HOW DOES AQUATIC INVERTEBRATE COMMUNITY STRUCTURE AND ABUNDANCE RELATE TO BROOK TROUT (*SALVELINUS FONTINALIS*) HABITAT QUALITY?

By

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ABSTRACT

Sixty-seven streams located south-west of Lake Nipigon, Ontario, were sampled for brook trout and macroinvertebrate orders. Sixteen orders were used to determine if their community structure significantly changed in different brook trout habitat and different brook trout densities (no brook trout, one to fifteen brook trout or more than fifteen brook trout). Though the orders *Odonata*, *Oligochaeta*, *Trichoptera*, *Chironomidae*, *Nematoda*, *Simuliidae*, *Gastropoda* and *Plecypoda* varied in response to brook trout habitat quality and abundance, the community structures did not change significantly. Pollutant sensitive orders, *Trichoptera*, *Ephemeroptera*, and *Plecoptera* that are used as indicators and share similar habitat to brook trout did not significantly change in response to brook trout densities. Future direction may involve identifying macroinvertebrates to family or genus level to detect community changes, or analyzing further data such as invertebrate biomass, diversity or richness in these streams.

Keywords

Aquatic macroinvertebrate community structure, brook trout, EPT index, Groundwater, habitat quality, predator and prey relationships, taxa abundance, taxa biomass, taxa diversity, stream health.

ACKNOWLEDGEMENTS

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INTRODUCTION

Streams have environmental and anthropogenic values (Meyer 1997). Environmental values include healthy aquatic ecosystems that are home to many organisms such as semi-aquatic mammals (Lacoul and Freedman 2006), like beavers, macroinvertebrates, and fish (Lacoul and Freedman 2006). Streams are also significant for fish spawning (Schlosser 1991).

Stream health and quality is reflected by an ecosystem that is sustainable and able to support and maintain ecological function over time while also serving human values (Meyer 1997). Stream health is determined by assessing for bacteria levels and pollution, which lowers the water quality (Meng *et al.* 2009). Anthropogenic values include recreation such as fishing, swimming, kayaking, provision of drinking water, (Gossling *et al.* 2012), education, and scientific study. If water quality becomes poor, important habitat and stream values can be lost (Able e*t al.* 1999).

Water quality is often at risk from agriculture, industry (Olness 1995) and recreational runoff (Green 1998). Natural ecosystem processes in streams may be altered by poor water quality, such as different usage by organisms, negative effects on reproduction, and die-off (Karr and Dudley 1981). If pollution worsens, habitat quality will degrade which may cause the entire ecosystem or food web of a stream to collapse (Cao *et al.* 2018), by decreasing prey numbers drastically so there is not enough to support predators, which die off as well (Karr and Dudley 1981). Conversely, wetlands, high groundwater discharge areas, and tributaries may help improve the quality of the water (Mitsch and Gosselink 2000).

Brook trout (*Salvelinus fontinalis*) are sensitive to pollution, degrading environmental conditions or altered habitat and are indicators for stream health (Smith and Sklarew 2013). When water is polluted, it can cause aquatic macroinvertebrates to die off, causing the brook trout to move to other streams (Smith and Sklarew 2013). Water that becomes contaminated from sources like sewage may cause brook trout to leave the area, as they depend on sight to hunt (Smith and Sklarew 2013). Therefore, the presence of brook trout can be used to indicate a healthy stream and good water quality (Smith and Sklarew 2013).

Brook trout prefer cold clear streams with cover but can occupy cold lakes (Kerr and Grant 2000). Adults spawn preferentially in streams during the day in the fall, usually in areas where there is groundwater discharge (Kerr and Grant 2000). Brook trout lay up to six-hundred eggs, which hatch around March when larvae macroinvertebrates are available to feed on (Kerr and Grant 2000). The temperature of the water and precipitation play a secondary role in where and when brook trout spawn (Kerr and Grant 2000).

Brook trout usually select spawning areas in relation to groundwater discharge (Curry and Noakes 1995). Groundwater discharge can be a source of nutrients for rivers and streams (Maavara *et al.* 2018) and is an important factor in brook trout spawning sites. Groundwater occurs beneath the water table, where water is trapped in sediment (Kornelson and Coulibaly 2014). Groundwater discharge is determined by the water table, surface water levels, and rate of flow of the area (Kornelson and Coulibaly 2014). Groundwater can purify water, maintain ecosystem health, and mitigate erosion (Kornelson and Coulibaly 2014).

Brook trout frequently choose spawning areas where there is groundwater seepage, often near headwaters (Witzel and Maccrimmon 1983). Upwelling groundwater also provides thermal conditions and oxygen needed for incubation in spawning areas (Nuhfer *et al.* 2015). Upwelling groundwater flow is also important for protecting spawning areas from cold surface waters in winter (Nuhfer *et al.* 2015). Upwelling groundwater also produces flowing water, chemical, and temperature gradients in surface waters that could be used by brook trout to identify ideal spawning sites (Curry and Noakes 1995).

Groundwater is warmer than surface waters during the winter (Nuhfer *et al.* 2015), and during these times, brook trout often forms aggregations in pools close to groundwater discharge (Cunjak and Power 1986). These groups of brook trout are increasingly common when water temperature decreases. Cunjak and Power (1986) reported that the temperature was up to six degrees warmer in these groundwater discharge areas compared to the rest of the stream. Brook trout can also be found in groups in summer using groundwater seepage to escape warmer streams (Cunjak and Power 1986).

In areas where groundwater flow is low, temperature becomes the main factor in determining brook trout population responses to habitat (Nuhfer *et al.* 2015). Brook trout populations can be self-sustaining in the presence of distinct groundwater discharge with spawning occurring in these areas (Curry and Noakes 1995). Brook trout and groundwater relationships can be used for management to stress the importance of groundwater hydrology protection (Curry and Noakes 1995).

Groundwater contains dissolved organic matter, which is transferred into streams when groundwater is discharged (Cooney and Simon 2009). Groundwater plays an important part in trophic level interactions (Cooney and Simon 2009). Aquatic invertebrate densities are often higher below a depth of ten centimeters (Hynes 1983). These high densities are often in areas where there is access to groundwater discharge and therefore dissolved organic matter that invertebrates can feed on (Cooney and Simon 2009). Aquatic invertebrates may also be found in these areas where they feed on microbial, which in turn feed on dissolved organic material (Cooney and Simon 2009).

Groundwater and surface interactions can influence the aquatic invertebrate abundance, richness and benthic productivity and high groundwater discharge sites can have up to 35% greater invertebrate abundance than sites where there is low groundwater discharge (Hunt *et al.* 2006). Sites with weak groundwater discharges were found to have up to 27% aquatic invertebrate abundance and 19% greater richness than sites that had only one groundwater discharge source (Hunt *et al.* 2006). Groundwater can increase algae growth, which can increase the numbers algae-feeding macroinvertebrates in these streams (Hunt *et al.* 2006).

Groundwater can also affect the riparian vegetation, which in turn provides resources and creates habitat (Swanson 1980). This vegetation helps determine what type of invertebrate feeding groups are found in certain streams and also the structure of the invertebrate community of the entire stream (Swanson 1980).

Brook trout are opportunistic predators, consuming a variety of invertebrate larvae, small fish, and crustaceans (Kerr and Grant 2000). Aquatic macroinvertebrates are important to trout (Kerr and Grant 2000). Mayflies (*Ephemeroptera*), stoneflies

(*Plecoptera*), caddisflies (*Trichoptera*) and other immature flies make up most of the brook trout diet and occupy the similar habitat of cold, clean streams (Allan 1981).

Aquatic macroinvertebrates are organisms that spend at least part of their life cycle in water and include *Hexapoda* (insects), *Gastropoda* (snails), phylum Annelida (leeches and worms), mussels, clams and *Crustacea* (crayfish and fairy shrimp). Most live on the bottom of lakes, rivers, and wetlands (Thorp and Covich 2001). Aquatic macroinvertebrates are usually found in the sediment, in vegetation or on rocks (Thorp and Covich 2001). They are important to the food chain of aquatic environments, playing an important role in processing and cycling of nutrients (Thorp and Covich 2001) as well as being a food source for aquatic animals. There are approximately 10,000 species of aquatic macroinvertebrates in stream systems in Ontario (Cummins and Merritt 2008).

Some aquatic macroinvertebrates spend their entire lives in water, such as crayfish and leeches (Contreras-Ramos 2010). Aquatic macroinvertebrates may either have complete or incomplete metamorphosis (Contreras-Ramos 2010). Incomplete metamorphosis has three stages: the egg stage, a nymph stage, and the adult stage (Contreras-Ramos 2010). Aquatic macroinvertebrates that go through incomplete metamorphosis include dragonflies, damselflies, mayflies, and stoneflies (Contreras-Ramos 2010).

Complete metamorphosis includes four distinct stages: the egg stage, the larval stage, the pupal stage, and the adult stage (Contreras-Ramos 2010). Most aquatic macroinvertebrates go through complete metamorphosis such as many types of flies (Contreras-Ramos 2010). Larvae of some species can spend up to five years in streams, then enter the pupal stage where the macroinvertebrates develop wings and structures for mating and reproduction (Contreras-Ramos 2010).

In some species, the pupal stage is completed on land, but others transfer to terrestrial life in their adult stage (Contreras-Ramos 2010). In many cases, the insects are adults for a very short time (Brittain and Sartori 2009). Many mayflies live in streams for up to several years but have a terrestrial adult phase of a few days (Brittain and Sartori 2009). Terrestrial adults' mate and lay their eggs in or near water so the cycle can continue (Contreras-Ramos 2010).

Aquatic macroinvertebrates that spend their whole lives in the water, such as water beetles, usually live for a couple of years while some species can live up to ten years, such as crayfish and some leeches (Ahmed *et al*. 2015). Leeches lay eggs in cocoons, which hatch into juvenile leeches that grow into adults who look identical to those in the juvenile stage (Ahmed *et al.* 2015). Many species of leeches die after reproduction (Ahmed *et al.* 2015). Aquatic worms have a lifespan between a couple of weeks to several years and can reproduce sexually or asexually (Edwards 1996). Worms have a similar lifecycle to leeches, where their eggs are laid in cocoons and hatch as juveniles (Edwards 1996).

Aquatic macroinvertebrates have different feeding guilds, including shredders, scrapers, collectors, filterers and predators (Goodyear and Mcneill 1999). Shredders, common of some crustacean species, break down plant material that has fallen into the streams and are found in areas of streams where there is a canopy cover (Goodyear and Mcneill 1999). Scrapers, a common feeding method of snails, scrape algae off rocks and are found in areas of streams that obtain enough light that causes algae to grow

(Goodyear and Mcneill 1999). Collectors like mayflies gather food such as algae, bacteria, and plants that are at the bottom of the stream (Goodyear and Mcneill 1999). Filterers like clams and some species of crayfish feed on suspended matter and bacteria (Goodyear and Mcneill 1999). Predators, such as stoneflies, dragonflies, and beetles, eat live organisms and can be found in different habitats in the stream (Goodyear and Mcneill 1999).

Aquatic macroinvertebrates have different habitat requirements. Habitat and physicochemical variables that aquatic invertebrates depend on are canopy cover, stream size and depth, oxygen levels, woody debris, and organic matter, substratum sediment, clarity, level of pollution, pH levels, temperature and flow speed, and slopes of the stream banks (LeCraw and Mackereth 2012). Aquatic macroinvertebrates usually prefer pools and riffles, where temperature and organic matter availability are primary factors for habitat selection (LeCraw and Mackereth 2012).

Aquatic macroinvertebrates serve as a biological indicator for stream health; identify cumulative environmental impacts and environmental changes (Joao *et al.* 2012). Macroinvertebrates are good indicators because they have little mobility, are abundant, spend a significant amount of time in streams, are primary food sources, and are easy to sample (Jao *et al.* 2012). Macroinvertebrates are affected by many environmental factors, such as sediment composition and quality, habitat loss, pollution, water quality, and hydrology (Joao *et al.* 2012).

Many macroinvertebrates are sensitive to changes in pH, dissolved oxygen, temperature, salinity, turbidity, and other changes in their habitat (Selvanayagam 2016). Pollution-sensitive species such as mayflies, stoneflies, and caddisflies require higher dissolved oxygen levels, neutral pH, and cold water (Chadde 2005). When present in large numbers, these macroinvertebrates suggest the stream is in good condition (Hodkinson and Jackson 2005).

Somewhat pollution tolerant species include dragonflies, crayfish, and damselflies (Chadde 2005). Pollution tolerant species can tolerate low oxygen; lower or higher pH and warmer water and include aquatic worms, leeches, blackfly larvae, and midge larvae (Chadde 2005). An abundance of these organisms suggests that environmental conditions have deteriorated (Hodkinson and Jackson 2005).

By looking at aquatic invertebrate abundance and distribution, knowledge of the water quality and water health can be gained (Hodkinson and Jackson 2005). Nematodes and caddisflies can be useful as indicators for heavy metals and toxicants (Fenske and Gunter 2001). Heavy metals can alter nematode behavior, migration activity, and numbers that migrate (Fenske and Gunter 2001). Dragonflies can be used to detect pesticides in aquatic ecosystems due to their long lifecycles, as dragonfly nymphs cannot recover their abundance as quickly as other larvae (Takamura *et al*. 1991).

Aquatic macroinvertebrates populations can be affected by many disturbances. Pesticides, pollution, deforestation, soil erosion, natural disasters such as floods and artificial stream modification are some problems that can change macroinvertebrate community structure (Hussain 2012). This can cause brook trout populations to decrease because the habitat becomes unsuitable, especially if there are no close-by suitable habitats for these fish to move to (Hussain 2012). Examining the effects that disturbances have on the macroinvertebrates populations and their responses can be used to determine the health of the stream and water quality (Hussain 2012). Factors such as aquatic macroinvertebrates tolerance to these changes can help determine brook trout survivability in different streams (Hussain 2012).

The EPT (*Ephemeroptera*, *Plecoptera,* and *Trichoptera*) index is based on the concept that higher abundance of species in these orders is indicative of higher water quality. *Ephemeroptera Plecoptera* and *Trichoptera* are common orders found in streams and rivers (Hamid and Rawi 2017). These orders are intolerant to pollution and therefore make good indicators of water quality (Hamid and Rawi 2017). Any habitat changes, pollutants and contaminants can cause observable effects in the life cycle of aquatic invertebrates both in the short-term and long-term (Hamid and Rawi 2017). Anthropogenic disturbances near streams can affect diversity and abundance of the EPT orders (Hamid and Rawi 2017). Stoneflies are the most sensitive to changes in water quality due to low motility (Hamid and Rawi 2017).

The EPT index predicts that high-quality waters are expected to have higher species richness while polluted water will have lower species richness (Hamid and Rawi 2017). This is because the pollution intolerant EPT orders will be in high-quality water and not in polluted waters (Hamid and Rawi 2017). If these orders are present, then the habitat characteristics are within tolerance limits of these species (Hamid and Rawi 2017).

The EPT index can be important to assess the effects of spills or runoff and the amount of damage that is caused to rivers and streams (Forrester 1992). Brook trout abundance is predicted to be higher when there are higher aquatic macroinvertebrate diversity and abundance. Brook trout are predicted to have higher numbers in streams where there are high numbers of EPT orders found.

The predator-prey relationship between the brook trout and aquatic invertebrates, especially those of the EPT index, should mean that the brook trout will be where their prey is available. Predator-prey relationships are more complex than this, however, and consumption behavior and presence in areas varies (Forrester 1992). Consumption behavior and occupancy of habitats can differ with prey density, abundance, distribution, and with the preferences of the trout (Forrester 1992).

OBJECTIVE

The objective of this thesis is to determine if there is a difference in invertebrate community structure between streams with three abundance classes of brook trout: no brook trout, low abundance of brook trout and high abundance of brook trout. The purpose of this study was to examine invertebrate taxa richness, abundance, and community structure for differences relating to brook trout abundance and stream sizes. A relationship between brook trout and the EPT orders will also be examined. Since the EPT orders are popular prey of brook trout, and they share similar habitats, it is hypothesized that the EPT orders can be used as an indicator for the presence of brook trout. Since the EPT orders are popular prey of brook trout, and they share similar habitats, it is hypothesized that the EPT abundance can be used as an indicator for the presence of brook trout and that EPT abundance will be positively related to brook trout abundance. It is hypothesized that there will be differences in macroinvertebrate composition, abundance, and richness, in different brook trout abundance classes based on different preferable stream characteristics and predation. Community composition will be examined to determine what differences there are among streams differing in brook trout abundance and stream size.

MATERIALS AND METHODS

Sixty-seven cold water stream systems were sampled South-west of Lake Nipigon (figure 1). Sampling took place between 1997 and 2003 in May through August. These streams were divided into four different size classes, depending on catchment size: 1-3, 5-10, 30-50 and 100+ sq. Km. There is no reported agriculture runoff or significant pollution, though there is forest management occurring in these areas.

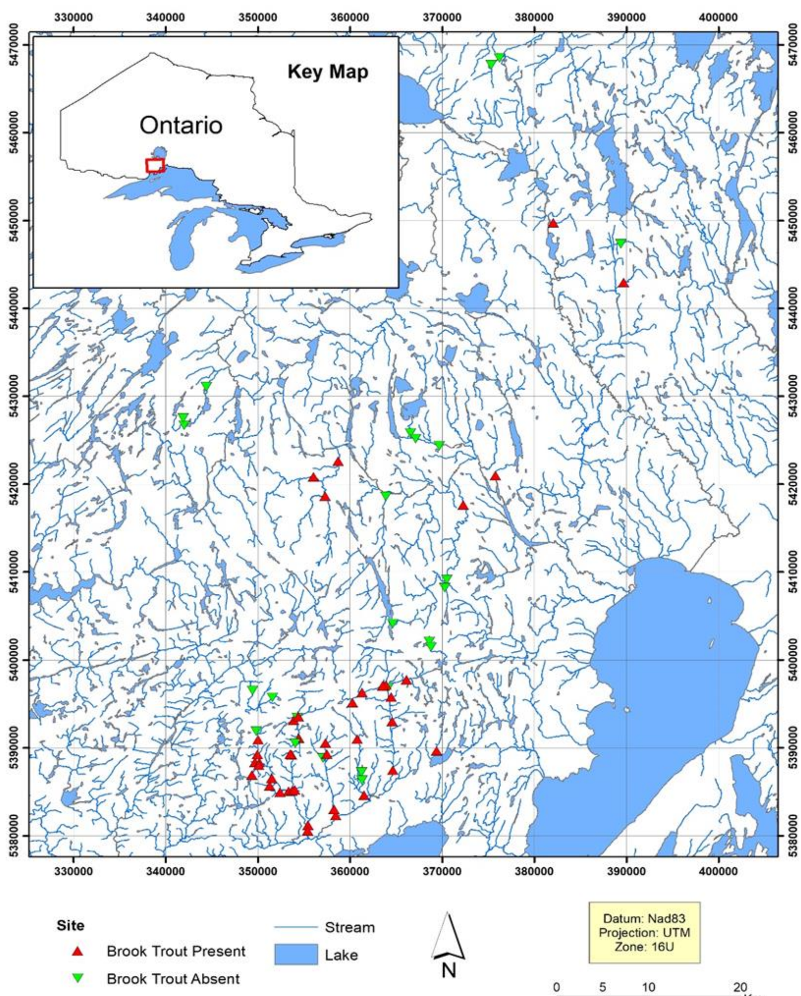

Figure 1. Location of the sixty-seven sampled streams.

All the streams, brook trout, and invertebrates were measured and caught following the Stream Survey Data Collection Manual (2011). This manual was developed by the Centre for Northern Forest Ecosystem Research for the Comparative Aquatic Effects Program.

Brook trout were sampled by single pass electrofishing. A fifteen to thirty-foot blocker net was placed across the streams to catch shocked fish that weren't originally caught after the original shock. Each brook trout had its weight and total length recorded, then it was released back into the stream. Streams were divided based on brook trout abundance: no brook trout present, 1-15 brook trout present, 15+ brook trout present. These abundance classes were created based on a catch per unit effort, which indirectly measures the abundance of brook trout; any change in this measurement can mean that brook trout abundance has changed.

Macroinvertebrates were caught using 30-centimeter sample squares; a D-net was placed downstream to collect the dislodged invertebrates. They were carried to shore and placed into a dishpan, then identified to order level though lab processing. Twenty-seven macroinvertebrate orders were sampled in these streams, but only sixteen significant aquatic macroinvertebrate orders were present and therefore used to analyze community structure changes.

The null hypotheses tested in the study were:

1. There is no difference in invertebrate taxa richness among the streams in the three brook trout abundance classes. That there is no difference in the taxa richness concerning four stream size classes. These hypotheses were tested using a two-way

ANOVA with brook trout abundance class as a fixed effect and stream size class as a random effect. The results can be seen in Figure 2, 3, 4, and Table 1.

2. That there is no difference in the EPT orders for the three brook trout classes. There are no significant differences in the *Trichoptera, Ephemeroptera,* and *Plecoptera* values concerning brook trout abundance. There are no significant differences in the *Trichoptera, Ephemeroptera,* and *Plecoptera* values concerning stream size class. There are no significant differences in the *Trichoptera, Ephemeroptera,* and *Plecoptera* values concerning brook trout abundances and stream size class interactions. These hypotheses were tested using a two-way ANOVA with brook trout abundance class as a fixed effect and stream size class as a random effect. The results can be seen in Figure 5, 6, 7, and Table 2.

3. That there is no difference in invertebrate abundance for the three brook trout abundances. That there are no significant differences in the number of aquatic invertebrate taxa concerning stream size classes. That there are no significant differences in the number of aquatic invertebrate taxa concerning brook trout abundances and stream size class interactions. These hypotheses were tested using a two-way ANOVA with brook trout abundance class as a fixed effect and stream size class as a random effect. The results can be seen in Figure 8, 9, 10, and Table 3.

4. There is no difference in invertebrate community composition among streams in the three brook trout abundance classes. This hypothesis was tested using a discriminate function analysis with the abundance of each of the twenty-two taxa of invertebrates as independent variables and brook trout abundance class as the categorical variable. The results can be seen in Figure 11.

The linear model for the experimental design for the Anovas is:

$Yijk = \mu + BTAi + SCj + BTA*SCij + \varepsilon(ij)k$.

i = 1, 2, 3; j = 1, 2,3,4; k = variable.

For Table 1, Yijk = the number of aquatic invertebrate taxa of the kth experimental unit in the jth level of factor SC and the ith level of factor BTA.

For Table 2, Yijk = the *Trichoptera, Ephemeroptera,* and *Plecoptera* values of the kth experimental unit in the jth level of factor SC and the ith level of factor BTA.

μ = the overall mean

BTAi = Brook Trout Abundance class; the fixed effect of the ith of a levels of factor BTA

SCj = Size Class; the fixed effect of the jth of b levels of factor SC,

BTA*SCij = the fixed effect of the ith level of factor BTA with the jth level of factor SC,

$\varepsilon(ij)k$ = the random effect of the kth plot in the ijth treatment combination. The $\varepsilon(ij)k$ are assumed to be iid N (0, σ2).

RESULTS

Taxa Richness:

There was no significant difference in taxa richness among stream in the three brook trout abundance classes ($F=0.583$, $p=0.591$, Fig 2). Taxa richness was variable within all brook trout abundance classes with the no brook trout streams showing the highest level of variability, ranging from 4-16 classes. The median taxa abundance in the high brook trout density sites was slightly lower than the other sites, although not significantly. There was also no significant difference in taxa richness among the different stream size classes ($F=0.297$, $p=0.839$, Fig 3). Taxa richness was highly variable within each size class. There was a significant interaction between brook trout abundance class and stream size class ($F=2.48$, $p=0.04$, Fig 4).

The most variability in taxa richness was seen in stream class three, from three to fifteen taxa (Fig 3). The smallest amount of variability was seen in stream class four, from nine to eleven. Three outliers are seen in stream class one.

The most variability in taxa richness in Fig 4 was in no brook trout abundance, size class 2 streams. Taxa ranged from six to sixteen. The lowest taxa richness was in a no brook trout abundance, size four stream with ten taxa. Taxa richness differed significantly as stream size class, and brook trout abundance increased. Taxa richness decreased as stream size class increased with high brook trout abundance. Taxa richness decreased by stream size class 3 in all three brook trout abundances.

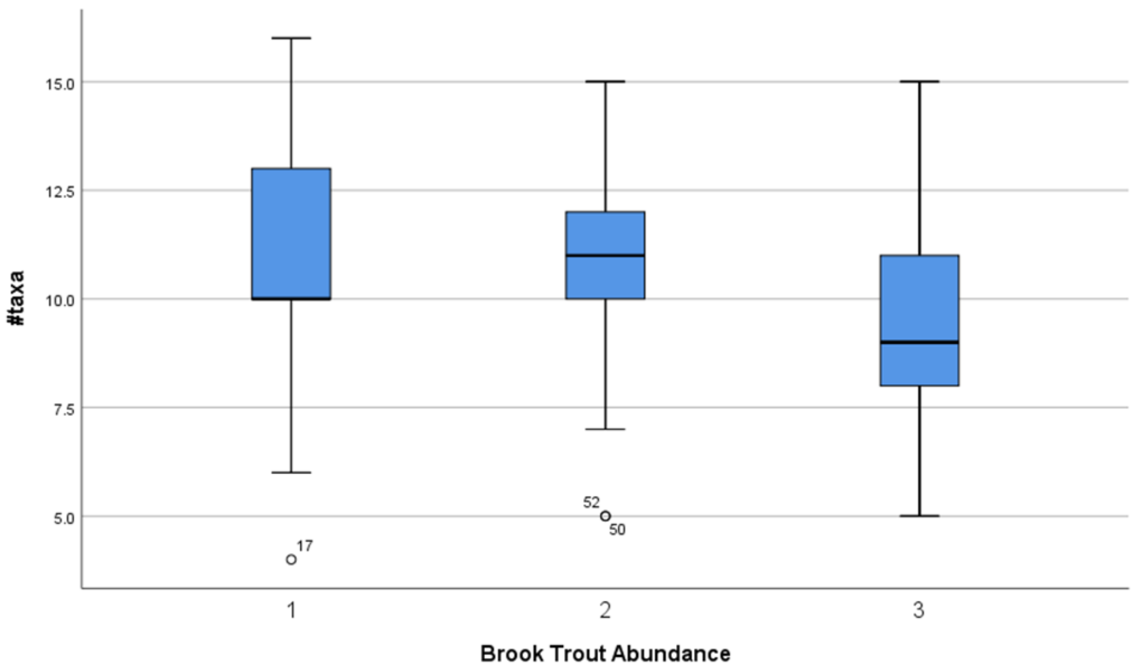

Fig 2. Taxa richness vs. brook trout abundance.

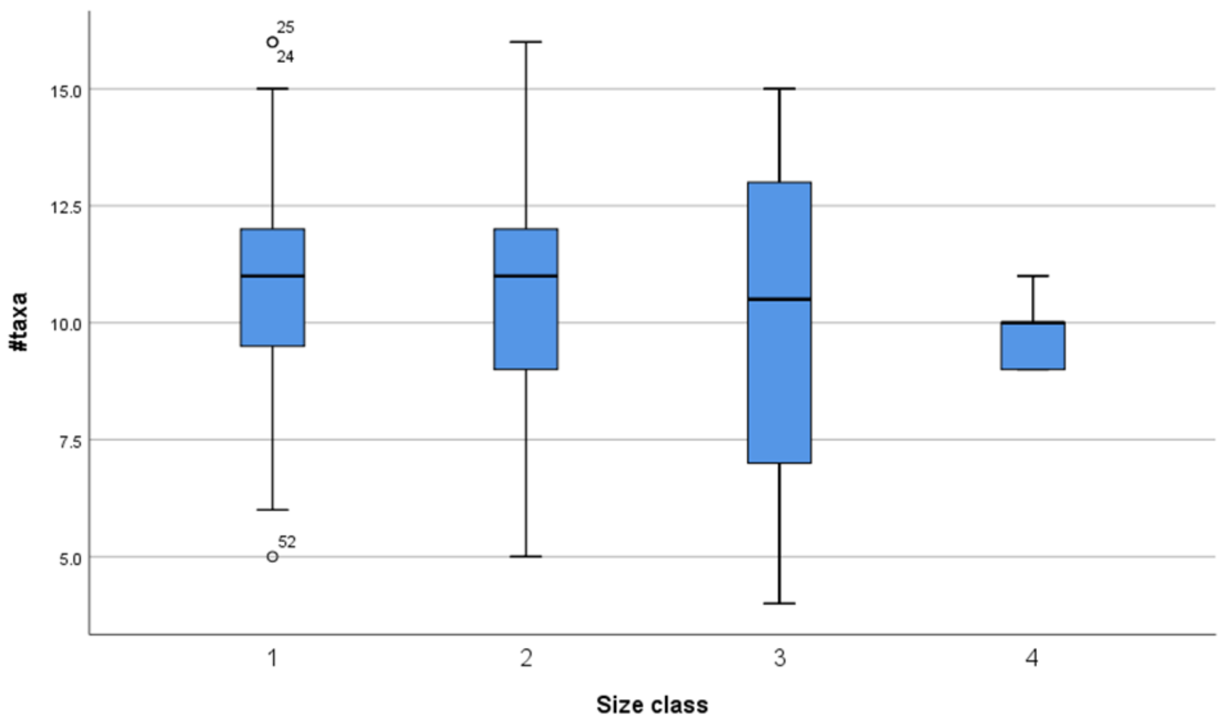

Fig 3. Taxa richness vs. stream size class.

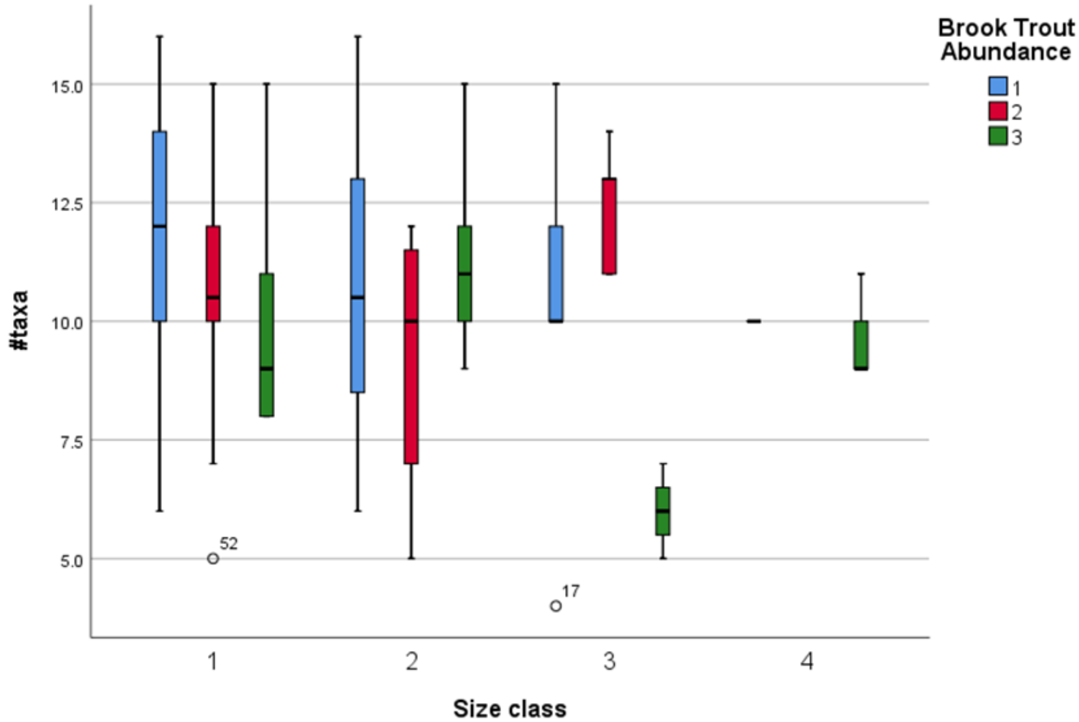

Fig 4. Taxa richness vs. stream size class and brook trout abundance interactions.

EPT Orders:

Ephemeroptera, *Plecoptera,* and *Trichoptera* numbers were similar in all abundance classes (Figure 5). Low brook trout abundance class exhibited the most variability and the highest amount of the EPT orders. The lowest sum of the EPT orders found in any of the streams was zero, in an abundance class 1 stream. The highest sum of the EPT orders found in any of the streams was five-hundred and ten, in an abundance class 2 stream. Upon visual inspection of the box plots, the hypothesis has failed to reject, meaning there were no significant differences.

The number of invertebrates in the EPT orders found in the different brook trout abundance streams varied between zero and five-hundred and ten taxa. Abundances of invertebrates varied between three and nine hundred seventy-seven in different brook trout abundance streams. Large streams had the largest range of taxa found, while very large streams had the smallest range of taxa.

There was no significant difference in the EPT orders among stream in the three brook trout abundance classes ($F=3.497$, $p=0.102$, Fig 5). EPT orders were variable within all brook trout abundance classes with the low abundance brook trout streams showing the highest level of variability, ranging from 0 to five-hundred and ten EPT taxa. The EPT orders in the high brook trout density and no brook trout absence sites were slightly lower than the other sites although not significantly. There was also no significant difference in EPT orders among the different stream size classes ($F=2.502$, $p=0.145$, Table 2). There was no significant interaction between brook trout abundances and stream size classes ($F=.733$, $p=.602$, Table 2).

The most variable stream size class was stream size class 2 (Fig 6), which ranged from zero EPT taxa to five-hundred and ten. The lowest variability was in stream size class 4, from twenty-nine to one-hundred and eighty-nine EPT taxa.

The most variable EPT orders occurred in low brook trout abundance in size class two streams (Fig 7). The least variable EPT orders occurred in high brook trout abundance, size class three streams.

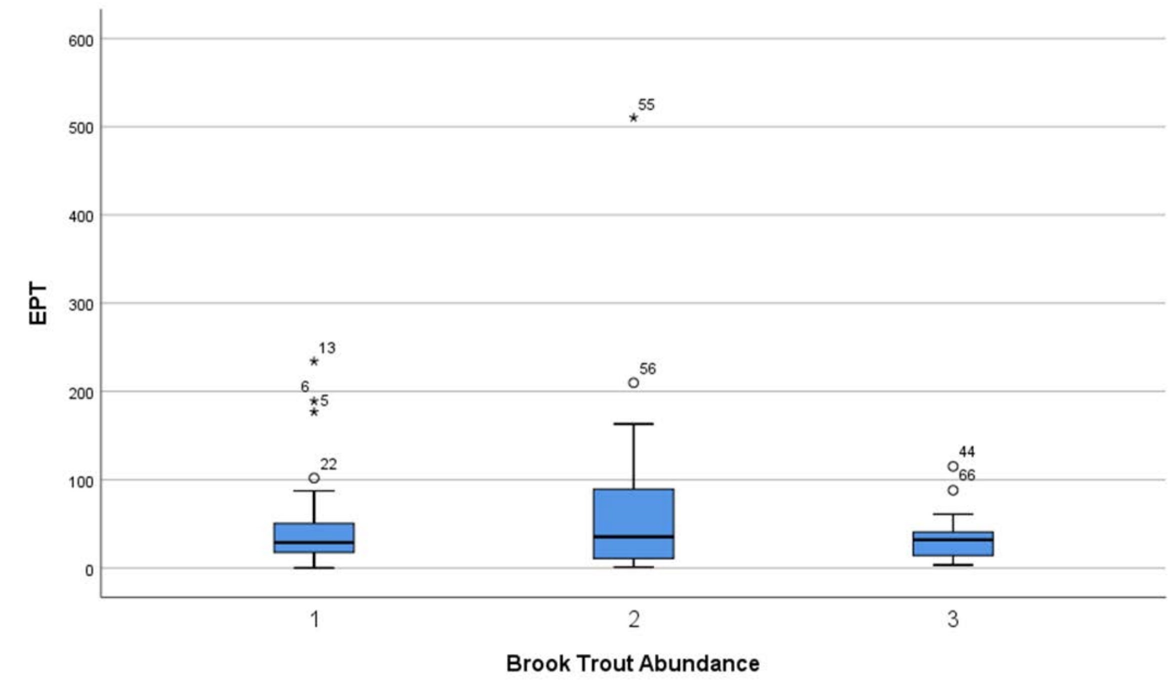

Fig 5. EPT orders vs. brook trout abundance

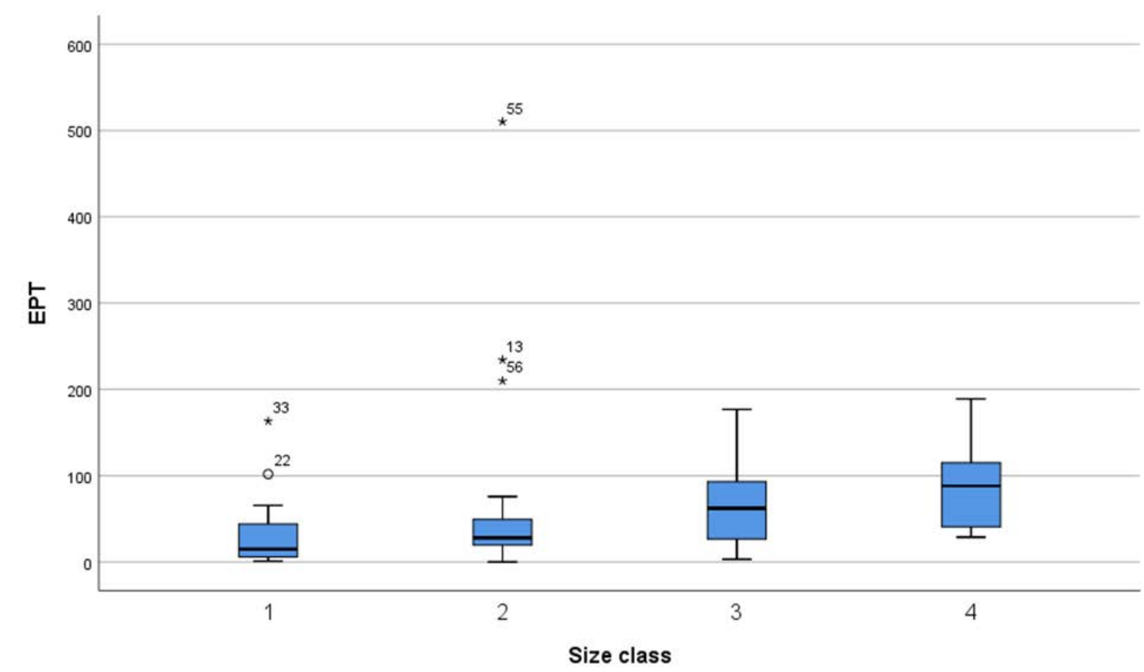

Fig 6. EPT orders vs. stream class size

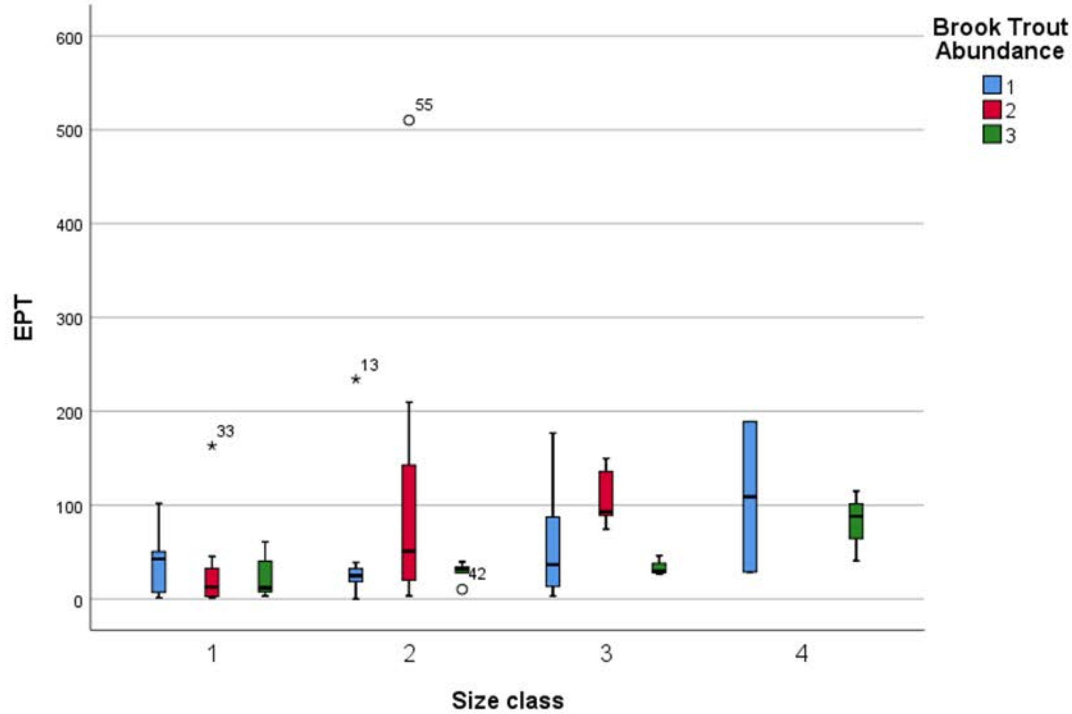

Fig 7. EPT orders vs. stream size class and brook trout abundance interactions

Invertebrate Abundance:

Macroinvertebrate abundance was most variable in abundance class one streams (Figure 8). There was a smaller abundance of macroinvertebrates in abundance class three streams. The lowest abundance was three, which was in an abundance class two stream. The highest abundance was nine hundred seventy-seven in an abundance class one stream. Upon visual inspection of the box plots, the hypothesis has failed to reject, meaning there were no significant differences.

There was no significant difference in aquatic invertebrate abundance among stream in the three brook trout abundance classes (F=0.328, p=0.731, Fig 8). Invertebrate abundance was variable within all brook trout abundance classes with the absent brook trout streams showing the highest level of variability, ranging from 9-977 taxa. The median invertebrate abundance in the high brook trout density sites was slightly lower than the other sites, although not significantly. There was also no significant difference in invertebrate abundance among the different stream size classes (F=.595, p=0.642, Table 3). There was no significant interaction between brook trout abundances and stream size classes (F=1.711, p=.147, Table 3).

The largest amount of variety was in stream size class two, from nineteen to nine-hundred and seventy-six (Fig 9). The other stream class sizes are similar values for invertebrate abundances. Outliers are seen in size class 1 and 2.

The highest variability of invertebrate abundance was in no abundance brook trout, size class 1 stream (Fig 10). The lowest variability was in a high abundance, size class 3 stream.

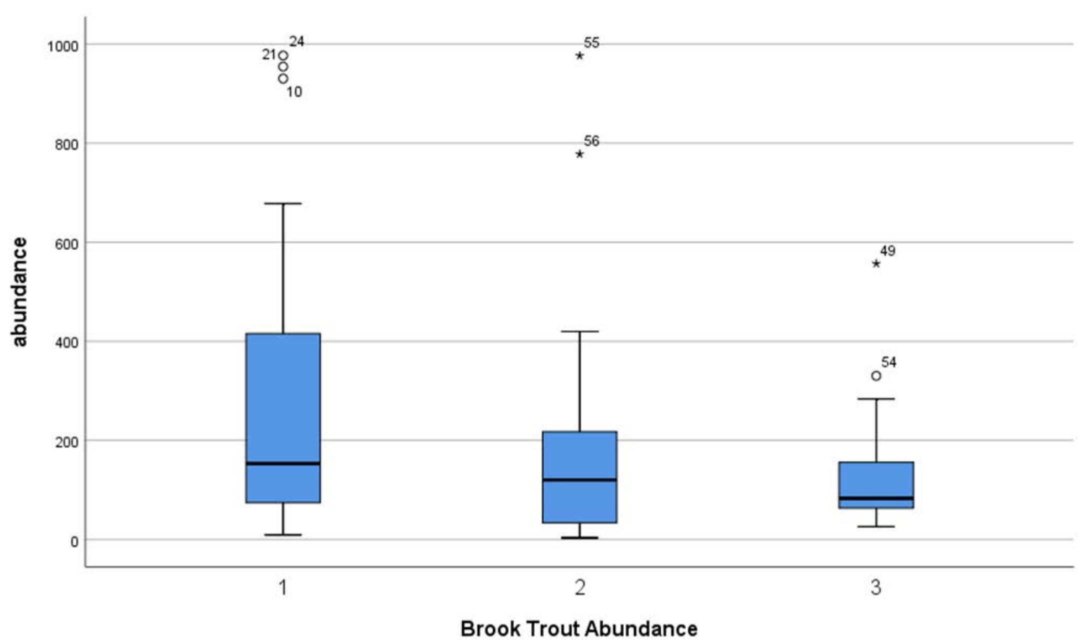

Fig 8. Invertebrate abundance vs. brook trout abundance.

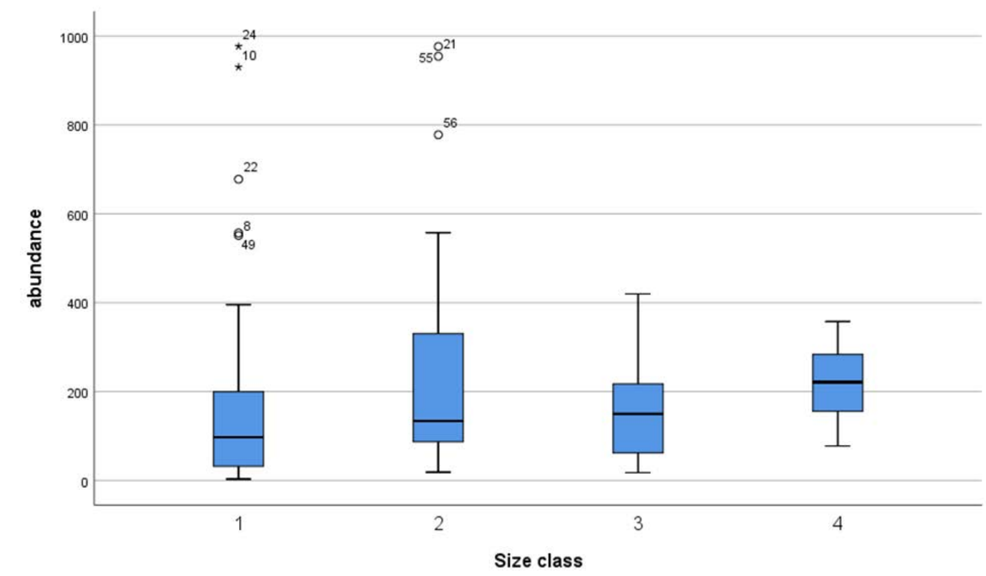

Fig 9. Invertebrate abundance vs. stream size class

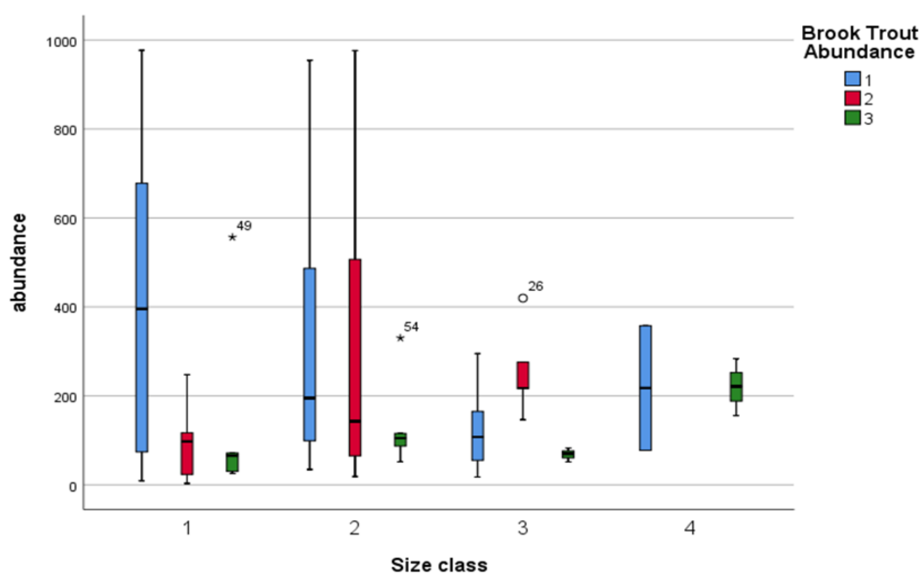

Fig 10. Invertebrate abundance vs. the stream size class and brook trout abundance interaction.

Invertebrate Community Structure:

Streams with no brook trout had higher proportions of black flies, snails and slugs, and mollusks. Streams with some brook trout abundance had a higher portion of dragonflies and damselflies, worms, and caddisflies. Streams with high brook trout abundance did not have higher proportions of any invertebrate orders but had some biased towards dragonflies and damselflies, caddisflies, nematodes, and non-biting midge's presence. Caddisflies, worms, non-biting midges, nematodes, and dragonflies were found in lower levels in streams with no brook trout. Streams with some brook trout had lower levels of black flies, snails, mollusks, dragonflies, nematodes, non-biting midges, and caddisflies.

There were some differences in the invertebrate community among streams in the brook trout abundance classes, but overall, the community differences were not significant (Wilks lambda= 0.544, p= 0.353). Although the difference among the groups is not significant the discriminate functions did summarize approximately 35% and 15% of community structure variability respectively (function 1: eigenvalue= 0.56, canonical correlation = 0.599, function 2: eigenvalue= 0.178, canonical correlation = 0.389). There is some separation of group 1 from 2 and 3 on axis 1 and group 3 from 1 and 2 on axis 2 (Fig 11).

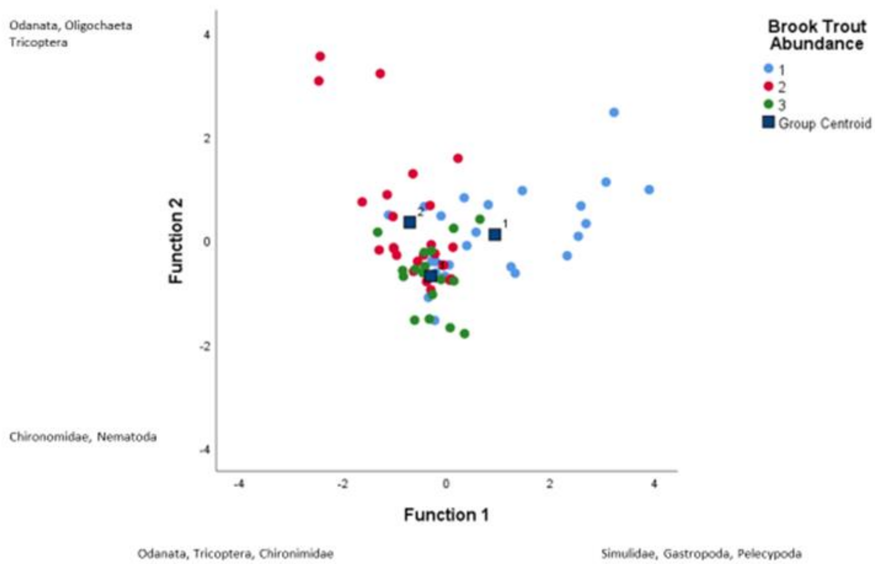

Fig 11. Discriminate functions illustrating differences in invertebrate community structure among streams in each brook trout abundance class.

DISCUSSION

There were no clear differences in invertebrate community structure, abundance or richness based on brook trout abundance and stream size class. EPT orders also did not differ significantly in different brook trout abundances or stream class sizes. The only significant difference that occurred was between taxa richness and brook trout abundance and stream size class.

Currently, only forest management occurs near the streams that were sampled. Factors that may have influenced brook trout presence and abundance are natural or anthropogenic landscape and climate changes to the water systems (Merriam *et al.* 2018). Habitat quality and habitat degradation would also be important based on brook trout's preference for cold, clear bodies of water (Merriam *et al.* 2018). Water systems near agriculture, residential and commercial development, or historic mining are also expected to have less brook trout occupying them (Merriam *et al.* 2018). Streams that were not occupied or had less abundance may have had historical degradation, and the habitat is yet to recover (Merriam *et al.* 2018).

EPT richness was found to be highest in streams with high forest cover, large catchments, and low temperatures in a study by Tonkin *et al.* (2015). EPT richness was also promoted in areas with high broadleaf tree cover, high elevation and slope, large catchment size, and low mean temperature (Tonkin *et al.* 2015). *Ephemeroptera* favoured areas with high forest cover, medium-to-large catchment sizes, and high-temperature seasonality (Tonkin *et al.* 2015). *Plecoptera* favored low-temperature seasonality, high slope and elevation, and rainfall (Tonkin *et al.* 2015). *Trichoptera* favoured high elevation areas, with high forest cover, and low mean temperature

(Tonkin *et al*. 2015). EPT orders did not differ among stream classes; this means that these preferences were not all met in the streams that were sampled. EPT orders in large catchment streams likely did not have other suitable characteristics such as forest cover or water temperature.

EPT order levels between low and high abundance brook trout streams likely did not differ significantly due to the preference of similar habitat (Vandusen *et al.* 2005). Brook trout and EPT orders prefer stable temperature streams with no disturbances. Both also prefer alkalinity, total dissolved solid amounts, and specific conductivity (Vandusen *et al.* 2005). EPT order levels were similar in no brook trout abundance streams indicating that the habitat is suitable for brook trout. However, their absence may be explained by several possibilities. There may have been recent, in the last thirty years, forest disturbances close to the streams that the EPT orders have recently recolonized, while brook trout have yet to recolonize (Vandusen *et al.* 2005). The streams with no brook trout may have harder accessibility that can be accessed by the EPT orders easier due to terrestrial adult stages (Vandusen *et al.* 2015). Lastly, brook trout occupation of the stream may have been missed by the timing of the sampling. Occupation may differ based on the age of the fish or stream characteristics that would make it preferable for different needs, such as spawning, or areas utilized by juveniles (Vandusen *et al.* 2015).

Fish predation has been more obvious in more mobile invertebrates than in smaller, less mobile taxa (Diehl 1992). However, stocking of non-native trout has been found to significantly alter community structure (Alexiades and Kraft 2016). The density changed for only a few macroinvertebrate taxa, *Diptera,* and Annelida (Alexiades and Kraft 2016). Their abundances increased after stocking. *Ephemeroptera, Coleoptera,* *Plecoptera, Trichoptera* were not altered significantly in the community structure (Alexiades and Kraft 2016). *Diptera* and Annelida were broken down into families for this study. Leeches were not found in any significant numbers in this study, while families within *Diptera* showed no significant changes within the community structure. Though the families, *Simuliidae* and *Chironomidae*, did demonstrate small variation in different trout densities, as seen in Figure 11, maybe due to habitat or stream characteristics.

Common perch (*Perca fluviatilis*) were stocked at three densities (no fish, and low and high natural densities), densities that were also used for the brook trout in this analysis, into pond enclosures with either dense vegetation present or absent in a study by Diehl (1992). The aquatic invertebrates were monitored, in the summer, in terms of biomass, abundance, community size and structure, and diversity (Diehl 1992). Perch stomach contents were also checked to observe what prey they consumed (Diehl 1992).

Community structure changes in the absence of the perch, with the herbivorous aquatic invertebrates decreasing as a result of increased invertebrate predation (Diehl 1992). Herbivorous macroinvertebrates that were found to increase in abundance in the presence of perch were mainly *Trichoptera* and *Diptera*, even though they were being preyed upon by non-fish. (Diehl 1992). Even with predation, their biomass did not decrease (Diehl 1992). *Trichoptera* were found in high amounts in many of the streams sampled in this study and could mean that brook trout at any abundance, though preying on this taxon, have no overall impact on abundance.

The abundance of invertebrates and taxa richness may not have differed significantly among stream size classes due to the lack of major filters that would block certain orders or significant amounts of invertebrates to enter a stream (Buendia *et al.* 2013). Such filters could be sediment, which can prevent certain taxa such as *Plecoptera* and *Simuliidae* from colonizing a stream (Buendia *et al.* 2013). Other filters could be geologic, climatic or riparian characteristics that would prevent access to a stream (Buendia *et al.* 2013). These would be absent from the streams sampled, allowing similar amounts of taxa and invertebrates abundance in all size streams. Riparian vegetation may also be present in the streams sampled in amounts that would mitigate filter effects (Buendia *et al.* 2013).

Decreasing taxa richness differed significantly in stream size class and brook trout abundance class interactions. Taxa richness significantly varied in stream size class 3 and 4. Larger stream sizes can decrease canopy cover that is available (Lenat 1983). Decreased canopy cover can create different niches that invertebrate taxa can utilize, favoring invertebrates depending on what feeding strategy is used (Lenat 1983). Temperature and organic matter is the primary factor (LeCraw and Mackereth 2012) that may be changed by forest cover. Shaded, clear areas of the large streams would be ideal hunting areas for brook trout (Kerr and Grant 2000), increasing predation. This may lower the number of taxa that are present in a large (size class 3 or 4) stream with a high abundance of brook trout.

Sediment in larger streams may create preferred habitat for brook trout but lower quality habitat for many invertebrates (Hartman and Hakala 2006), lowering their richness in the streams. Taxa richness was higher in no brook trout abundant streams in smaller sizes (class 1 and 2) likely due to canopy cover, creating appropriate niches for different types of feeding strategists (Lenat 1983). Small streams may offer refugia from predation such as brook trout that can decrease invertebrate richness (Lenat 1983). Competition may also be reduced if there are enough resources created by canopy cover for niches (Lenat 1983).

Black flies, snails, mollusks, dragonflies, nematodes, non-biting midges, and caddisflies (from Fig 11) usually prefer high water quality habitat that brook trout are also found in (Clifford 1991). Aquatic invertebrate families have more specific habitat requirements than at the order level, so the streams with low levels of invertebrate orders may have been lacking characteristics that invertebrate families need (Dijkstra and Lewington 2015) such as the speed of running water, if a stream is temporary or permanent, tolerance to water level changes, or changes in temperatures. Amount and type of vegetation in these streams may also be important in determining why macroinvertebrate orders levels were lower (Dijkstra and Lewington 2015). Stream characteristics can also influence whether invertebrate orders or families are found in higher levels (Dijkstra and Lewington 2015).

Proportions of non-biting midges and aquatic worms could be related to food availability in streams (Saether 1979). Non-biting midges are also used to examine water quality, such as pollution and oxygen levels (Saether 1979). There also may be increased organic matter in these high brook trout abundance streams (Saether 1979). Non-biting midges dominated streams that change to an oligochaete dominated community can be a sign of eutrophication (Saether 1979). Aquatic worm dominated streams were not found in this study, so it can be assumed that high brook trout abundant streams are likely of good quality or have enough food resources to cause proportions of the oligochaete worms and non-biting midges orders. Streams with no brook trout that had higher levels of aquatic worm taxa probably mean that there was enough food resources or higher quality of habitat for the oligochaete worms, but the habitat was not suitable enough for brook trout. There was likely no sign of eutrophication in any streams where proportions of aquatic worm taxa were found.

Community structure varied mainly with the orders blackflies, snails, mollusks, dragonflies, damselflies, nematodes, non-biting midges, aquatic worms, and caddisflies. Black flies, snails, and mollusks are usually generalists, but the specific families may prefer habitat that would be unsuitable for brook trout (Clifford 1991). This could explain why these orders are in higher proportions in streams with no brook trout (Clifford 1991).

Diversity of macroinvertebrates are likely to be decreased by the density of predators rather than simply the abundance of them (Diehl 1992). Only brook trout abundance was looked at in this study, which may not have had any significant effects on macroinvertebrates. The ability of macroinvertebrates to be resilient to fish predation is also an important feature depending on the co-evolution of the species (Diehl 1992). Community structure may not have changed significantly as the macroinvertebrates sampled in the sixty-seven streams may have co-evolved with brook trout and have a strong resilience against their predation or presence

Biomass of *Ephemeroptera* and *Chironomidae* larvae decreased significantly in the study by Diehl (1992), which may have been caused by their timing of emergence into the streams, allowing for greater predation. Macroinvertebrate biomass was not sampled in this study, so while there were no significant differences in community structure, biomass may have been altered significantly by brook trout.

The frequencies and magnitudes of droughts within streams may also influence invertebrate community structure differences, abundance, or number of taxa (Bogan *et al.* 2017). The factors may contribute to similar structures in temporary, permanent streams, and different size streams (Bogan *et al.* 2017). Shorter drought periods and continuous water flow in temporary streams may allow for higher macroinvertebrate diversity, as these conditions create longer recolonization times (Bogan *et al*. 2017). The streams that were sampled likely do not undergo significant droughts as the community structure did not significantly differ and because of the abundance brook trout that were found would indicate likely permanent streams. No abundance brook trout streams are likely permanent too based on the invertebrate abundance.

Species richness recovered completely after each drought season; a study done by Bogan *et al.* (2017) found. Aquatic invertebrate communities recovered in less than six months in temporary pools (Bogan *et al.* 2017). These recoveries are assisted by hyporheic zones, areas saturated with a mixture of local and regional groundwater and stream water, that can provide refuge to aquatic invertebrates when stream flow ceases (Bogan *et al.* 2017). Permanent pools left when a stream flow ceases may also be important for invertebrates for survival and may be a contributing factor in addition to temporary pools. Also, aquatic invertebrates may travel upstream to recolonize from pools quickly (Bogan *et al.* 2017). Hyporheic zones would allow taxa richness in the sampled streams in this study to be significantly affected by periods of droughts and allow them to recolonize streams. This would happen independently from brook trout abundance, which would likely recolonize much later, and their presence would not affect taxa richness.

High macroinvertebrate diversity is found usually in permanent groundwater-fed streams with various catchments (Crossman *et al.* 2011). Also, permanent streams create stability in environmental conditions, which allows for an increased distribution of diversity found within these streams (Crossman *et al.* 2011). Permanent groundwater-fed streams of any size likely caused similar macroinvertebrate abundance. Any temporary groundwater-fed streams that were sampled likely compensated by having preferable habitat for brook trout and invertebrates.

Variation in the community structure of aquatic macroinvertebrates may be a result of uneven distribution within the system while sampling, anthropogenic effects such as runoff, or macroinvertebrates response to predators (Alexiades and Kraft 2016). Depending on the water system, the community structure has been found to vary in response to trout (Alexiades and Kraft 2016).

The variability seen in the macroinvertebrate community structure could relate to water and sediment chemistry variables (Cai *et al.* 2012). Water may be hypertrophic, due to eutrophication or runoff from machinery, or pollution (Cai *et al.* 2012). These conditions create unsuitable environments for aquatic macroinvertebrates with changes to community structure, often being due to eutrophication (Cai *et al.* 2012). In Meiliang Bay, of Lake Taihu, China, Cai *et al.* (2012) found that benthic aquatic invertebrate communities shifted from the dominant species being *C. fluminea*, the Asian clam, and gastropods to a dominant community of tubificids (aquatic oligochaete worms), and non-biting midges due to eutrophication (Cai *et al.* 2012). These changes demonstrate the increase of macroinvertebrates that are gatherers instead of filterers (Cai *et al.* 2012). Gathers take advantage of the organic pollution in the water systems, which could increase their abundance in the community (Cai *et al.* 2012). Pollution may be present in some streams sampled, but not insignificant amounts to cause major community differences, but rather some variability. The orders that varied the most in this study as listed above and found in Fig 6 covered a variety of different feeding types, further evidence at a lack of any significant pollution in the streams.

Brook trout had little effect on prey populations in two streams in a study by Reice and Edwards (1986). In one of the streams, nearly 50 of the recorded 65 taxa had decreased mean abundance (Reice and Edwards 1986). When the trout were present, abundance was reduced across the stream (Reice and Edwards 1986). Though the abundance was decreased, brook trout did not significantly alter the community structure (Reice and Edwards 1986). Species diversity and richness were not affected by brook trout (Reice and Edwards 1986). Only the genus *Oxyethira* belonging to the *Trichoptera* order was shown to be significantly reduced in the other sampled stream, while invertebrate density remained relatively stable (Reice and Edwards 1986). Feeding tendencies of brook trout are general, and the availability of prey minimize the effect of trout on invertebrate taxa (Reice and Edwards 1986).

A relationship between prey migration and the impacts of local predatory brook trout density on local prey abundance was examined in Cooper *et al.* (1990). The spatial distribution of predators may affect the abundance of the invertebrates (Cooper *et al*. 1990). Predation can lower migration rates of invertebrates into habitats, which can cause abundance to decrease (Cooper *et al.* 1990). The usually less mobile, non-migratory taxa in this study would be less susceptible to predation of this type but still may have caused variation if there were migratory taxa.

Vegetation may alter the flow in the stream, which can also change the invertebrate community structure (Diehl 1992). Streams with vegetation generally have higher species richness, as it favors both herbivorous and predatory invertebrates by offering protection and ambush locations, respectively (Diehl 1992). Structural complexity created by vegetation can reduce foraging efficiency, but ambush predators such as dragonflies may have high foraging efficiency (Diehl 1992). Vegetation also provides resources for the invertebrate community (Diehl 1992). Vegetation can promote algae growth, which favors invertebrate grazers (Diehl 1992). Different types or abundance of vegetation may also be responsible for community structure changes, as grazers tend to be detritivores (Diehl 1992). Their presence may increase the number of predatory macroinvertebrates, or of other predators (Diehl 1992). This can also inversely shift grazer populations in the community (Diehl 1992). Grazers such as snails varied in all densities of brook trout in this analysis, which could be related to available appropriate vegetation, or predation rates in areas with brook trout.

Vegetation also promoted species richness of all macroinvertebrates (Diehl 1992). When it comes to native predators, predation may have more to do with effecting invertebrate diversity, biomass, and individual taxonomic order abundance than it does with changing the community structure (Diehl 1992).

CONCLUSION

Significant differences in aquatic invertebrate structure, abundance, taxa richness, or EPT orders did not occur among the streams that differed in brook trout abundances or stream size class in this study, though there was variation. There were, however, significant differences between taxa richness and stream class size and brook trout abundance interactions. Other studies indicate that community structure has co-evolved with predatory fish and may be more resilient towards predation. Aquatic invertebrate biomass, diversity, and abundance have also been shown to differ due to predation, rather than the community structure. Future studies may be needed to examine these characteristics in relation to brook trout in the streams for a relationship. Timing of sampling may also play an important role, as brook trout consumption of invertebrates may have previously altered the community structure before sampling occurred, and the community may have stabilized during the sampling periods. Examining invertebrates at genus, or family level could provide specific evidence of a community structure, biomass, abundance or richness change that isn’t obvious from an order level.

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APPENDICES

Table 1. ANOVA for taxa richness, brook trout abundance and size classes.

Tests of Between-Subjects Effects

Dependent Variable: #taxa

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	4854.045	1	4854.045	855.013	.000
	Error	34.999	6.165	5.677[a]		
BrookTroutAbundance	Hypothesis	20.849	2	10.424	.583	.591
	Error	93.239	5.211	17.891[b]		
Sizeclass	Hypothesis	14.386	3	4.795	.279	.839
	Error	94.992	5.524	17.195[c]		
BrookTroutAbundance* Sizeclass	Hypothesis	92.166	5	18.433	2.477	.043
	Error	416.789	56	7.443[d]		

a. .817 MS(Sizeclass_A) + .036 MS(BrookTroutAbundance_A * Sizeclass_A) + .147 MS(Error)

b. .951 MS(BrookTroutAbundance_A * Sizeclass_A) + .049 MS(Error)

c. .887 MS(BrookTroutAbundance_A * Sizeclass_A) + .113 MS(Error)

d. MS(Error)

Table 2. ANOVA for *Trichoptera*, *Ephemeroptera*, and *Plecoptera* orders, brook trout abundance and size classes.

Tests of Between-Subjects Effects

Dependent Variable: EPT

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	202259.624	1	202259.624	20.365	.013
	Error	36758.430	3.701	9931.956[a]		
BrookTroutAbundance	Hypothesis	29897.656	2	14948.828	3.497	.102
	Error	24499.741	5.731	4275.169[b]		
Sizeclass	Hypothesis	32819.179	3	10939.726	2.502	.145
	Error	30011.897	6.864	4372.211[c]		
BrookTroutAbundance * Sizeclass	Hypothesis	20997.814	5	4199.563	.733	.602
	Error	321016.523	56	5732.438[d]		

a. .817 MS(Sizeclass) + .036 MS(BrookTroutAbundance * Sizeclass) + .147 MS(Error)

b. .951 MS(BrookTroutAbundance * Sizeclass) + .049 MS(Error)

c. .887 MS(BrookTroutAbundance * Sizeclass) + .113 MS(Error)

d. MS(Error)

Table 3. Total aquatic invertebrate abundance in each Brook trout abundance and size.

Tests of Between-Subjects Effects

Dependent Variable: Ln Abun

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	1102.560	1	1102.560	841.045	.000
	Error	6.241	4.761	1.311[a]		
BrookTroutAbundance	Hypothesis	1.441	2	.721	.328	.734
	Error	11.645	5.307	2.194[b]		
Sizeclass	Hypothesis	3.810	3	1.270	.595	.642
	Error	12.313	5.767	2.135[c]		
BrookTroutAbundance * Sizeclass	Hypothesis	11.200	5	2.240	1.711	.147
	Error	73.329	56	1.309[d]		

a. .817 MS(Sizeclass) + .036 MS(BrookTroutAbundance * Sizeclass) + .147 MS(Error)

b. .951 MS(BrookTroutAbundance * Sizeclass) + .049 MS(Error)

c. .887 MS(BrookTroutAbundance * Sizeclass) + .113 MS(Error)

d. MS(Error)

Table 4. Discriminate Analysis Results testing the null hypothesis.

Box's Test of Equality of Covariance Matrices

Test Results

Box's M		1792.809
F	Approx.	4.022
	df1	272
	df2	8248.338
	Sig.	.000

Tests null hypothesis of equal population covariance matrices.

Table 5. Eigenvalues

Eigenvalues

Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	.560[a]	75.8	75.8	.599
2	.178[a]	24.2	100.0	.389

a. First 2 canonical discriminant functions were used in the analysis.

Table 6. Lambda probability distribution of brook trout abundance.

Wilks' Lambda

Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	.544	34.405	32	.353
2	.849	9.274	15	.863

Table 7. Standardized Canonical Discriminant Function Coefficients of the Invertebrate Orders

x

Standardized Canonical Discriminant Function Coefficients

	Function	
	1	2
Ceratopogonidae	.409	.244
Chironimidae	-.254	-.393
Coleoptera	.077	.642
Collembola	-.279	.490
Ephemeroptera	.100	-.176
Gastropoda	.488	-.198
Hemiptera	.078	.481
Hydracarina	-.098	-1.181
Nematoda	.078	-.294
Odonata-Anisoptera	-.564	.453
Oligochaeta	.661	.920
Pelecypoda	.442	-.012
Plecoptera	.279	-.149
Simulidae	.507	.020
Terrestrial	-.319	.107
Trichoptera	-.356	.270

Table 8. Structure matrix of the invertebrate orders.

Structure Matrix

	Function 1	Function 2
Simulidae	.508*	.133
Gastropoda	.399*	.106
Pelecypoda	.330*	.133
Hemiptera	.267*	.209
Nematoda	.255*	.100
Hydracarina	.151*	.096
Terrestrial	.145*	.036
Odonata-Anisoptera	-.086	.607*
Ephemeroptera	.026	.463*
Coleoptera	.156	.446*
Trichoptera	-.161	.403*
Ceratopogonidae	.193	.396*
Collembola	-.189	.386*
Oligochaeta	.248	.284*
Chironimidae	.018	.164*
Plecoptera	.037	.085*

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions

Variables ordered by absolute size of correlation within function.

*. Largest absolute correlation between each variable and any discriminant function

Tabe 9. Functions at Group Centroids showing brook trout abundance differences

Functions at Group Centroids

Brook Trout Abundance	Function 1	Function 2
1	.926	.115
2	-.715	.352
3	-.311	-.686

Unstandardized canonical discriminant functions evaluated at group means