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This study seeks to establish a relationship between a fish's total length to the size of various bony structures of prey fishes. Three important prey fish species in Minnesota were selected for examination in this study, including Black Crappie Pomoxis nigromaculatus, Bluegill Lepomis macrochirus, and Yellow Perch Perca flavescens. Length and weight dimensions of each fish's otoliths, cleithra, and vertebrae were associated to total lengths of the fish they came from. In many dietary studies, the need for these relationships is strongly related to the disfigurement or degradation of prey fish in stomach samples. With accurate enough regression equations, researchers can formulate cheaper and more efficient methods to collecting data regarding piscivorous predator diets. Data collected from these species could predict the original fish's length with a range of 92-99% confidence using the bony structure regressions. The cleithrum weight was the most useful measurement among all three species with an average of 98.3% accuracy of predicting total length. Additionally, all other relationships between total length and bony structure dimensions were highly significant (P<0.001). Upon success, these relationships may be implemented to reconstruct the size of a fish when length data is not available.

Faculty Sponsor: Dr. Andrew W. Hafs

Introduction

Intricate knowledge of fish diets is crucial for research, management, and the protection of fisheries. Researchers can use diet evaluations to understand and monitor trophic level and food web interactions (Manko 2016). Fisheries biologist can identify prey species in diet contents by recognizing unique bony structures of a particular specimen (Granadeiro and Silva 2000). Fish eating habits can also help fisheries biologists to increase the abundance of piscivorous predators. In the past dietary studies have even been able to indicate the presence of exotic species in waterbodies thought to be exotic or invasive-free (Chips and Garvey 2007). However, in order for biologist to execute management plans regarding piscivores, diet examinations must be as accurate as possible.

In the past, diet studies have been conducted using dissection, gastroscopes, emetics, stomach flushing, and even physically pulling food out of the stomach with forceps (Manko 2016). Though accurate, these methods all have variability due to the digestive states of the stomach contents. If the prey specimen is too digested, it can affect the accuracy and precision of a diet analysis (Manko 2016). Bony structures such as otoliths, vertebrae, and cleithra are relatively indigestible (Garman 1982). These structures are commonly found in the gut when all other prey tissue has been broken down by stomach acid. Therefore, a precise total length formula using bony structures will increase availability and accuracy of piscivorous fish diets.

Several studies have examined the relationship between bony dimensions and a fish's total length (Brown and Pierce 1997; Granadeiro and Silva 2000; Vigliola and Meekan 2009). This study aims to develop a relationship that determines the total length from bony structures of prey fish commonly found in freshwater lakes in Minnesota. The total length estimate will be calculated from precise length and weight dimensions of the otolith, cleithrum, and vertebrae. An accurate diet analysis can provide information regarding the predator's energy intake, habitat usage, behavior, and condition that can help us better understand fisheries (Manko 2016).

Methods

Fish Collection

Most of the samples in this study were collected in conjunction with an ongoing diet research in

Lake Name	DOW Number	County	Lake Acreage
Bald Eagle	62000200	Ramsey	1049.09
Bemidji	04013002	Beltrami	6595.96
Deer	04023000	Beltrami	297.79
Grace	29007100	Hubbard	859.82
Little Boy	11016700	Cass	1451.68
Miltona	21008300	Douglas	5724.3
Ten Mile	11041300	Cass	5080.43
White Fish	04013700	Beltrami	385

Table 1. All of the lakes used throughout this study listed with their specific identification, location and size.

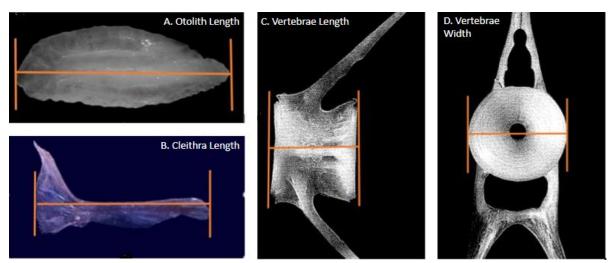


Figure 1. The measurment parameters for each bony structure outlined with orange dimension brackets.

Minnesota lakes. These lakes include Bald Eagle, Bemidji, Deer, Grace, Little Boy, Miltona, and Ten Mile Lake. An additional waterbody, White Fish Lake was included in this study for the purpose of angling fish that did not reach the target sample size from the initial lake sampling. All waterbodies specific identification, location and size are shown in Table 1. Black Crappie Pomoxis nigromaculatus, Bluegill Lepomis macrochirus, and Yellow Perch Perca flavescens were collected for examination using diet extraction, electrofishing, and angling techniques. Diet extraction was completed by gastric lavage or stomach dissection of piscivorous fishes in conjunction with an ongoing study. Samples that were collected by examining stomach contents were preserved in Whirl-pak bags and submerged in 70% ethanol. Other sampling methods such as electrofishing and angling involve direct capture of the subject fish. Individuals that were captured using electrofishing or angling techniques were sacrificed and frozen to avoid decay.

Dissection

Total length of each prey fish was recorded before any dissection process could begin. Once the total length was recorded, the cleithra were removed from the posterior arch of the gill. It was essential to remove the cleithra first, so that no damage could be done to them in the following procedures. Next, the otoliths were removed from the inner ear within the fish's skull. The otolith removal process was explained in Secor et al. (1992). Once otolith and cleithrum are removed, the fish was filleted to expose the spine of the fish. The vertebra that was removed was the anterior most vertebra that does not support a rib. All bony structures were then cleaned of any remaining flesh using a dry cloth and tweezers.

Analysis

Once the bones were clean, the length of every individual bone was measured using a micrometer. The otoliths were measured from tip to tip at the longest point (Figure 1A). Cleithra were measured from the tip of the anterior arm to the middle of the posterior lobe (Figure 1B). Additionally, otoliths and cleithra were weighed to the nearest ten thousandths of a gram. The vertebrae were not able to be weighed because the vertebral protrusions were often too fragile to remove without inflicting damage to the bone. However, the width of the vertebrae was rarely affected by removal and was used in place of vertebrae weight (Figure 1C and 1D). After all skeletal dimensions were completed, regression models were developed to relate total length to bony structure dimensions. Length-based measurements were analyzed by a linear regression equation while weight-based measurements were analyzed using power regressions.

Results

Overall, Black Crappie formulas were most accurate at predicting total length and Bluegill were least accurate. However, each species' regression equations (Table 2) were significant at predicting fish length (P < 0.001). All equations explained at least 92% of the variation in original fish ($R^2 > 0.92$).

Black Crappie

All regressions that predict total lengths of Black Crappie were calculated using bony structures from individuals ranging in length from 59-333 mm. Black Crappies total lengths were best predicted using cleithrum weights, vertebrae lengths and vertebrae widths ($R^2 = 0.99$, P < 0.001). Other Black Crappie bone regressions were also very good predictors of total length including otolith length ($R^2 = 0.95$, P < 0.001, Figure 2), otolith weight ($R^2 = 0.96$, P < 0.001) and cleithrum length ($R^2 = 0.98$, P < 0.001).

Bluegill

All regressions to predict total lengths of Bluegill were calculated using bony structures from individuals ranging in length from 25-183 mm Bluegill regressions were less accurate than the other species but were still able to predict total length very precisely with the right bone dimensions. The most accurate estimator for Bluegill length was the vertebrae length regression ($R^2 = 0.98$, P < 0.001; Figure 3). The cleithrum length and weight ($R^2 = 0.97$, P < 0.001) were also very good at calculating fish lengths. Otolith lengths, otolith weights and vertebrae width regressions (Table 2) were less accurate, but they can still provide valuable data when other bone measurements are not accessible.

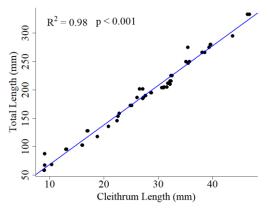


Figure 2. The relationship between Black Crappie total length and cleithrum length using linear regression with a line of best fit.

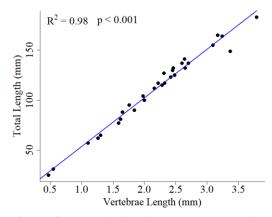


Figure 3. The relationship between Bluegill total length and vertebrae length using a linear regression with a line of best fit.

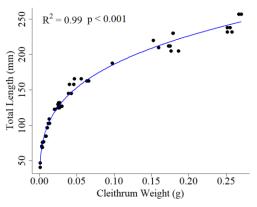


Figure 4. The relationship between Yellow Perch total length and cleithrum weight using power regressions with a line of best fit.

Species	Structure Dimension	Regression Equations	R ²
Black Crappie	otolith length	y = 30.736x - 33.394	0.95
Black Crappie	cleithrum length	y = 6.9372x - 0.4273	0.98
Black Crappie	vertebrae length	y = 60.89x - 1.5487	0.99
Black Crappie	vertebrae width	y = 50.672x + 28.82	0.99
Black Crappie	otolith weight	$y = 705.02x^{0.4218}$	0.96
Black Crappie	cleithrum weight	$y = 363.48x^{0.301}$	0.99
Bluegill	otolith length	y = 26.201x - 8.9971	0.93
Bluegill	cleithrum length	y = 5.1796x + 13.163	0.97
Bluegill	vertebrae length	y = 48.66x + 5.008	0.98
Bluegill	vertebrae width	y = 50.084x + 21.649	0.93
Bluegill	otolith weight	$y = 755.3x^{0.4309}$	0.92
Bluegill	cleithrum weight	$y = 270.32x^{0.2529}$	0.97
Yellow Perch	otolith length	y = 33.012x - 14.967	0.97
Yellow Perch	cleithrum length	y = 6.906x + 9.0065	0.97
Yellow Perch	vertebrae length	y = 56.029x + 4.2342	0.97
Yellow Perch	vertebrae width	y = 60.598x + 23.743	0.96
Yellow Perch	otolith weight	$y = 1162x^{0.4565}$	0.96
Yellow Perch	cleithrum weight	$y = 362.16x^{0.2865}$	0.99

Table 2. All bony regression equations for each species posted with their accuracy. The most accurate regression equations for each species are labeled with an asterisk in the structure dimension column. All regressions produced a p value of < 0.01.

Yellow Perch

The regressions used to predict the total lengths of Yellow Perch were calculated using bony structures from individuals ranging in length from 41-257 mm. The regression that can best predict Yellow Perch length uses cleithrum weight for regression analysis ($R^2 = 0.99$, P < 0.001; Figure 4). The accuracy of other bony structures such as vertebrae length, cleithrum length and otolith length follow closely behind ($R^2 = 0.97$, P < 0.001). Regressions using some alternate dimensions of these bones including otolith weight and vertebrae width are just slightly less accurate ($R^2 = 0.96$, P < 0.001; Table 2).

Discussion

It was hypothesized that at least one of these bony structures would have a strong enough relationship to determine total length for each fish species. However, all regressions of bony structures were precise enough to predict total length within 92-99% accuracy. This is similar to another study that observed a 90-99% accuracy when testing the total length relationship to otolith length and vertebra length (Granadeiro and Silva 2000). In this study each species has at least one regression that can predict total length within 98% accuracy or greater. Researchers should look for bones that can generate the most accurate total lengths before using a less accurate regression. This allows fisheries ecologists to have multiple options when looking for bones inside a fish's stomach.

According to this study, using cleithra weight for regressions is the most accurate method of total length prediction among all the tested species. The cleithrum weights observed from Black Crappie Bluegill, and Yellow Perch generated regressions with an average accuracy of 98.3%. This would imply that cleithra are the most important structure for dietary analysts to look for when sorting through the contents of a stomach. Most studies in the past have been focused on cleithrum length rather than cleithrum weight (Yazicioğlu et al. 2017; Gaygusuz et al. 2008). This study supports a testament for future data to be collected in length and weightbased measurements for bony regressions.

Otolith based regressions were highly variable among Bluegills. The otolith length regression (R^2 =0.93) and otolith weight regression (R^2 =0.92) from Bluegills were the least accurate of all the regressions formulated in this study. The lack of accuracy in Bluegill regressions could be due to growth rate fluctuations seen across lakes with differing population dynamics (Hilborn and Minte-Vera 2008). These results were still similar when compared among other studies that have backcalculated the total length of a fish using otolith dimensions (Granadeiro and Silva 2000; Yilmaz et al. 2015). Many studies have had at least a few regressions that are less accurate than others, this does not mean that the regressions are useless, just that Bluegill bony structure dimensions are slightly more variable among individuals. However, regression equations with an R² value of greater than 0.92 are still very powerful. Especially considering how little information can be present inside of a fish's stomach.

Overall, this study provides a guideline for fish diet research in Minnesota. Ecologists can use this study to support or formulate data from future diets of piscivores. These regressions will be used in coordination with the ongoing diet study at Bemidji State University to improve available fisheries data. In order to further solidify the accuracy of predicting stomach content analysis, many more Minnesota native fish species should be processed for regressions. However, this research has paved a way to predict some of the most common prey species in Minnesota lakes.

Acknowledgements

This study could not have been completed without the extra efforts of my supervisors and DNR employees. I would like to thank my immediate supervisor and current graduate student at Bemidji State University, Kamden Glade. I would also like to express my gratitude to my advisor, professor, and project supervisor Andrew Hafs. Additionally, I would like to thank the Minnesota Department of Natural Resources with a special thanks to Brian Herwig, the research biologist at the Bemidji Fisheries department.

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