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Seasonal variation in condition, body lipid, reproductive investment, and diet in brook stickleback (*Culaea inconstans*)

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Buffalo State University
State University of New York

Seasonal variation in condition, body lipid, reproductive investment, and diet in brook
stickleback (*Culaea inconstans*)

A Thesis in
Great Lakes Environmental Science

by

Matthew P. Basista

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Arts
May 2023

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Abstract

North-temperate fishes are subject to significant changes in abiotic and biotic conditions across seasons, which are likely reflected in temporal differences in energy dynamics, reproductive investment, and diet. This study explores seasonal changes in body lipid content, female reproductive investment (GSI), body condition (Fulton's K), and diet in brook sticklebacks (*Culaea inconstans*) in Western New York. I expected body lipid content and body condition would decline during the breeding season reflecting energy expenditure for reproduction, and these parameters would increase in the summer and fall prior to the onset of winter. Based on previous studies, I also expected the diet of sticklebacks would vary seasonally, reflecting a broad and flexible feeding strategy. GSI of female brook sticklebacks was highest during the spring, declined and remained low during the summer, and gradually increased during the fall; Fulton's K showed a similar pattern. In contrast, body lipid content increased during the spring in male and female sticklebacks before declining in the summer and increasing in the fall, and Fulton's K overall was not a reliable predictor of female body lipid. The diet of brook sticklebacks was broad in terms of the number of taxa consumed but was focused primarily on aquatic insects and crustaceans. The feeding strategy was seasonally variable, expressing a generalist feeding strategy in the spring and more specialized in the summer and fall. An enhanced understanding of prey fish energetics may aid in informing the management and conservation of native freshwater fish communities, sport fisheries management, and waterfowl conservation.

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Introduction

The organisms of north-temperate aquatic ecosystems are subject to changing abiotic and biotic conditions across seasons, leading organisms to endure variable amounts of physiological stress. The physiological response of organisms to changing conditions may vary depending upon thermoregulation type, habitat type, habitat use, latitudinal location, and life history. Ectotherms such as fish have a metabolism which is strongly dependent upon water temperatures and therefore the metabolic needs of fish are also strongly tied to abiotic conditions (Fike et al. 2007). Many north-temperate fish do not undergo prolonged dormancy so, physiological processes such as metabolism, organ function, and cellular maintenance remain as active energetic costs during the winter period, presenting a temporal challenge (Chavin 1973; Adams 1999; Snyder et al. 2011). This physiological stress may be mediated by winter foraging and subsidized by the metabolism of lipid reserves. In contrast, some freshwater tropical fish accumulate lipid reserves over the winter period when metabolic requirements are low, and forage is abundant as these habitats present less pronounced winter constraints. Instead, the fish in tropical habitats may experience metabolic stress during the summer when reproductive maturation and behavior occurs alongside elevated fish metabolism that is prompted by warm water conditions, and therefore rely in part on lipid reserves accumulated in the previous winter (Karapanagiotidis et al. 2010).

As the challenge of physiological stress is mediated and seasonally predictable, fish species have various life history strategies and express seasonal variation in growth and energy storage. By consuming more calories than needed and storing excess energy as lipids during favorable conditions, fish are able to overcome future periods

where energy demand may outweigh dietary contribution (Adams 1999; Fike et al. 2007). If an organism can optimize energy allocation during periods of physiological stress, future survival and reproduction may be increased. On the other hand, if an organism does not have enough energy to maintain its physiological processes during a period of metabolic stress, the likely result is mortality (Adams 1999; Hurst 2007).

Fish acquire important biomolecules such as lipids, proteins, and carbohydrates from dietary items which are absorbed through digestion and assimilated into bodily tissues for growth, metabolism, or energy storage. Lipids are essential for most physiological processes in fish and serve as a primary source of stored energy in the form of fat tissues and fish oil. Apart from the cellular components that require proteins, by mass proteins primarily constitute muscle tissues, enabling muscle function, and thereby, are of importance to the swimming ability of fish. Due to naturally low levels of insulin, carbohydrates are often less bioavailable to fish in comparison to lipids and proteins but nonetheless provide nutrition to fish (Adams 1999; Fike et al. 2007). Of the various types of lipids (fats, phospholipids, and steroids), triglycerides are most energetically relevant, being distributed towards fish metabolism, growth, and energy storage. The portion of triglycerides obtained from digestion which are not immediately used for tissue growth or metabolism are stored in the mesenteries, muscle, liver, or subdermal tissues (Adams 1999; Hurst 2007). The usage of stored triglycerides is generally triggered during times of physiological stress or when diet is low in lipid content (Langley 1965; Chavin 1973; Biga et al. 2019). The mobilization of stored triglycerides may also be beneficial for maintaining foraging, migratory, and reproductive behaviors (Chellappa et al. 1995).

An established fisheries biology concept is that fish lipid reserves positively correlate with greater probability of overwintering survival (Adams 1999; Hurst 2007). The latter half of winter can require that fish survive an extended period of food scarcity or elevated energetic foraging cost, relying in part on lipid reserves (Chavin 1973; Adams 1999; Snyder et al. 2011). If an organism does not have enough energy via dietary intake or lipid reserves to maintain its physiological processes over the winter period, the likely result is winter mortality (Adams 1999; Hurst 2007). It is also possible that the body condition and reproductive investment of fish may be lower as a result of overwintering food deprivation (Wootton 1977).

Spring spawning is a life history strategy of many north-temperate fish as it has benefits for offspring. Spring larvae and juveniles may have access to early season resources while also maximizing time spent as young of the year (YOY) allowing for an extended period to develop, grow, and store energy prior to exposure to winter conditions (Adams 1999). For example, the early stocking of fathead minnow (*Pimephales promelas*) into ponds in Alberta, Canada, led to increased length and weight of fish compared to the fish in control ponds, which were stocked three weeks later. Offspring of fish which reproduce before conspecifics often have a growth, energy storage, and time to maturation advantage (Divino and Tonn 2007).

Stored triglycerides are used to develop and contribute to the maturation of the gonads of adult fish. For female fish which reproduce in the spring, triglycerides are mobilized to maintain metabolism and develop reproductive tissue during winter and early spring (Adams 1999; Hurst 2007). For nine-spine sticklebacks (*Pungitius pungitius*) in Poland, female gonadosomatic index (GSI) increased three months prior to

the breeding period, reaching a peak during the breeding period. After the cessation of the breeding period in July, female GSI values were the lowest (Sokolowska and Skóra 2002). Older and larger individuals, likely in better condition, may be capable of greater maternal lipid investment into eggs compared to younger and smaller individuals. Fish which develop from eggs of lower lipid content may be put into an energetic deficit at early life stages, possibly having implications on the future fitness and probability of an individual's survival (Divino and Tonn 2007).

Maternal lipid content is relevant to larval success and fitness of offspring. The energy necessary to develop and mature female gonads is higher compared to males. Female yellow perch (*Perca flavescens*) require 2.7-4.5 times more energy to develop and mature ovaries than males require to develop sperm. Also, in many fishes, older females may express higher fecundity at the expense of somatic reserves (Craig 1977). Larvae that hatch from eggs containing a high lipid content can withstand longer periods of scarce resources and expend more energy foraging than cohort conspecifics of eggs containing lower levels of fish oil. If fewer lipids are allocated to female gonad development and maturation, proper oocyte development may be impaired, and the morphological development of offspring impacted (Heming and Buddington 1988). Since lipids are acquired through the diet, and lipids impact all phases of reproduction, the lipid content of reproductive-age fish within a population will impact the recruitment success of offspring and, consequently, population structure and growth (Adams 1999).

The development and maturation of female fish gonads may occur prior to and during the spawning period. Female gonadal development is likely associated with female lipid content and may be partially subsidized by dietary intake in late winter and

early spring. Observations of female central mudminnow (*Umbra limi*) expressing higher spring body condition than in the prior fall support such a notion (Chapman 2011). The restriction of threespine stickleback (*Gasterosteus aculeatus*) diets during the reproductive period appeared to significantly reduce ovarian weight (Wootton 1977; Ali and Wootton 1999). Such results indicate that there may be an important link between reproductive investment and female dietary intake prior to and during the breeding season for small-bodied north-temperate fish.

The studies cited above indicate that diet may play an important role in supporting egg development in sticklebacks during the breeding season. Various methods have been developed to assess the consumption of prey in wild and captive fish populations using stomach content analysis. One approach to assessing the importance of prey items in populations of predators is by using the frequency of occurrence in individual predators and the relative abundance of prey items in a sampled population (volumetric contribution, weight, or total count.). The method associated with obtaining the count of dietary items has been adopted by many biologists as the data are accurate, easy to obtain, and simple to interpret (Amundsen et al 1996). Other methods such as volumetric assessment may be either subjective or, if precise, are time consuming for small prey items such as invertebrates, and the same may be said for the precise measure of the weight of small invertebrates such as zooplankton.

Given the importance that fish condition and energy content have on fish populations, it is not surprising that significant time and effort have been spent determining how to best measure these characteristics in natural populations. The

common route to determining the condition or health of an individual fish is to use weight-length relationships (WLR). Fulton's K condition factor falls within this WLR category and is commonly used. Fulton's K operates upon the assumption that the greater the weight of an individual fish for a given length, the better condition an individual expresses. A higher relative weight is often used to indicate condition because increasing fish weight frequently positively correlates with lipid content, which is hence associated with better overall health. Fulton's K divides the weight of a fish in grams by its length in centimeters cubed; this value is then multiplied by a constant value of 100 to normalize the index score close to a value of 1 (Froese 2006; Nash et al. 2006). Fulton's K is used to infer the condition of fish wherein low values within the range describe poor condition and high values indicate excellent condition (Froese 2006).

The advantage of using WLR techniques is that data can be quickly obtained, and organisms can be released back into the population they were sampled from. For this reason, Fulton's K and other WLR indices have been widely used by the fisheries management community despite their flaws (Froese 2006; Nash et al. 2006). Although Fulton's K condition factor has been used for a wide range of applications, it has been criticized in several studies (see Weber et al. 2003; Froese 2006; Cone 2011; Morton and Routledge 2014; Schloesser and Fabrizio 2017; Bavčević et al. 2020). One significant disadvantage of Fulton's K is that it does not separate the structure of a fish from its energy reserves, so factors that increase the weight of a fish such as increased water retention could inflate Fulton's K even if energy reserves remain low (Bavčević et al. 2020).

Various biochemical techniques may be used to directly measure important biological macromolecules such as triglycerides, fatty acids, and proteins. These analytical methods offer a more precise measurement of fish condition than traditional WLR techniques (Weber et al. 2003). The drawback to chemical analysis is that sampled fish must be sacrificed in order to run these tests. Other procedures such as lipid extraction homogenize samples of wet tissue in organic solvents to draw lipids out of a sample, and after filtration and drying the lipid contents can be retained and measured. These analytical techniques can accurately measure the stored energy content of fish, but these analyses can be costly, time consuming, and require sacrificing fish (Lee et al. 1996; Snyder et al. 2011).

Non-game fish serve as an important trophic link between primary producers, insects, and top predators of aquatic and terrestrial environments (Helfrich and Neves 2019). Brook stickleback have been found to be a valuable resource for wading birds and mink (Reist and Carmichael 2007). In recent years, it has become understood that there are gaps in understanding related to the life histories and current status of non-game fish species. In light of increasing stress on aquatic ecosystems and decreasing freshwater fish biodiversity further research into non-game fish is warranted (Dudgeon 2019; Helfrich and Neves 2019).

Brook stickleback are a cold-water non-game fish found in wetlands, ponds, lakes, and streams of the Northeast and Midwest of North America. Brook sticklebacks are often found in clear and quiet waters that offer well vegetated margins with clay, silt, and or organic matter substrates (Stewart et al. 2007). Brook stickleback have been found to commonly consume insects, crustations, and during the breeding season,

express filial cannibalism with their eggs (Reist and Carmichael 2007). Brook stickleback reproduction occurs during early spring (May to mid-June) when they move from cool waters to comparatively warm margins (8 °C to 19 °C) with vegetation (used for male nest construction), clear waters (for courtship displays), and soft substrate for cover (burrowing predator avoidance behavior). Both sexes express intricate nuptial coloration where females express a light green coloration and males turn jet black with accentuated pelvic and anal spines of reddish orange coloration (Stewart et al. 2007).

Brook sticklebacks have complex reproductive behaviors. At the beginning of the spawning period, males establish and defend territories, accumulate pieces of vegetation and organic matter which they attach to firm substrates (rocks, downed woody debris, or rooted macrophytes) using a glue protein that is formed in the kidneys and excreted as a thread. Once constructed, males will court females to enter their constructed nest where females lay a clutch of eggs and are followed by the male which will fertilize the eggs. Males will then chase the female away from the nest, protect the brood, and capture/return larvae to the nest as they hatch to entangle larvae, eventually abandoning brood care once he can no longer keep up with the number of larvae which are escaping (Stewart et al. 2007). The energetic investment of males is often associated with the preparation of spawning habitat and brood care whereas females' energetic investment is primarily associated with extensive investment into gonad development, maturation, and egg production as females school and feed. Lastly, since nuptial coloration is apparent in both sexes, it is likely that some energetic investment is associated with the production and mobilization of hormones in the spring (Chavin 1973; Reist and Carmichael 2007; Stewart et al. 2007).

Brook sticklebacks have interesting metabolic adaptations which reduce their demand for dissolved oxygen and allow for their seasonal use of winter habitats which may approach anoxia. Although some small-bodied fishes may have an increased metabolic rate, it has been observed that under low oxygen conditions, brook sticklebacks had very low metabolic rates. In comparison to other species which are also capable of surviving low oxygen conditions (central mudminnow and fathead minnow), brook stickleback expressed the lowest weight adjusted metabolic rate at all temperatures (5 °C; 10 °C; and 15 °C) (Klinger et al. 1982). At low oxygen concentrations (0.5 and 0.25 mg/L) brook sticklebacks were found to exhibit the lowest activity level, a factor which is likely in part responsible for their low metabolic rate. Furthermore, brook sticklebacks are also found to be capable of detecting and occupying high oxygen micro-zones (nearby the ice water interface) of elevated dissolved oxygen through upward movement in the water column and appear to have the best head shape for utilizing these micro-zones (Klinger et al. 1982). Such adaptations may be responsible for the ability of brook stickleback to colonize areas following fish kills or to endure the conditions that may otherwise be uninhabitable to other fish species.

As brook sticklebacks have interesting life history and metabolic strategies to overcome periods of physiological stress, further study of their seasonal use of lipids, reproductive investment and diet is necessary. My study investigated seasonal variation in body condition, body lipid content, reproductive investment, and diet of brook stickleback. The first objective of this study was to explore changes in female reproductive investment (using the gonadosomatic index, GSI) during and after the

spring spawning period. Similarly, I investigated the relationship between somatic lipid and female GSI, which would be expected if significant energy reserves were mobilized to support egg production. Another objective of this study was to determine if spring and fall Fulton's K values would be comparable. I expected Fulton's K to be high in the spring, low in summer, and high in fall as fish accumulated lipid reserves prior to winter. I also examined the reliability of the Fulton's K index for predicting the percent lipid content in brook stickleback. Lastly, I carried out a detailed analysis of the brook stickleback diet to determine how the feeding strategy and diet breadth changed seasonally, and to compare my results with existing literature.

Methods

Site Description

Sampling of brook sticklebacks occurred in western New York at Reinstein Woods Nature Preserve, a 294-acre protected area within the Buffalo River watershed (Figure 1). Reinstein Woods was naturally a flat wooded area which received water input from two tributaries of Slate Bottom Creek (tributary of Cayuga Creek). Prior to its dedication as a nature preserve, the previous landowner Dr. Reinstein artificially created 9 ponds and 11 wetlands via various damming techniques of two Slate Bottom Creek tributaries. Dr. Reinstein also installed pipes and overflow weirs to connect the various ponds and wetlands to control flooding and protect gravel roads which were installed around the property (Pataki and Sheehan 2006). Stickleba27cks were sampled from Hidden Pond, a permanent pond located at the northwestern area of Reinstein Woods which receives input from Secluded Wetland and drains into the Lower Pond via an overflow weir (Figure 2).

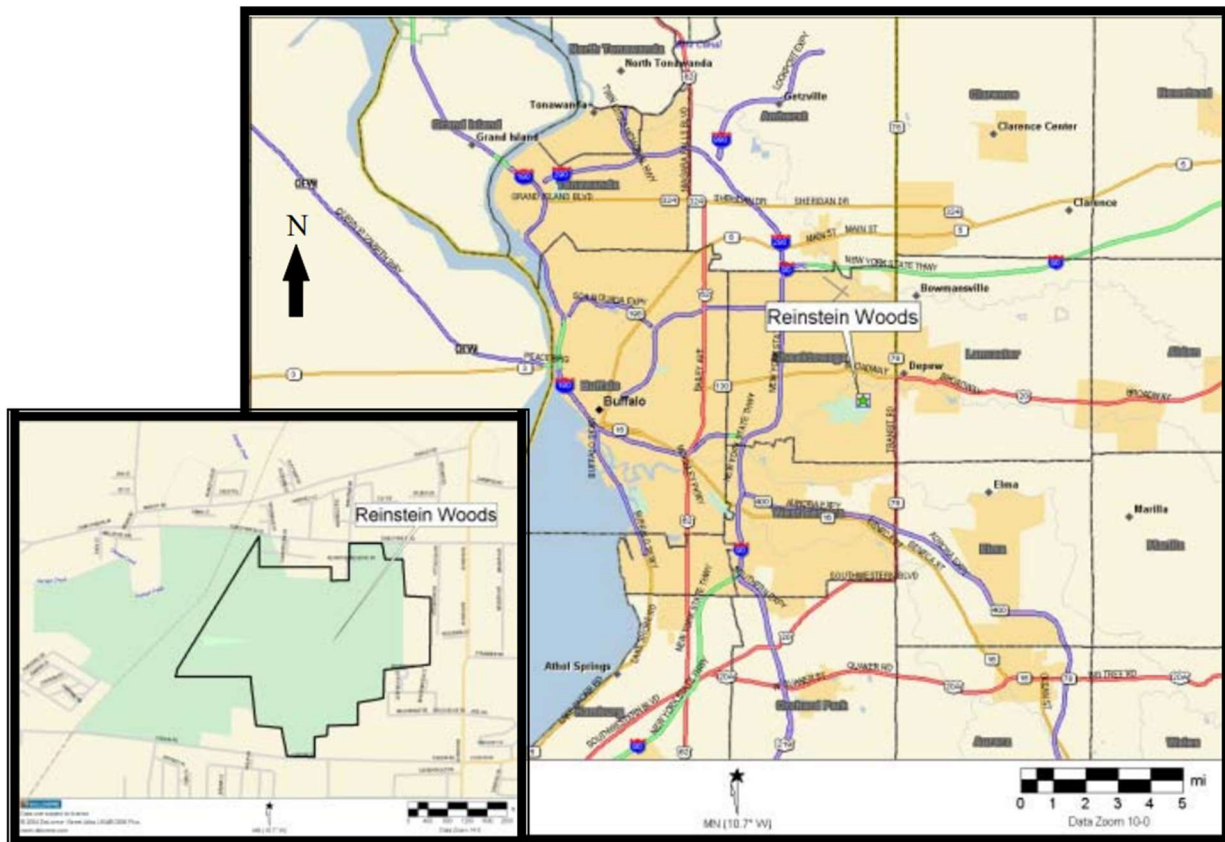


Figure 1: Location of Reinstein Woods Nature Preserve, 77 Honorine Drive, Depew NY 14043 within Erie County NY. Map provided by the New York State Department of Environmental Conservation Unit Management Plan (Pataki and Sheehan 2006).

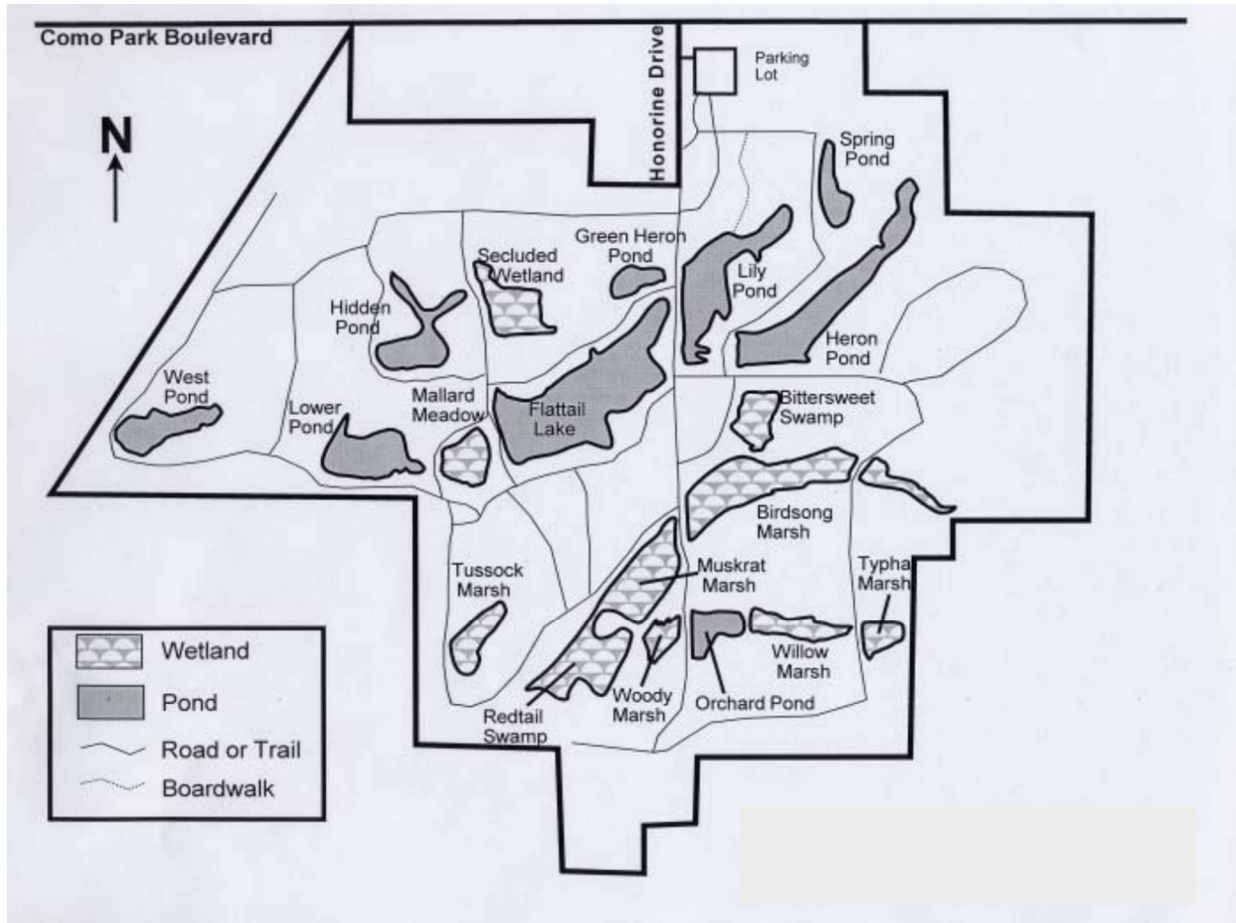


Figure 2: Map indicating aquatic habitats within Reinstein Woods Nature Preserve located at 93 Honorine Dr, Depew, NY 14043. Map provided by the New York State Department of Environmental Conservation Unit Management Plan (Pataki and Sheehan 2006).

Sampling Procedure

Sticklebacks were obtained via quarter-inch gee minnow traps set in the evening and retrieved in the morning on a bi-weekly schedule during the spring spawning season and on a monthly basis during the summer and fall periods. Fish were sorted in a bucket using minnow nets and approximately 20 brook sticklebacks were separated into a ten-liter cooler which was filled with two liters of water. An anesthetic dose of 75 mg L^{-1} of tricaine methanesulfonate (MS-222) and an equivalent amount of sodium bicarbonate (as a pH buffer) was added in powder form to the cooler on site. This dose was deemed adequate for sedating 3g fathead minnows (*Pimephales promelas*) into stage 3 anesthesia (Palić et al. 2006). After sedation had been achieved, a lethal dose (250 mg L^{-1}) of MS-222 was introduced to euthanize the sticklebacks (Buffalo State University IACUC Permit # 59). For the July and August sampling dates the traps were deployed in the morning and retrieved in the evening to avoid fish exposure to night-time hypoxia (lack of primary production) in the warm, near-shore sampling areas.

Sample Processing and Dissection

On the same day they were collected, sticklebacks were placed into labeled resealable plastic bags which were half filled with deionized water and frozen at $-20\text{ }^{\circ}\text{C}$ on the Buffalo State University campus. For the determination of condition, energy content, reproductive investment, and diet, fish were thawed in a tub which was filled with cold tap water. Individual fish were then blotted dry and measured for length (mm) and weight (g).

Individual fish underwent dissection beginning with a longitudinal cut on the ventral side of the fish extending from the posterior of the isthmus to the posterior of the

anal fin. Two transverse cuts were made at both ends of the initial cut to expose the viscera. The gastrointestinal tract (GI tract) was separated from other internal organs and cut at the esophagus and vent. GI tracts were removed and preserved in labeled vials with 10% buffered formalin. Stomachs were later removed from their vials and dietary items were enumerated using a small Barkov sorting chamber using an Olympus SZH10 microscope at a range of 10x to 50x magnification. Ovaries were carefully removed from each female, blotted dry, weighed to the nearest 0.001g, and stored in individually labeled vials and frozen at -20 °C (Manko 2016). Male testes were left within the carcass and all carcasses were frozen in individually labeled vials.

Lipid Extraction

The following procedure was adapted and scaled down from the procedure used by Lee et al. (1999). Thawed fish were weighed to obtain a post-dissection weight. Individual fish were cut into several pieces and placed into 25 mL homogenization tubes and suspended with 18 mL of a 2:1 hexane isopropyl alcohol solvent. Fish were blended using a homogenizer at a moderate speed for one minute and filtered using vacuum filtration. The liquid yielded from vacuum filtration was combined with 12 mL of a NaCl solution (30g per liter) and thoroughly mixed. The organic and aqueous layers were separated by draining the lower aqueous layer using the stopcock and pouring the organic layer into pre-weighed tins. The organic layer was evaporated to dryness at 60 °C in pre-weighed tins which were weighed once more to determine the net lipid content of each fish.

Data Summaries and Indices

To estimate female reproductive investment, the gonadosomatic index (GSI) of individual fish was calculated by equation 1,

(1)

$$GSI = \left(\frac{G}{W} \right) 100$$

where (G) is the ovary weight (g) and (W) is the wet weight (g) of the individual female fish (Korsøen et al. 2013). Fulton's K was calculated by equation 2,

(2)

$$K = \left(\frac{W}{L^3} \right) 100$$

where (W) is the wet weight (g) and (L) is the length of an individual fish in centimeters (Weber et al. 2003). Percent lipid was calculated by equation 3,

(3)

$$\text{Percent Lipid} = \left(\frac{L}{P} \right) 100$$

where (L) is the net weight in lipid yielded by lipid extraction (g) and (P) is the post dissection wet weight (g) of an individual fish. The frequency of occurrence of dietary items (F_i) was calculated by equation 4,

(4)

$$F_i = \left(\frac{N_i}{N} \right)$$

where (N_i) is the number of predators with prey type (i) in their stomach and (N) is the total number of predators sampled (Amundsen et al. 1996). Prey specific abundance (P_i) was calculated by equation 5,

(5)

$$P_i = \left(\frac{\sum S_i}{\sum S_{t_i}} \right) 100$$

where (S_i) is the stomach content amount (number) of prey i , and (S_{t_i}) is the total stomach content of only the predators which contain prey i (Amundsen et al. 1996).

Fish samples were grouped into seasonal categories of spring, summer, and fall. All samples which contained visibly gravid females were grouped into the spring category which occurred from early May to mid-June. Samples collected from late June to late August were grouped into the summer category. The fall category was composed of three sampling events which occurred from late September to the first of December. Fish which were collected between May and the end of June were collected on a bi-weekly basis. Upon the first observation of absent gravid females within a sample (which occurred on 06/30/2022), subsequent samples were collected on a monthly basis.

Statistical Analyses

Seasonal differences in morphology (i.e., length and weight) and the various indices were determined using one-way ANOVA and Tukey HSD tests in the statistical software R and calculated using the tidyverse package. Data from all individuals were used to examine seasonal variation in length and weight, while only data from female sticklebacks were used to examine seasonal trends in GSI. One-way ANOVA was used to determine if there were seasonal differences in Fulton's K and percent lipid and constrained to within sex comparisons. Time series graphics with standard error bars were created in Microsoft excel and were used to display variation in Fulton's K, percent

lipid content, and GSI between sampling dates. Single linear regressions were used to determine the relationship between Fulton's K and percent lipid content in using the tidyverse package. This relationship was plotted for each season, for the entirety of the sampled population, and constrained by sex.

Costello Plots Interpretation and Procedure:

A graphical method for analyzing predator feeding strategy has been developed based on the relationship between prey specific abundance and frequency of occurrence (Costello 1990). With this method the prey importance of a dietary item within a population of predators can be determined by the use of a plot (now often referred to as a Costello plot) which examines the relationship of percent frequency of occurrence and prey specific abundance. Prey items that are found to be high in both frequency and abundance are thought to be dominant or important prey items and the opposite is true for items low in frequency and abundance (Figure 3b). Costello (1990) also suggested that it is possible to determine the feeding strategy of a predator through the same graphical analysis of prey- specific abundance and relative frequency.

Amundsen et al. (1996) recommended that the true measurement of prey-specific relative abundance should be constrained to only those stomachs where those items are present, and the graphical interpretation of prey items based on the position of dietary items be amended. Amundsen et al. (1996) also suggested that the feeding strategy of a population can be determined by inspecting the position of prey items along the y-axis. For instance, populations of predators can be assumed to specialize in consuming prey types when they are in the upper portion of the y-axis (high in prey

specific abundance) and can be assumed to have a general affinity to prey items are that are located in the lower half of the y-axis of the diagram. Prey items which are located in the upper left or lower right corner represent similar overall contributions to a population of predators but are indicative of different strategies at the level of the individual. Prey items positioned in the upper left portion of a Costello plot are consumed by a few individuals which are or have specialized in that item, labeled as high between-phenotype components (Figure 3a). Items which are frequently consumed at low abundances are noted as a high within-phenotype dietary component as most phenotypes utilize the prey item as a resource (Figure 3c).

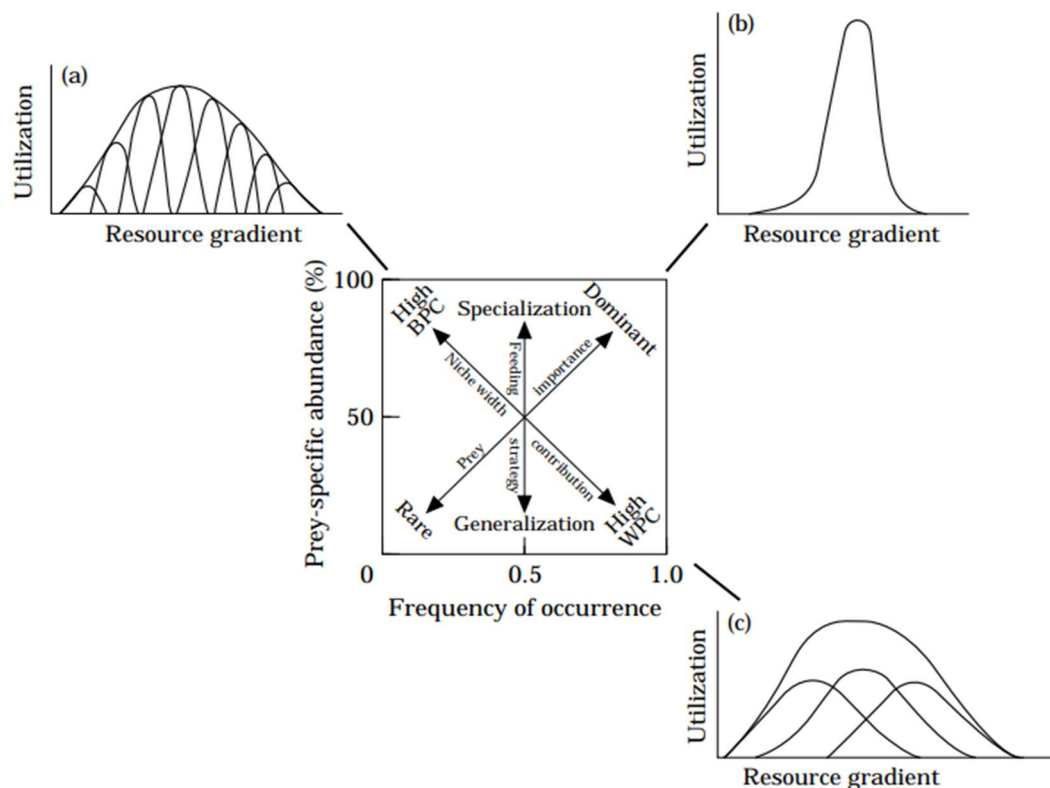


Figure 3: Explanatory diagram (center) for interpretation of feeding strategy, niche width contribution and prey importance from the proposed method, together with characteristic niche utilization curves. (a) High between-phenotype component to niche width, (b) narrow niche width and (c) high within-phenotype component (Amundsen et al. 1996).

Costello plots were used to assess the feeding strategy of brook stickleback and to investigate whether there was seasonal variation in prey importance. This analysis was done by using the count of dietary items observed in brook stickleback stomach contents. Fish which were found to have empty stomachs were eliminated from the dataset used for dietary analysis. The feeding strategy and dietary importance of prey items was determined by graphical inspection of plots and followed the procedure outlined by Amundsen et al. 1996.

Results

Table 1: Sample Sizes, mean length, weight, gonadosomatic index (GSI), percent lipid and Fulton's K (\pm standard errors) of brook sticklebacks sampled over three seasons from Hidden Pond of Reinstein Woods, NY during the sampling season of 2022.

Category	N	Length (mm)	Weight (g)	GSI	Percent Lipid	Fulton's K
Female	46	40.5 \pm 0.41	0.90 \pm 0.025	9.76 \pm 0.742	1.98 \pm 0.154	1.34 \pm 0.018
Male	4	40.9 \pm 0.32	0.91 \pm 0.039	N/A	2.69 \pm 0.546	1.33 \pm 0.035
Spring	50	40.6 \pm 0.38	0.90 \pm 0.023	N/A	2.03 \pm 0.149	1.34 \pm 0.016
Female	24	40.1 \pm 0.45	0.73 \pm 0.031	1.92 \pm 1.120	3.36 \pm 0.284	1.12 \pm 0.024
Male	21	40.8 \pm 0.41	0.81 \pm 0.040	N/A	2.52 \pm 0.309	1.18 \pm 0.039
Summer	45	40.4 \pm 0.31	0.77 \pm 0.025	N/A	2.98 \pm 0.216	1.15 \pm 0.022
Female	18	38.1 \pm 0.75	0.61 \pm 0.029	2.36 \pm 0.114	2.50 \pm 0.163	1.10 \pm 0.024
Male	17	38.8 \pm 0.80	0.68 \pm 0.038	N/A	2.28 \pm 0.172	1.15 \pm 0.028
Fall	35	38.4 \pm 0.54	0.65 \pm 0.024	N/A	2.39 \pm 0.118	1.13 \pm 0.018

Length and Weight

Average length of all sticklebacks significantly varied across seasons (ANOVA: $F = 7.798$, $P < 0.001$). Sticklebacks sampled in the fall were significantly smaller than those in the spring and summer sampling periods (Tukey HSD Test: $P = 0.001$; $P = 0.003$). Average length of the sticklebacks did not vary between spring and summer sampling periods (Tukey HSD Test: $P =$

0.971) (Fig. 4a). The average weight of all sticklebacks seasonally varied (ANOVA: $F = 23.82$, $P < 0.001$). Average weight of spring stickleback was significantly higher than both summer and fall fish and average summer weight was significantly higher than fall weight (Tukey HSD Test: spring and summer, $P < 0.001$; spring and fall, $P < 0.001$; and summer and fall, $P = 0.003$) (Figure 4b).

Condition Factor and Percent Lipid

GSI of female brook sticklebacks showed a distinct pattern of a decrease over time with a steep drop during the last spring and first summer sampling dates, followed by a flat stabilization of low GSI during the summer and a slow buildup of GSI during the fall period (Figure 7a). Average female GSI significantly varied between seasons ($F = 35.84$, $P < 0.001$). Specifically, spring GSI varied significantly from that of summer and fall (Tukey HSD Test: $P < 0.001$ for both comparisons), while GSI did not vary significantly between summer and fall sampling periods (Tukey HSD Test: $P = 0.942$) (Figure 5).

Average body condition (Fulton's K) decreased as spring and summer sampling progressed, followed by an increase during the fall period (Figure 7b). Fulton's K varied significantly across seasons for females (ANOVA: $F = 42.63$, $P < 0.001$) while for males it did not (ANOVA: $F = 2.47$, $P = 0.098$). For females, Fulton's K in spring was different from both summer and fall periods (Tukey HSD Test: $P < 0.001$ for both comparisons) but body condition did not vary significantly between summer and fall (Tukey HSD Test: $P = 0.882$) (Figure 6a).

Percent lipid in female brook stickleback showed a large increase in the spring period and a peak during the first sampling date of summer at 4 percent. After this peak, female percent lipid varied without a clear trend until a small increase in percent lipid occurred between the last two sampling dates in fall. Percent lipid in male brook stickleback displayed an overall increase in the spring period, a sharp decrease during the summer period, and gradual increase during the fall (Figure 7c). Percent lipid in females differed significantly among seasons (ANOVA: $F = 42.63$, $P < 0.001$) whereas percent lipid in males did not (ANOVA: $F = 0.327$, $P = 0.723$). Female percent lipid in the summer was significantly higher than spring and fall (Tukey HSD Test: $P < 0.001$; $P = 0.036$). Female percent lipid did not vary significantly between spring and fall (Tukey HSD Test: $P = 0.203$) (Figure 6b).

Regression Analysis

Fulton's K did not accurately predict percent lipid in spring females or males (Female: $y = 0.007x + 1.9683$, $r_2 < 0.001$, $P = 0.952$; Male: $y = -2.8376x + 6.4674$, $r_2 = 0.0337$, $P = 0.817$) (Figure 8a) but was positively related to percent lipid in summer males and females (Female: $y = 6.3788x - 3.7848$, $r_2 = 0.647$, $P < 0.001$; Male: $y = 6.3633x - 4.9801$, $r_2 = 0.302$, $P = 0.005$) (Figure 8b). Fulton's K positively predicted percent lipid in fall males ($y = 4.5609x - 2.9821$, $r_2 = 0.532$, $P < 0.001$) while it did not for fall females ($y = 2.2475x + 0.0231$, $r_2 = 0.110$, $P = 0.180$) (Figure 8c). Overall, Fulton's K was a strong predictor of percent lipid in males ($y = 5.3141x - 3.851$, $r_2 = 0.524$, $P < 0.001$) but did not accurately predict percent lipid in females ($y = -1.053x + 3.759$, $r_2 = 0.019$, $P = 0.196$) (Figure 8d).

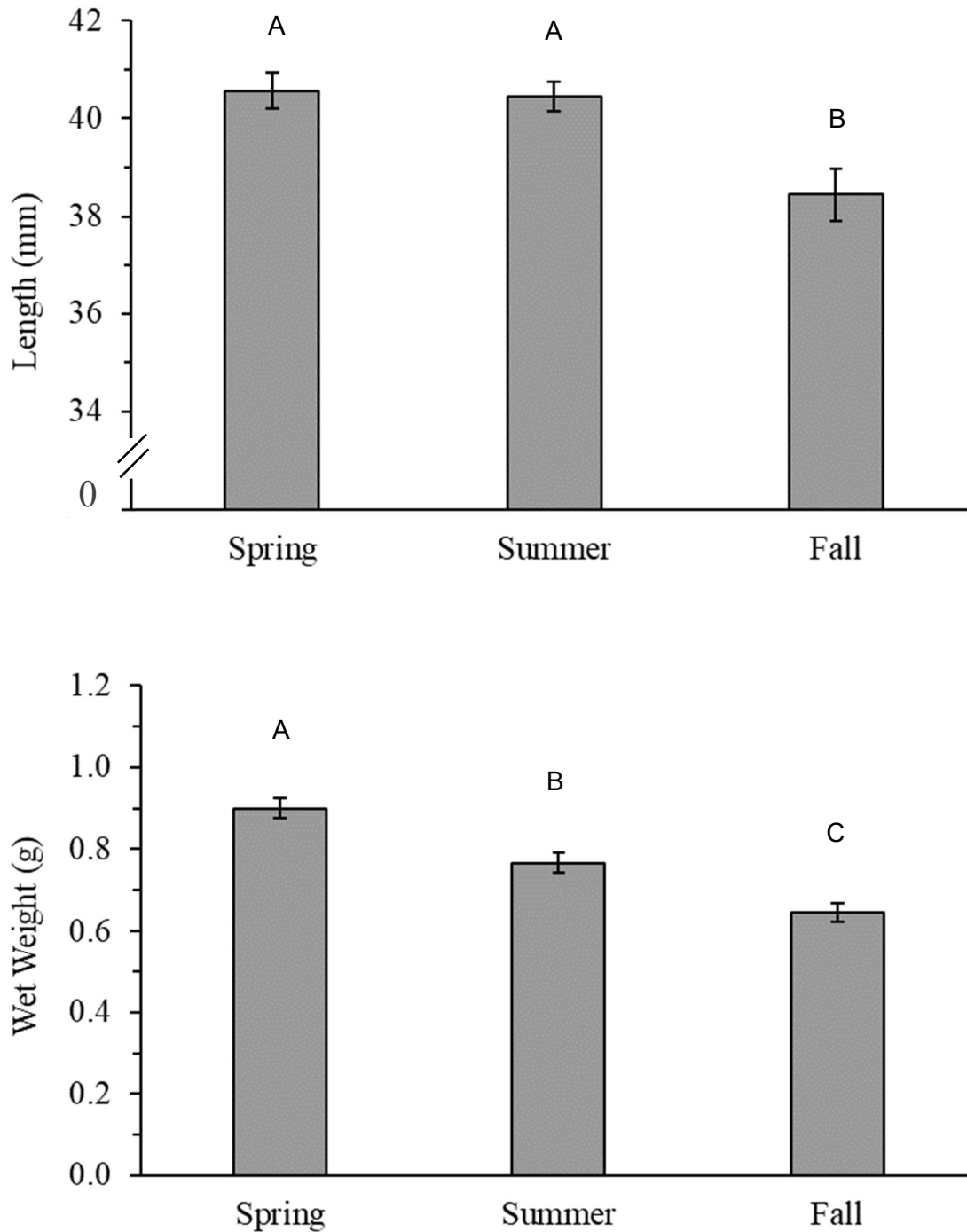


Figure 4: Seasonal relationships of mean length (a) and weight (b) of all brook sticklebacks. Capital letters indicate statistical differences between means of all individuals within seasonal groups using ANOVA (\pm standard errors). Note that the y-axis of figure 4a contains a break.

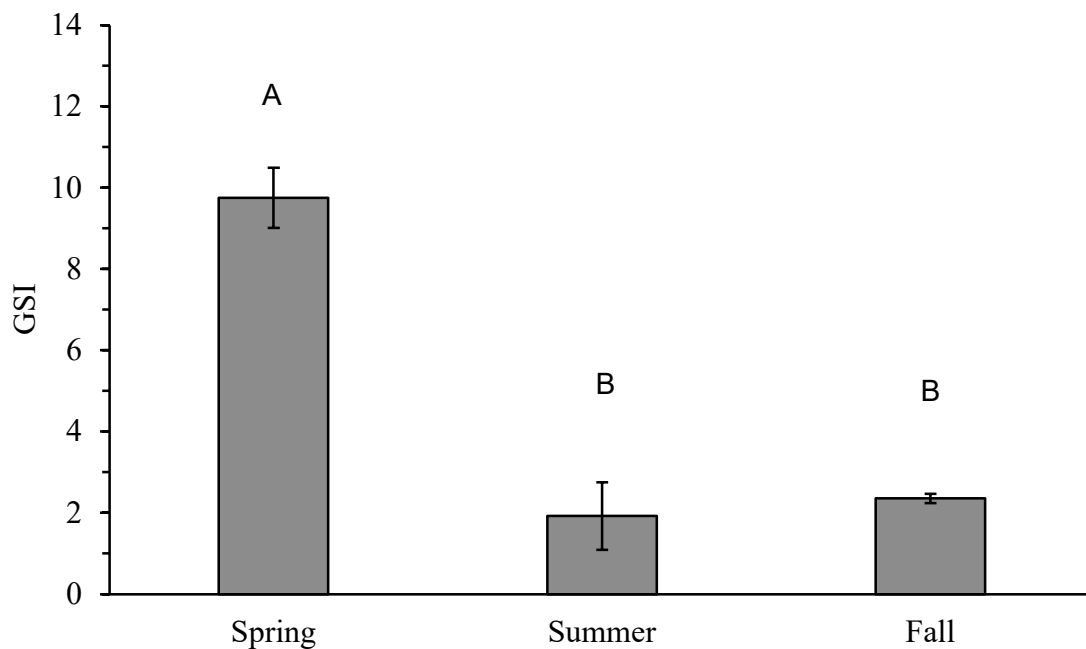


Figure 5: Seasonal relationship of mean gonadosomatic index of brook sticklebacks sampled over three seasons. Capital letters indicate statistical differences between means of all individuals within seasonal groups using ANOVA (\pm standard errors).

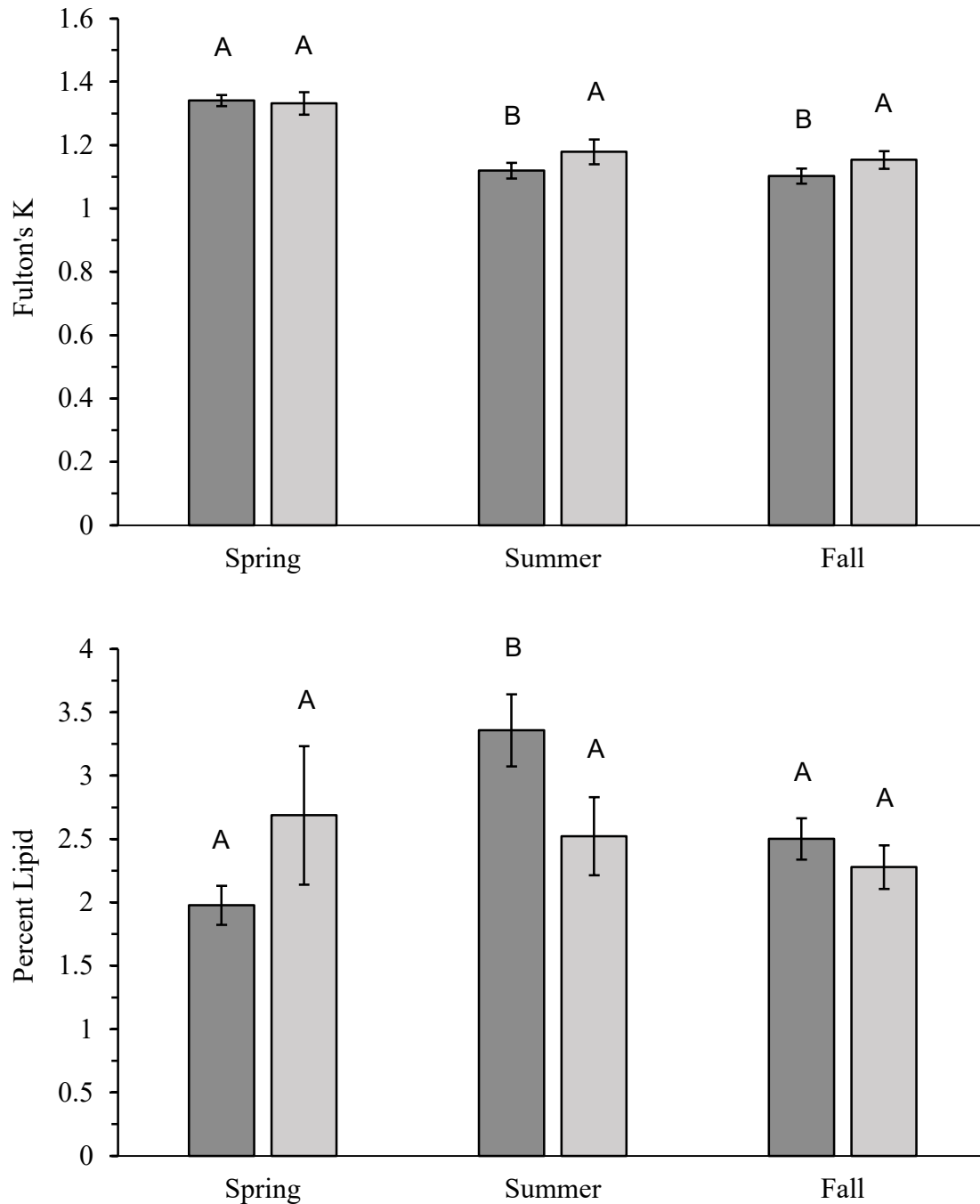


Figure 6: Seasonal relationships of mean Fulton's K (a) and percent lipid (b) of brook sticklebacks. Capital letters indicate statistical differences between means of all individuals within seasonal and sex groups using ANOVA (\pm standard errors). Dark grey bars represent the mean of female individuals (left) and light grey bars indicate

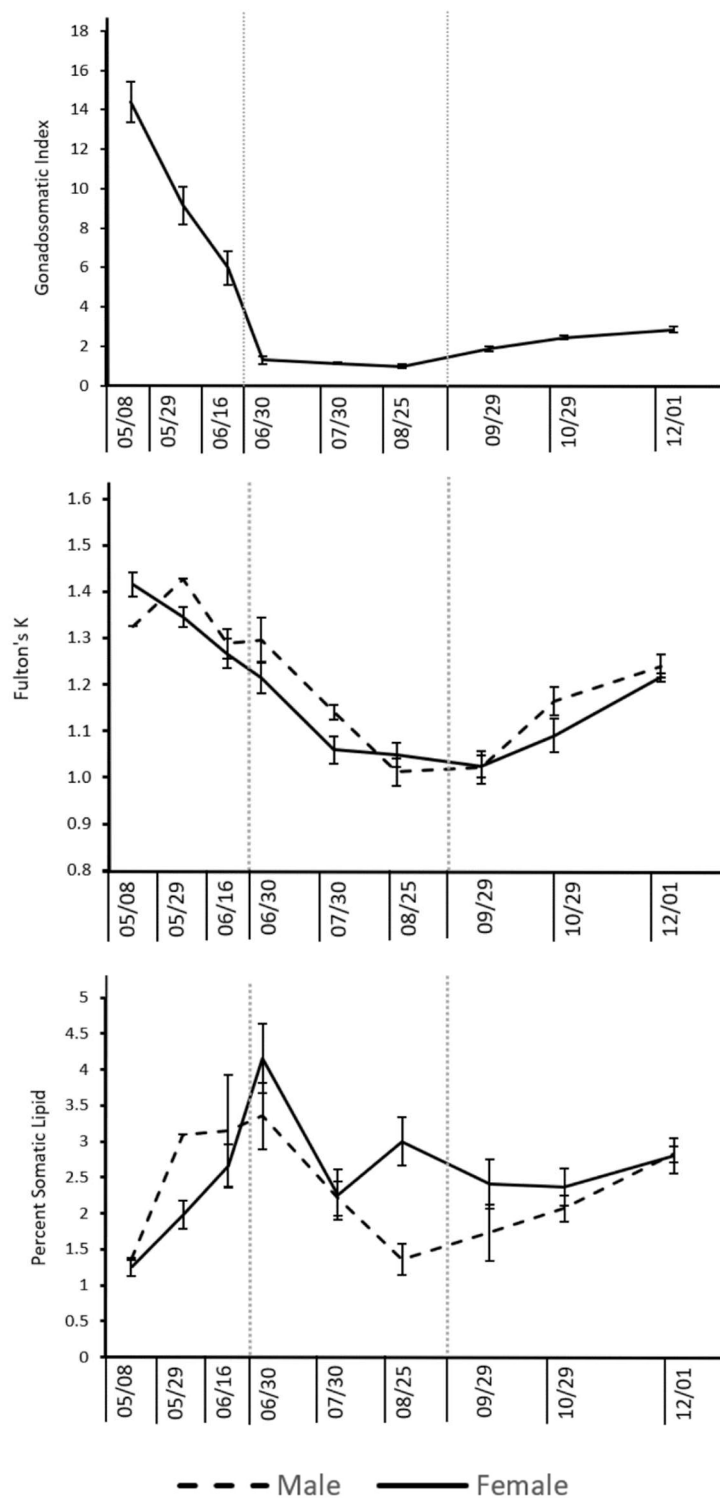


Figure 7: Time series depicting seasonal changes in mean female gonadosomatic index (a), as well as mean changes of both male and female Fulton's K (b), and percent somatic lipid with standard errors. Dashed vertical breaks are used as a visual aid to indicate seasonal categories. Note that the y-axis of figure 7b contains a

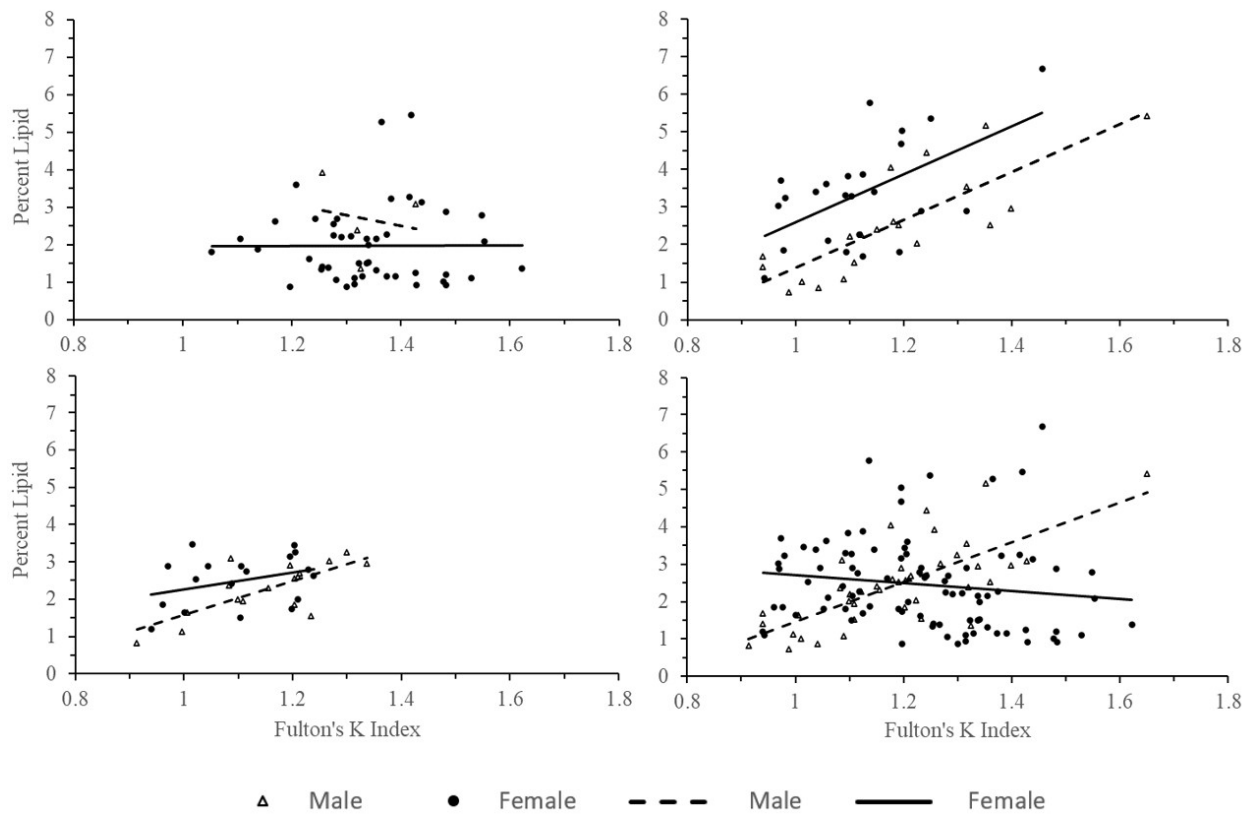


Figure 8: Single linear regressions of Fulton's K as a predictor of percent lipid of brook sticklebacks sampled in spring (a), summer (b), fall (c) and all seasons (d). Note that the x-axis contains a break for all regressions.

Costello Plots

In the spring, the sticklebacks showed a mostly generalist feeding strategy as most prey items plotted in the lower half of the y-axis (Figure 9a). The spring Costello plot suggests that a high number of prey items were occasionally consumed with the exception of fish eggs, which were consumed at higher abundance ($F = 0.675$; $Pi = 49.1\%$) than others and were likely abundant during the stickleback spawning period. Many other dietary items such as *Bosmina* ($F = 0.60$; $Pi = 24.5\%$), *Daphnia* ($F = 0.50$; $Pi = 8.9\%$), copepods ($F = 0.50$; $Pi = 38.0\%$), chironomids ($F = 0.55$; $Pi = 13.6\%$), and ostracods ($F = 0.37$; $Pi = 9.3\%$) expressed moderate prey importance (Figure 9a).

In the summer, the sticklebacks followed a more specialist feeding strategy consisting mainly of chironomids as they were plotted in the upper portion of the diagram. Chironomids were the most frequent ($F = 0.73$) and abundant ($Pi = 85.9\%$) prey item, representing high importance. Other prey like *Bosmina* ($F = 0.15$; $Pi = 5.9\%$), *Daphnia* ($F = 0.35$; $Pi = 15.3\%$), and copepods ($F = 0.42$; $Pi = 18.6\%$) decreased in frequency of occurrence and prey specific abundance, and therefore were less important prey items (Figure 9b). One item, ostracod ($F = 0.35$; $Pi = 32.2\%$) apart from chironomid increased in both relative frequency and prey-specific abundance but this effect was not large enough to be distinguishable from the rest of the items clustered in the lower left corner.

In the fall, the sticklebacks displayed a specialist feeding strategy for copepods. copepods expressed high frequency ($F = 0.97$) and abundance ($Pi = 67.8\%$) displaying high prey importance while most other prey items displayed a very low prey specific abundance. Although many items could be described as rare (lower left corner) some

items appeared in the lower right corner of the diagram such as *Bosmina* ($F = 0.85$; $Pi = 17.3\%$), chironomids ($F = 0.94$; $Pi = 6.6\%$), and ostracods ($F = 0.69$; $Pi = 5.4\%$). This pattern is indicative of a high within-phenotype component where these items were frequently encountered in stomachs but were not very abundant (Figure 9c).

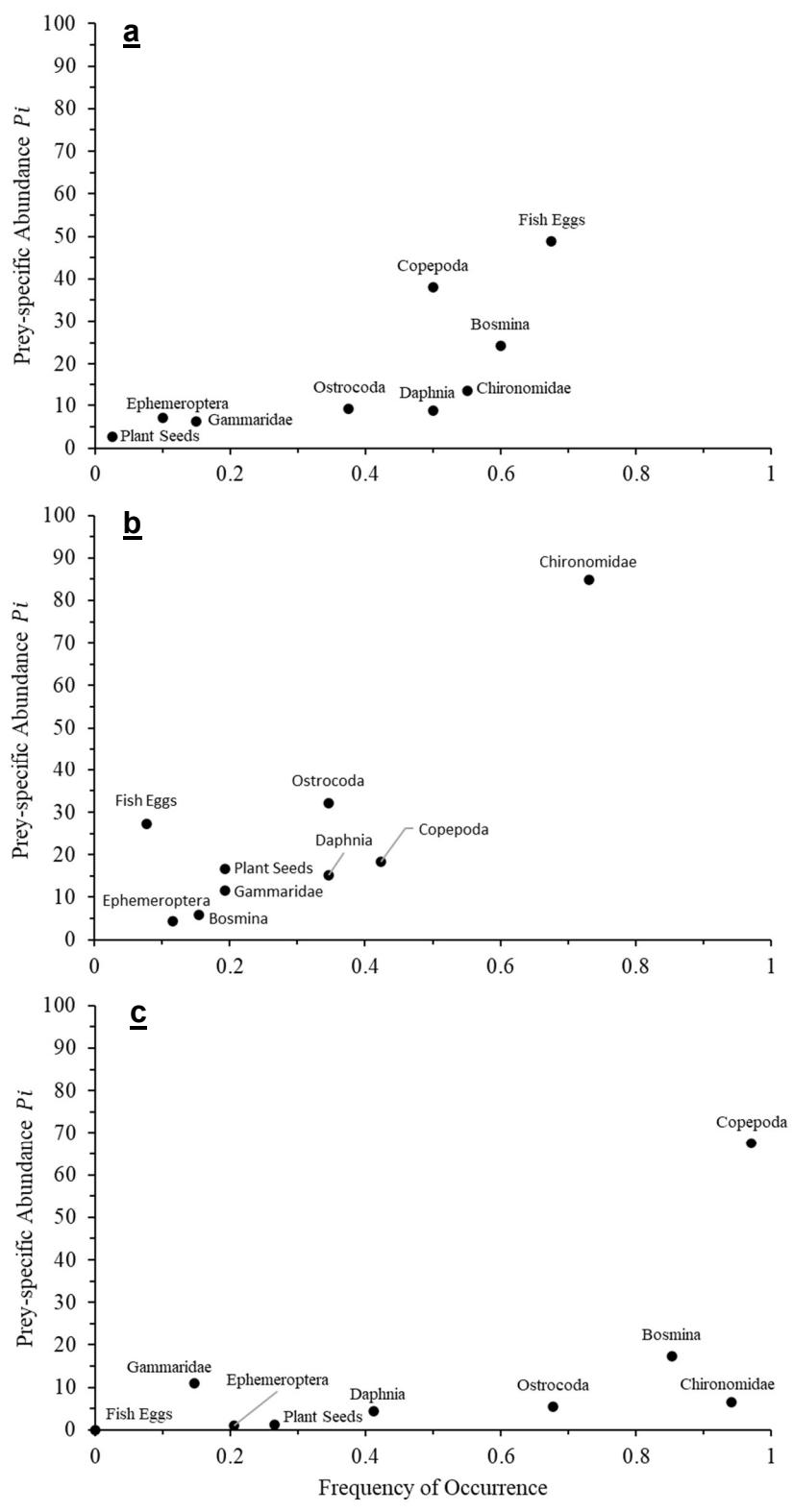


Figure 9: Costello plots comparing frequency of occurrence against prey-specific abundance (P_i) of brook stickleback populations in Spring (a), Summer (b), and Summer (c).

Discussion

Brook sticklebacks primarily utilize nearshore habitats in the spring for spawning and foraging, moving to deeper refugia during the summer and winter months (Stewart et al. 2007). In my study, brook sticklebacks were collected from nearshore habitats from spring to fall (May - December) indicating that nearshore habitats are, at least partially, utilized by sticklebacks year-round but, at lower abundances following spring reproduction. Previous literature suggests that brook sticklebacks are flexible predators capable of optimally foraging based upon prey distribution and abundance (Reist and Carmichael 2007). Although my study cannot provide resolution as to the true selectivity of prey based upon environmental abundance, my results support the idea that brook sticklebacks express dietary flexibility as indicated by significant seasonal variation in prey choice and diet composition.

The diet of the fish in my study was broad in terms of the number of taxa consumed with a focus on insects and crustaceans (Figure 7), in agreement with previous assessments of brook stickleback's diets in the Northwest of North America (Stewart et al. 2007). Sticklebacks in my study were generalist feeders in the spring, but more specialized in the summer and fall periods (Figure 9). This corroborates previous findings from a Wisconsin stream in which brook sticklebacks consumed a broad range of dietary items in the spring and showed a strong preference toward crustaceans in the fall (Hlavec 1971).

In the spring, the sticklebacks in my study expressed a generalist feeding strategy indicated by a cluster of low importance dietary items (lower left), a cluster of

relatively important prey items (lower center) and fish eggs which were of high importance (middle right) (Figure 9a). This suggests that at this time of year, the sticklebacks had a large niche width as a variety of prey items were commonly consumed at moderate levels (Amundsen 1996). Previous research suggests that brook stickleback eggs are 1mm in diameter (Barker 1918). The fish eggs that were consumed by the stickleback in my study were indeed 1mm in diameter and comparable in size to eggs that were removed from the ovaries of gravid females from the same population (Appendix 8). Therefore, my results are consistent with previous studies which indicated that sticklebacks could cannibalize eggs during the breeding season (Moodie 1986). In my study, both male and female fish consumed stickleback eggs, indicating that both sexes may benefit energetically from consuming this high fat item in the spring (Appendix 2).

The feeding habits of stickleback in the summer were more specialized than spring. The majority of fish consumed chironomids ($F = 0.73$) and of those sticklebacks that consumed chironomids, most of their stomach contents consisted of this prey item ($P_i = 86\%$). Other prey items such as ostracods, copepods, and *Daphnia* (noted in the lower left corner of figure 9b) were less common but these items still comprised a moderate proportion of the diet. In a previous study using environmental sampling and a selectivity index, brook sticklebacks were found to show preference for chironomids (Thompkins and Gee 1983).

In the fall, the sticklebacks also followed a specialist feeding strategy, but they consumed mainly copepods instead of chironomids. The fall sticklebacks expressed a high within-phenotype component, as most individuals consumed high numbers of

copepods and a large proportion of fish consumed chironomids, ostracods, and *Bosmina*, but did so at lower quantities (Figure 9c). Thus, although copepods made up a large portion of the diet, other items were also providing nutrition to the majority of sticklebacks in the population, suggesting most fish were utilizing many resource types simultaneously (Amundsen 1996).

Overall, my study found strong evidence to suggest that the diet and feeding strategy of the brook stickleback varied between seasons. This seasonal variation in diet can be attributed to some combination of changes in habitat use, prey selectivity, and prey availability. The sticklebacks consumed a variety of prey in the spring but also tended to focus on fish eggs, a nutritious item that most likely was locally abundant at the time. In the summer and fall, the diet of the sticklebacks was more specialized. It is possible that chironomids were the most frequently encountered item in the summer and because of it, the preference of sticklebacks for this prey item increased in the summer. The dietary shift from summer to fall indicates that brook sticklebacks are capable of switching from one dominant prey item to another (i.e., from chironomids to copepods). This change was also accompanied by most individuals consuming low abundances of a diversity of prey items, subsequently increasing the breadth of the fall diet and potentially the realized nutritional intake of the fish.

Reproduction in brook sticklebacks occurred in early May and ceased by the end of June. Brook stickleback reproductive investment, as measured by the gonadosomatic index (GSI) in females, varied between seasons, being highest in the spring and lowest in the summer (Figure 5). The GSI of females steadily decreased as the spring period progressed (Figure 7a). As water temperatures increased and the breeding period

progressed, female egg production continued until a thermal limit was reached. Pre-spawning adult brook stickleback prefer a temperature range of 14.9 - 20.2 °C and female egg resorption occurs after a prolonged exposure of 20-22 °C (Braekevelt and McMillan 1967). The water temperature at my collection site increased 1°C between the first two sampling events (18°C to 19°C) and 8°C between the second and third sampling events (19°C to 27°C) of spring (Appendix Table 1). Due to these circumstances, female stickleback may have produced eggs leading up to and between the first two sampling events. However, females likely stopped producing eggs after laying a last brood or began resorbing eggs after the third sampling event (6/16/2022) as average female GSI substantially declined and did not recover after this sampling event (Figure 7a).

It is understood that many female north-temperate fish use lipid reserves throughout the year for survival, maturation, and reproduction (Adams et al 1999; Hurst et al. 2007). In my study, female percent body lipid increased steadily during May and June while the GSI of females declined during this period. This may be due to lipid being allocated toward other physiological needs as the breeding period reaches its peak. However, this trend may also be due in part to the dietary intake of brook stickleback in the early spring. Moodie (1986) observed that some gravid female brook stickleback which were stripped of their eggs and placed into 20.5 °C surface waters with “abundant food” were gravid upon recapture six days later (20% of fish). After repeating the experiment at daily intervals, Moodie concluded that female brook stickleback may reproduce up to 7 times in one reproductive period. If female stickleback were to reproduce at this frequency (producing 214 eggs every three days

for 28 days) they would generate 120% of the maximum female weight in eggs per breeding period. Ali and Wootton (1999) demonstrated that female fecundity of threespine stickleback responded to food treatments. The total fecundity of three consecutive spawning intervals significantly increased under two successive high-food treatments (16% of body weight per day) and a less emphasized effect was observed for a low (4% of body weight per day) -to-high treatment. Such an effect was not observed in two successive low-food treatments nor a high-to-low treatment. Lastly, in another study, the level of food available to spring threespine stickleback had a profound effect on the weight of the ovaries within fish during the reproductive period (Wootton 1977). Therefore, in brook stickleback, female reproductive investment may be less dependent on stored lipid than the availability of food during the breeding season.

Average summer and fall GSI were not statistically different yet a slow and steady increase in GSI can be observed as fall progressed (Figure 7a). Although fall GSI is low in comparison to spring values, females may have been beginning to energetically invest into the following year's reproduction as mean GSI successively increased during the fall period (Figure 7a). Female brook stickleback that are able to begin the process of egg development in the fall may be at an advantage during the following breeding season when more energy becomes available for reproduction from stored body lipid and nutritional contributions from prey (Wootton 1977; Moodie 1986).

Female Fulton's K in the spring was significantly higher than in summer and fall, whereas male condition did not significantly differ across seasons. In contrast, overall, both male and female Fulton's K remained fairly low (less than 1.5) (Figures 6a and 7b),

suggesting that brook sticklebacks are generally streamlined in comparison to many other north-temperate fish (Feroese 2006; Nash et al. 2006). The observed seasonal variation in Fulton's K for females is likely due to increased ovary weight during the reproductive season, which influenced spring index scores (Figures 5 and 6a). Females for the rest of the study expressed lower condition and this expression may be tied to decreased reproductive investment. Chapman (2011) demonstrated that the central mudminnow (*Umbra limi*) entered the spring period in higher condition than fish collected during the following fall. These results support the conclusion that spring condition values for female north-temperate fish may often be inflated due to increased ovarian weight.

Female percent somatic lipid increased as spring sampling dates progressed reaching its peak in the first sampling date of summer (Figure 7c). Such increases in stored body lipid may result from an increase in the quantity and quality of food being consumed by the fish. However, the trend of increasing somatic lipid shows an inverse relationship with female GSI. The peak of female percent lipid in summer may be related to resorption of ovarian lipid into somatic tissue following the reproductive period (Figures 7a and 7c) (Hayes and Taylor 1994; Adams 1999). It may therefore be possible that the combination of a decrease in reproductive investment and the resorption of ovarian lipid contributed to the increase in percent lipid during the summer period.

Female percent lipid was significantly higher in the summer than in the fall period (Figure 6c). This result was surprising and could imply that fall somatic lipid is less important for cool water fish such as brook stickleback than it is for other north-

temperate fishes. The prioritization of growth over lipid storage has been suggested to have life history advantages for YOY threespine stickleback, a species with an average lifespan of 3.6 years. Threespine sticklebacks that have achieved the greatest length in the fall had an increased winter survival rate and reproductive success, potentially at the expense of mortality before the following reproductive period (Ab Ghani and Merilä 2015; Baker et al. 2015). Greater body lengths may increase the ability of threespine sticklebacks to compete for scarce winter resources (Baker et al. 2015). Large body size has been shown to strongly predict reproductive output of females (GSI and fecundity), better territory defense in males, and a higher female encounter rate with males during the breeding period (Candolin and Vigot 2001). Therefore, for the short-lived brook stickleback which reach a maximum of three years of age, it may be possible that summer and fall growth in length is prioritized over lipid storage.

Fulton's K is a strong predictor of percent lipid in male brook stickleback but a weak predictor of female percent lipid (Figure 8d). Though Fulton's K predicted somatic lipid in females during the summer period, it did so with low confidence ($R^2 = 0.30$) (Figure 8b). My results support the idea that Fulton's K may not be uniformly applicable as a measure of condition or overall health. Such an idea may especially apply to fish species which replace stored lipid with water as fat reserves are mobilized (Cone 2011; Morton and Routledge 2014; Schloesser and Fabrizio 2017; Bavčević et al. 2020).

The mean length of fish sampled in the spring and summer was similar, but the average length decreased by approximately two millimeters in the fall (Table 1). This difference was statistically significant (Figure 4a) and could be attributed to a number of factors. As brook stickleback are relatively short-lived fish living between 1-3 years of

age, it may be that the oldest and largest individuals experienced mortality after the spawning season and prior to the fall (Stewart et al. 2007). Therefore, the fall population of brook stickleback may contain a greater share of age-two fish and new YOY recruits which are likely to be smaller than age-three fishes. It is not implausible that the individuals captured in the fall would have grown over the coming winter to reach lengths comparable to the individuals I captured in the spring prior (Froese 2006). Another reasonable explanation for the fall decrease in mean length is seasonal variation in habitat use. Brook sticklebacks overwinter, and on occasion spend the summers in deeper water. Hence, it is possible that larger individuals moved to deep-water habitats sooner than smaller individuals (Stewart et al. 2007), and my method of near shore sampling would not have included individuals who had left those shallow-water areas.

Average brook stickleback weight varied between all seasons, being the highest in spring and lowest in the fall (Table 1 and Figure 4b). The statistical difference between summer and fall was associated with the increase in mean length of fish sampled. However, the difference in length of fish between spring and summer was minimal, therefore, the difference in weight cannot be attributed to a change in length. Because the GSI of fish was significantly higher in the spring than in the summer, the difference in the gonad weight of females may be responsible for this change (Figure 5). Another potential explanation for this change in weight could be that the fish that were sampled during the summer were actively growing, a concept that is supported by evidence of rapid compensatory growth of threespine stickleback following the breeding period (Ab Ghani and Merilä 2015; Baker et al. 2015).

The scope of my study is limited to brook sticklebacks from a single location, Reinstein Woods Nature Preserve in Depew, New York (Figures 1 and 2). Initially, brook sticklebacks were sampled from two other locations that were intended to be used in this study (The Buffalo River Oxbow Lake located in West Seneca, New York and Tift Nature Preserve located in Buffalo, New York). However, brook sticklebacks were found at low abundance in the Buffalo River Oxbow Lake and highly variable hydrologic conditions of the marsh at Tift Nature Preserve as a result of a hydrologic study made sampling there difficult and unreliable.

The sampling of brook stickleback occurred in shallow vegetated margins of the inlet of a permanent pond using minnow traps, and as such was limited to sampling fish that were utilizing nearshore habitats. This sampling technique appeared to be quite biased toward females (F:M sex ratio: 0.92:0.08) in the spring which may be tied to sexually distinct life history traits of brook stickleback during the reproductive period, where males establish and defend territories while females form roaming schools (Reist and Carmichael 2007; Stewart et al. 2007). The sex ratio of summer and fall samples was approximately equal, coinciding with the relief of male brood care during the spring breeding season (Summer sex ratio: 0.53:0.47; Fall sex ratio: 0.51:0.49) (Table 1).

Stickleback capture rates were highest in the spring but showed no distinct pattern with temperature. The first two spring sampling dates had high capture rates in comparison to other dates. A subset of brook sticklebacks was kept from samples in which stickleback were highly abundant, and when sampling efforts yielded less than 20 sticklebacks all individuals were kept (Appendix 1). Such sampling efforts may have led

to variability in terms of how randomly the individual representatives of the population were sampled.

A total number of 1282 dietary items were consumed in spring by 45 fish (28.5 items per fish) (Appendix 2). By contrast, in summer, 319 dietary items were enumerated for 27 fish which were analyzed for stomach content (11.8 items per fish) and a total count of 3669 dietary items were recorded for 30 fish which were sampled in fall (122.3 items per fish) (Appendix 2). Although these differences might seem staggering, the importance of prey items and feeding strategy did change as expressed by the Costello plots and the size of prey items likely influenced available stomach volume. For example, fish eggs and chironomids are larger in size, so the count of items in full stomachs where these items are dominant would be lower than that of a full stomach where copepods were the dominant item.

The use of Fulton's K condition factor as an index for fish condition is tied to its ability to predict the lipid content of fish. My research suggests that Fulton's K condition factor was sexually and seasonally variable for predicting percent lipid in brook stickleback. If my results are replicable in other brook stickleback populations, the application of Fulton's K may need to be limited to evaluating the condition of only male brook stickleback. Such an application would require that sampled stickleback be sexed in the field based upon sex specific morphological expression (barred eyes, black nuptial color, and orange spine coloration) and potentially further constrained from late winter to summer when such expressions are reliably observable (Stewart et al. 2007). Other indexes may be more reliable for both male and female brook sticklebacks (for

example RNA-DNA ratios), but such metrics must be evaluated and validated using chemical analysis.

The seasonal use of lipids by north-temperate fish is a highly researched topic and is established in the existing literature. The most common hypothesis for explaining the seasonal variability of lipid content is that freshwater fish must overcome energetic challenges during early life stages via maternal lipid and also must ration energy on a temporal scale as adults. Most freshwater fish hatch as fry with a finite amount of maternal lipid which they use to develop, forage, and grow as larvae, before self-sustaining their metabolic needs, growth, and development as a juvenile into an adult (Heming and Buddington 1988). The above hypothesis suggests that after development, fish balance metabolism, growth, reproductive investment, and in turn build somatic lipid in preparation for the winter period (Adams 1999; Hurst 2007). To the contrary, Black et al. (2014) found that whole body lipid levels of threespine stickleback increased throughout the winter and prior to breeding. Although the above hypotheses may be true for many fish species, it may also be possible that some fish do not follow this trend and, therefore, the reliance upon such notions may need to be reconsidered.

My research suggests that brook sticklebacks may not require heavy lipid reserves moving into the winter period. The body lipid that males (2.3 %) and females (2.5%) had entering the winter period was modest (Table 1). Such a value is fairly low compared to the central mudminnow, another small-bodied fish outside of the family Gasterosteidae which was noted as containing a fall average of 5.5% lipid (Chapman 2011). The observed levels of brook stickleback percent lipid are not likely indicative of future winter mortality but rather a life history strategy of favoring growth over

reproduction as observed in freshwater threespine stickleback (Baker et al. 2015; Reyes and Baker 2015).

The very high reproductive output of species within the family Gasterosteidae may be tied to their low accumulation of temporal lipid reserve and nonconformity to Fulton's K index. Brook stickleback and threespine stickleback females have been noted to repeatedly spawn during the breeding period (Moodie 1986; Baker et al. 2008) and subsequent broods of stripped female threespine stickleback had lower fecundity in treatments with lower food rations (Wootton 1977; Ali and Wootton 1999). Therefore, the reproductive investment of female brook stickleback may be more clearly tied to dietary intake than fall lipid reserves. Apart from avoiding predation, encountering males, and laying eggs, females have the ability to obtain energy to continue to develop eggs while males undertake the energetic cost of brood care (Reist and Carmichael 2007; Stewart et al. 2007). The sexual differences in breeding behaviors, spring female foraging, and life history strategies of brook stickleback may be responsible for the unusual pattern of seasonal lipid dynamics that they display.

For north-temperate fish, physiological stress is expected over winter and metabolic maintenance is seasonally variable. Since the overall metabolism of a cool water fish such as brook stickleback lowers with decreasing temperatures it can be expected that the sole energetic cost of metabolism may also be lower when experiencing winter conditions as observed by Klinger et al. 1982. Respiration and fecal loss were most responsible for the metabolic loss of overwintering threespine stickleback (Wootton et al. 1980; Moodie 1986). Little growth was recorded over the winter period with a notable increase of somatic and ovarian growth in the spring,

leading researchers to conclude that the difference in food availability between these two periods was responsible for changes in growth (Wootton et al. 1980; Moodie 1986).

Conclusions

My study found that the average length of fish decreased in the fall which suggests that the demographic of the sampled population changed (Figure 4a). During this period female GSI was increasing, possibly indicating that the new demographic of smaller females was preparing for reproduction the following spring (Figure 5; Figure 7a). If true, these females entered the winter period with 2.5% (± 0.163) somatic lipid, a fairly low lipid reserve with respect to other north-temperate fish, but nonetheless a greater amount than females sampled the spring prior which had only 1.98% (± 0.154) somatic lipid (Table 1). It is likely that sticklebacks use fall lipid reserves to support winter metabolism and spring reproduction. Although this is probably true, such an effect may not be detectable through the use of Fulton's K condition factor since sticklebacks express unique metabolic adaptations to winter physiological stress and retain a relatively low somatic lipid reserves as they enter the winter period.

My study found that Fulton's K condition factor only predicted percent body lipid of summer females, failing to predict the somatic lipid content of females collected in the spring and fall. As the metabolism of fall lipid reserves is understood to support the winter survival and spring reproduction of many north-temperate fish, it may be necessary to evaluate the lipid content of fish before they enter the winter period of physiological stress. This may be of particular importance to fish with short lifespans such as brook stickleback, where the reproductive capacity of the population may be

reliant on age-two fish and new recruits (relating to the age of fish as they enter the winter period). Since Fulton's K did not predict the lipid content of fall female brook stickleback, it may be necessary to deploy the use of fall fish sampling for chemical analysis for the conservation of non-game fish like stickleback. Because non-game fish such as stickleback serve as an important link to migratory waterfowl and sport fish, the successful management of native non-game fish resources may have benefits to both aquatic and terrestrial ecosystems.

It may be possible that fish species or phenotypes with more common life history strategies adhere closely to commonly accepted concepts while other less common strategies stray further from the assumptions which are the basis of such concepts and are used to justify levels of measurement. This presents an interesting paradox: to what extent do north-temperate fish rely on lipid reserves in comparison to daily dietary nutrition over the winter and early in the spring? Similarly, how does dietary intake influence reproductive investment and the body condition of fish, and lastly, is such an effect variable from species to species? The proposed questions above may be viable prompts for further investigation into the topics of aquatic ecology, fish bioenergetics, and the effect of climate on north-temperate fish. Such efforts may be warranted to understand the connection between life history strategies and temporal energetic expenditure.

References

- Ab Ghani I.N. and Merilä J. (2015). Population divergence in compensatory growth responses and their costs in sticklebacks. *Ecology and Evolution*, 5, 7-23. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4298429/>
- Adams S.M. (1999). Ecological Role of Lipids in the Health and Success of Fish Populations. In: Arts M.T., Wainman B.C. (Eds.). *Lipids in Freshwater Ecosystems*, 132-160. New York, New York: Springer Publishing. https://sci-hubtw.hkvisa.net/10.1007/978-1-4612-0547-0_8
- Ali M. and Wootton R.J. (1999). Effect of food levels on reproductive performance of breeding female three-spined sticklebacks. *Journal of Fish Biology* 55, 1040-1053. <https://sci-hub.ru/10.1111/j.1095-8649.1999.tb00739.x>
- Amundsen P.A., Gabler H.M., and Staldvik F.J. (1996). A new approach to graphical analysis of feeding strategy from stomach contents data – modification of the Costello (1990) method. *Journal of Fish Biology*, 48, 607-614. <https://sci-hub.se/10.1111/j.1095-8649.1996.tb01455.x>
- Bavčević L., Petrović S., Karamarko V., Luzzanad U., and Klanjšček T. (2020). Estimating fish energy content and gain from length and wet weight. *Ecological Modeling*, 436. <https://sci-hubtw.hkvisa.net/10.1016/j.ecolmodel.2020.109280>
- Baker J.A., Heins D.C., Foster S.A., and King R.W. (2008). An overview of life-history variation in female threespine stickleback. *Behavior*, 145, 579-602. <https://sci-hub.ru/10.1163/156853908792451539>

- Baker J.A., Wund M.A., Heins D.C., King R.W., Reyes M.L., and Foster S.A. (2015). Life-history plasticity in female threespine stickleback. *Heredity*, 115, 322-334. <https://sci-hub.ru/10.1038/hdy.2015.65>
- Barker E.E. (1918). The Brook Stickleback. *Scientific Monthly*, 6 (6), 526-529. <https://www.jstor.org/stable/22598?seq=1>
- Biga L., Dawson, S., Harwell, A., Hopkins, R., Kaufmann, J., LeMaster, M., Matern, P., Morrison-Graham, K., Quick, D., and Runyeon J. (2019). Lipid Metabolism. In: *Anatomy and Physiology 1st Edition*. Open Oregon State, Oregon State University. Houston, Texas: OpenStax. <https://open.oregonstate.education/aandp/chapter/24-3-lipid-metabolism/>
- Black C.A., Scott R.J., Bernards M.A. (2014). Seasonal changes in carotenoid and lipid storage by threespine stickleback, *Gasterosteus aculeatus*. *Environmental Biology of Fishes*, 97, 209-214. <https://link.springer.com/article/10.1007/s10641-013-0130-x>
- Braekevelt C.R., and McMillan D.B. (1967). Cyclic changes in the ovary of the brook stickleback *Eucaliainconstans* (Kirtland). *J. Morphol.* 123: 373-396. <https://pubmed.ncbi.nlm.nih.gov/5583345/>
- Candolin U. and Vigot H.R. (2001). Correlation between male size and territory quality: consequence of male competition or predation susceptibility? *Oikos*, 95, 225-230. <https://sci-hub.ru/10.1034/j.1600-0706.2001.950204.x>
- Chapman J.M. (2011). Effects of watershed land use on the seasonal energy dynamics, condition, and parasite communities in stream fish. Master's Thesis, Carleton

University, Ottawa, Canada. <https://curve.carleton.ca/28d46cb1-b153-4023-8d80-27bfeb7a3803>

Chavin W. (1973). *Responses of Fish to Environmental Changes*. Springfield, Illinois: Charles C Thomas Publishing.

Cone R.S. (2011). The Need to Reconsider the Use of Condition Indices in Fishery Science. *Transactions of the American Fisheries Society*, 118 (5), 510-514. <https://www.tandfonline.com/doi/abs/10.1577/1548-8659%281989%29118%3C0511%3ATNTRTU%3E2.3.CO%3B2>

Craig J.F. (1977). The Body Composition of Adult Perch, *Perca fluviatilis* in Windermere, With Reference to Seasonal Changes and Reproduction. *Journal of Animal Ecology*, 46, 617-632. https://www.jstor.org/stable/3834?seq=2#metadata_info_tab_contents

Divino J.N., and Tonn W.M. (2007). Effects of reproductive timing and hatch date on fathead minnow recruitment. *Ecology of Freshwater Fish* 16, 165-176. <https://sci-hub.hkvisa.net/10.1111/j.1600-0633.2007.00208.x>

Dudgeon D. (2019). Multiple threats imperil freshwater biodiversity in the Anthropocene. *Cell Press: Current Biology*, 29 (19), 960-967. <https://www.sciencedirect.com/science/article/pii/S0960982219310140>

Fike A.D., Myrick C.A., and Hansen (2007). Potential impacts of global climate change on freshwater fisheries. *Reviews in Fish Biology and Fisheries*, 17, 581-613. <https://link.springer.com/article/10.1007/s11160-007-9059-5>

- Froese R. (2006). Cube law, condition factor and weight length relationships: history, meta-analysis, and recommendations. *Journal of Applied Ichthyology*, 22, 241-253.
https://www.waterboards.ca.gov/waterrights/water_issues/programs/hearings/elsur_ranch/docs/exhbts/esr_62.pdf
- Hayes D.B., and Taylor W.W. (1994). Changes in the Composition of Somatic and Gonadal Tissues of Yellow Perch following White Sucker Removal. *Transactions of the American Fisheries Society* 123, 204-216. [https://sci-hub.hkvisa.net/10.1577/1548-8659\(1994\)123%3C0204:citcos%3E2.3.co;2](https://sci-hub.hkvisa.net/10.1577/1548-8659(1994)123%3C0204:citcos%3E2.3.co;2)
- Helfrich L.A., Neves R.J. (2019). Sustaining America's Aquatic Biodiversity: Freshwater Fish Biodiversity and Conservation. Virginia Cooperative Extension, Virginia Tech, Publication 420-525. <https://www.pubs.ext.vt.edu/420/420-525/420-525.html>
- Heming T.A., and Buddington R.K. (1988). Yolk absorption in embryonic and larval fishes. In: Hoar W.S., Randall D.J. (Eds.). *Fish Physiology*, vol. XIA. The Physiology of Developing Fish, 407-446. San Diego, California: Academic Press.
[https://sci-hub.hkvisa.net/10.1016/s1546-5098\(08\)60203-4](https://sci-hub.hkvisa.net/10.1016/s1546-5098(08)60203-4)
- Hlavec R. (1971). The seasonal dietary variation of the brook stickleback (*Eucalia inconstans*) in a southeastern Wisconsin stream. University of Wisconsin-Milwaukee, *Field Station Bulletin* 4 (2): 7-10.
https://dc.uwm.edu/fieldstation_bulletins/29/

- Hurst T.P. (2007). Causes and consequences of winter mortality in fishes. *Journal of Fish Biology* 71, 315-345. <https://sci-hub.hkvisa.net/10.1111/j.1095-8649.2007.01596.x>
- Karapanagiotidis I.T., Yakupitiyage A., Little D.C., Bell M.V., and Mente E. (2010). The Nutritional value of lipids in various tropical aquatic animals from rice-fish farming systems in northeast Thailand. *Journal of Food Composition and Analysis*, 23, 1-8. <https://sci-hub.ru/10.1016/j.jfca.2009.08.001>
- Klinger S.A., Magnuson J.J., and Gallepp G.W. (1982). Survival mechanisms of the central mudminnow (*Umbra limi*), fathead minnow (*Pimephales promelas*) and brook stickleback (*Culaea inconstans*) for low oxygen in winter. *Environmental Biology of Fishes*, 7 (2), 113-120. <https://sci-hub.ru/10.1007/bf00001781>
- Langley L.L. (1965). *Outline of Physiology*. New York, New York: Blackiston Division, McGraw-Hill Publishing.
- Lee C.M., Trevino B., and Chaiyawat M. (1996). A Simple and Rapid Solvent Extraction Method for Determining Total Lipids in Fish Tissue. *Journal of AOAC International*, 79 (2), 487-492.
re <https://www.researchgate.net/publication/14287264>
- Manko P. (2016). Stomach content analysis in freshwater fish feeding ecology 116. *Prešov, Slovakia: University of Prešov*.
<https://www.researchgate.net/profile/Peter-Manko/publication/312383934>

- Moodie G.E.E. (1986). The population biology of *Culaea inconstans*, the brook stickleback, in a small prairie lake. *Canadian Journal of Zoology*, 64, 1909-1717. <https://sci-hub.ru/10.1139/z86-258>
- Morton A., and Routledge R.D. (2014). Fulton's Condition Factor: Is it a Valid Measure of Sea Lice Impact on Juvenile Salmon?. *North American Journal of Fisheries Management*, 26 (1), 56-62. <https://sci-hub.st/10.1577/m05-068.1>
- Nash R., Valencia A.H., and Geffen A.J. (2006). The origin of Fulton's condition factor – Setting the record straight. *American Fisheries Society: Fisheries*, 31 (5), 236-238. <https://www.researchgate.net/publication/233388972>
- Palić D., Herolt D.M., Andreasen C.B., Menzel B.W., and Roth J.A. (2006). Anesthetic efficacy of tricaine methanesulfonate, metomidate and eugenol: Effects on plasma cortisol concentration and neutrophil function in fathead minnows (*Pimephales promelas* Rafinesque, 1820). *Journal of Aquaculture* 254, 675-685. <https://dr.lib.iastate.edu/entities/publication/1ae77500-b5ea-4578-9d46-c31beaf4404c>
- Pataki G.E., and Sheehan D.M. (2006). Dr. Victor Reinstein Woods Nature Preserve Unit Management Plan. New York State Department of Environmental Conservation. <https://www.dec.ny.gov/lands/22619.html#:~:text=The%20approved%20UMP%20for%20Reinstein,forester%2C%20and%20communication%20services%20staff.>
- Reist J.D., and Carmichael T. (2007). Fish diets and food webs in the Northwest Territories: brook stickleback (*culaea inconstans*). *Fisheries and Oceans*

Canada: *Canadian Manuscript Report of Fisheries and Aquatic Sciences* 2798.

<https://www.researchgate.net/publication/252775668>

Reyes M.L. and Baker J.A. (2015). The consequences of diet limitation in juvenile threespine stickleback: growth, lipid storage and the phenomenon of compensatory growth. *Ecology of Freshwater Fish*, 26, (2), 301-312. <https://scihub.ru/10.1111/eff.12276>

Schloesser R.W., and Fabrizio M.C. (2017). Condition Indices as Surrogates of Energy Density and Lipid Content in Juveniles of Three Fish Species. *American Fisheries Society*, 146, 1058-1069.

<https://afspubs.onlinelibrary.wiley.com/doi/full/10.1080/00028487.2017.1324523>

Snyder R.J., DeMarche C.J., and Honeyfield D.C. (2011). Impacts of changing food webs in Lake Ontario: Implications of dietary fatty acids on growth of Alewives. *Aquatic Ecosystem Health and Management*, 14 (3), 231-238.

<https://www.researchgate.net/publication/254272622>

Sokolowska E., and Skóra K.E. (2002). Reproductive cycle and the related spatial and temporal distribution of the nine-spine stickleback (*Pungitius pungitius* L.) in Puck Bay. *Journal of Oceanologia* 44 (4), 475-490.

<https://www.researchgate.net/publication/26393643>

Stewart D.B., Reist J.D., Carmichael T.J., Sawatzky C.D., and Mochnacz N.J. (2007). Fish life History and habitat use in the Northwest Territories: brook stickleback (*culaea inconstans*). *Fisheries and Oceans Canada: Canadian Manuscript*

Report of Fisheries and Aquatic Sciences 2799.

<https://www.researchgate.net/publication/242720112>

Weber L.P., Higgins P.S., Carlson R.I., and Janz D.M. (2003). Development and Validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. *Journal of Fish Biology*, 63, 637-658. <https://scihubtw.hkvisa.net/10.1046/j.1095-8649.2003.00178.x>

Wootton R.J. (1977). Effect of Food Limitation During the Breeding Season on the Size, Body Components and Egg Production of Female Sticklebacks (*Gasterosteus aculeatus*). *Journal of Animal Ecology*, 46 (3), 823-834. <https://scihub.ru/10.2307/3643>

Wootton R.J., Allen R.M., and Cole S.J. (1980). Energetics of the annual reproductive cycle in female sticklebacks, *Gasterosteus aculeatus* L. *Journal of Fish Biology*, 17 (4). <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1095-8649.1980.tb02773.x>

Appendices

Appendix 1: Table of sampling date, morning water temperatures, and number of fish captured by the deployment of three, one-quarter-inch gee minnow traps. Fish species include *Culaea inconstans* (Stickleback), *Culaea inconstans* yong of the year (YOY), *Campostoma anomalum* (Central Stoneroller), fish of the family *Centrarchidae* (Sunfish), and *Umbra limi* (Central Mudminnow).

Sampling Date	Temperature (°C)	Stickleback	YOY	Central Stoneroller	Sunfish	Central Mudminnow
05/08/2022	18	110	0	5	3	0
05/28/2022	19	140	0	6	0	0
06/16/2022	27	50	0	0	0	0
06/30/2022	22	19	0	1	0	1
08/02/2022	21	51	20	0	0	0
08/25/2022	27	16	20	0	0	3
09/29/2022	14	19	40	9	0	11
10/28/2022	11	61	0	9	0	7
12/01/2022	4	13	0	2	0	2

Appendix 2: Stomach contents of brook stickleback individuals sampled in the spring for the dietary items *Bosmina* (Bosm), chironomids (Chir), copepods (Cope), *Daphnia* (Daph), Ephemeroptera (Ephe), Gammaridae (Gamm), Fish Eggs (Eggs), ostracods (Ostr), plant seeds (Seed) with totals for individuals (as a column) and for prey types (as a row at the bottom).

Sample Date	Sex	Bosm	Chir	Cope	Daph	Ephe	Gamm	Eggs	Ostr	Seed	Total
05/08/2022	F	5	4	51	1	0	0	11	5	0	77
05/08/2022	F	25	0	71	3	0	0	3	3	0	105
05/08/2022	M	5	0	4	1	0	0	2	0	0	12
05/08/2022	F	1	0	0	0	0	0	0	0	0	1
05/08/2022	F	6	0	57	0	0	0	10	1	2	76
05/08/2022	F	2	2	45	0	0	3	0	5	0	57
05/08/2022	F	0	0	0	0	0	0	0	0	0	0
05/08/2022	F	13	6	8	3	0	0	2	5	0	37
05/08/2022	F	0	0	0	0	0	0	40	0	0	40
05/08/2022	F	12	0	4	2	0	0	0	0	0	18
05/08/2022	F	0	0	0	1	0	0	0	0	0	1
05/08/2022	F	0	0	0	0	0	0	5	0	0	5
05/08/2022	F	0	0	0	0	0	0	34	0	0	34
05/08/2022	F	27	7	4	8	0	4	0	0	0	50
05/08/2022	F	48	1	2	3	0	1	2	1	0	58
05/08/2022	F	3	0	0	0	0	5	2	0	0	10
05/29/2022	F	2	3	2	6	4	0	5	4	0	26
05/29/2022	F	11	5	18	3	3	1	12	5	0	58
05/29/2022	F	5	6	2	3	2	0	0	23	0	41
05/29/2022	F	5	0	6	0	0	0	21	0	0	32
05/29/2022	M	2	2	1	1	0	0	40	2	0	48
05/29/2022	F	0	4	0	2	0	0	24	0	0	30
05/29/2022	F	1	0	0	1	0	0	0	3	0	5
05/29/2022	F	0	0	0	0	0	0	0	0	0	0
05/29/2022	F	0	0	0	0	0	0	41	0	0	41
05/29/2022	F	0	1	0	0	0	0	30	0	0	31
05/29/2022	F	0	1	0	0	0	0	0	0	0	1
05/29/2022	F	17	27	10	2	0	0	0	3	0	59
05/29/2022	F	1	13	0	4	0	0	3	4	0	25
05/29/2022	F	2	4	8	2	1	0	0	0	0	17
05/29/2022	F	2	0	1	0	0	0	0	0	0	3
05/29/2022	F	0	0	0	0	0	0	0	0	0	0
06/16/2022	F	4	4	9	8	0	0	2	1	0	28
06/16/2022	F	0	0	0	0	0	0	0	0	0	0
06/16/2022	F	0	0	0	0	0	0	27	0	0	27
06/16/2022	M	0	0	0	0	0	0	24	0	0	24
06/16/2022	F	0	0	0	0	0	0	11	0	0	11

06/16/2022	F	4	3	0	0	0	1	0	1	0	9
06/16/2022	F	0	0	0	0	0	0	0	0	0	0
06/16/2022	F	10	0	7	0	0	0	0	0	0	17
06/16/2022	F	0	0	0	0	0	0	0	0	0	0
06/16/2022	F	1	1	0	3	0	0	0	0	0	5
06/16/2022	F	0	12	0	6	0	0	0	0	0	18
06/16/2022	F	0	1	0	0	0	0	16	0	0	17
06/16/2022	F	0	0	0	0	0	0	9	0	0	9
06/16/2022	F	0	3	9	2	0	0	0	0	0	14
06/16/2022	F	0	2	0	0	0	0	73	0	0	75
06/16/2022	F	0	1	0	0	0	0	12	0	0	13
06/16/2022	M	0	0	0	0	0	0	10	0	0	10
Total		214	113	319	65	10	15	471	66	2	1275

Appendix 3: Stomach contents of brook stickleback individuals sampled in the summer for the dietary items *Bosmina* (Bosm), chironomids (Chir), copepods (Cope), *Daphnia* (Daph), Ephemeroptera (Ephe), Gammaridae (Gamm), Fish Eggs (Eggs), ostracods (Ostr), plant seeds (Seed) with totals for individuals (as a column) and for prey types (as a row at the bottom).

Sample Date	Sex	Bosm	Chir	Cope	Daph	Ephe	Gamm	Eggs	Ostr	Seed	Total
6/30/2022	F	0	11	4	1	0	0	0	1	0	17
6/30/2022	M	0	0	0	0	0	0	0	0	0	0
6/30/2022	F	0	0	0	0	0	0	0	0	0	0
6/30/2022	M	0	0	0	0	0	0	0	0	0	0
6/30/2022	F	0	0	0	0	0	0	0	0	0	0
6/30/2022	F	0	0	0	0	0	0	0	0	0	0
6/30/2022	F	1	50	0	3	0	0	0	7	0	61
6/30/2022	F	0	0	0	0	0	0	0	0	0	0
6/30/2022	M	0	1	0	0	0	0	0	0	0	1
6/30/2022	M	0	0	0	0	0	0	0	0	0	0
6/30/2022	M	0	0	0	0	0	0	0	0	0	0
6/30/2022	M	0	0	0	0	0	0	0	0	0	0
6/30/2022	F	0	3	0	0	0	0	0	0	0	3
6/30/2022	F	0	11	2	7	0	0	0	7	0	27
6/30/2022	F	0	1	0	0	0	0	1	0	0	2
6/30/2022	M	0	1	0	0	1	0	0	1	0	3
6/30/2022	M	0	0	0	0	0	0	0	0	0	0
6/30/2022	M	2	0	0	0	0	0	0	0	0	2
6/30/2022	F	0	7	0	2	0	0	0	1	0	10
6/30/2022	M	1	3	0	1	1	0	0	0	0	6
6/30/2022	M	0	0	0	0	0	0	0	0	0	0
7/30/2022	M	0	0	0	0	0	0	0	0	0	0
7/30/2022	F	0	0	0	0	0	0	0	0	0	0
7/30/2022	M	0	0	0	1	0	0	0	0	0	1
7/30/2022	M	0	0	0	0	0	0	0	0	0	0
7/30/2022	F	0	0	0	0	0	0	0	0	0	0
7/30/2022	F	0	31	0	0	0	0	0	0	0	31
7/30/2022	M	0	0	0	0	0	0	0	0	0	0
8/25/2022	F	0	0	0	0	0	0	0	0	0	0
8/25/2022	M	0	8	0	0	0	0	0	0	0	8
8/25/2022	M	0	3	5	0	0	2	0	1	0	11
8/25/2022	F	0	0	1	2	0	0	0	0	7	10
8/25/2022	M	0	4	5	0	1	3	0	42	3	58
8/25/2022	M	0	8	2	0	0	0	0	0	0	10
8/25/2022	M	0	3	3	0	0	0	0	0	0	6
8/25/2022	F	0	0	0	0	0	0	0	0	0	0
8/25/2022	F	0	0	1	0	0	1	0	0	0	2

8/25/2022	F	0	0	0	0	0	0	0	0	0	0
8/25/2022	F	1	0	5	2	0	2	0	5	1	16
8/25/2022	F	0	0	0	0	0	0	0	0	0	0
8/25/2022	F	0	3	4	0	0	0	0	4	0	11
8/25/2022	F	0	1	1	5	0	0	2	0	0	9
8/25/2022	M	0	1	0	0	0	3	0	0	3	7
8/25/2022	F	0	2	0	0	0	0	0	0	2	4
Total		5	152	33	24	3	11	3	69	16	316

Appendix 4: Stomach contents of brook stickleback individuals sampled in the fall for the dietary items *Bosmina* (Bosm), chironomids (Chir), copepods (Cope), *Daphnia* (Daph), Ephemeroptera (Ephe), Gammaridae (Gamm), Fish Eggs (Eggs), ostracods (Ostr), plant seeds (Seed) with totals for individuals (as a column) and for prey types (as a row at the bottom).

Sample Date	Sex	Bosm	Chir	Cope	Daph	Ephe	Gamm	Eggs	Ostr	Seed	Total
9/29/2022	M	2	19	24	0	0	0	0	11	0	56
9/29/2022	F	0	0	0	0	0	0	0	0	0	0
9/29/2022	F	0	4	129	0	0	0	0	2	0	135
9/29/2022	M	1	1	1	0	0	0	0	0	0	3
9/29/2022	F	1	0	3	0	0	0	0	0	0	4
9/29/2022	F	0	2	2	0	0	0	0	0	0	4
9/29/2022	F	6	6	77	0	0	0	0	5	0	94
9/29/2022	M	0	1	0	0	0	0	0	0	0	1
9/29/2022	M	0	0	0	0	0	0	0	0	0	0
9/29/2022	M	0	0	0	0	0	0	0	0	0	0
9/29/2022	F	4	3	2	2	0	0	0	2	2	15
9/29/2022	F	1	0	1	0	0	0	0	2	1	5
9/29/2022	M	0	0	0	0	0	0	0	0	0	0
9/29/2022	F	9	14	99	0	0	0	0	2	0	124
10/29/2022	F	37	21	322	45	0	0	0	5	0	430
10/29/2022	F	63	7	56	5	1	0	0	8	0	140
10/29/2022	F	64	15	53	5	2	0	0	4	3	146
10/29/2022	M	3	5	14	2	1	0	0	0	0	25
10/29/2022	M	7	19	69	1	1	0	0	7	0	104
10/29/2022	M	37	9	106	12	1	0	0	4	0	169
10/29/2022	M	75	9	47	4	1	0	0	1	3	140
10/29/2022	M	0	0	0	0	0	0	0	0	0	0
10/29/2022	F	9	12	78	1	0	0	0	14	0	114
10/29/2022	M	40	7	80	4	0	0	0	15	1	147
10/29/2022	F	3	3	15	0	0	0	0	1	0	22
10/29/2022	F	73	4	22	6	1	0	0	9	0	115
10/29/2022	F	93	4	197	11	0	0	0	20	2	327
12/1/2022	M	6	20	38	0	0	0	0	12	0	76
12/1/2022	M	9	6	169	0	0	0	0	10	2	196
12/1/2022	F	3	3	102	0	0	0	0	14	0	122
12/1/2022	F	0	6	17	0	0	0	0	0	0	23
12/1/2022	M	18	6	222	2	0	0	0	6	2	256
12/1/2022	F	12	5	27	0	0	0	0	0	0	44
12/1/2022	M	6	6	150	0	0	0	0	0	0	162
12/1/2022	F	0	1	75	0	0	4	0	0	0	80
12/1/2022	M	1	15	45	0	0	3	0	5	0	69
12/1/2022	F	1	3	127	3	0	6	0	0	2	142
12/1/2022	M	5	1	60	0	0	5	0	0	0	71

12/1/2022	M	2	4	48	0	0	33	0	9	0	96
Total		591	241	2477	103	8	51	0	168	18	3657

Appendix 5: Frequency of occurrence and prey specific abundance of dietary items *Bosmina* (Bosm), chironomids (Chir), copepods (Cope), *Daphnia* (Daph), Ephemeroptera (Ephe), Gammeridae (Gamm), Fish Eggs (Eggs), ostracods (Ostr), plant seeds (Seed) of spring brook stickleback.

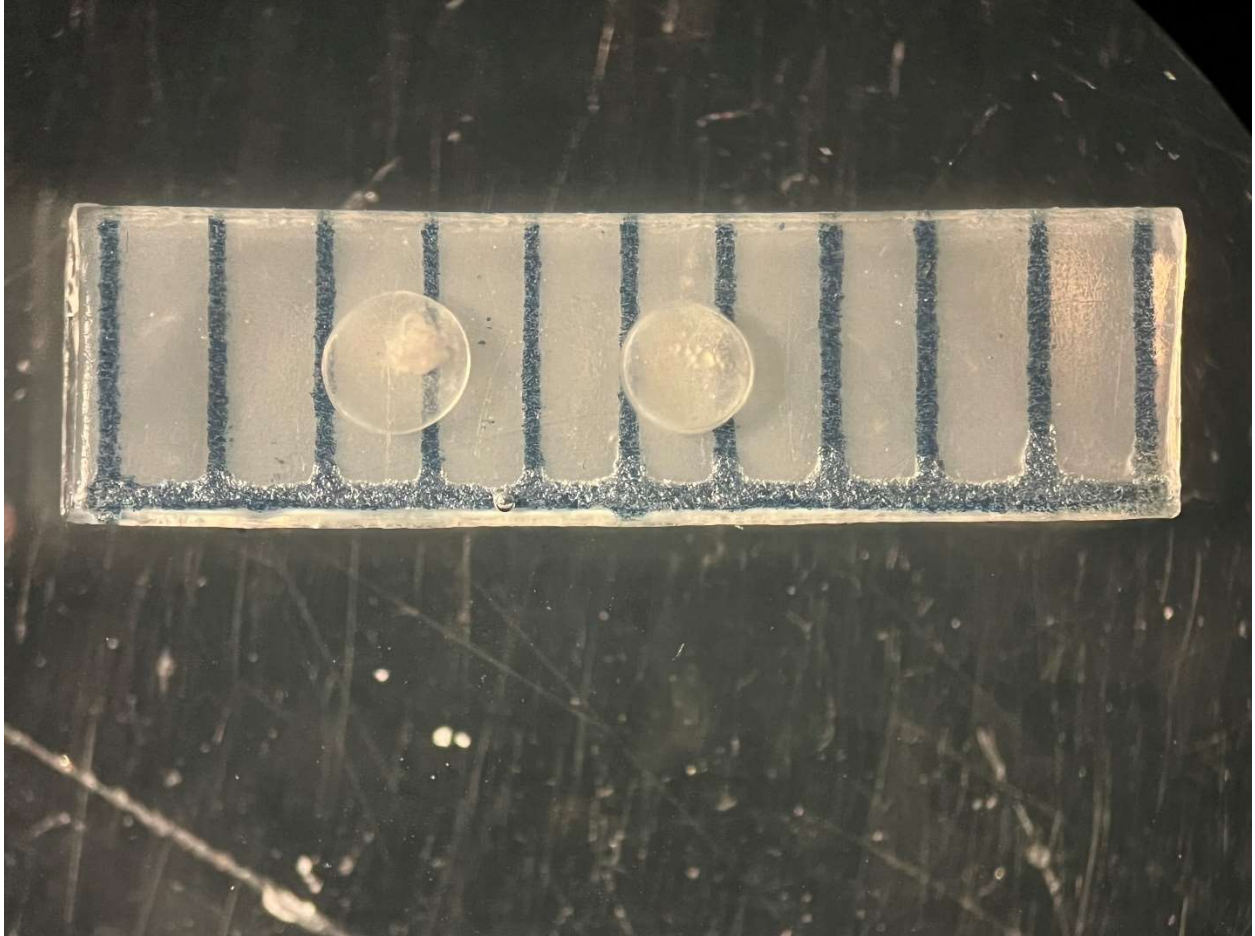
Prey Type	<i>Bosm</i>	<i>Chir</i>	<i>Cope</i>	<i>Daph</i>	<i>Ephe</i>	<i>Gamm</i>	<i>Eggs</i>	<i>Ostr</i>	Seed
Frequency	60.0	55.0	50.0	50.0	10.0	15.0	67.5	37.5	2.5
Abundance	24.5	13.6	38.0	8.9	7.0	6.2	49.1	9.3	2.6

Appendix 6: Frequency of occurrence and prey specific abundance of dietary items *Bosmina* (Bosm), chironomids (Chir), copepods (Cope), *Daphnia* (Daph), Ephemeroptera (Ephe), Gammeridae (Gamm), Fish Eggs (Eggs), ostracods (Ostr), plant seeds (Seed) of summer brook stickleback.

Prey Type	<i>Bosm</i>	<i>Chir</i>	<i>Cope</i>	<i>Daph</i>	<i>Ephe</i>	<i>Gamm</i>	<i>Eggs</i>	<i>Ostr</i>	Seed
Frequency	15.4	73.1	42.3	34.6	11.5	19.2	7.7	34.6	19.2
Abundance	5.9	85.9	18.6	15.3	4.5	11.7	27.3	32.2	16.8

Appendix 7: Frequency of occurrence and prey specific abundance of dietary items *Bosmina* (Bosm), chironomids (Chir), copepods (Cope), *Daphnia* (Daph), Ephemeroptera (Ephe), Gammeridae (Gamm), Fish Eggs (Eggs), ostracods (Ostr), plant seeds (Seed) of fall brook stickleback.

Prey Type	<i>Bosm</i>	<i>Chir</i>	<i>Cope</i>	<i>Daph</i>	<i>Ephe</i>	<i>Gamm</i>	<i>Eggs</i>	<i>Ostr</i>	Seed
Frequency	85.3	94.1	97.1	41.2	20.6	14.7	0.0	67.6	26.5
Abundance	17.3	6.6	67.8	4.5	1.0	11.1	0.0	5.4	1.3



Appendix 8: Image of a fish egg removed from the stomach of a male brook stickleback (left) compared to an egg removed from the ovary of a gravid female (right), both fish were sampled on the same day (5/8/2022). A metric ruler (mm) was placed below the eggs for reference of scale.