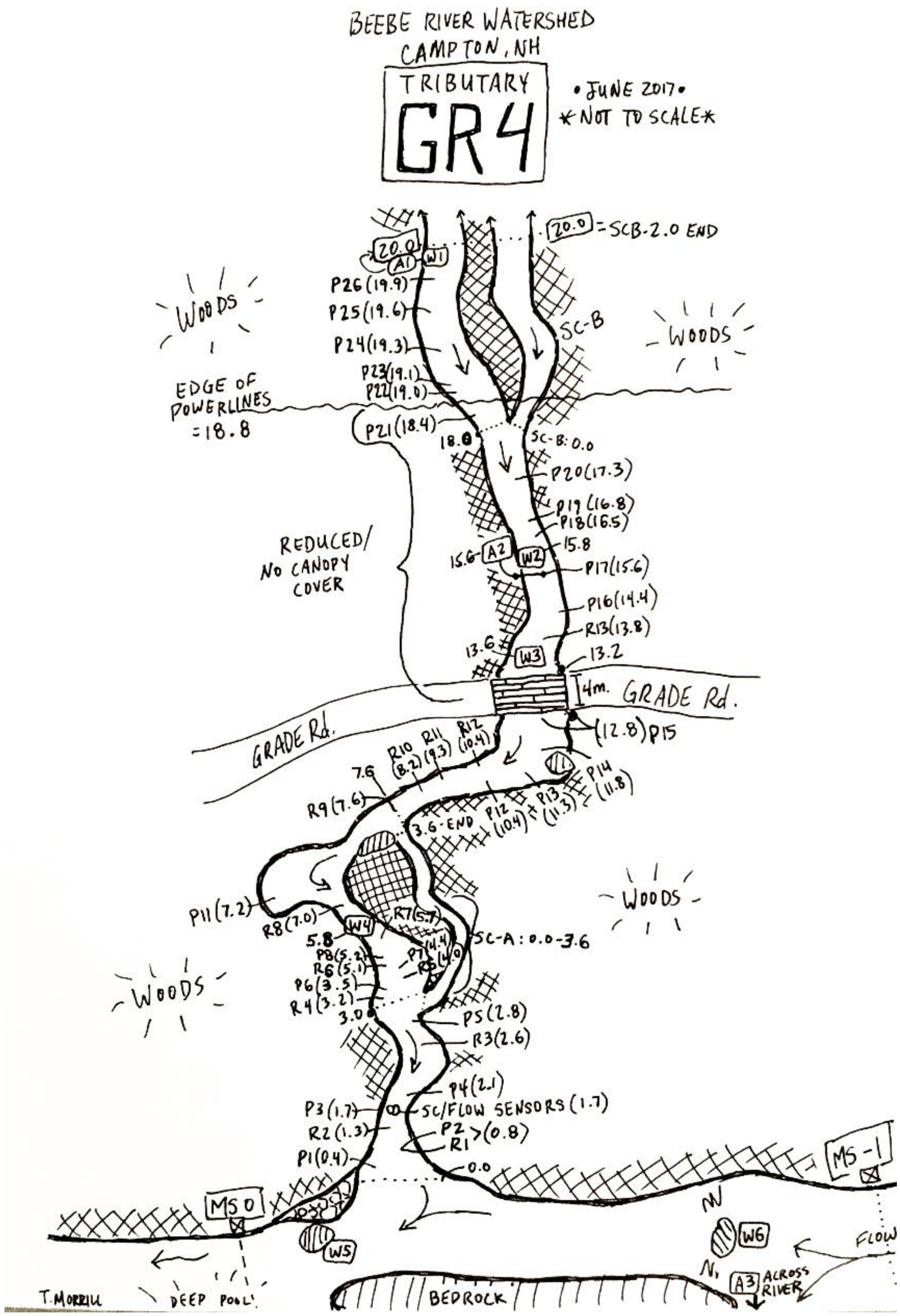


Fig. A3. Map of stream layout including sites and transects for electrofishing, macroinvertebrate sampling and temperature loggers within tributary GR4.

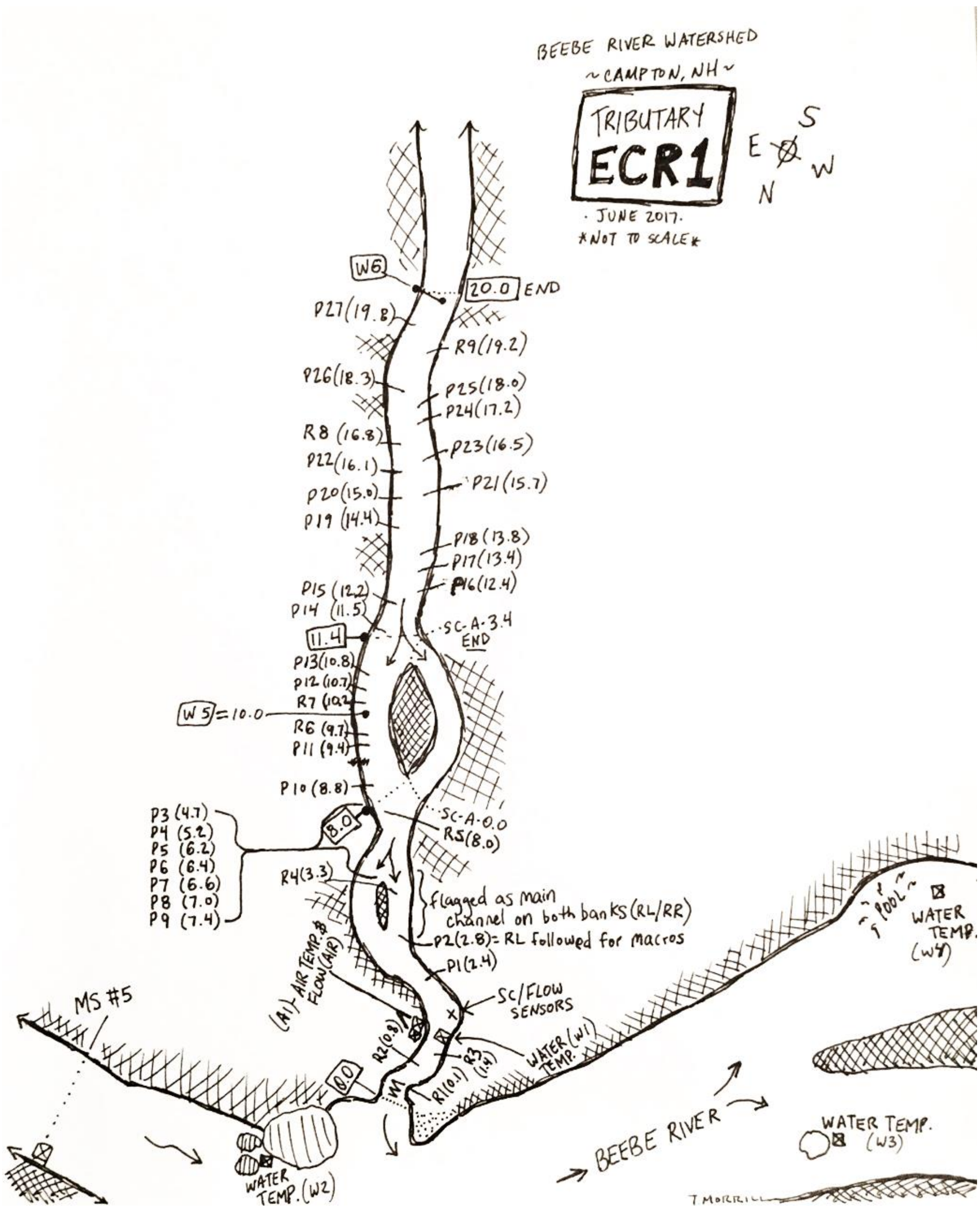


Fig. A4. Map of stream layout including sites and transects for electrofishing, macroinvertebrate sampling and temperature loggers within tributary ECR1.

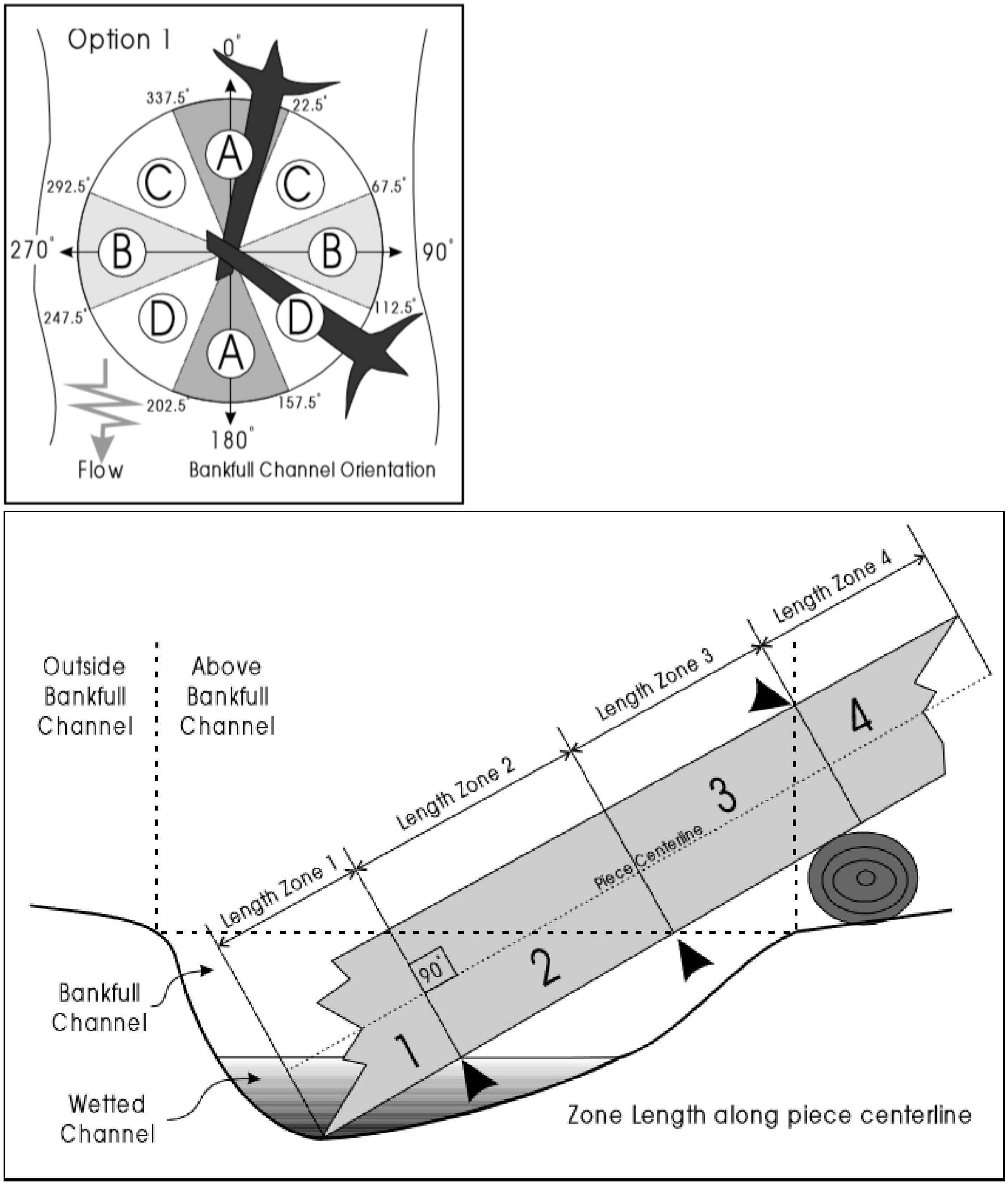


Fig. A5. Wood survey directional and orientation schematics

APPENDIX TABLES

Table A2.1. Metadata of sources used to assess hydrologic conditions located closest to focal study streams within the Beebe River watershed.

Data Type	Station Name	Station #	Latitude	Longitude	Location	Station location in reference to Beebe R. watershed outlet	Date data retrieved	Source
2015-2017 precipitation	Plymouth 1.6 NNE	NH-GR-11	43.760317	-71.688856	Plymouth State University: Boyd Science Center (Plymouth, NH, U.S.A.)	6.98km SSW	06/28/2018	cocorahs.org/ViewData/StationPrecipSummary.aspx
Historic precipitation	Plymouth	N / A	43.760317	-71.688856	Plymouth State University: Boyd Science Center (Plymouth, NH, U.S.A.)	6.98km SSW	06/28/2018	unh.edu/stateclimatologist/NHnormals.htm
Discharge	Pemigewasset River (Pemi. R.) at Plymouth, NH	01016500	43.759167	-71.686111	61m downstream of Route 175A crossing Pemi. R. on town line of Plymouth & Holderness, NH, U.S.A.	6.53km SSW	06/28/2018	waterdata.usgs.gov/nwis/wys_rpt?dv_ts_ids=64017&wys_water_yr=2017&site_no=01076500&agency_cd=USGS&adr_water_years=2006%2C2007%2C2008%2C2009%2C2010%2C2011%2C2012%2C2013%2C2014%2C2015%2C2016%2C2017&referred_module=
Groundwater	NH-CVW-315	431527071312401	43.831111	-71.6525	Off Beebe R. Road, 1.3km upstream of Pemi. R. confluence with the of Beebe R.	Within watershed	06/28/2018	nwis.waterdata.usgs.gov/nwis/inventory/?site_no=431527071312401&agency_cd=USGS

Table A2.2. Water temperature (°C) within each focal transect seven days prior to fish sampling within the Beebe River watershed. Recordings included daily max temperature and analysis of logger's temperatures reaching ≥20 were not cumulative.

			GR3	GR3 MS	GR4	GR4 MS	ECR1	ECR1 MS
		mean	17.44	21.72	17.18	22.75	17.41	22.5
July	2016	median	17.65	17.65	17.03	17.51	17.51	22.4
		days ≥20	0	7	0	7	0	7
		mean	10.87	10.70	11.02	12.62	9.89	10.14
October	2016	median	10.49	10.49	10.48	12.87	9.53	9.63
		days ≥20	0	0	0	0	0	0
		mean	14.81	18.72	15.52	19.8	14.23	20.92
June	2017	median	14.62	19.00	15.44	19.97	14.27	20.67
		days ≥20	0	3	0	4	0	4
		mean	14.88	17.99	15.73	20.2	15.19	19.5
July	2017	median	14.73	17.95	15.22	20.03	15.01	19.56
		days ≥20	0	1	0	4	0	2
		mean	14.94	17.62	16.19	17.27	15.76	18.57
September	2017	median	15.23	18.00	16.75	17.64	15.84	18.77
		days ≥20	0	0	0	0	0	0

Table A2.3. Compiled descriptive statistics of estimated Brook Trout capture probability (%) ±standard error (SE) in focal tributaries of the Beebe River watershed calculated using Trout Count Lite. Also included, age-specific count of untagged Age-1+ (Adult) fish captured within tributaries and the proportion (%) of these fish captured per sampling event. Ages not captured during sampling events are indicated by ‘0’ and empty cells represent no analysis available for the corresponding metrics.

		July 2016	October 2016	May 2017	June 2017		July 2017		September 2017	
		% captured / ±SE	# / % untagged captures	% captured / ±SE	% captured / ±SE	# / % untagged adult captures	% captured / ±SE	# / % untagged adult captures	% captured / ±SE	# / % untagged adult captures
GR3	YOY	81.82 / ±0.16		N / A	95.45 / ±0.11		100 / ±0.03		100 / ±0.08	
	≥Age-1	98.78 / ±0.04		26.88 / ±0.09	100 / ±0.05		100 / ±0.04		100 / ±0.08	
	Age-1					4 / 67%		2 / 40%		2 / 40%
	Age-2					2 / 11%		1 / 7%		0
	Age-3					2 / 67%		0		1 / 33%
	Total	96.12 / ±0.05	40 / 35%	26.88 / ±0.09	98.15 / ±0.06	8 / 29%	100 / ±0.02	3 / 14%	94.87 / ±0.09	3 / 17%
GR4	YOY	96.43 / ±0.09		N / A	93.33 / ±0.14		100 / ±0.00		97.14 / ±0.08	
	≥Age-1	100 / ±0.06		100 / ±0.05	100 / ±0.09		100 / ±0.05		100 / ±0.05	
	Age-1					5 / 63%		6 / 60%		4 / 20%
	Age-2					2 / 33%		2 / 22%		1 / 17%
	Age-3					1 / 100%		0		0
	Age-4					1 / 100%		0		0
	Total	96.43 / ±0.67	38 / 61%	100 / ±0.05	96 / ±0.1	9 / 60%	100 / ±0.02	8 / 82%	98.36 / ±0.05	5 / 37%
ECR1	YOY	89.92 / ±0.07		N / A	100 / ±0.13		100 / ±0.00		100 / ±0.06	
	≥Age-1	96.97 / ±0.08		76.32 / ±0.15	100 / ±0.06		100 / ±0.03		100 / ±0.06	
	Age-1					3 / 25%		6 / 27%		2 / 11%
	Age-2					0		1 / 11%		1 / 14%
	Age-3					0		2 / 100%		0
	Total	91.36 / ±0.06	51 / 69%	76.32 / ±0.15	76 / ±0.14	3 / 14%	100 / ±0.05	9 / 27%	98.15 / ±0.06	3 / 11%

Table A2.4. Population attributes of wild Brook Trout statewide and in Beebe River focal tributaries. Analysis includes statewide biomass in pounds/acre (n=225), condition in pounds/fish (n=255) and density in count of fish/acre (n=276) while ranking % is the percent of populations with larger values than each study stream.

	Biomass	Condition	Density
Statewide (mean / range)	16.78 / 0.61-125.49	0.03 / 0.01-0.26	17.03 / 4.01-147.19
GR3 (value / ranking %)	14.12 / 35.11%	0.03 / 34.51%	94.5 / 2.17%
GR4 (value / ranking %)	1.9 / 92.44%	0.01 / 94.9%	230.36 / 0.04%
ECR1 (value / ranking %)	13.93 / 36.44%	0.01 / 96.47%	239.61 / 0%

Table A2.5. Brook Trout biomass in focal tributaries of the Beebe River watershed compared to biomass standards initiating additional wild Brook Trout management across NH, VT and ME. Values compared to NH and ME standards represent biomass relative to state criteria above (bold) or below (-) criteria. VT standards display values either meeting a category's minimum range (bold) or a biomass value not meeting a category's minimum.

	NH standards			VT standards			ME standards		
	Lbs./acre			Lbs./acre			Fish/mile		
	GR3	GR4	ECR1	GR3	GR4	ECR1	GR3	GR4	ECR1
July 2016	**9.52**	-8.19	**3.18**	**W2**	W4 -15.19	W4 -3.82	-521.19	-899.38	-46.43
June 2017	**0.87**	-9.12	-0.91	W4 -6.13	W4 -16.12	W4 -7.91	-915.48	-1148.83	-947.66
July 2017	-10.43	-11.79	**0.30**	W4 -17.43	W4 -18.79	W4 -6.7	-883.29	-995.94	-810.87
Sept. 2017	-10.42	-10.22	-0.90	W4 -17.42	W4 -17.22	W4 -7.9	-1036.18	-859.15	-915.48

Table A3.1. Mean movement characteristics of potamodromous, age 1+ wild Brook Trout inhabiting 1st-3rd order streams across their native range. Studies include fish assemblages dominated by Brook Trout populations that have not been reintroduced or translocated, although the limited presence of introduced salmonids may occur in these systems. 'Months tracked' are calendar months and ± error measurements are standard error in Petty, Hansbarger, Huntsman & Mazik, 2012 while other studies report standard deviation. Superscript ' a ' denotes calculations completed with data provided in publications or supplemental material, and superscript ' b ' denotes a recapture rate provided for the entire study, not unique to individual monitoring events.

Reach length	Months tracked	Method	% recaptures / n fish monitored	Records per fish	Net	Cumulative	Home range	Author
5.6 km	5-6	Radio tag	80% / 16	18 ±3.61	337 ±406.1	1,137 ±1,045.5	637 ±647.7	Hartman & Nel Logan, 2010
5.6 km	3-5	Radio tag	100% / 20	19 ±1.67	27 ±189.4	777 ±1,004.3	301 ±369.6	Hartman & Nel Logan, 2010
4 km	6-8	Radio tag	100% / 8	2-11	41 ±37	133 ±30.4		Petty, Hansbarger, Huntsman & Mazik, 2012
4 km	9-11	Radio tag	100% / 16	2-11	194 ±129	202 ±129		Petty, Hansbarger, Huntsman & Mazik, 2012
4 km	6-8	Radio tag	80% / 4	2-11	158 ±106	175 ±121		Petty, Hansbarger, Huntsman & Mazik, 2012
5 km	9-01	Radio tag	100% / 10	[a]16 ±2	50 ±115	[a]803 ±745	[a]260 ±239	Mollenhauer, Wagner, Kepler & Sweka, 2013
5 km	9-01	Radio tag	100% / 6	[a]22 ±5	150 ±350	[a]3,286 ±2,792	[a]918 ±1,058	Mollenhauer, Wagner, Kepler & Sweka, 2013
	9-01	Radio tag	100% / 9	[a]9 ±4.53	20 ±40	[a]158 ±135.05	[a]73 ±79	Mollenhauer, Wagner, Kepler & Sweka, 2013
21.05 km	9-02	Radio tag	55	55		1,769 ± 2,194		Davis, Wagner & Bartron, 2015
330 m	3-5	PIT tag	[b]34% / 6	6	9 ±20.3			Anglin & Grossman, 2018
330 m	5-8	PIT tag	[b]34% / 5		11.4 ±13.2			Anglin & Grossman, 2018
330 m	8-5	PIT tag	[b]34% / 3		6 ±22.8			Anglin & Grossman, 2018

Table A1. Abundance of male and female wild Brook Trout based on visible gonad development during September 2017 sampling in focal tributaries within the Beebe River watershed. Sex determination was quantified by presence of milt or ovipositor visible supported by state biologist(s) expertise.

	Sex	Count	Percent
Age 1	Male	9	21 %
	Female	29	67 %
	Unknown	5	12 %
	Total	43	100 %
Age 2	Male	12	46 %
	Female	12	46 %
	Unknown	2	8 %
	Total	26	100 %
Age 3+	Male	4	36 %
	Female	6	55 %
	Unknown	1	9 %
	Total	11	100 %

Table A2. Large woody debris survey criteria & code sheet

Large Woody Debris Survey Criteria & Code Sheet

Criteria **Log** (L)
1. Dead - No chance of survival
2. Roots no longer support weight of log
3. Minimum 10 cm dia. FOR AT LEAST 2 m length
4. Enters Zone 1 or 2 (optional Zone 3)

Codes **Stability** (choose up to three or unstable)
- R Root system is attached to piece
- B Greater than 50% diameter buried at some point along length
- P Piece is pinned between vertical live or dead structures
- U Unstable (if NONE of the above)

Criteria **Rootwad** (R)
1. Dead & roots have detached from original location
2. Total length less than 2 m
3. Minimum 20 cm diameter where bole meets root collar
4. Enters Zone 1 or 2 (optional Zone 3)

Codes **Wood type** (choose one)
- C Conifer species (100% confident)
- D Deciduous species (100% confident)
- U Unknown species (Less than 100% confident)

Criteria **Debris Jam**
1. Minimum 10 qualifying pieces LWD touching or assoc. w/jam structure
2. Minimum 1 piece enters Zone 1 or 2 (optional Zone 3)

Codes **Pool forming function** (choose one)
- Y Piece associated with adjacent pool unit formation
- N No pool unit or it doesn't meet minimum Habitat criteria (estimated only)

Criteria **Zone system**
- Zone 1: Within wetted portion of channel
- Zone 2: From water surface to a line connecting the bankfull edges
- Zone 3: Directly above Zone 2 to infinity
- Zone 4: Outside of bankfull channel

Mean Seg BFW (m)	Min m^2	Min RPD	Mean Seg BFW (ft.)	Min ft.^{2}	Min RPD (feet/tenths)
< 2.5	0.5	0.10	< 8.2	5.4	0.33
2.5 - 5.0	1.0	0.20	8.2 - 16.4	10.8	0.66
5.0 - 10	2.0	0.25	16.4 - 32.8	21.5	0.82
10 - 15	3.0	0.30	32.8 - 49.2	32.3	0.98
15 - 20	4.0	0.35	49.2 - 65.6	43.1	1.15
> 20	5.0	0.40	=/> 65.6	53.8	1.31

Lumpers & Splitters

- > Green leaves: do not count as part of core data
- > Nurse logs count unless root systems of live growth provide piece stability
- > Floaters can be part of, but CANNOT *make* a Jam
- > Disregard branches
- > Forks: measure the one with most bankfull influence and/or largest diameter or length - disregard other
- > Never assume what you can't confirm by measurement
- > Ea. piece has only one total length or volume - inaccurate lengths = inaccurate volumes
- > Minimum 0.10 m piece length to call in a zone
- > Discharge measurements are required when doing a LWD survey

Metric to Feet Conversion		
	10 cm = 0.1 m = 4 inches	2 m = 6.6 feet
	20 cm = 0.2 m = 8 inches	
	50 cm = 0.5 m = 20 inches	

Meter/Feet conversion: meters x 3.281 = feet

Table A3. Wood survey declass class criteria

Decay Class	Bark	Limbs	Surface Texture	Center
1	Intact	Present	Firm	Solid
2	Intact	Absent	Firm	Solid
3	Loose or absent	Absent	Firm	Solid
4	Absent	Absent	Slightly rotted	Solid
5	Absent	Absent	Extensively rotted	Solid
6	Absent	Absent	Completely Rotted	Solid
7	Absent	Absent	Completely Rotted	Rotted