Environmental and Human Factors Affecting the Population Biology of Nova Scotia Brook Trout (*Salvelinus fontinalis*)

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Introduction

Anthropogenic activities such as angling, logging, agricultural and residential development, and industrial manufacturing can have a negative impact on the health of brook trout *(Salvelinus fontinalis)* populations. In addition to the direct impacts on habitat, such as water quality and spawning area, accessibility to remote lakes may increase. For example, access roads associated with logging can increase angler pressure and negatively impact trout populations (Gunn and Sein, 2000). Broad et al. (2002) assumed that easier access usually corresponded with more intense exploitation. All terrain vehicles (ATV), boats, motors, paths, and cabins facilitate greater angling opportunities to areas that at one time may have been difficult to access. There is some indication that lake accessibility can have a positive influence on angling effort, however, general support for this hypothesis is lacking for many regions. In Nova Scotia, there are very few areas of the province that can be considered remote from paved roads, logging roads, and ATV trails; most of the more than 6500 lakes in the small province are readily accessible.

Brook trout are very sensitive to habitat degradation. It is a well-known fact that acid rain has increased the acidity of surface waters in regions of Nova Scotia (Kerekes et al., 1982). Emissions of sulphur and nitrogen oxides have increased the acidity of surface waters (Rodhe et al., 1995). Acid rain facilitates the acidification of lakes and rivers resulting in damage of aquatic ecosystems, including fish habitat (Ikuta et al., 2003). Marschall and Crowder (1996) reported that habitat alterations, such as increased acidity and sedimentation, have a negative impact on trout populations. Water quality, specifically acidified waters, is thought to have had a significant impact on trout populations in Nova Scotia.

There is growing concern that increased lake accessibility and decreasing pH (acidification) are posing a threat to the population size, size and age structure of brook trout, as well as to the genetic diversity of populations on which the future adaptability of the species depends. There have been few attempts in Nova Scotia to measure the impact of lake accessibility and surface water acidification on trout populations.

Wilderness areas were created to protect representative examples of the province's natural landscapes, the native biological diversity, and outstanding natural features (Wilderness Areas, 2006). However, brook trout are not granted any extra protection in these areas. Tangier Grand Lake Wilderness Area (TGLWA) has experienced a long period of intensive recreational fishing for brook trout and there is concern that trout populations are being over-exploited. Use of motor vessels, storage of vessels on different lakes, ATV's, and old cabin leases in TGLWA facilitate easier access. Trout Nova Scotia (TNS), a non-profit, non-government trout conservation organization had proposed that TGWLA be classified as a Special Management Area. Special Management Areas can include the following fishery management techniques regarding a specific area or body of water: the maximum allowable catch can be reduced or increased, size restrictions can be placed on retainable fish (usually only smaller fish are allowed to be killed), angling method or gear restrictions can be implemented, and length of fishing season can be altered. One reason Special Management Areas were created was to help manage vulnerable freshwater fish stocks. TNS proposed regulation changes including reducing the bag limit from five trout at any length to three trout less than 30 cm. The changes were aimed at reducing trout harvest and increasing the number of older individuals in the wilderness area. Older individuals are spawners and larger females generally produce more eggs than small spawners. The Nova Scotia Department of Fisheries and Aquaculture, Inland Fisheries Division rejected the suggested regulation changes to the brook trout fishery in TGLWA due to a lack of scientific information supporting the changes.

The objective of this study was to examine the potential role that several factors may play in influencing trout population biology. Specifically, I examined the associations between environmental (lake size, pH) or human factors (lake accessibility, proxy of fishing activity (mean vessel (boat and canoe) presence and proportion of total observed anglers) and trout population biology (catch per unit effort (CPUE, a proxy for trout abundance), trout length, and trout age). I hypothesized that there would be negative associations between lake access difficulty and measures of fishing activity (mean vessel presence and the proportion of total observed anglers). For example, as lake access difficulty increased, proxies of fishing activity would decrease. I hypothesized that there would be positive associations between lake accessibility and measures of trout population biology. For example, as lake access difficulty increased so would factors of trout population biology (CPUE, age, and length). Similarly, positive associations were expected between pH and measures of brook trout population biology. For example, as pH increased toward neutral conditions (better trout habitat), it was expected that factors of trout population biology (CPUE, age, and length) would also increase.

The results generated by this research will facilitate appropriate management decisions regarding the issues of accessibility, pH, fishing activity, and sustainable fisheries in TGLWA. This information may also have broad implications for fisheries management, both in Nova Scotia and elsewhere in North America.

Literature Review

Fishing has been an important human activity for thousands of years (Pringle, 1997). World wide, fishing provides employment opportunities, food, and recreational activities for many cultures. Economic and social gains motivate humans to exploit fish stocks (Hutchings et al., 1997). Commercial fisheries have received extensive academic and media attention with papers and articles examining fish population declines and extinctions (Myers et al., 1997). Recreational fisheries have also received considerable attention; however, the potential effects of angling on fish populations have not been scientifically examined to the extent that commercial fisheries have (Cooke and Cowx, 2004).

Post et al. (2002) believe that a recreational fishing collapse has already started in Canada, with evidence of dramatic declines in certain fish populations, which has largely gone unnoticed by fishery scientists, managers and the public. A study by Pearse (1998) concluded that brook trout, Atlantic salmon (*Salmo salar*), walleye (*Sander vitreus*), and northern pike (*Esox lucius*) populations in Canadian water bodies that drain into the Atlantic Ocean are declining due to overfishing and habitat deterioration. In Alberta, in the 1990's, northern pike catches were 15% of what they were 20 years ago (Sullivan, 1999). A reduction in average age, size, and year classes are associated with lower catch rates (Sullivan, 1999). In southern Ontario, 60% of the natural lake trout *(Salvelinus namaycush)* population are maintained by stocking (Evans and Wilcox, 1991). Only 1% of lakes are considered to need a stocking program in northern Ontario, away from the large population centres in the southern regions of the province.

Nova Scotia's most sought after recreational sport fish is the brook trout (MacMillan and Crandlemere, 2005). Annual catches have ranged between 800,000 and 2.2 million over the last 25 years and the annual catch has decreased approximately by 60 percent in Nova Scotia (MacMillan and Crandlemere, 2005). There are many factors that may be responsible for this decrease including habitat changes and lower angling pressure; however, previous studies and many anglers have indicated that over-fishing has occurred (Gunn and Sein, 2000; Post et al., 2002; MacMillan and Crandlemere, 2005). The Nova Scotia Department of Fisheries and Aquaculture, Inland Fisheries Division, found very low densities of trout in two TGLWA lakes (MacMillan and Crandlemere, 2005). Low trout densities often indicate poor water quality or over fishing which are both likely implicated in trout declines in Nova Scotia (MacMillan and Crandlemere, 2005).

Very little literature exists that examines the relationships between lake accessibility, measures of trout population biology (trout abundance, trout age, and trout length), and fishing activity. There are several studies that conclude the degree of accessibility can influence the amount of fishing pressure a particular body of water receives (Gunn and Sein, 2000; Broad et al. 2002). Gunn and Sein (2000) examined the exploitation of lake trout in a lake in Ontario that previously did not have direct road access and had been closed to angling. The relative abundance of trout in the lake was calculated before and after the change took place. CPUE fell from 1.23 trout/ net/ 2 hours to 0.37 trout / net/ 2 hours after one year of angling. Gunn and Sein (2000) concluded that easier access facilitates fishing pressure that could have a substantial impact on trout populations. They suggested that fishery managers needed to give more attention to the impact that motor vehicle access to a lake can have on fish populations. Broad et al. (2002) found that more accessible angling locations are likely to experience more intense fishing pressure than locations that are difficult to access. They concluded that increased exploitation altered the natural population structure in long-fin eels *(Anguilla dieffenbachia)*. At sites that were difficult to access, long-fin eels had a normally distributed length-frequency relationship. Eels sampled from easily accessible sites had a non-normal distribution and were skewed to smaller size classes (Broad et al., 2002). Heavily exploited populations were characterized by smaller mean lengths. Contrastingly, in their study of stocked cutthroat trout *(Oncorhtnchus clarki),* Bailey and Hubert (2003) found that as lake access difficulty increased, CPUE decreased. However, they concluded this was due to the fact that easily accessible lakes were stocked with trout more often. They also found that as lake access difficulty increased, mean total length and survival of trout increased. Bailey and Hubert (2003) concluded that exploitation prevented the majority of trout from aging over two years, consequently resulting in many short lived fish in many of their study locations.

Low pH in freshwater systems also negatively affects trout populations. Acidification has led to the local extinction of populations of salmonid fishes such as the

Atlantic salmon and the brook trout from many rivers and lakes (Beamish 1976; Schofield 1976; Hesthagen et al., 1999). Exposure to low pH kills fish directly by discharge of sodium and chloride ions from body fluid (Ikuta et al., 2003). Aluminum leached from surrounding soils due to low pH intensifies this effect on gill membranes (Leivestad and Muniz, 1976). Many studies have examined the effects of reduced or low pH on fish populations. For example, recruitment failure can occur when a population is not able to produce naturally viable offspring as a consequence of biological or physical factors. Low pH can facilitate recruitment failure by reducing the survival of trout eggs, alevins, and parr, and by reducing or eliminating spawning and food sources. Warren et al. (2005) found there was a strong connection between groundwater pH and brook trout egg survival. Redds (trout nests) supplied with groundwater with a pH under 5 contributed to trout egg mortality. Lachance et al. (2000) found that brook trout eggs and fingerlings exposed to acidic conditions (between 4.1- 6.0) experienced mortality rates between 60-85%. Brook trout eggs experienced 100% mortality in waters with a pH below 4.5 (Hunn et al., 1987). Brook trout respond to decreases in pH with decreased egg to larva survival rates, decreased survival rates for small fish, and with decreased growth rates in all size classes (Marschall and Crowder, 1996). Schindler et al (1985) conducted a study on lake trout in an experimental lake in Ontario in which they slowly decreased the pH from 6.8 to 5 over an eight-year period. Midway through the experiment recruitment failure resulted and continued through to the end of the experiment. In a study of lakes ranging in pH from 4.7-6.6, Hesthagen et al. (1999) reported that the mean age of brown trout increased with decreasing abundance in lakes with low pH. They concluded that low recruitment rate was responsible for an ageing population. High mortality at the sensitive egg and alevin stages seems to be responsible for aging fish populations (Schofield, 1976; Rosseland et al., 1980; Lachance et al., 2000).

In some lakes, juvenilization has occurred; older individuals occur at low abundance or are absent altogether. Rosseland et al. (1980) found that after acidic episodes, juvenilization occurred, because there was an increased mortality in post spawning brown trout. These acidic episodes did not seem to affect trout eggs. Beamish et al. (1975) found that constantly high acidic conditions resulted in spawning failure in several species such as brown bullhead (*Ictalurus nebulosus*) and northern pike. Ikuta et al. (2003) found that salmonids did not dig nests or spawn in extremely acidic conditions and concluded that this could be the most significant cause in the reduction of salmonid populations.

Trout condition also deteriorates with acidification. Trout that are adversely affected by acidic conditions tend to weigh less at a certain length than trout that are not affected. There is a positive relationship between brown trout condition and pH; condition increases as pH increases (Rosseland et al., 1980). Schindler et al. (1985) found the condition of lake trout started to decrease after four years and became very poor after eight years. They concluded the severe disruption of the food web caused by pH reduction caused poor trout condition, characterized by emaciated trout. Lack of food can also cause an increase in cannibalism on younger cohorts of lake trout (Schindler et al., 1985).

Periods of low pH can initiate the emigration of adult fish (Gloss et al., 1989) leading to lower brook trout densities (Baker et al., 1996). In a study by Gloss et al. (1989), a previously limed lake that was stocked with brook trout sustained a population of trout until the lake began to reacidify. The pH dropped from 6.5 to 5 and there was a large-scale emigration of brook trout from the lake. Brook trout living in connected streams also emigrate from areas of low pH to areas with better water quality (Baker et al., 1996). Radio telemetry was used to track the movement of brook trout emigrating from streams experiencing acidic episodes to streams with a higher pH. Streams with low pH usually had lower trout densities (Baker et al., 1996). Salmonids may avoid acidic environments when choosing a spawning site (Ikuta et al., 2003). Ikuta et al. (2003) conducted a study in which brown trout were given a choice of channel to enter to reach a spawning ground, one channel with close to neutral pH and one with a pH of 5. Ikuta et al. (2003) found that brown trout, when given a choice of route to spawning grounds, would chose water with more neutral pH to swim in. Similarly, Johnson and Webster (1977) found that brook trout chose to spawn in areas of lakes with neutral or slightly alkaline upwelling water and clearly avoided spawning over groundwater with a pH from 4.0 to 4.5.

Methods

Study Area

TGLWA is one of 34 Wilderness Areas in Nova Scotia and is located on the Eastern Shore, approximately 100 km east of Halifax. It has over 100 lakes and streams in its 15800 hectares. TGLWA is typical of Nova Scotia's Eastern Shore granite ridge natural landscape. Due to the region's geology, the region's lakes have low nutrient levels and a reduced buffering capacity against acidity. The study focused on 12 lakes representing the range of sizes and access difficulty in TGLWA; all of the lakes in the study are considered oligotrophic. The lakes chosen for the study ranged from 4 - 97 hectares (Figure 1). Brook trout are native to all of the lakes in the study. Other fish species found in TGLWA include white sucker (*Catostomus commersoni*), brown bullhead, golden shiner (*Notemigonus crysoleucas*), gaspereau (*Alosa pseudoharengus*), and yellow perch (*Morone americana*). With no internal road access or introduced species, and little residential or agricultural development, TGLWA harbours some of the last near-pristine brook trout habitat in Nova Scotia.

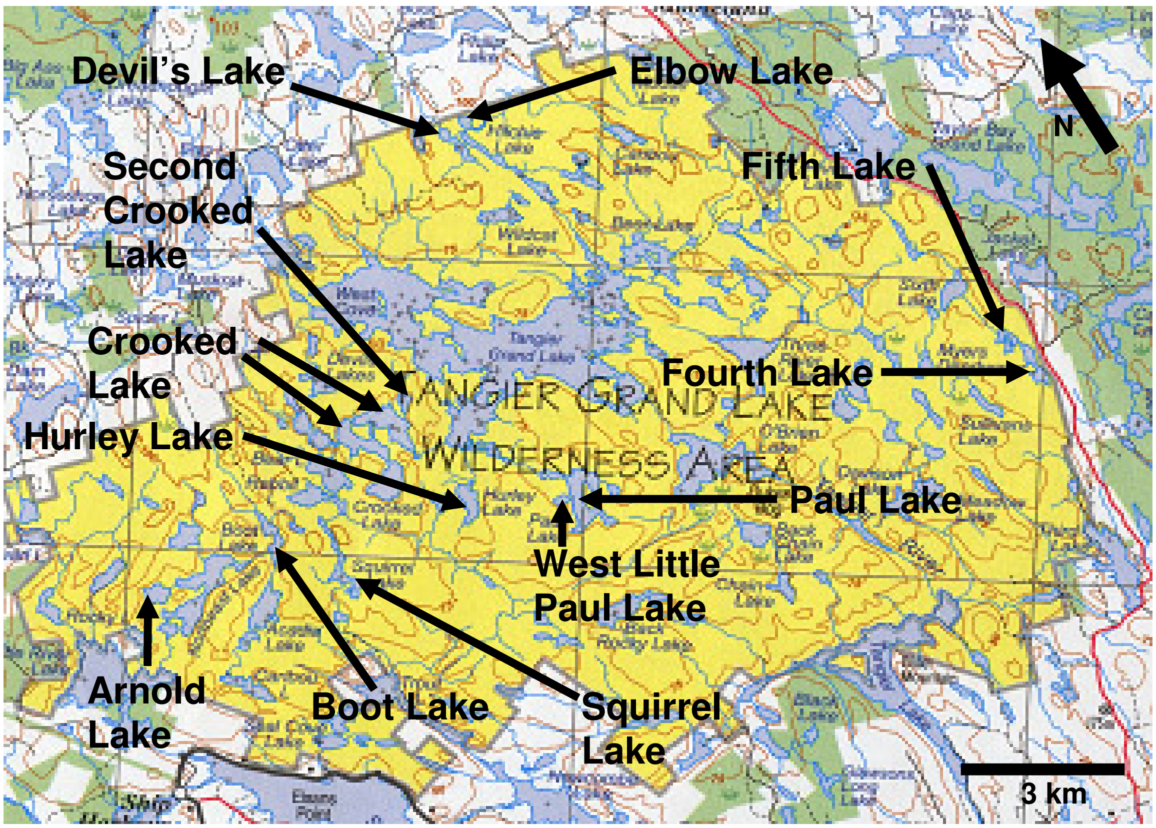

Figure 1. Tangier Grand Lake Wilderness Area in yellow (Service Nova Scotia and Municipal Relations, 2006). Individual lakes chosen for the study.

Permits

Preceding the field season of this project, application forms were filled out and submitted to the appropriate organizations to obtain the necessary permits required. An Animals for Research and Study Permit was required by Dalhousie University to ensure the ethical handling of trout (see Appendix A). A Licence to Conduct Scientific Research in a Wilderness Area permit was required by the Nova Scotia Department of Environment and Labour (see Appendix B). A permit to collect species of fish for artificial breeding and scientific purposes was required for sampling by the Department of Fisheries and Oceans. The privileges of this permit were extended to Dalhousie University from the Nova Scotia Department of Fisheries and Aquaculture, Inland Fisheries Division, who had already obtained the permit and were conducting a similar study in TGLWA.

Sampling/ Data Collection

Twelve lakes representing the range of sizes and access difficulty of lakes within TGLWA were chosen for the study (Table 1). The field season was from April 15, 2007 to June 15, 2007. This sampling period was chosen because trout are less stressed during handling while water temperatures are cool. Small groups of lakes were sampled from specific base locations (Figure 2). Three sampling bases were strategically chosen and were visited two or three times each. Arnold, Boot, and Squirrel Lakes were sampled from sampling base 1. Crooked, Second Crooked, Paul, West Little Paul, and Hurley Lakes were sampled from base 2. Devil's and Elbow Lakes were sampled from base 3.

Table 1. Values for trout population biology measures and environmental and human factors for 12 study lakes in TGLWA.

Lake	Latitude and longitude	Lake area (Ha)	Mean pH	Lake access difficulty score	Total visits to lake	Total nets set	Total brook trout netted	Proportion total observed anglers per lake	Mean vessel presence (vessels)	Mean CPUE trout per net per hour (± 1 standard error)	Mean fork length (cm) (± 1 standard error)	Mean age (years) (± 1 standard error)	Proportion of older trout caught in nets
Arnold	44° 50' 29" N 62° 53' 35" W	12	5.13	4.35	4	18	33	0	3	1.83 (± 0.27)	23.2 (± 0.4)	2.1 (± 0)	0.05
Boot	44° 51' 2" N 62° 52' 5" W	16	4.64	5.2	4	37	12	0.09	2	0.3 (± 0.13)	28.2 (± 1.2)	2.8 (± 0.1)	0.77
Crooked	44° 52' 9" N 62° 50' 23" W	93	5.28	4.78	6	53	75	0.2	2.8	1.42 (± 0.27)	21.8 (± 0.6)	1.7 (± 0.1)	0.11
Devils	44° 55' 16" N 62° 50' 7" W	11	5.16	1.1	4	20	12	0	0	0.61 (± 0.20)	25.9 (± 1.1)	2.2 (± 0.2)	0.11
Elbow	44° 55' 19" N 62° 49' 46" W	7	4.75	2	5	25	3	0	0	0.12 (± 0.01)	24.5 (± 4.1)	2 (± 0.6)	0.33
Fifth	44° 53' 24" N 62° 42' 24" W	8	4.94	1	6	37	40	0.17	2	1.05 (± 0.28)	25.1 (± 0.9)	2 (± 0.1)	0.25
Fourth	44° 53' 4" N 62° 42' 2" W	13	5.08	0.6	6	32	49	0.11	7	1.5 (± 0.43)	21.3 (± 0.8)	1.7 (± 0.1)	0.1
Hurley	44° 51' 42" N 62° 49' 34" W	16	5.05	4.35	1	9	14	0	5	1.41 (± 0.46)	25.4 (± 1.2)	2.3 (± 0.2)	0.23
Paul	44° 51' 44" N 62° 48' 8" W	51	5.2	4.45	6	50	94	0.2	4.8	1.68 (± 0.26)	23.3 (± 0.5)	1.9 (± 0.1)	0.22
Second Crooked	44° 52' 34" N 62° 50' 25" W	6	4.87	5.15	4	18	40	0.03	2.8	1.82 (± 0.46)	25.1 (± 0.9)	1.9 (± 0.1)	0.17
Squirrel	44° 51' 1" N 62° 51' 43" W	24	4.73	4.98	4	31	48	0.2	5	1.52 (± 0.39)	23.6 (± 0.5)	2.2 (± 0.1)	0.23
West Little Paul	44° 51' 38" N 62° 48' 17" W	4	5.27	4.85	3	14	39	0	4.8	2.64 (± 0.60)	23.6 (± 0.7)	2.2 (± 0.1)	0.2

Fourth and Fifth Lakes were accessed from a paved road that bordered the Wilderness Area and were sampled opportunistically within each period.

Three rounds of sampling were planned for mid spring, late spring, and early summer (so data could be compared across temporal periods). We attempted to visit each lake twice during each period, however, this turned out to be impossible for several reasons. Three of the twelve lakes were opportunistically added to the study as it progressed in an attempt to increase the lake sample size; this resulted in some lakes not being sampled as extensively as others. Also, due to logistical reasons, half of the lakes were not sampled during the last period (early summer).

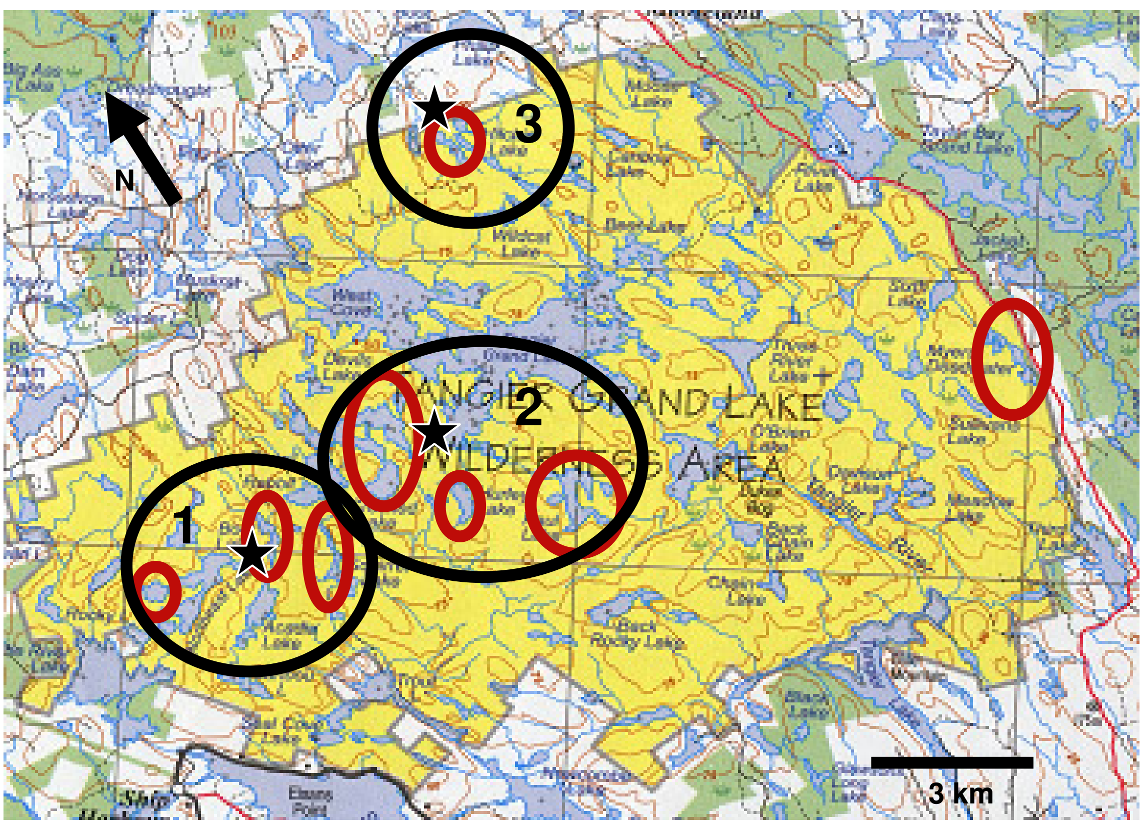

Figure 2. Tangier Grand Lake Wilderness Area in yellow (Service Nova Scotia and Municipal Relations, 2006). Stars and numbers represent sampling base locations. Black ovals indicate area accessed from sampling bases. Red ovals indicate lakes or groups of lakes sampled.

The pH was measured for each lake during each sampling period. The pH was measured at a random location in each lake with a hand held Hanna HI 98129.

The number of anglers and vessels we observed were counted during each visit to each lake. These counts were taken to help confirm the validity of our lake access difficulty ratings. I assumed these counts would be negatively correlated with accessibility; as access difficulty increased, the amount of anglers and vessels would decrease. Anglers were counted individually in boats and on shore only while we were sampling that specific lake. We did not count anglers who told us where they were fishing unless we observed them doing so. Vessels included boats and canoes; all floatable vessels were counted around the perimeter of the lake as well as vessels that anglers were occupying if they were not already identified in the perimeter count. The proportion of total observed anglers visiting each lake during the study (angler presence) and the mean number of vessels per lake (vessel presence) were calculated over the entire study period. Mean vessel presence and the proportion of total observed anglers were used as relative measures of angler activity or exploitation on a lake.

A standardized netting technique was used to make results across the lakes comparable. The research nets used in the study were monofilament nets intended to capture trout non-lethally by the mouth parts; 50 feet by 8 feet (15.24 metres by 2.43 metres) and mesh sizes 1.5”, 2”, and 2.5” (3.8, 5.1, and 6.4 cm). The use of different mesh sizes was an attempt to catch trout of different year classes. During each visit, each lake received proportionally the same amount of sampling effort across the different nets: 1.5’ net- 44% effort, 2” net- 44% effort, and 2.5” net- 12% effort. The 2.5” net effort was intentionally low in an attempt to avoid mortalities involving larger trout.

Nets were randomly set in each of the lakes. This was achieved by dividing the perimeter of a lake into 200 metre sections on a map and numbering them. Numbers were randomly drawn to determine in what sections the nets would be set. However, this process was limited; there were instances when the originally chosen net set location was rejected due to unsafe windy conditions or extreme lake surface vegetation. Another section was randomly chosen when conditions prevented effective sampling.

The larger lakes had proportionally more nets set to ensure that lakes received a comparable amount of netting effort in relation to their size. One or two nets were set simultaneously for an hour and were checked every 20 minutes for trout. A total of five to six nets were set per lake per visit for smaller lakes and ten to twelve nets were set per lake per visit for larger lakes. The netting procedure involved non-lethal sampling, however, there were a small number of trout mortalities.

Trout were retrieved from the research nets and placed in a live holding container. Scales were collected to age each trout. Scale samples were taken from either lateral side of the trout, slightly anterior of the dorsal fin. Fork lengths (from the tip of the mouth to the edge of the centre of the caudal fin) were measured to the nearest millimetre. The adipose fin was clipped from each trout to obtain a tissue sample and stored in 95% ethanol. Trout were allowed to recover in the holding tank before release back into the lake. Tissue samples and lengths were taken for possible future projects that could examine trout population genetics, growth rates, and size-at-age distributions.

The study examines trout population biology to assess the impact of lake accessibility pH, and fishing activity. We measured trout population biology among lakes using four dependent variables describing trout samples; mean CPUE, mean age, the proportion of older individuals, and mean fork length. These variables were calculated for trout in each lake over the entire study. CPUE was calculated as the mean number of trout per net per hour and was considered a measure of relative trout abundance. Lakes with higher mean CPUE are consider more productive. Brook trout scales were used to age the fish in the study from which the proportion of older fish was calculated. The proportion of older individuals is a measure of relative trout population age structure. Three years old or older were considered the older fish. Mean length and mean age of trout were calculated and compared across lakes. A Nikon stereomicroscope, model SMZ 1500, was used to magnify the scale samples, and approximately five pictures were taken of different scales from each trout. The method described by Bagnal and Tesch (1978) guided the analysis. Scales were aged by identifying annuli which are compact areas of growth rings that are separated by rings (circuli) with more space in-between them. The annuli are formed during slow growth in the winter and each annulus represents one year of growth. Local federal and provincial fisheries biologists and technicians also aided in the analysis by providing a second opinion on ages for a small proportion of the scales that were aged.

Accessibility

A lake accessibility scale was required to test the hypotheses. Five factors were considered when rating the accessibility of individual lakes: (1) the distance that had to be travelled from the nearest paved road to a parking area adjacent to the wilderness area boundary from which a lake could be accessed by trail (road distance points [RDP]; < 100 metres= 0 points, 100 metres-10 km= 0.5 point, 10.1 km- 20 km= 1 point, and > 20 km= 1.5 points); (2) the sum of each segment of trail distance (km) multiplied by its difficulty rating that had to be hiked to reach a lake (segment hike points [SHP]; difficulty rating (Z), easy= 1 point, moderate= 2 points, hard= 3 points); (3) the number of lakes that had to be crossed during the hike to reach a lake multiplied by a set coefficient (lakes crossed points [LCP]); (4) the total length (km) of the boat rides that had to be taken to reach a lake multiplied by a set coefficient (boat ride points [BRP]); and (5) the sum of the estimated proportion of anglers accessing the same lake by different routes multiplied by the sum of all other variables (proportion of anglers by route [PAR]). All of the measurements (km) were estimated using a 1:50000 topographical map (11D/15). The factors were aggregated to get an access difficulty [AD] score where:

$$AD = \sum_{i=1}^{n} PAR_i \left(RDP + \sum_{i=1}^{n} SHP_i \ (Z) + LCP\ (0.2) + BRP\ (0.1) \right)$$

The access difficulty equation assumes: (1) the use of a 4wd vehicle to the access point of the wilderness area; (2) that anglers using the wilderness area have vessels stored at every lake; and (3) that every angler uses a motor when using a vessel. These assumptions and the values given to coefficients, difficulty ratings, and driven distances, were based on my field experience and observations, as well as the experience and observations of provincial wilderness area and federal fishery officers that police the wilderness area. For example, a lake that could be accessed from a paved road and a short walk would receive a lower score than a lake that required travelling on a logging road, paddling across several lakes, and lengthy portages (Table 1).

Statistical analysis

Linear and multiple regression analyses were used to identify relationships between lake features (lake area and pH), lake access difficulty, descriptors of trout population biology (CPUE, proportion of older trout, mean age, and mean fork length), and proxies of fishing activity. Independent variables were lake features, lake access difficulty, and vessel and angler presence. Multiple regression models were accepted if all partial regression coefficients were significant. Scatter plots and correlation analysis were used to identify relationships between independent variables assessed in my regression models. The analytical approach identifies the percentage of the variance that is accounted for by the relationship between the variables. Minitab 15 was used for the statistical analysis and significance was determined at p-values less than 0.05 for all tests.

Results

Fifty- three trips were made to lakes and 344 nets were set in which all of the trout samples were collected. This resulted in 459 trout samples being collected among the 12 sample lakes.

Multiple regression models were not accepted because there were not any analyses in which all the partial regression coefficients were significantly different than zero.

Trout Population Biology

Trout ranged between 1 and 4 years of age with a mean age of 1.96 years ± 0.03 years standard error (Table 1). Mean age among lakes ranged from 1.7 years in Crooked Lake and Fourth Lake to 2.8 years in Boot Lake. However, the mean ages for the majority of study lakes were similar, and ranged between 1.9 and 2.3 years. The proportion of older trout in the study, 0.19 (19%), was calculated using three and four year olds (Table 1). The proportion of older trout found in the study lakes ranged from 0.05 (5%) in Arnold Lake to 0.77 (77%) in Boot Lake. The proportions of older trout among the majority of study lakes were similar, and ranged between 0.11 (11%) and 0.25 (25%). Trout ranged between 14.3 cm and 40.4 cm in the study lakes (Table 1). The mean fork length of all trout sampled was 23.5 cm ± .2 cm standard error. Mean fork length ranged between 21.3 cm in Fourth Lake to 28.2 cm in Boot Lake. The mean lengths for the majority of study lakes were similar, and ranged between 23.2 and 25.1 cm. Mean hourly CPUE ranged from 0.12 trout per net in Elbow Lake to 2.64 trout per net in West Little Paul Lake (Table 1).

Trout biology factors mean length, mean age and the proportion of older trout were highly associated with each another (all P values less than 0.003 and all r^2 values greater than 60.1%). Mean CPUE was not associated with mean length, mean age or the proportion of older trout (all P values greater than 0.072 and all r^2 values less than 28.7%).

Environmental and human factors

Mean lake pH ranged from 4.64 in Boot Lake up to 5.28 in Crooked Lake. The proportion total observed anglers over the study period varied from none in Devil's, Elbow, Arnold, Hurley, and West Little Paul lakes up to 0.20 (20%) in Paul and Crooked lakes. We only counted 35 anglers during our field season due to our protocol of only counting anglers that visited lakes the same times we did. We were only at each lake for a short period of time, and our angler counts did not reflect the actual angling activity in the study area. We decided that vessel presence was a more accurate method of estimating angler activity and exploitation. Vessel presence stayed fairly constant over all visits to lakes and ranged from none at Devil's and Elbow Lakes to seven at Fourth Lake. West Little Paul Lake and Second Crooked Lake had no vessels stored around their perimeter. However, we made an assumption that anglers would move their boats from Paul Lake to West Little Paul Lake and from Crooked Lake to Second Crooked Lake due to the relative ease of this and by observing anglers do this. Therefore, the mean vessel presence for West Little Paul Lake and Second Crooked Lake were the same as the vessel count for the lakes from which anglers gained access from adjacent/connected lakes.

Environmental factors (lake area and pH) were not correlated with each other or with human factors. Lake access difficulty and proxy of fishing pressure (proportion of total observed anglers and mean vessel presence) were also not associated with each other.

Mean CPUE

Mean trout CPUE was positively associated with pH (Figure 3) but was not related to lake access difficulty, lake size, or the proportion total observed anglers (Table 2). Thus, in general, the higher the pH was in a lake (less acidic conditions), the greater the mean CPUE of trout was for that lake.

Mean age

The proportion of older trout was negatively associated with pH (Figure 3) but was not associated with lake access difficulty, lake area, mean vessel presence, or the proportion total observed anglers (Table 2). Therefore, lakes with lower pH had greater

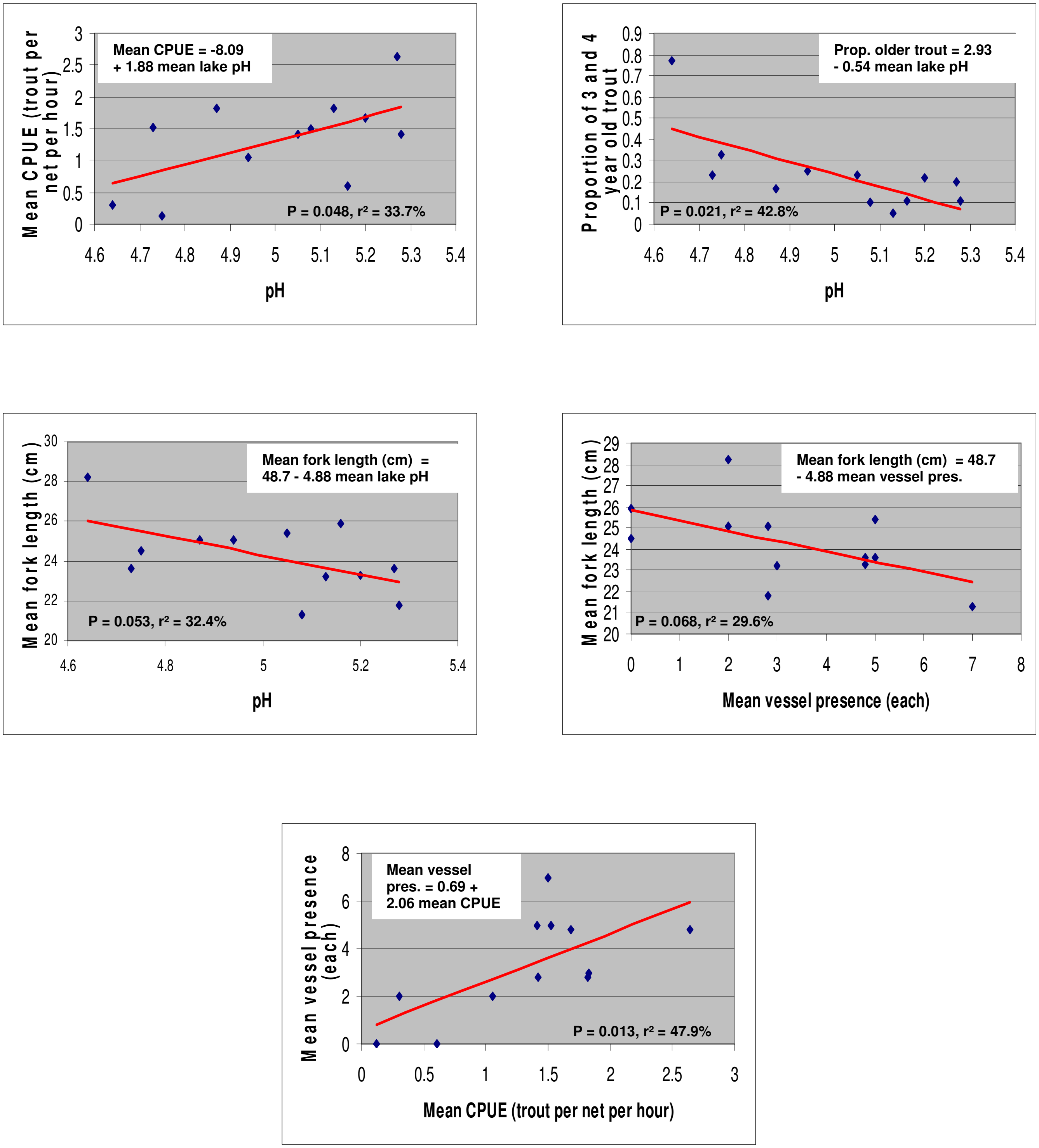

Figure 3. Scatter plots with regression lines for measures of environmental and human factors that were most strongly associated with variables used to describe trout population biology for brook trout in 12 lakes in Tangier Grand Lake Wilderness Area.

proportions of older (three and four year old) trout. The mean age of trout was not associated with pH, lake access difficulty, lake area, mean vessel presence, or the proportion total observed anglers (Table 2).

Mean fork length

There were also negative associations, albeit marginal ones statistically, between mean fork length (for each lake) and either pH or mean vessel presence (Figure 3). Mean fork length was not associated with lake access difficulty, lake area, or the proportion total observed anglers (Table 2). Thus, overall, trout were longer in lakes with lower pH and fewer vessels stored on them.

Mean vessel presence

Mean vessel presence was positively associated with CPUE (Figure 3). Thus, in general, there were more vessels present at lakes that had higher CPUE of trout.

Table 2. Regression analyses that were not significant

Dependent variable	versus	Independent variable	P value	r^2 value
Mean CPUE	versus	Lake Area	0.821	0.5%
Mean CPUE	versus	Proportion of total observed anglers	0.901	0.2%
Mean CPUE	versus	Lake access difficulty	0.186	16.8%
Mean age	versus	pH	0.106	24.0%
Mean age	versus	Lake access difficulty	0.3	10.7%
Mean age	versus	Lake Area	0.213	15.0%
Mean age	versus	Proportion of total observed anglers	0.319	9.9%
Mean age	versus	Mean vessel presence	0.471	5.3%
Proportion of 3 and 4 year olds	versus	Lake access difficulty	0.367	8.2%
Proportion of 3 and 4 year olds	versus	Lake Area	0.566	3.4%
Proportion of 3 and 4 year olds	versus	Proportion of total observed anglers	0.852	0.4%
Proportion of 3 and 4 year olds	versus	Mean vessel presence	0.417	6.7%
Mean fork length	versus	Lake access difficulty	0.715	1.4%
Mean fork length	versus	Lake Area	0.155	19.2%
Mean fork length	versus	Proportion of total observed anglers	0.269	12.1%

Discussion

Lake access difficulty

I hypothesized that measures of fishing pressure (mean vessel presence and the proportion of total observed anglers) would decrease as lake access difficulty increased. Rather, I found that fishing pressure was not associated with lake access difficulty. I was not able to find another study that found lake access difficulty not to be related to angler exploitation. However, there are several studies that have found fishing pressure to be negatively associated with lake access difficulty (Gunn and Sein, 2000; Broad et al., 2002; Bailey and Hubert, 2003; Schill et al., 2007). I also hypothesized that measures of trout population biology, such as mean CPUE, mean age, the proportion of older trout, and mean fork length would increase with increased lake access difficulty. Namely, more accessible angling locations are likely to experience more intense fishing pressure than locations that are difficult to access (Gunn and Sein, 2000; Broad et al., 2002) reducing measures of trout population biology. However, there was no relationship between the degree of lake accessibility and mean CPUE, mean age, the proportion of older trout, and mean fork in TGLWA lakes. Again, I was not able to find another study that had similar results. In contrast, several studies have found positive associations between lake access difficulty and mean age and mean length (Broad et al., 2002; Bailey and Hubert, 2003).

There are several possible explanations for the discrepancies between studies. The difference in findings may be due to the fact that the accessibility of Gunn and Seins' (2000) study lake increased from one year to the next. TGLWA study lakes have been accessed through relatively unchanged roads and trail systems for many years. If access difficulty was decreased in TGLWA (by allowing ATV use in the wilderness area), or if access difficulty was increased (by not allowing vessels to be stored or use of boat motors), perhaps a future study of TGLWA lakes would conclude there is a positive association between access difficulty and mean age and length as Gunn and Sein (2000) did. However, the most likely reason for the difference in findings between this study and others (Gunn and Sein, 2000; Broad et al., 2002; Bailey and Hubert, 2003) regarding lake accessibility and trout population biology (mean age and mean length) is the difference in proxy of fishing activity between study areas related to lake access difficulty. I assumed, when creating the accessibility scale, there was a strong negative association between increasing lake access difficulty and angler exploitation. For example, as lake access difficulty increased, angler exploitation decreased. This was true in the aforementioned studies, but not in my own. My accessibility scale was a reasonable measure of effort needed to reach destined lakes, however, it did not accurately estimate the amount of angler activity or exploitation TGLWA lakes receive. Allowing the storage of vessels (see below), camps, and the use of motors boats in the wilderness area increases the area's accessibility; perhaps to the point that all lakes in the wilderness area are relatively easy to access by anglers. During the field season, many vessels and anglers were seen at easy to access lakes as difficult to access lakes. This observation was confirmed by the lack of any significant relationship between mean vessel presence, the proportion of total observed anglers and lake access difficulty (Table 2).

Mean vessel presence (proxy of fishing activity)

There was a strong association between CPUE and mean vessel presence (Figure 3). It could be interpreted that vessels are placed at certain lakes because anglers that are using the resource know where the more productive (abundant) trout populations are.

Throughout our field season in TGLWA, we had many conversations with local anglers who told us what lakes we would find trout in, where we would find large trout, and where we would catch lots of trout. Our results pertaining to mean CPUE and mean length confirmed much of the information communicated to us by anglers. Therefore, angler knowledge of productive lakes for fishing is likely the factor driving fishing activity and exploitation in TGLWA, rather than lake access difficulty.

There was a negative trend between the proportion of older trout, mean fork length, mean age and mean vessel presence; there were smaller, younger trout in lakes with more fishing activity (Figure 3). Bailey and Hubert (2003) found that fishing activity prevented the majority of cutthroat trout from aging over two years, consequently resulting in many short lived fish in many of their study locations. Eighty percent of the fish netted in my study were under two years old. It is possible that fishing activity is also preventing the majority of brook trout from aging over two years. However, cutthroat trout live up to three times as long as brook trout in TGLWA, and this difference in life span cannot be ignored.

Mean fork length was the only factor marginally associated with mean vessel presence, whereas the proportion of older individuals and the mean age of trout in each lake were not. Perhaps this is because angling is selective of larger trout, but not necessarily older trout (some grow faster than others). The fork length of trout decreased as the number of vessels increased among study lakes. Exploitation can alter natural population structures by reducing the amount of larger individuals in the population (Broad et al., 2002) and it is possible that this is happening in TGLWA. It is interesting that mean fork length of brook trout decreased among TGLWA lakes as the pH decreased, while mean length also decreased as a measure of fishing activity (mean vessel presence) increased. Thus, although environmental factors (pH) may be contributing to the relationships between CPUE and trout length (see below), these results suggest that angling exploitation may also be resulting in size-selective harvesting of brook trout in the more productive TGLWA lakes.

Acidification and trout population biology

The result that lakes with higher pH had a higher mean CPUE of trout (Figure 3) is consistent with previous research (Hesthagen et al. 1999). This result can be interpreted in several ways: (1) less acidic waters have higher survival rates for trout eggs, alevins, and fingerlings which could increase trout abundance (CPUE) and (2) acidic freshwater conditions can initiate the emigration of trout to water bodies with less acidic conditions leading to lower densities. Several studies that have examined the effect of pH on brook trout survival show that there are higher survival rates in juvenile stages in less acidic conditions (Marschall and Crowder, 1996; Lachance et al., 2000; Warren et al., 2005). Several studies indicate that brook trout will emigrate from very acidic habitats (Gloss et al., 1989; Baker et al., 1996) which can lower brook trout densities (Baker et al., 1996) in acidic waters.

I hypothesized that the proportion of older trout would increase as pH increased. Rather, I found that the proportion of older trout in a sample increased as lake water acidity (pH) decreased. This has been observed in several fish species in acidified waters (Schindler et al 1985; Marschall and Crowder, 1996; Lachance et al., 2000; Warren et al., 2005). A possible explanation may be recruitment failure. Low pH can facilitate recruitment failure by reducing survival at young and sensitive juvenile stages. As well, a reduction or elimination of spawning can lead to an aging of the population. Brook trout avoid acidic environments when spawning which could lead to a lower recruitment rate. This response has been observed in several studies that examine the effect of pH on brook trout spawning behaviour (Johnson and Webster 1977; Ikuta et al., 2003). Several species experience spawning failure in constantly high acidic conditions (Beamish et al. 1975, Ikuta et al. 2003) which could increase the mean age of the population. Not surprisingly, lake water acidity (pH) was also negatively associated with mean fork length of trout in individual lakes and the relationship was marginally significant (Figure 3). Hesthagen et al. (1999) found similar results in their study of brown trout and concluded that recruitment failure had occurred in lakes that were impacted by acidic conditions. However, my results indicated that fishing activity was also negatively associated with mean fork length in TGLWA. Therefore, it cannot be discounted that human exploitation (in addition to acidification) may be driving the patterns observed in this study between age structure, mean length and pH.

Conclusions

This study examined several associations between environmental (e.g. lake size, pH) or human factors (e.g. lake accessibility, proxy of fishing activity (e.g. mean vessel (boat and canoe) presence and proportion of total observed angler presence) and trout population biology (e.g. trout catch per unit effort (CPUE, a proxy for trout abundance), trout length, and trout age).

Lake access difficulty and trout abundance

Lake accessibility was not the driving force influencing fishing activity; greater trout abundance seemed to encourage fishing activity. Anglers know where the more productive trout lakes are, and that is where they fish.

Mean vessel presence (Proxy of fishing activity)

It is possible that over fishing has decreased the size of trout because the more-fished lakes also have smaller trout. However, lakes that have the highest fishing activity are those with the least acidic pH, highest CPUE, and also the smallest mean length of trout. It is possible that both fishing activity and less acidic conditions (see below) are responsible for smaller mean trout length in TGLWA lakes.

Acidification

There are higher CPUE's of trout and younger, smaller trout in less acidic lakes. It is possible that acidification has influenced the age structure in TGLWA lakes. Acidification is possibly one reason why there are aging populations in more acidic lakes and younger populations in less acidic lakes.

Recommendation

My results show that fishing activity is higher on study lakes with higher trout abundance, higher pH and that the mean length of trout is smallest in those lakes (Figure 3). It is possible that size-selective harvest of large trout by anglers (human factor) and higher pH (environmental factor) has influenced these populations towards a smaller average size. The degradation of mean trout size by size-selective angling is a matter of serious conservation concern, given that the negative effects of size-selective fishing are known to have adverse effects on harvested stocks of other species (Stokes and Law

2000; Hutchings and Fraser 2008). These negative effects can lead to reduced productivity, lower maximum sustainable yields, and slower rates of population growth (Hutchings and Fraser 2008).

The previously proposed TNS regulation change (bag limit of three trout under 30 cm) derives from anecdotal reports of degraded trout populations or habitat without any measurable scientific basis. The results of my research can be used to reveal the extent to which fishing activity explains the observed trends. This can be achieved through the implementation of experimental management regimes on several study lakes with follow-up surveys on these lakes. The implementation of a maximum retainable trout length (26 cm) to protect larger trout in a number of lakes can be used to assess the degree to which the protection of larger trout changes trout population biology (mean length, mean age, the proportion of older trout, and mean CPUE of populations). Lakes with and without the suggested length maximum would be re-assessed after a five-year period (after 2 trout generations). With this recommendation, there is an opportunity to disentangle the effects of size-selective harvest by angling from habitat degradation (acidification). This approach has the potential to yield important results that can be applied to trout management in TGLWA, throughout the province, and to brook trout management in general.

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DALHOUSIE UNIVERSITY
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FORM A

Appendix A

(original + 14 copies)

UNIVERSITY COMMITTEE ON LABORATORY ANIMALS

APPLICATION TO USE ANIMALS FOR RESEARCH or TEACHING - ETHICS REVIEW

Principal Investigator (must have Faculty appointment): Dr. Jeff Hutchings

Faculty Position: Professor **Department:** Biology

Building: Life Science Centre **Phone:** 494-2687

E-Mail: jeff.hutchings@dal.ca

Project Title: Tangier Grand Lake Wilderness Area (TGLWA) Brook Trout Conservation Project

Renewal of Previous Protocol? Yes: ☐ **No:** ☒ **Previous Protocol Number:**

Is this protocol for: Research: ☒ **Teaching:** ☐ **Testing:** ☐

Is the proposed work currently funded? Yes: ☒ **No:** ☐ **Pending:** ☐

Name of funding source(s): NSERC

Have or will the concepts for the experiments proposed in this protocol be peer reviewed by the funding agency?

Yes: ☒ **No:** ☐ * **Pending:** ☐ *

* **All protocols must be reviewed for scientific merit. If this project has not been reviewed, or was reviewed but not funded, please see *APPENDIX 2*.**

Is this protocol a contract *or* part of a contract? Yes: ☐ **No:** ☒

Proposed Start Date: April 11/07 **Proposed End Date:** August 11/07

<u>EMERGENCY CONTACTS</u>: ***MUST* include phone numbers for *PRINCIPAL INVESTIGATOR* <u>*and*</u> *ONE OTHER PERSON* designated to handle animal emergencies or media inquiries. Must be available for evenings and weekends.**

Name	Certificate #	Department	Position	After hours phone
1. Dr. Jeff Hutchings	2009-Dec-270	Biology	Professor	454-4240
2. Dr. Dylan Fraser	2010-May-079	Biology	Post Doctoral Researc	454 6409

***<u>DECLARATION</u>*:** **All animals used in this research project will be cared for in accordance with the recommendations of the Canadian Council on Animal Care and the policies of Dalhousie University.**

SIGNATURE: ____ **DATE:** March 8, 2007

Principal investigator or course director

SECTION 2

RESEARCH STAFF: **Certification numbers are valid for five years, at which time, re-certification is required. Please ensure that certification numbers are current. Principal Investigator *MUST* have a current certification number. Add additional personnel on a separate sheet.**

Name	Certificate #	Department	Position	Phone
1. Dr. Jeff Hutchings	2009-Dec-270	Biology	Professor	494-2687
2. Dr. Dylan Fraser	2010-May-079	Biology	Post Doctoral Researc	494-6279
3. Dave Hardie	2010-may-078	Biology	PhD Candidate	494-6279
4. Anthony Heggelin	Pending	Environmental Science	Work Term	494-6279
5.				

<u>RESEARCH PERSONNEL TRAINING</u>:

Everyone handling laboratory animals is required to be listed as research staff and have a certification number in addition to *practical training* for the species they are using (see Certification/Training in information section, or contact the University Veterinarian's office for more information). Add additional personnel on a separate sheet.

For research staff required to handle live animals, check off which workshops/training have been attended.
Acronym description of practical workshops currently offered is provided below:

NAME	RH	MH	ANES	AS	FH	Custom Session Describe below *	Other Describe below *
1. Dr. Jeff Hutchings	☐	☐	☐	☐	☐	☐	☐
2. Dr. Dylan Fraser	☐	☐	☐	☐	☐	☐	☐
3. Dave Hardie	☐	☐	☐	☐	☐	☐	☐
4. Anthony Heggelin	☐	☐	☐	☐	☐	☐	☐
5.	☐	☐	☐	☐	☐	☐	☐

Describe *<u>CUSTOM SESSION with Lab Animal Training Coordinator</u>* or *<u>OTHER</u>* Training here:

RH = Rat Handling : Recommended Tech Procedures — **Familiarization with handing, restraint, injection and blood collection procedures in the rat.**
MH = Mouse Handlng : Recommended Tech Procedures — **Familiarization with handling, restraint, injection and blood collection procedures in the mouse**
ANES = Intro to Anesthesia — **Principles of Anesthesia**
AS = Intro to Aseptic Surgical Technique — **Aseptic surgical tech, post-op care, analgesia, scrub, gown, glove tech, suture**
FH = Fish Handling and Water Quality — **Evaluation of water quality parameters. Anesthesia, tagging, blood collection & surgical techniques**
Custom Session = — **Individualized sessions with Dalhousie Lab Animal Training Coordinator**
Other = — **eg. formal training from other institutions, or training from other qualified personnel**

Will anyone listed on the protocol require new/extra training for some procedures? **Yes** ☐ **No** ☒
If "*yes*", please describe what procedures:

SECTION 3: **Project Objectives: *Briefly* describe the objectives of the proposed work using animals.**

The objectives are: to evaluate the overall health (age and size distributions) of different trout populations in the TGLWA, to assess the degree to which accessability impacts trout population health, and to use this research to facilitate proper management decisions (such as angling regulations) regarding the issue of accesssibility and sustainable recreational fisheries in the TGLWA.

SECTION 4: **What is the potential benefit that might result from your research? If applicable, please indicate the disease process that this work will investigate.**

The potential benefits are healthy, more genetically diverse populations of trout due to changes in angling regulations.

SECTION 5 (PART 1) CHECKLIST:

This YES / NO checklist will provide assistance in determining which Attachments will be required.
Check YES or NO for each item below, and complete the required attachment for each YES.

5a. * Does this project involve surgery and/or administration of anesthetics? YES ☒ NO ☐
If **yes** , complete and include ***ATTACHMENT A***

* It is **NOT** necessary to complete *Attachment A* if an anesthetic ***overdose*** is being administered solely as a method of euthanasia, without any surgical manipulations, recordings, etc. performed on anesthetized animals.

5b. **Is this protocol a renewal of a previously approved protocol?** **YES** ☐ **NO** ☒
If *yes*, complete and include *ATTACHMENT B*

5c. **Will these animals be used for teaching?** **YES** ☐ **NO** ☒
If *yes*, complete and include *ATTACHMENT C*

5d. **Is this a field study?** **YES** ☒ **NO** ☐
If *yes*, complete and include *ATTACHMENT D*

5e. **Are agents hazardous to humans or animals, such as radioisotopes, biohazardous materials, chemicals or radiation administered to animals?** **YES** ☐ **NO** ☒
If *yes*, complete and include *ATTACHMENT E*

5f. **Is breeding required as part of this protocol?** **YES** ☐ **NO** ☒
If *yes*, complete and include *ATTACHMENT F* *
***All breeding colonies must be covered under an approved protocol. Breeding animals can be included as part of this research protocol by completing Attachment F. Alternatively, if many breeding colonies are maintained, or if a breeding colony supports many different experimental protocols, a separate breeding protocol can be submitted.**

5g. **Are animals being immunized in this project?** **YES** ☐ **NO** ☒
If *yes*, complete and include *ATTACHMENT G*

5h. **Does this project involve the injection or implantation of tumours, stem cells or other biological materials (other than tissue transplants) into rodents?** **YES** ☐ **NO** ☒

If *yes*, complete and include *ATTACHMENT H*

5i. **Does this project involve safety/efficacy/toxicity testing for *regulatory* purposes?** **YES** ☐ **NO** ☒
If *yes*, complete and include *ATTACHMENT I*

SECTION 5 (PART 2): PROJECT DESCRIPTION

Provide a description of all procedures to which animals will be exposed. *Use language that non-specialists can understand*. Please remember that not all committee members are scientists. Very clear and simple language (lay language) is expected. All procedures performed in animals must be described *in detail* here, unless the details are included in one of the attachments. Do not excerpt pages from a grant application. Include all anticipated effects of the procedures on the animals. If animals do not all undergo the same procedures, be sure to clarify the number of animals undergoing each procedure, and the sequence of events. Unless this is a very simple project, a *FLOW CHART* will be required. *FLOW CHARTS* should include the number of animals in each group, the number of replicates, procedures , timelines and endpoints.
(this answer section will expand)

The trout will be caught by tooth fished nets and angling. The procedure using tooth fished nets is non lethal. A net will be set for 30 minutes, the trout will be collected from the net, anesthetized, sampled, and be placed in an aerated recovery bath until they have regained their equilibrium. At this point they will be released back into the lake. Fish will be angled with fishing rods using artificial flies and lures. The angled fish will be anesthitized, sampled, rehabilitated and released using the same procedure as the tooth netted fish. Sampling will include measuring the length of each fish on a measuring board and obtaining scales from each individual (for ageing) by rubbing a scalpel along the surface of the fish in the area adjacent to the dorsal fin. This procedure does not cut or make the fish bleed and only several scales will be taken from each fish. As well, each trout will have its adipose fin clipped with scissors. This does not make the fish bleed and is a low impact way to mark a fish and obtain DNA information. All of these procedures are non lethal. These are very common procedures used by the Department of Fisheries and Oceans Canada and the Nova Scotia Department of Agriculture and Fisheries to sample fish.

SECTION 6: ANIMALS

6a. Justify the number of animals based on the experimental design of this project. Indicate how the number of animals was determined by breaking their use down into experimental groups. Include a statistical justification if possible, or a yield of tissue needed per animal.

If the project is expected to take more than one year to complete, justify the total experimental design here, and then indicate how many you expect to use in the *FIRST YEAR*. If additional animals are required before the expiry date of this protocol, a FORM B may be submitted requesting additional animals. *(this answer section will expand)*

The experiment will include 12 lakes that have varying degrees of accessibility. Lakes will range in accessibility as follows: paved access, logging road access (2wd), logging road access (4wd), ATV trail access, portage trail, and no defined trail. Each lake will be sampled 3 times, once in the early spring, late spring, and early summer. We are hoping to get a combined total of 50 fish per lake for the three visits per lake. Fifty is a reasonable number of trout to determine the age and size ditributions of each population as well as genetic samples from each population. Therefore, the amount of fish we hope to sample is 600. We would obtain the adipose fin from each animal which is a non lethal procedure.

6b. Indicate the number of animals you will use for the next 1 (one) year period.

SPECIES	STRAIN	WEIGHT or AGE	SEX	ANNUAL TOTAL	SUPPLIER

Brook trout		all	both	600	TGLWA

6c. **If animals for this study are produced by in house breeding, you must include *ATTACHMENT F* or provide the number of your currently approved breeding protocol. *PROTOCOL #***

6d. **ANIMAL HOLDING FACILITY: BUILDING: ROOM:**

EXPERIMENTAL STUDIES: BUILDING: ROOM:

6e. **Why must animals be used in this study?**
If alternatives to animals are available, indicate why they cannot be used in this study.

It is the only means of distaining the information we require for each lake population.

6f. Explain choice of animal model or animals species.

Trout are the most sought-after sport fish in the province. We require scientific data on how accessibility affects trout populations (age and size distribution).The data collected will be used to implement changes in regulations that will protect and benefit trout populations.

6g. Can normal environmental enrichment devices be provided to captive animals on this study?
YES ☐ NO ☒ If *NO*, please justify withholding these items.

The fish caught by angling will be caught, anesthetized, sampled, and released. For the tooth fished net, the fish will remain in the water in the net for less than 30 minutes. Thirty minutes is required because the area that the net is in must be undisturbed in order to invite the fish into the area where the net is. When the fish is taken from the net, it will be anesthetized, sampled, and released as fast as possible. There is not any captivity other than what is described in this paragraph.

SECTION 7 : HUMANE ENDPOINTS

Some experimental manipulations or phenotype abnormalities can be expected to produce a degree of unavoidable pain, distress or illness in experimental animals. CCAC guidelines require that adverse effects "be minimized or alleviated by choosing the earliest endpoint consistent with the scientific objectives of the research.

7a. What is the expected time course of the study? (ie., how long are animals maintained from the first experimental manipulation until the end of the experiment or planned euthanasia?) For field studies what is the period of observation?

Observation will include anesthesia, sampling, and recovery in an aerated bath. The entire procedure is estimated to take less than five minutes, however, we will not release the fish until it has fully regained its equilibrium. This may extent the observation period.

7b. Do you expect this study to cause any pain, distress or illness? YES ☒ NO ☐
If *yes*, please describe, including time course. ***(this answer section will expand)***

The study will stress the fish. The fish will experience up to 30 minutes of stress in the tooth fished net and less that two minutes of stress if caught by angling. The anesthetic bath the fish will be placed in prior to sampling should help aleviate some of the stress the trout would experience during sampling.

7c. What clinical signs / observations may indicate that animals used in this project are experiencing pain, distress or illness? (e.g. behavioural changes such as decreased grooming, vocalization or postural changes, or physical abnormalities such as anorexia, dehydration, diarrhea, etc.) ***(this answer section will expand)***

When caught by the net, they may try to swim away or escape until they are exhausted. They may float belly up (a temporary loss of their equilibrium) when exhausted

When are these signs expected to occur and when would they be most severe?
(this answer section will expand)

When they are caught in the tooth fished net, it is possible that they may become exhausted. This would be the only time that this would happen.

7d. How frequently will animals be monitored during the course of the study as well as during the critical times when signs are most severe? ***(this answer section will expand)***

The tooth fished net will be checked every 30 minutes. They will be monitored constantly in the anesthetic bath, during sampling, and the recovery bath.

How/where will monitoring be recorded? ***(this answer section will expand)***

The contents of the net will be recorded in a waterproof notebook each time the net is checked. The recording will take place at the net site. The net will be checked from a canoe.

Who will monitor the animals? ***(this answer section will expand)***

All of the research staff will take turns monitoring the net , the anesthetic bath, and the recovery bath. The people who set the net will be responsible to check the net.

7e. What criteria, appropriate to the species, will trigger the decision to humanely euthanize an animal before the experimental objective is achieved? (examples could include the following: a weight loss limit as a percentage of body weight, allowable durations of anorexia, ulcerative skin lesions). ***(this answer section will expand)***

We will have a recovery bath in the canoe. After sampling, each fish will be given as much time as they need to recover. If a trout is not breathing, determined by observing the movement of the operculum and gills, or is bleeding from the gills, it will be euthanized.

7f. The veterinarian has the authority to euthanize animals in distress; however, a reasonable attempt will be made to communicate with the research group before taking action. Who in the research group can authorize the euthanasia of animals?

N/A

7g. **Animals that die unexpectedly or are euthanized may be submitted for post-mortem examination by the veterinarian. Are there any special instructions for sample collection from the animal at the time of euthanasia?** **YES ☐ NO ☒**
If *yes*, please describe and staff will attempt to accommodate this request.

SECTION 8 - CCAC CATEGORIES OF INVASIVENESS

Using *APPENDIX 1* (For wildlife use *APPENDIX 1B*)
indicate the *Level of Invasiveness* that best identifies this protocol.

B ☐ C ☒ D ☐ E ☐

For LEVEL A experiments - the use of invertebrates (excluding higher invertebrates eg. all cephalopods), eggs, protozoa or other single-celled organisms, tissue obtained from sources such as a slaughterhouse or from another source, please use a Form C.

PURPOSE OF ANIMAL USE: **This is a CCAC requirement. Please indicate which single statement *<u>BEST</u>* describes the purpose of animal use in this protocol.**

0. ☐ **Breeding *ONLY***
1. ☒ **Studies of a fundamental nature in sciences relating to essential structure or function(e.g. biology, psychology, biochemistry, pharmacology, physiology).**
2. ☐ **Studies for medical purposes, including veterinary medicine, that relate to human or animal diseases or disorders**
3. ☐ **Studies for regulatory testing of products, for the protection of humans, animals, or the environment.**
4. ☐ **Studies for the development of products or appliances for human or veterinary medicine.**
5. ☐ **Education and training of individuals in post-secondary institutions or facilities**

<u>CCAC Animal Care Classification Category:</u>

An acute study is one in which animals are euthanized without an extended holding period or prior invasive manipulations, unless these are performed under anesthesia without recovery. This would include animals euthanized for tissue collection, or anesthetized for a surgical procedure and euthanized before waking up. A chronic study involves experimental manipulations on conscious animals, or recovery from anesthesia.

Is this study *ACUTE* ☐ *or CHRONIC* ☒ ?

SECTION 9 - EUTHANASIA

9a. METHODS OF EUTHANASIA

SPECIES	METHOD	DRUG / ROUTE / DOSE
Brook trout	**Anesthesia overdose**	clove oil
	Exsanguination with Anesthesia	
	Decapitation *WITH* Sedation or Anesthesia	
	Decapitation *WITHOUT* Sedation or Anesthesia ***(see 9b.)****	**N/A**
	Cervical Dislocation *WITH* Sedation or Anesthesia	
	Cervical Dislocation *WITHOUT* Sedation or Anesthesia ***(see 9b.)****	**N/A**
	CO 2 Chamber	**N/A**
	Other methods of Euthanasia (specify):	

9b. **<u>IMPORTANT</u>:**

If decapitation or cervical dislocation *WITHOUT* sedation or anesthesia was indicated above, provide *scientific justification* (with references where available) for why sedation or anesthesia cannot be used:

(this answer section will expand)

Describe the training or experience of personnel performing cervical dislocation or decapitation.

(this answer section will expand)

9c. **If animals are *<u>not</u>* to be euthanized at the completion of the study, please indicate what will happen to them:**

They will be released back into their habitat.

Appendix B

SCOTIA

Environment & Labour

Halifax, Nova Scotia
B3J 2T8

Fax (902) 424-0501

Our File Number:
RL200703WA

Protected Areas Branch

LICENCE TO CONDUCT SCIENTIFIC RESEARCH IN A WILDERNESS AREA

Pursuant to Section 21 of the *Wilderness Areas Protection Act* and subject to the terms and conditions contained on the reverse side of this Licence, this Licence is issued to Jeff Hutchings as a Licence holder to conduct scientific research as described in the attached application in the Tangier Grand Lake Wilderness Area.

This Licence expires on 31 July 2007, and is subject to cancellation by the Minister, or person authorized by the Minister, if it is determined that the research is contributing to the degradation of the Wilderness Area, or for non-compliance with the *Wilderness Areas Protection Act*, its regulations, or any terms and conditions of this Licence by the Licence holder.

The Licence holder may apply for renewal of this Licence by submitting a written request and progress report, but renewal is not automatic and may be subject to additional terms and conditions.

This Licence issued at Halifax, in the Province of Nova Scotia this 4th day of April, 2007.

Minister or Person Authorized by the Minister

2007
Licence No. RL200703WA

The Licence holder and additional members of the research team must carry a copy of this Licence, including the terms and conditions at all times while in the Wilderness Area.

This Licence is subject to the following terms and conditions:

1. This Licence permits the Licence holder, and other research team members identified in the attached application, to conduct scientific research as outlined in the attached application.

2. A copy of all data, publications, reports, or other products of the research shall be forwarded to the Manager, Protected Areas Branch. A detailed list of any material collected from the Wilderness Area shall be provided to the Manager and shall include information on the archival disposition of specimens.

3. For multi-year projects, an annual progress report shall be submitted to the Manager, Protected Areas Branch, within one year of the issuance of this Licence, along with the request for renewal of this research Licence.

4. The Licence holder shall comply with the *Wilderness Areas Protection Act* and Regulations and any other relevant Act and Regulations.

5. The Province of Nova Scotia, including the Department of Environment and Labour and the Minister of Environment and Labour, shall not be liable for any injury or damage (including death) to the person covered by this Licence, or for the loss or damage to the property of the Licence holder attributable in any way to the performance of any act under this Licence.

6. The Holder shall travel and camp in a manner consistent with the Leave No Trace Principles presented in provincial Keep it Wild brochure, and the Order on Camping and Lighting of Fires in Designated Wilderness Areas.

7. This Licence does not allow the use of motorized vehicles within a Wilderness Area

Additional terms and conditions:

8. This Licence permits the Licence holder, and other research team members identified in the attached application, to capture live brook trout (*Salvelinus fontinalus*) by means of angling and tooth nets and remove and retai n the adipose fin of each fish caught as outlined in the attached application.

The Licence holder is reminded that Wilderness Areas are protected under the *Wilderness Area Protection Act*. It is an offence to litter, cut trees or construct unauthorized campsites.

Failure to comply with the terms and conditions of this Licence constitutes an offence under the *Wilderness Areas Protection Act*. Penalties for a first offence by an individual are fined up to $500,000, 6 months imprisonment, or both.

Please help keep the Wilderness Wild.