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MOVEMENT AND HABITAT USE OF SELECTED NONGAME FISHES IN A
MINNESOTA LAKE

By

Michael D. Habrat

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
For the Degree of Master of Science
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in the Department of Wildlife and Fisheries

Mississippi State, Mississippi

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MOVEMENT AND HABITAT USE OF SELECTED NONGAME FISHES IN A
MINNESOTA LAKE

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Aquatic vegetation provides important habitats for fish, but these habitats are increasingly being altered anthropogenically. My research evaluated the movement and habitat use of three small rare fish species, the blackchin shiner *Notropis heterodon* (BCS), blacknose shiner *Notropis heterolepis* (BNS), and banded killifish *Fundulus diaphanous*, in a Minnesota lake. BCS and BNS traveled farther in spring than summer, but selection of habitat based upon macrophyte biovolume did not explain these differences. All three fish species traveled long distances (> 1,800 m) and were capable of reaching all available habitats in Square Lake. Macrophyte species richness and prey (zooplankton) abundance were not correlated with fish abundance; however, ordination techniques suggested several macrophyte species were important to the habitat use of these fishes in Square Lake. Proactive management for the conservation of these sensitive fish species in Square Lake should focus on protecting vegetated habitats and preserving water quality.

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CHAPTER I

INTRODUCTION

Aquatic macrophytes are unique in structure and provide structural complexity for fish habitats (Dibble et al. 1996). These vegetated habitats play a major role in structuring fish assemblages (Tonn and Magnuson 1982) by providing fish with foraging sites (Crowder and Cooper 1982), a direct food source (Lodge et al. 1988), protection from predation (Savino and Stein 1982), and spawning areas (Becker 1983). In many aquatic systems a direct correlation exists between macrophytes and fish abundance and diversity (Tonn and Magnuson 1982), and the heterogeneous nature of macrophyte beds leads to spatial heterogeneity in fish assemblages (Weaver et al. 1997). Macrophytes also mediate abiotic interactions within lakes such as nutrient cycling, dissolved oxygen concentrations, buffering water movements, creating temperature gradients, and mitigating sedimentation (Carpenter and Lodge 1986; Engel 1988). The removal of aquatic macrophytes can have profound effects biologically (i.e. trophic cascades) and chemically (Engel 1985).

Recent increases in lakeshore development have raised concern for fishery managers as many lake users view aquatic macrophytes as a nuisance (Jennings et al. 2003). Emergent and floating-leaved macrophytes have particularly been shown to be reduced at developed lakeshore sites (Radomski and Goeman 2001; Jennings et al. 2003).

Reductions in coarse woody debris and increases in substrate embeddedness are also correlated with lakeshore development (Jennings et al. 2003). Alterations to littoral zone fish habitats by homeowners are incremental and typically occur at the scale of individual dwellings (Jennings et al. 1999). As a result, these small shoreline fragments are viewed to be ecologically insignificant. This viewpoint creates a problem for fishery managers as these alterations are cumulative and can have basin-wide effects on water quality and intolerant fish species (Jennings et al. 1999). An understanding of these cumulative effects is vital to managers as fish community responses to habitat fragmentation are often times nonlinear (Ryall and Fahrig 2006). Fish communities may appear unaffected by initial losses of habitat and fragmentation, but a threshold may eventually be reached whereby further alterations to the available habitat can have drastic effects. Fragmented habitats may be suitable to support a fish species, but limitations such as patch size and connectivity may inhibit use of the remaining patch matrix (MacArthur and Wilson 1967). This threshold is particularly important to predator-prey interactions, and if reached, one or both of the interacting species may go extinct (Ryall and Fahrig 2006).

Freshwater fish use vegetated habitats for a variety of reasons (i.e. foraging, spawning, predator avoidance), and the use of these habitats changes depending upon the reason for use. For example, northern pike (*Esox Lucius*) use seasonally flooded shallow wetlands as spawning sites in early spring but frequently forage near submersed vegetation edges in deeper water the remainder of the year (Becker 1983). Littoral zone macrophyte beds often are composed of several macrophyte species (Chick and McIvor 1994), and because individual macrophyte species differ in spatial complexity (Dibble et al. 1996), the littoral zone can be viewed as a mosaic of different habitat patches.

Understanding of the ecological processes of fish-habitat relations must incorporate fish movement (Skalski and Gilliam 2000). Relationships between fish distribution, movement/dispersal patterns, and habitat variables allow inferences to be made on the mechanisms regulating community structure (Weaver et al. 1996; Weaver et al. 1997). Determining factors of fish movement are interrelated with fish habitat use; particularly in heterogeneous or patchy environments. Suitable habitats may be within physical reach of a species, but a lack of patch connectivity may prevent that species from successfully reaching those habitats (Turner et al. 2001). Habitat patch connectivity can not be viewed as an absolute; rather, connectivity is a function of habitat abundance, the habitat patch matrix, and an organism's movement capabilities (Turner et al. 2001; Hein et al. 2004). Fish species that exist as subpopulations in a patchy environment stand a greater risk of local extinction than those species existing as a continuous population. One way to determine if a fish species exists as a subpopulation is through mark/recapture. Theoretically, if a fish species exists as a set of subpopulations, the majority of marked individuals should be recaptured within the same habitat unit in which they were marked (Johnston 2000). Past research has shown small non-game fishes move relatively short distances (Chitty and Able 2004; Jacobus and Webb 2005; Able et al. 2006) with many stream fishes remaining in the habitat patch where they were released (Johnston 2000; Rodríguez 2002).

The Minnesota Department of Natural Resources has identified four fish species whose numbers are in decline and believed to be sensitive to habitat destruction: blacknose shiner *Notropis heterolepis* (BNS), blackchin shiner *Notropis heterodon* (BCS), banded killifish *Fundulus diaphanous* (BKF), and Iowa darter *Etheostoma exile*

(IOD). These four species prefer vegetated shallow habitats (<1.5 m) of clear water glacial lakes and slow moving rivers and streams (Becker 1983; Lyons 1987; Houston 1990; Carlson 1997). The shiners, in particular, prefer extremely shallow habitats (0.1-0.5 m), require a substrate of clean gravel or sand, and respond quickly to disturbances in habitat. Degradation of water quality predominantly in the form of increased siltation has been linked to the extirpation of these species (Becker 1983; Houston 1996; Bernstein et al. 2000).

The overall goal of my research was to describe the movements and habitat use of BCS, BNS, BKF, and IOD in a Minnesota lake where these species currently remain abundant. In Chapter II, I conducted a two-year mark/recapture study on these four fish species. I investigated the hypothesis that movement of these species was determined by habitat selection and a temporal factor such as season. I also examined the dispersal patterns of these species by using the general statistical properties of the movement distributions. In Chapter III, I investigated the habitat use of BCS, BNS, and BKF at a small scale through underwater observation, and the relationship between the abundance of these fish species and available prey. I hypothesized that BCS, BNS, and BKF abundance was influenced by the small scale macrophyte community and density of preferred prey.

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CHAPTER II
SEASONALITY AND HABITAT SELECTION EFFECTS UPON
MOVEMENT OF FOUR SMALL NON-GAME FISH
SPECIES IN A MINNESOTA LAKE

Introduction

Small non-game fishes are important constituents of fish communities in freshwater lakes both biologically and anthropogenically; whether it be providing prey for desired game species or acting as indicators to pollution and habitat loss. Accordingly, high conservation priority has been given to small bodied lotic (Angermeier 1995; Warren et al. 2000) and lentic (Houston 1990, 1996; Roberts et al. 2006) fish species in North America, yet methodologies for tracking these populations have lagged behind the need for information about the movement and population-size of these fishes (Roberts and Angermeier 2004). Many of these species are used as indicators of ecosystem health as they are often classified as intolerant to poor water quality and habitat degradation (Drake and Pereira 2002). The persistence of these non-game fish species is of increasing concern in light of eminent increases in watershed and lakeshore development.

Lakeshore development directly impacts aquatic ecosystems and habitats necessary for fish survival (Radomski and Goeman 2001). These impacts generally take

the form of nutrient runoff, sedimentation, and aquatic macrophyte removal. Aquatic macrophytes provide important habitat for many fishes, yet the small scale alteration of aquatic macrophytes is common adjacent to lakeshore homes (Beauchamp et al. 1994; Engel and Pederson Jr. 1998; Radomski and Goeman 2001). The loss of macrophytes in this way may lead to fragmentation of aquatic habitats. These fragmented habitats may still be suitable in supporting a fish species, but limitations such as patch size and connectivity to other suitable patches may inhibit use of critical aquatic habitat and prevent permanent colonization of that species (MacArthur and Wilson 1967). In addition, alterations in habitat affect species differentially (Jacobus and Webb 2005). Large piscivorous fish may be unaffected by minor changes in habitat as they typically have larger home ranges than small non-game fishes. Before population dynamics and habitat patch characteristics can be fully understood, managers need a better understanding of specific habitat features and the requirements of single species (Summerfelt 1999). More specifically, data are needed that establish whether a fish population exhibits metapopulations dynamics where the species is restricted to habitat patches that are linked by limited dispersal or as populations with relatively unrestricted dispersal (Johnston 2000).

Quantitative research illustrating patterns of organism movement rates can be useful in a wide array of ecological applications (Skalski and Gilliam 2000). Relationships between fish distribution, movement patterns, and habitat variables allow inferences to be made on the mechanisms regulating fish community structure (Weaver et al. 1996; Weaver et al. 1997). Accordingly, insight into the mechanistic interactions of individuals and their system can foster a detailed understanding of higher level processes

(Real 1982; Stein et al. 1988) and identify species in need of proactive conservation (Angermeier 1995). Determining the movement of small fishes is difficult due to the size incompatibility of available tags (Buckley et al. 1994). Methods used to mark small fish allow recognition of individuals and increase accuracy in estimates of population dynamics (Jones 1990), which aids managers in conserving threatened non-game species.

Previous investigations on the movement of small non-game fishes has demonstrated species move relatively short distances (Chitty and Able 2004; Jacobus and Webb 2005; Able et al. 2006), and many stream fishes remain in the habitat patch where released (Johnston 2000; Rodri'guez 2002). The objective of this study was to determine the movement patterns of the blacknose shiner *Notropis heterolepis* (BNS), blackchin shiner *Notropis heterodon* (BCS), banded killifish *Fundulus diaphanous* (BKF), and Iowa darter *Etheostoma exile* (IOD) by using the general statistical properties of the movement distributions to gain insight into how these species may be affected by habitat fragmentation. Specifically I was interested in whether these four small-sized fish species were capable of moving relatively long distances to utilize habitat in a fragmented environment. I investigated the hypothesis that movement was determined by habitat selection and a temporal factor such as season. I also tested the general statistical properties of the movement distributions.

Methods

Study Site

Square Lake (79 ha) is located in the St. Croix River basin in the North Central Hardwood Forest of Washington County, Minnesota. Watersheds in this area are mostly

agricultural and contain large residences with well manicured lawns that facilitate nutrient runoff. Accordingly, lakes in this region are typically shallow, eutrophic, and highly developed. Square Lake is unique from other area lakes in the region because of its large maximum depth (20.7 m), mesotrophic classification, and relative minimal shoreline development. Data from the Minnesota Pollution Control Agency (MPCA 2004) indicate water quality within Square Lake averages 120 ppm alkalinity and 12 ppb total phosphorous, and that secchi disk averages 6.4 m. The littoral zone is home to diverse temperate macrophyte species (Table 2.1), and growth of these species have been recorded in Square Lake at depths near 8 meters (Valley et al. 2005). Square Lake supports a two-story fishery consisting of warm and cool-water species characteristic of the region, rainbow trout (*Oncorhynchus mykiss*) that have been stocked biannually since 1998, and contains several species listed by the Minnesota Department of Natural Resources as species of special concern and in statewide decline including the blacknose shiner, blackchin shiner, banded killifish, and Iowa darter.

Sample Design

Fish and macrophyte sample locations were stratified within the littoral zone and sampling activities occurred in a two-stage process from May through August 2005 and 2006. The geographic information system (GIS) ArcGIS ArcMap Version 9.1 (Environmental Research Systems Institute Inc., Redlands, California) was used to create random sample points within Square Lake. Points were then uploaded to a hand held GPS (Garmin Map 76, Garmin International Inc., Olathe, Kansas) and used to navigate to sample sites in the field. Random points were sampled across the littoral zone of Square

Lake the first week of each month, both vegetated and non-vegetated. From these samples, aggregations discerned using catch-per-unit of effort of the target species were found. The remainder of each month was spent sampling at random points within the areas of these aggregations. This approach allowed monitoring seasonal aggregations of target species while concentrating effort in areas where these species were most abundant.

Purse and bag seines (3.175 mm mesh) were used to collect fish from a 25 m² sample area. Purse seines were used where depths did not allow use of the bag seine. Bag seines were 5 m long and were pulled parallel to shore for 5 m. Purse seines were 17.7 m long and deployed and retrieved from a boat. A 4 m tall purse seine was used to sample depths ≤ 4 m, while a 8 m tall purse seine was used for sampling depths > 4 m. BCS, BNS, BKF, and IOD were enumerated in each haul and a GPS coordinate and depth was recorded at the center of each haul. Seines are a proven gear for sampling small fish in the littoral zone (Lyons 1986; Lyons 1987; Dewey et al. 1989; Weaver et al. 1993; Pierce et al. 2001; Drake and Pereira 2002), and are often advantageous over other gears in the littoral zone as sampling with them is easy and rapid while causing minimal harm to the fish captured (Hayes et al. 1996). Beach seines, designed for sampling shallow-shoreline areas, and purse seines, designed for pelagic sampling, are the most commonly used of the encircling nets (Hayes et al. 1996).

Macrophyte biovolume (BV), expressed as plant height/water depth, was recorded using a Biosonics DE-6000 echosounder equipped with a 430 kHz 6° split-beam transducer (Biosonics Inc. 2002) mounted on a 16-foot flat-bottom boat per Valley et al. (2005). BV was recorded in July 2005 and in May and July in 2006. May and July were

recorded separately because BV in Square Lake increased considerably after May (Valley et al. 2005). Krigging was used to spatially interpolate the BV data and create a spatially-referenced BV map of Square Lake (Valley et al. 2005). Data collection and interpolation was performed at 25 m² to allow the fish sampling and biovolume scales to correspond. These data and map were created by Ray Valley, Minnesota Department of Natural Resources. I assumed that the BV data recorded in July were representative of June, July, and August as Valley et al. (2005) found no appreciable differences throughout these months. I also assumed BV data recorded in May 2006 were representative of May 2005 (Valley et al. 2005).

Fish Marking

A visible implant elastomer (VIE; Northwest Marine Technologies, Inc., Shaw Island, Washington) was used to mark the target fish species. VIE is a fluorescent material injected subcutaneously as a liquid that soon cures into a pliable, bio-compatible solid which can be recognized at some distance, much like an external tag. Mortality of VIE tagged individuals is negligible and in some cases non-existent (Buckley et al. 1994; Haines and Modde 1996; Willis and Babcock 1998; Roberts and Angermeier 2004). VIE has been used to tag coral reef fishes as small as 8 mm in length (Frederick 1997). Despite this evidence I chose to determine VIE retention and mortality for the target species in a preliminary aquarium experiment. Unfortunately, the geographical range of BCS, BKF, BNS, and IOD did not allow capture of these species near Mississippi State University, so three surrogate genera were used. Specimens used in this experiment came from species similar taxonomically and/or in body form and behaviors to the

Minnesota target species: *Notropis spp.* were used to represent BCS and BNS, *Etheostoma spp.* were used to represent IOD, and *Gambusia spp.* were used to represent BKF. I randomly assigned two treatment groups and one control group to individual fish. Group 1 individuals were subcutaneously injected with a 2-3 mm VIE mark using a 0.3-cc syringe into the fleshy area just below the dorsal fin (Roberts and Angermeier 2004). Group 2 individuals were tagged in the same manner as group 1, but retagged at days 15 and 30 for a total of three tags. The second and third tags were injected just below the dorsal fin on the opposite side and next to the first tag respectively. All fish from each group were anesthetized with 60 mg/L tricaine methanesulfonate (Hill and Grossman 1978) during each marking event. The tagging needle was inserted into fish from the control group, but no mark was injected. Aquaria were inspected daily for fish mortality and bi-weekly (to reduce handling) for loss of marks over a 120 day period. Mark retention and fish mortality were similar to those reported in the bulk of the literature; VIE marks had high retention and mortality was negligible (Table 2.2).

BCS, BKF, BNS, and IOD caught in the seine hauls were marked using the protocol developed in the preliminary aquarium experiment. A unique batch mark was given to each fish in the sample by using one color (eight possible) at one or more tagging locations. Upon recapture of a marked fish, this unique mark allowed me to reference the GPS position where the mark was applied. A minimum of 32 unique marks was possible using one mark, four locations, and four colors. The possible number of unique marks greatly increased through the use of multiple colors and multiple marks per fish. Fish were marked and recaptured during the seine sampling as described above.

Fish were marked May-July 2005 and 2006, but the recapture period extended into August of both years.

Data Analysis

Data Preparation

Movement analysis was performed for recaptured fish using ArcMap. To reduce bias in calculating distances traveled, it was assumed fish traveled along a 0.25 m depth contour nearest to the average depth of the mark and recapture locations. Choosing one movement path for all the fish recaptured may have greatly under/over-estimated the distances traveled because the littoral zone of Square Lake is generally gently sloping. For example, the perimeter of the shoreline around Square Lake is 3,960 m while the perimeter of the 2.5 m contour is 3,576 m, a difference of ~10%. One-quarter meter depth contours were chosen as they were the smallest increment accurately created using the depths recorded at each BV sample point. I assumed a fish traveled the shortest distance possible, i.e. if a fish was recaptured on the opposite end of the lake it was assumed the fish traveled the shortest path along the 0.25 m depth contour. Movement paths were created for each recaptured fish in a three step process: 1) A line was digitized (drawn) from the marking location perpendicular to the associated depth contour, 2) then traced along that depth contour, and finally 3) continued perpendicularly away from the depth contour terminating at the recapture location (Figure 2.1). The length of this line was used as the traveled distance of each recapture. The shoreline of Square Lake was used as a depth contour if a fish was marked and recaptured in depths < 0.25 m as the

average contour would have been 0 m. I calculated the line lengths using the “add length field to table” option in the free downloadable ArcMap extension Hawth’s Tools (Beyer 2004).

Biovolume was calculated at each mark and recapture location using the interpolated BV map. The “Extract values to points” function in the Spatial Analyst extension in ArcMap overlaid the capture locations upon the BV map and each capture location (point) was then assigned the underlying BV value.

Temporal Model

Several variables were examined using Analysis of Covariance (ANCOVA) to determine if any temporal variables were correlated with movement. Type III sums of squares were used due to the unbalanced design (Goldberg and Scheiner 2001), and all significance tests were performed at $\alpha = 0.05$. Separate models were used for each of the fish species. Year and season were included as independent class variables and days-at-large as a covariate. Year was included to determine whether distances traveled varied between 2005 and 2006. Season was defined as the month in which a recaptured fish was tagged. Season was considered an important variable because aquatic macrophyte biomass increases throughout the growing season (Wetzel 1983), and the habitats created by them provide services to the fish community (Weaver et al. 1996) that are seasonally dependent. Abiotic factors such as temperature and dissolved oxygen also change through time (Wetzel 1983) and these factors can further affect the services provided by the littoral zone (Hayes et al. 1999). Days-at-large, here forth referred to as days, was calculated as the number of calender days between marking and recapture events.

This method allowed me to test for the effects of year and season on distance traveled while controlling for the number of days a fish could have traveled. A preliminary data analysis was performed to determine if days was linearly related to distance traveled (Petraitis et al. 2001). The ANCOVA was collapsed to two-way Analysis of Variance (ANOVA) when days was not related to distance traveled. Full models including all interactions were tested first. If an interaction term was non-significant, it was dropped from the model and the test was repeated to investigate the main effects (Hatcher and Stephanski 1994; Goldberg and Scheiner 2001).

Parametric model assumptions are often not met in ecological data (Petraitis et al. 2001). However, Shirley (1981) and Freund and Wilson (2003) acknowledge parametric methods using least squares are known to be robust against these violations. Petraitis et al. (2001) point out that ANCOVA is sensitive to unbalanced designs resulting in unreliable results and suggest the use of nonparametric methods in such cases. Two distinct approaches have emerged to handle violations of parametric assumptions: 1) transformation of data, and 2) using a distribution free procedure (Conover and Iman 1981). However, these two approaches are not without limitations. Transformation of data may alleviate parametric assumption violations, but may change the relationship between dependent and independent variables and change additive effects to multiplicative effects (Petraitis et al. 2001). Distribution free procedures (i.e. nonparametric) have been shown to have reduced power over their parametric counterparts (Freund and Wilson 2003), and some statistical software packages lack the ability to perform further analysis such as post-hoc comparisons (Conover 1999).

Conover and Iman (1981) addressed these limitations and suggest using a rank transformation approach, commonly referred to as the RT approach. This method calls for transforming data into ranks, thereby creating distribution free data, and then using the ranked data in standard parametric tests. Conover and Iman (1981) further explain that common parametric multiple comparison techniques (i.e. Tukey's, Duncan's, Fishers) may be applied using the RT approach, and are more robust and powerful for nonnormal populations over nonranked data. The RT technique is popular because of its simplicity and accessibility in statistical packages, and numerous studies have concluded the technique is powerful and robust (see Mansouri and Chang 1995 for a review). Mansouri and Chang (1995) caution the RT method may inflate Type I error rates, and suggest using normal scores of the ranks when interactions are used. This suggestion makes sense because although data created by ranks is distribution free, the "apparent distribution" appears symmetrical as a result of the evenly spaced ranks, i.e. 1, 2, 3... (Conover 1999). Normal scores can be used in place of ranks for parametric procedures and are generally distributed symmetrically around zero giving the appearance of a "perfect normal sample" (Conover 1999). Several variations of normal scores are available, but all are derived by replacing the ranks with quantiles from a Z normal distribution (Conover 1999); the variations arise from how the quantiles are calculated. SAS Version 9.1 (SAS Institute Inc., Cary, North Carolina) has the ability to compute Blom, Tukey, and van der Waerden normal scores in the PROC RANK procedure, but the Blom version appears to fit best (Blom 1958; SAS 2000).

PROC GLM in SAS was used to perform ANCOVA, or when applicable ANOVA, with Blom normal scores for distances traveled as the dependent variable, year

and season as class variables, and days as a covariate. Least-squares means were used for post-hoc comparisons of groups if a significant effect of a dependent variable was found. Least-squares means are predicted population margins that account for unbalanced designs and missing values (SAS 2000). The use of the LSMEANS statement in PROC GLM produced p-values for each group comparison and allowed me to see the strength of the differences. Graphical comparisons were used to determine the effects of the covariate days if a significant interaction of a dependent variable and the covariate occurred.

Movement Through Time

An organism is said to spread constantly through time if the variances of the movement distributions increase linearly with time (Skellam 1951; Skalski and Gilliam 2000). To test this I regressed the variances of distances traveled against time with the equation $y = c_0 + c_1t + c_2t^2$, where y is the variance of distance traveled and t is time (Skalski and Gilliam 2000). Spread through time is constant when $c_0 = 0$, $c_1 > 0$, and $c_2 = 0$. Time was defined as four day intervals for two reasons: 1) to ensure there was an adequate number of distances traveled for each time category to calculate a variance, and 2) fish were not usually sampled Friday-Sunday. Grouping movement times into four day intervals ensured a fish that was tagged on Thursday could be captured within the first time interval. PROC REG was used to fit the polynomial model for each species; significance was assumed at $\alpha = 0.05$.

Habitat Model

One-way ANOVA was used to determine if distance traveled was dependent upon habitat selection, and type III sums of squares were used due to the unbalanced design (Goldberg and Scheiner 2001). Again, significance was assumed at $\alpha = 0.05$. Blom normal scores for the ranks of distances traveled were used as the independent variable and change in habitat selectivity values was used as the dependent variable. Reasoning for this technique was discussed under the temporal model. Change in habitat selectivity values were defined in three categories. A fish was assigned to category 1 if the fish was recaptured in a habitat with a selectivity value significantly greater than the selectivity value of the marking location, category 0 was assigned when there was no significant difference between selectivity values of the recapture and marking locations, and category -1 was assigned when a fish was recaptured in a significantly lower selectivity value than the marking location selectivity value. BV was used as a measurement of habitat because aquatic macrophytes were the primary structure in the littoral zone of Square Lake (personal observation). The BV map of Square Lake used BV data in a continuous-integer scale, so computing selectivity values for each integer of BV would have been excessive. Moreover, my random sample design did not bring me to sample fish in all of the BV integer values that would have been necessary to create selectivity values for each BV value. Therefore, BV was grouped into 10% increments to create a reasonable number of habitats and ensure those habitats were sampled.

Habitat selectivity requires knowledge of habitat availability and the abundance of a species in each habitat type. Estimates of abundance from standard fish sampling techniques inherently include biases to some degree, and these biases are increased when

multiple sampling gears are used (Weaver et al. 1993). Lyons (1986) and Pierce et al. (1990) provided capture efficiencies of common littoral fish species from beach seines that ranged from < 10% to nearly 100%. During the summer of 2006, my technician, Luke Kusilek, investigated the species specific capture efficiencies of the bag and purse seines used for the sampling of fish. He also tested whether aquatic macrophyte biomass affected the capture efficiencies of these gears, and found no such effect (Kusilek et al. In Preparation). The abundance estimates of BCS, BKF, BNS, and IOD were corrected using the capture efficiencies found by Kusilek et al. (In Preparation). This technique enabled me to remove potential gear biases in my abundance estimates, and increased the accuracy of my selectivity index.

Jacobs D (1974) selectivity index was used to assign habitat selectivity values to each 10% BV habitat in Square Lake for each fish species in May and July of each year. The different selectivity values were calculated for May and July because BV in Square Lake increased considerably after May (Valley et al. 2005) and conceivably could have altered habitat selectivity of the target fish species. Selectivity values for BNS in May 2005 were not created due to improper identification of a similar species. As a result, these species were undistinguished and the exact number of BNS sampled during this period was unknown. I calculated habitat selectivity as $D = r-p/[(r+p)-2rp]$, where r is the proportion of the resources used and p is the proportion of available resources (Jacobs 1974). The proportion of available habitat was calculated as the number of 25 m² habitats in each BV category divided by the number of all 25 m² habitats. This analysis was restricted to the littoral zone of water depths of ≤ 2.5 m as no fish were tagged and

recaptured from deeper waters. Habitats with selectivity values >0 are considered to be selected for while habitat values <0 are avoided.

Selectivity values are useful but lack measures of precision, making it difficult to interpret them (Dixon 2001). Resampling Stats Excel add-in version 3.2 (Resampling Stats, Arlington, Virginia) was used to bootstrap D and create 95% confidence limits for each D value. Bootstrapping estimates the sampling distribution of a test statistic by first estimating the unknown population distribution from the sample data, and then repeatedly sampling the estimated population many times (Dixon 2001), each time recalculating the test statistic. One-thousand bootstrap replicates were used to estimate the confidence intervals for each selectivity value (Efron 1987). These confidence limits were then used to determine if a recaptured fish was recaptured in a location of significantly different habitat selectivity. This was used to examine if fish movement was linked to habitat selectivity. If the 95% confidence limits overlapped I assumed there to be no significant difference in habitat selection values.

Population Heterogeneity

The normality of the movement distributions were tested using the Shapiro-Wilk test (SAS 2000). Kurtosis of the movement distributions were also tested using the b_2 , the fourth standardized sample moment, statistic (Zarr 1999). The kurtosis estimates were corrected so that a positive value indicates leptokurtosis (heavy tailed) and a negative value indicates platykurtosis (light tailed) where $kurtosis = b_2 - 3(n-1)/(n+1)$ (D'Agostino et al. 1990). The hypothesis that the movement distributions lacked kurtosis was tested by comparing the kurtosis estimates to tabled critical values at $\alpha = 0.05$ (Table

9.5: D'Agostino and Stephens 1986). Leptokurtic distributions can result from the composite of two normal distributions (Zar 1999), and Fraser et al. (2001) suggest leptokurtic movement distributions may result from a population heterogeneity. If significant kurtosis existed, the data were divided into more homogeneous sub-groups in an attempt to remove any heterogeneity (Fraser et al. 2001). The sub-groups were created by breaking each species' movement distribution down into two factors: year and season. Only groups containing >7 individuals were used for testing (D'Agostino and Stephens 1986). Days was also used as a covariate in each subgroup. Within each subgroup distance traveled was regressed against days and kurtosis of the residuals was calculated (Zar 1999; Fraser et al. 2001). I again tested the significance of the resulting kurtosis estimates using tabled critical values at $\alpha = 0.05$.

Results

Seines captured 7,238 target fish species from 590 hauls in 2005 and 9,729 target fish species from 569 hauls in 2006. From these captures, 3,356 fish were marked in 2005 and 4,666 fish were marked in 2006. Fifty-nine (1.8%) fish from 44 locations were recaptured in 2005 (Figure 2.2) and 99 (2.1%) fish were recaptured from 57 locations in 2006 (Figure 2.3) for an overall recapture rate of 2.0%. The recaptures in 2005 originated from 31 locations and the recaptures in 2006 originated from 32 locations. A summary of the number of fish sampled, number of fish marked, number of recaptures, percent recaptured, number of locations marked, number of locations recaptured, and number of locations of marking from which the recaptures originated, by species and year, can be found in Table 2.3.

The movement distributions for BCS, BKF, and BNS shared a similar trend in that they appeared to be right-skewed (Figure 2.4). The majority of fish recaptured traveled short distances ($\leq 50\text{m}$) while some individuals traveled to the other end of the lake ($>1,800\text{ m}$). Only three (0.96%) IOD were recaptured in 2005 out of a total of 313 marked individuals in both years. Interestingly these three recaptures originated from the same marking location and were recaptured together 23 m away. Consequently, a movement distribution for IOD was not determined and no analyses were performed on this species.

Combining data from both years, BKF traveled farthest on average (562 m), followed by BNS (372 m) and BCS (310 m; Table 2.4). This trend was also true for the median distances traveled of each species, where BKF = 127 m, BNS = 106 m, and BCS = 36 m. No significant difference in distance traveled by species was found (Kruskal-Wallis: $\chi^2 = 7.13$; $df = 3$; $P = 0.0678$). The minimum and maximum distance traveled for each species were very similar and only varied by 3 m and 32 m respectively (Table 2.4). These trends in central tendency measures also held true each year, except in 2005 mean distance traveled for BCS was greater than that of BNS by 6 m (Table 2.4).

Temporal Model

A preliminary analysis was performed to determine if the covariate days was linearly related to distance traveled for each species and found no relationship for BCS and BKF. Therefore, a two-way ANOVA was used to test if distance traveled differed between season and year for BCS and BKF. Season was significantly different ($F_{(2,61)} = 16.62$; $P < 0.0001$) for BCS while year was not ($F_{(1,61)} = 2.24$; $P = 0.1397$). Post-hoc

comparisons showed distance traveled was significantly greater for those BCS tagged in May than those tagged in June ($P < 0.0001$) and July ($P < 0.0001$). The majority of recaptured BCS tagged in May traveled from one side of the lake to the other, with most of those fish moving from the East basin to the West basin (Figure 2.5). Distance traveled was not significantly different by season or year for BKF ($F_{(3,33)} = 1.92$; $P = 0.1462$). The preliminary analysis for BNS revealed days was linearly related to distance traveled; therefore, a two-way ANCOVA was used to test if distance traveled differed significantly by season and year using days as a covariate. A significant main effect of year was found for BNS ($F_{(1,48)} = 7.14$; $P = 0.0103$), and post-hoc comparisons showed BNS traveled significantly farther in 2006 than in 2005 ($P = 0.0103$). The interaction of the main effect season and covariate days was also significant ($F_{(1,48)} = 8.58$; $P = .0052$) for BNS. Linear regression in PROC REG revealed distance traveled for BNS increased linearly with days for fish tagged in May ($t = 3.63$; $P = 0.0040$) but did not in June ($t = -0.67$; $P = 0.139$; Figure 2.6). No BNS tagged in July were recaptured.

Movement Through Time

The premises described by Skellam (1951) for constant spread through time did not conform to the movement of BCS, BNS, and BKF. Each species violated at least one of the assumptions (zero intercept, positive slope, and zero quadratic term) for constant spread through time (Table 2.5), and the relationships of variance of distance traveled through time was nonlinear (Figure 2.7).

Habitat Model

Habitat selectivities for the 10% BV categories followed similar patterns in July of 2005 and 2006 for BCS and BKF. Figure 2.8 illustrates how both species tended to avoid habitats of $BV \leq 50-60\%$ and selected for habitats $> 60\%$ BV. Conversely, no clear trend existed for May in either species in either year (Figure 2.8). Habitat selectivity of BNS also showed no clear trends in July of 2005 and 2006 and in May 2005 (Figure 2.8).

No significant effect of habitat selection on distance traveled were found for BCS ($F_{(2,62)}=1.59$; $p<.2117$), and BKF ($F_{(2,34)} = 0.62$; $P = 0.5440$). Distance traveled by BNS was significantly different ($F_{(2,10)} = 6.56$; $P = 0.0036$) based upon habitat selection values where BNS traveled farther to habitats with smaller selectivity values. This analysis was performed for BNS only in 2006, while the analysis for BCS and BKF included both years.

Population Heterogeneity

The movement distributions of BCS, BKF, and BNS all deviated significantly from normality (for each case: $P < 0.0001$; Table 2.6). Similarly, the kurtosis estimates for the movement distributions of BCS and BNS were significantly leptokurtic, and the movement distribution of BKF was platykurtic, although not significant (Table 2.6).

Only seven of the possible 10 sub-groups were tested for kurtosis because tabled critical values are not available where $n < 7$. None of the subgroups for BKF were tested because the kurtosis estimate of the original movement distribution was not significant. The kurtosis estimates were positive (leptokurtic) for all of the sub-groups with the exception of the negative (platykurtic) estimate (-0.73) for the group of BNS tagged in

May of 2005 (Table 2.6). Significant leptokurtosis remained in four of the seven groups tested (Table 2.6). Consequently, the distributions of these groups also deviated significantly from normality. Two of the remaining three kurtosis-free distributions did not deviate significantly from normality (Table 2.6).

Discussion

My overall recapture rate was lower than those reported by similar studies that examined movements of minnow species (Chitty and Able 2004; Jacobus and Webb 2005; Able et al. 2006). This may be an artifact of high mortality as BCS, BKF, and BNS are short lived (2 years: Becker 1983). The low recapture rate I observed may also be indicative of very large populations of these species in Square Lake. Intrigued by this possibility, I performed an ad hoc population estimate for BNS during a two week period in June and found the estimated population size to be 173,282 (95% CL: {67,680 , 278,884}). To my knowledge, no other study has investigated the movement of these species in an inland lake; hence any amount of recapture data for these species is useful. The single recapture event for IOD is not surprising as Jacobus and Webb (2005) were also unable to recapture marked IOD. This may be because IOD is considered a territorial sedentary species that frequently associate in and under structure (Becker 1983) and are difficult to capture with seines (Lyons 1986). The majority of IOD were captured and tagged during spring before aquatic macrophyte growth peaked. The increased aquatic macrophyte biomass and complexity likely hindered capture of IOD during the remainder of the sampling period.

Although no significant difference was noted in distance traveled among species, the observed movements of BKF were contradictory to known behaviors of BKF. Able et al. (2006) found the average movements of two salt-marsh killifishes to be small (29 m) with most of the recaptures of *Fundulus luciae* occurring in the area of the release site. Becker (1983) described the BKF as a “ghost” in an aquarium setting due to its quiet behavior and camouflage. However, in my study BKF traveled the farthest on average with some movements in excess of 1,800 m.

The maximum distance traveled for BCS, BKF, and BNS were quite similar with at least one individual of each species moving > 1,800 m, a distance equivalent to moving to the opposite end of Square Lake. This suggests that these species are potentially capable of emigrating from habitats that become inhospitable due to disturbances, predation, water chemistry, etc., and move throughout Square Lake, i.e. any available habitat. This statement must be met with caution however. Although these species may move long distances, the effects of the overall habitat matrix and the facilitation of habitat corridors was unknown as I did not test for connectedness of suitable habitat. Suitable habitats may be within physical reach of a species, but a lack of patch connectivity may prevent that species from successfully reaching those habitats; this is particularly true if the patch matrix is separated by inhospitable environments (Turner et al. 2001). Gilliam and Fraser (2001) found an increase in physical structure in corridors facilitated movement in stream fish and increased the proportion of fish colonizing an adjacent river tributary. Sufficient patch connectivity requirements could be described both physically (distance between patches, amount of cover between patches) and biologically (predation, water quality, food availability). Since species perceive

fragmented habitats differentially (Pickett and Rogers 1997; Jacobus and Webb 2005), it can probably be assumed the required patch connectivity is not species specific. The required patch connectivity can not be considered a constant as the required degree of connectivity is a function of habitat abundance, the habitat patch matrix, and an organism's movement capabilities (Turner et al. 2001; Hein et al. 2004), and these movement capabilities can change according to habitat quality (Johnston 2000). Habitats can remain functionally connected in a fragmented landscape if a species is capable of crossing boundaries of unsuitable habitat (With et al. 1999). Albanese et al. (2004) suggest the long distances traveled by *Nocomis leptcephalus* in a Virginia stream were the result of this species moving through unsuitable habitat. Habitat fragmentation may not be a severe threat to BCS, BKF, and BNS in Square Lake as the movement transects I created for recaptured fish crossed through areas of no aquatic vegetation. These open areas were typically hard-bottom sandy areas near shore. I must point out that these movements are based upon my assumption of an individual moving along a specific depth contour. Conceivably, a fish could have traveled away from shore to a depth where vegetation was present and traveled along that depth until that individual traveled shallow again where it was recaptured. I did not investigate habitat connectedness between my observed moves, but such data would be useful for determining the importance of habitat corridors to these fish species. However, I am confident in my original assumption as relatively few fish were sampled in such locations (unpublished data).

Habitat disturbances can adversely affect fish populations even in an ideal situation where fragmented habitats are sufficiently connected. The obvious physical loss of habitat patches will reduce the overall carrying capacity of the system, and

consequently reduce the populations of fish in that system. Moreover, if habitats are lost, fish populations are forced to occupy the remaining habitats where fish density will be increased. An increase in fish density can reduce a once highly suitable habitat patch to one with reduced suitability (Fretwell and Lucas 1970). Theoretically then, the overall fitness of a fish species may be reduced as the species is unable to occupy habitats with the same return in fitness found pre-disturbance (Pulliam 1988). Habitat loss and fragmentation will also affect the prey items of BCS, BKF, and BNS. Chydorid cladocerans have been shown to be principal diet contributors for BCS (Becker 1983) and BNS (Roberts et al. 2006), and Williams (1982) found chydorid density drastically declined in mud areas where the aquatic macrophyte *Chara spp.* had receded in winter. Habitat loss and fragmentation may affect chydorid abundance in a similar fashion and result in an overall reduction of prey items for BCS and BNS.

Temporal movements were different for the three species I investigated. Distance traveled was significantly greater for BCS tagged in May than those tagged in June and July. The majority of long distance moves for recaptured BCS were from fish that were tagged in May, and consisted of individuals moving from the east end of Square Lake to the west end (Figure 2.5). Fish species commonly utilize the littoral zone for feeding and spawning and the areas of the littoral zone utilized can be dependent upon the reasons for use and/or time of year (Weaver et al. 1996). Therefore, the recaptured BCS in Square Lake may have been spawning in the east end of the lake when they were tagged and traveled to the west end of the lake after spawning. The overall BV and aquatic macrophyte richness of Square Lake increased from spring to summer (unpublished data), and, conceivably, habitats preferred by BCS may not have been available in May

lending BCS to habitat generalists until the preferred habitats were available. This would further explain the observed cross-lake movements of fish tagged in May. BNS exhibited a similar relationship of distance traveled for fish tagged in May. The interaction of days and season revealed that distance traveled significantly increased as the number of days between marking and recapture increased for BNS tagged in May. In other words, as the season progressed I recaptured those BNS tagged in May farther away from the location they were marked. This relationship was not true for BNS tagged in June as distance traveled and days was not linearly related. The interaction found in May conform to the results of those of BCS moving farthest in May, and suggest that BNS were also acting as habitat generalists in May and the observed longer moves were the result of BNS moving to optimal habitats when they became available (Matthews 1998).

Food availability may have also explained the longer distances that fish traveled in May. The chydoridae cladocera family of zooplankton are found in near shore vegetated habitats (Williams 1982) and their abundances are associated to the abundance and species composition of aquatic macrophytes (Quade 1969) and the extent of the littoral zone (Harmsworth and Whiteside 1968). Roberts et al. (2006) found chydorids to be the principal item in the diet of BNS followed by bosminidae, cladocera, and ostracoda. Becker (1983) summarized several studies of BCS diets and also found chydorids to be important food items for BCS. Roberts et al. (2006) found chydorids to be the largest portion of the BNS diet in summer and fall while ostracods were dominant in spring. Since chydorid populations peak in summer (Williams 1982) and the littoral zone is the broadest in the west end of Square Lake (Figure 2.2), it can be suggested that

BNS and BCS traveled long distances in May to the west end of Square Lake to feed on chydorids as they became abundant in summer.

BCS and BKF generally avoided habitats with little BV and selected habitats of higher BV in June and July of 2005 and 2006. Numerous studies previously have recognized a connection between BCS and BKF and aquatic vegetation. Carlson (1997) suspected reduced catches of BCS in the St. Lawrence River was likely due to the reduced occurrence of aquatic vegetation. Suitable and preferred habitats of BCS and BKF have been typically described as clear shallow waters with submerged aquatic vegetation (Scott and Crossman 1973; Houston 1990; Houston 1996). Interestingly, Becker (1983) found BCS to associate with aquatic vegetation in Wisconsin, but found BKF to have a strong preference for open sandy areas in the vicinity of sparse vegetation. Data on BNS in Square Lake suggested that this species selected greater BV in June 2005, but selected less BV in 2006. This change in habitat selection in June 2006 was intriguing as aquatic vegetation has been shown to be important to populations of BNS (Trautman 1957; Becker 1983; Roberts et al. 2006). In addition, distance traveled by BNS was different between years with the greatest distance traveled in 2006, which suggests that some other factor(s) may have caused BNS in Square Lake in 2006 to behave differently than BCS and BKF. One explanation may be that availability of the preferred food of BNS differed in 2006. If chydorids were not as abundant or distributed in 2006, BNS may have been traveling farther and using those habitats where chydorids were found whether vegetation existed or not. Although this is possible, it is unlikely as BCS have also been shown to rely on chydorids and no change in distance traveled or habitat selectivity was observed for BCS. BNS, like BCS and BKF, are protractive

spawners with spawning observed from May to July and are suspected to spawn over sandy locations (Becker 1983; Roberts et al. 2006). The pattern of selecting habitats of lesser BV in 2006 would then be expected if BNS were protractively spawning throughout the entire summer of 2006, but not in 2005. Becker (1983) observed a similar phenomenon of BNS habitat use in a central Wisconsin lake where no BNS were captured along a particular shoreline where the same sampling captured BNS “by the thousands” the previous year.

No clear trend in habitats selection was seen for either of the species investigated in May. Several factors such as water temperature, dissolved oxygen, aquatic macrophyte growth, and food availability all change during this time period (Wetzel 1983). As these environmental factors change, so should the behaviors of the fish species that interact with them. The lack of a pattern in habitat selection across the BV gradient in spring may be the result of BCS, BKF, and BNS selecting habitats based upon factors other than BV. The fact that these target species are also spawning during this time adds complexity to the relationship of the changing environment and habitat use in May.

Distance traveled of BCS, BKF, and BNS appeared not to depend upon habitat selection. Several explanations are possible for this result. Distance of the nearest habitat of each category was not accounted for. For example, perhaps the fish were moving to areas of higher habitat selectivity values but the nearest habitat with a higher selectivity value was close; hence large changes in selectivity values would be associated with short distances traveled. Fish may have also been captured in transit. If so, the recaptured fish can not be assumed to have selected the habitat they were caught in, but merely using that habitat as a corridor to a destination they will select. Lastly, the

motivations for movement of these species may not be driven by habitat selection. Animal movement can result from a variety of variables: food availability (Charnov 1976), territoriality (Fretwell and Lucas 1970), day length and water temperature (Albanese et al. 2004), and habitat complexity and presence of predators (Gilliam and Fraser 2001).

The movement distributions of BCS and BNS contained significant leptokurtosis. Leptokurtosis in movement is important for maintaining metapopulations and because kurtosis exists in BCS, BKF, and BNS they may be buffered against habitat loss and fragmentation. Leptokurtic distributions can result from the composite of two normal distributions (Zar 1999). Leptokurtic movement distributions in fish may result from population heterogeneity in movement, and this heterogeneity is related to heterogeneity in other traits (Skalski and Gilliam 2000). This heterogeneity can result from size, growth, gender, age, predators, genetics, individual behavior, and environmental factors (Fraser et al. 2001). I did not collect data about these traits. Fraser et al. (2001) found distance traveled of a stream killifish (*Rivulus hartii*) was correlated with boldness, a behavior trait. When released into a natural stream, those fish that exhibited bold characteristics in a preliminary laboratory experiment were found to move farther than fish that were not identified as bold. Even though I was able to remove heterogeneity in the movement distributions of some of the sub-groups by further dividing the overall movement distributions by year and season, heterogeneity remained in the other groups. This suggests that year and season did not explain the cause for the population heterogeneity in BCS and BNS. I lacked sufficient data to test these effects on the

heterogeneity of the movement distribution of BKF. I also lacked the data necessary to test the possible causes of heterogeneity described by Fraser et al. (2001).

My data suggests that these small bodied fishes in Square Lake, Minnesota may be able to persist in an environment of fragmented habitats. However, managers should remain cautious towards human activities that threaten littoral zone vegetation because it is difficult to predict potential thresholds of too much fragmentation and how it might affect these sensitive species. Managers must also be attentive to the other impacts of human disturbances affecting lake watersheds and the land-water interface. Human disturbances can increase lake sedimentation through increased erosion and sediment transport and BCS and BNS are known to be particularly sensitive to sedimentation and turbidity (Trautman 1957; Houston 1996; Roberts et al. 2006). Individual lake ecosystems are unique biotically and abiotically, so management of these three species with regards to habitat fragmentation should be viewed on an individual lake basis. There is no doubt that BCS, BKF, and BNS are sensitive to environmental disturbances and in light of the imminent increased human development of aquatic ecosystems, the risk of extirpation is high.

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Table 2.1. Occurrence of aquatic macrophyte species at sample points found by point intercept sampling with a two-sided rake in Square Lake, Washington County, Minnesota in 2005 and 2006.

Macrophyte Species	Occurrence
Coontail - <i>Ceratophyllum demersum</i>	24%
Muskgrass - <i>Chara Spp.</i>	87%
American waterweed - <i>Elodea Canadensis</i>	10%
Northern-water milfoil - <i>Myriophyllum sibiricum</i>	23%
Slender naiad - <i>Najas flexilis</i>	4%
Stonewort - <i>Nitella Spp.</i>	16%
Water lilly - <i>Nuphar Spp.</i>	5%
Largeleaf pondweed - <i>Potamogeton amplifolius</i>	2%
Flat-stalked pondweed - <i>Potamogeton friesii</i>	4%
Variableleaf pondweed - <i>Potamogeton gramineus</i>	4%
Illinois Pondweed - <i>Potamogeton illinoensis</i>	20%
Floating pondweed - <i>Potamogeton natans</i>	1%
White-stemmed pondweed - <i>Potamogeton praelongus</i>	3%
Small pondweed - <i>Potamogeton pusillus</i>	1%
Rhichardson's pondweed - <i>Potamogeton richardsonii</i>	22%
Narrowleaf pondweed - <i>Potamogeton strictifolius</i>	1%
Robbin's pondweed - <i>Potamogeton robbinsii</i>	3%
Flat-stem pondweed - <i>Potamogeton zosteriformis</i>	17%
Water buttercup - <i>Ranunculus longirostris</i>	6%
Bulrush - <i>Scirpus americana</i>	1%
Soft-stem bulrush - <i>Scirpus validus</i>	1%
Sago pondweed - <i>Stuckenia pectinata</i>	8%
Water celery - <i>Vallisneria americana</i>	9%
Water stargrass - <i>Zosterella dubia</i>	3%

Table 2.2. Survival and mark retention of fish marked with a visible implant elastomer in a laboratory experiment for 120 days. The fish used in the experiment represent surrogates of the fish species investigated in Square Lake, Washington County, Minnesota.

Genera	<i>N</i>	Survival (%)	Retention (%)
<i>Notropis spp.</i>	73	94.5	98.6
<i>Etheostoma spp.</i>	72	100	94.4
<i>Gambusia spp.</i>	75	98.7	100

Table 2.3. Total number of fish collected, marked, and recaptured, percent recaptured, number of locations where fish were marked and recaptured, and number of marking locations the recaptures in Square Lake, Washington County, Minnesota during 2005 and 2006. Fish species codes are: BCS = blackchin shiner (*Notropis heterodon*), BNS = blacknose shiner (*Notropis heterolepis*), BKF = banded killifish (*Fundulus diaphanous*), and IOD = Iowa Darter (*Etheostoma exile*).

	2005				2006			
	BCS	BNS	BKF	IOD	BCS	BNS	BKF	IOD
Collected	2,547	2,664	1,730	297	2,162	5,608	1,830	129
Marked	894	1,646	662	154	1,006	2,736	865	59
Recaptured	36	13	7	3	29	40	30	0
Number of Sites With Fish Recaptured	4.0	0.8	1.1	1.9	2.9	1.5	3.5	0
Number of Sites With fish Marked	56	53	53	26	51	69	64	20
Locations Recaptured	28	13	7	1	18	25	21	0
Number of Marking Sites From Which Recaptures Originated	22	9	6	1	14	12	17	0

Table 2.4. Measures of central tendency of distances traveled (m) for blackchin shiners *Notropis heterodon* (BCS), banded killifish *Fundulus diaphanous* (BKF), blacknose shiners *Notropis heterolepis* (BNS), and Iowa darters *Etheostoma exile* (IOD) recaptured in Square Lake, Washington County, Minnesota during 2005 and 2006.

	Year	Mean	Std. Dev.	Median	Minimum	Maximum	N
BCS	2005	276	435	34	2	1721	36
	2006	351	548	77	9	1815	29
	Combined	310	486	36	2	1815	65
BKF	2005	366	423	229	4	1137	7
	2006	608	763	127	7	1813	30
	Combined	562	712	177	4	1813	37
BNS	2005	270	478	44	5	1703	13
	2006	405	535	135	18	1845	40
	Combined	372	520	106	5	1845	53
IOD	2005*	23	0	23	23	23	3

*Iowa darters were only recaptured in 2005.

Table 2.5. Parameters from the fitted quadratic equation to test for the constant spatial spread of blackchin shiners *Notropis heterodon* (BCS), blacknose shiners *Notropis heterolepis* (BNS), and banded killifish *Fundulus diaphanous* (BKF) through time in Square Lake, Washington County, Minnesota in 2005 and 2006. The equation was fitted where: Variance of distance moved = $c_0 + c_1(\text{Time}) + c_2(\text{Time})^2$. A negative t value indicates parabolic relationship.

	Intercept (c_0)		Slope (c_1)		Quadratic Term (c_2)	
	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
BCS	2.62	0.0278*	-2.02	0.0737*	3.00	0.0149*
BKF	1.85	0.1242	-2.44	0.0587*	3.93	0.0111*
BNS	0.02	0.9828	0.33	0.7497*	-0.21	0.8440

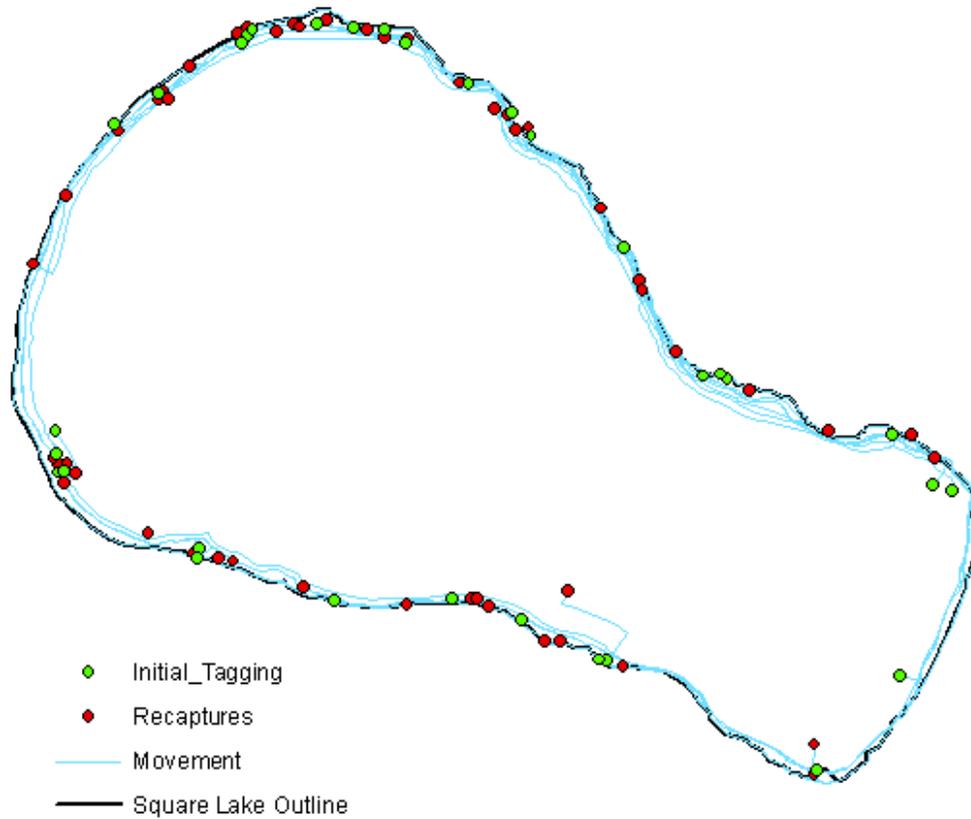
* Violation of assumption for constant spread through time.

Table 2.6. Estimates of normality (W) and kurtosis of the distances traveled (m) for blackchin shiners *Notropis heterodon* (BCS), banded killifish *Fundulus diaphanous* (BKF), and blacknose shiners *Notropis heterolepis* (BNS) recaptured in Square Lake, Washington County, Minnesota during 2005 and 2006.

SPP	Year	Season	n	W	Kurtosis
BCS	Combined		65	0.6713*	1.66*
BKF	Combined		37	0.7304*	-0.73
BNS	Combined		53	0.7041*	1.66*
BCS	2005	June	14	0.7781*	0.10
		July	18	0.4642*	5.26*
	2006	May	12	0.8705	1.40
		June	13	0.5131*	7.56*
BNS	2005	June	9	0.6971*	2.69*
	2006	June	16	0.9342	-0.80
		June	24	0.6007*	4.06*

*Estimates indicate significant kurtosis ($P < 0.05$).

A.



B.

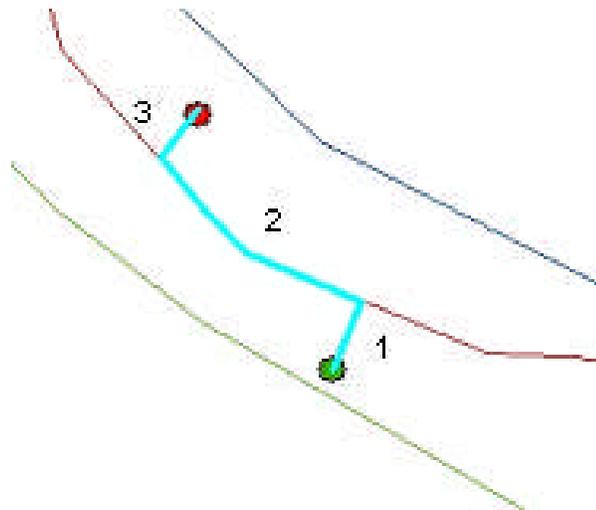


Figure 2.1. (A) Map of movement corridors used to calculate distance traveled for recaptured fish from Square Lake, Washington County, Minnesota in 2006. (B) Diagram depicting the steps used in ArcMap to create a movement path (light blue line) from 1) a marking location (green dot) to 3) a recapture location (red dot) 2) along a depth contour (grey, purple, and blue lines).

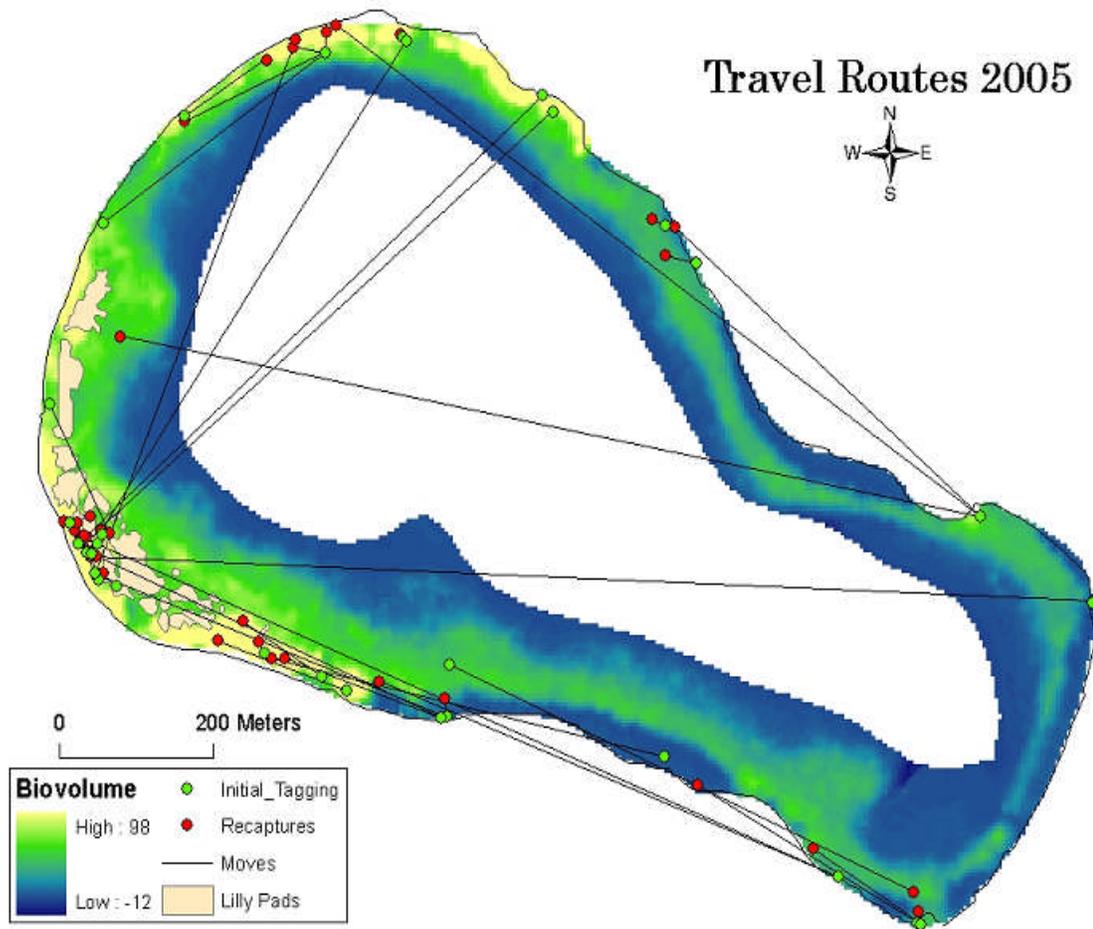


Figure 2.2. Map of mark and recapture locations overlaid upon the biovolume (%) of the littoral zone for fish recaptured from Square Lake, Washington County, Minnesota in 2005. Biovolume was interpolated using Kriging from data recorded hydroacoustically and represents the percent of the water column occupied by aquatic macrophytes. Moves” are for illustration only and connect mark and recapture locations.

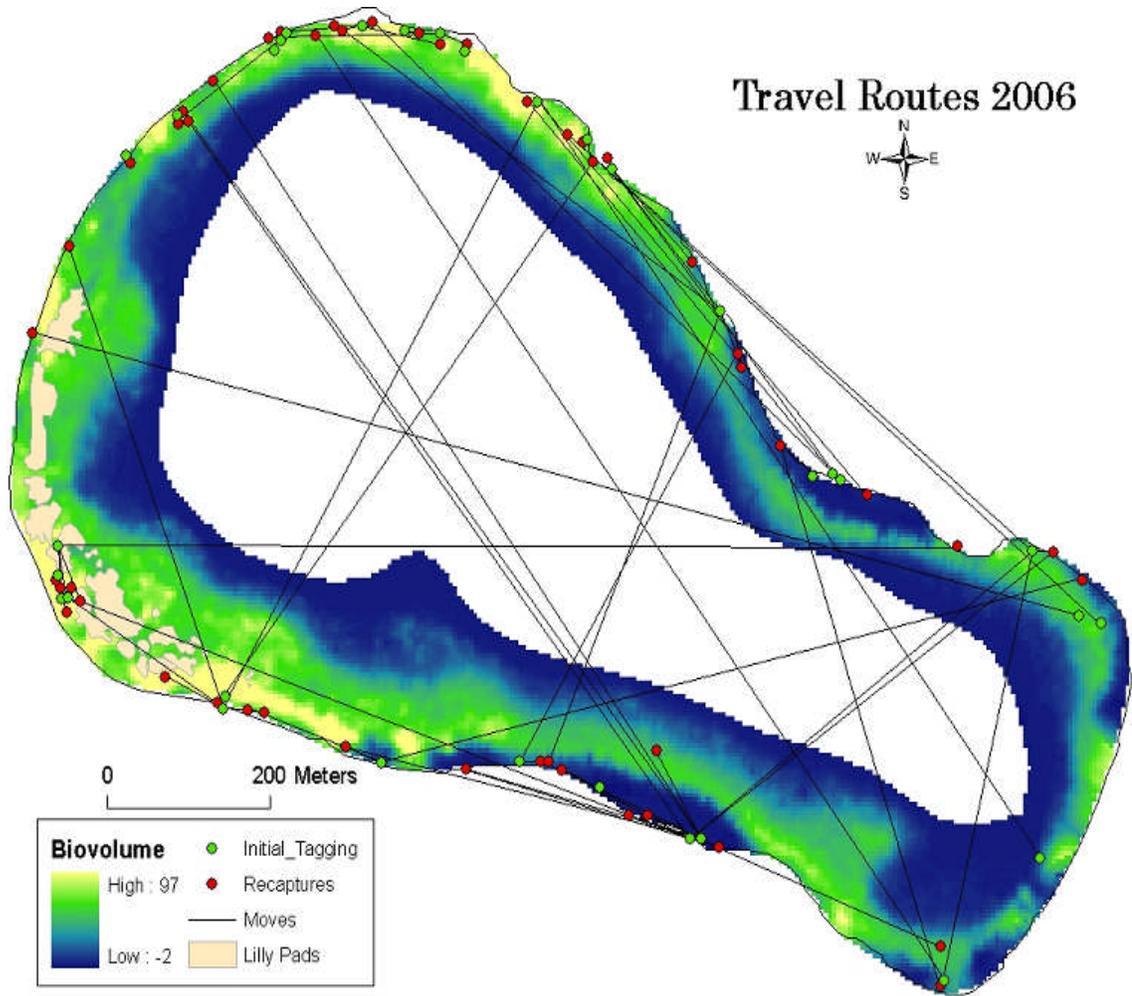


Figure 2.3. Map of mark and recapture locations overlaid upon the biovolume (%) of the littoral zone for fish recaptured from Square Lake, Washington County, Minnesota in 2006. Biovolume was interpolated using Kriging from data recorded hydroacoustically and represents the percent of the water column occupied by aquatic macrophytes. Moves” are for illustration only and connect mark and recapture locations.

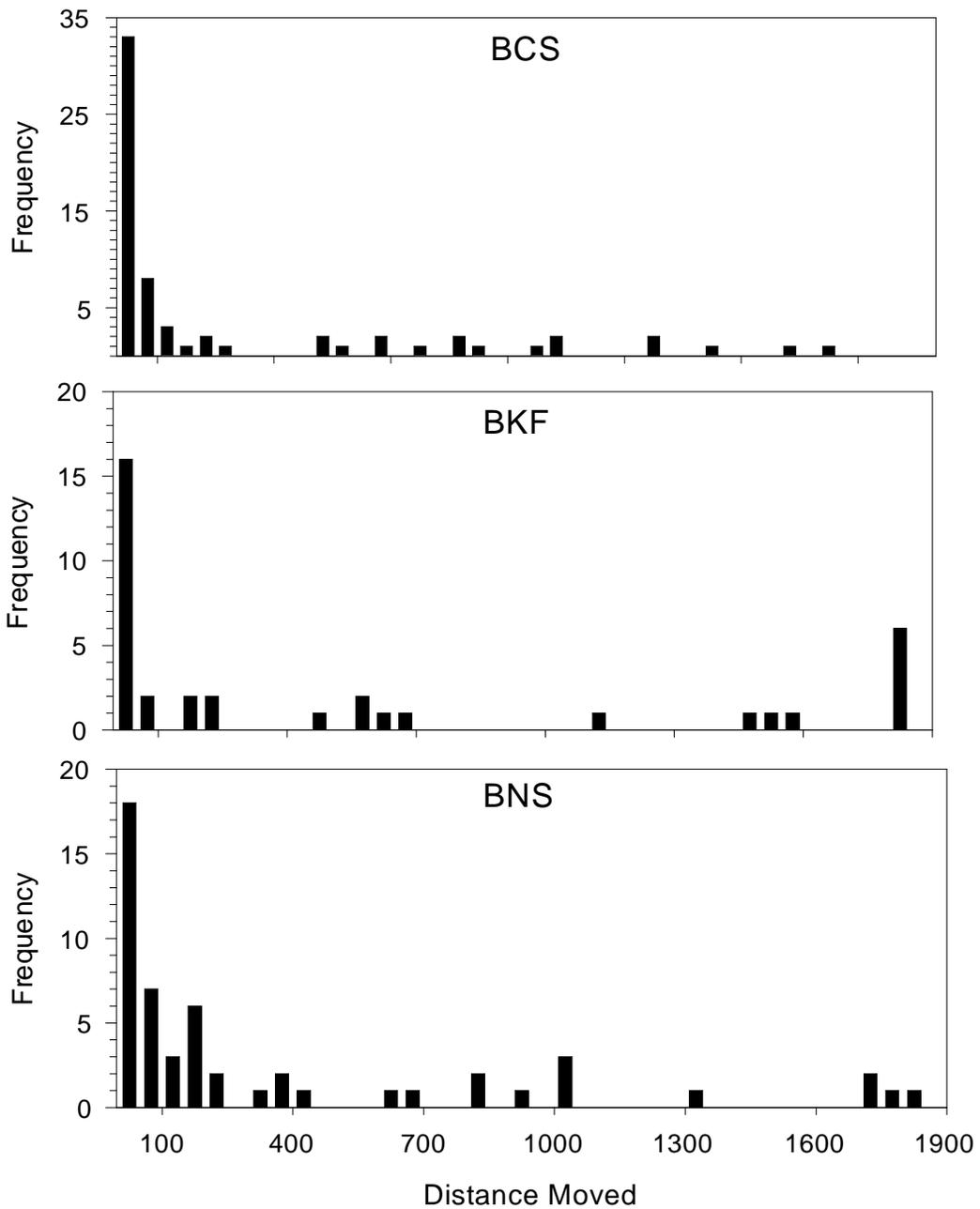


Figure 2.4. Distributions of distance traveled for blackchin shiners *Notropis heterodon* (BCS), banded killifish *Fundulus diaphanous* (BKF), and blacknose shiners *Notropis heterolepis* (BNS) in Square Lake, Washington County, Minnesota during 2005 and 2006. Distances traveled represent the midpoint of each 50m increment.

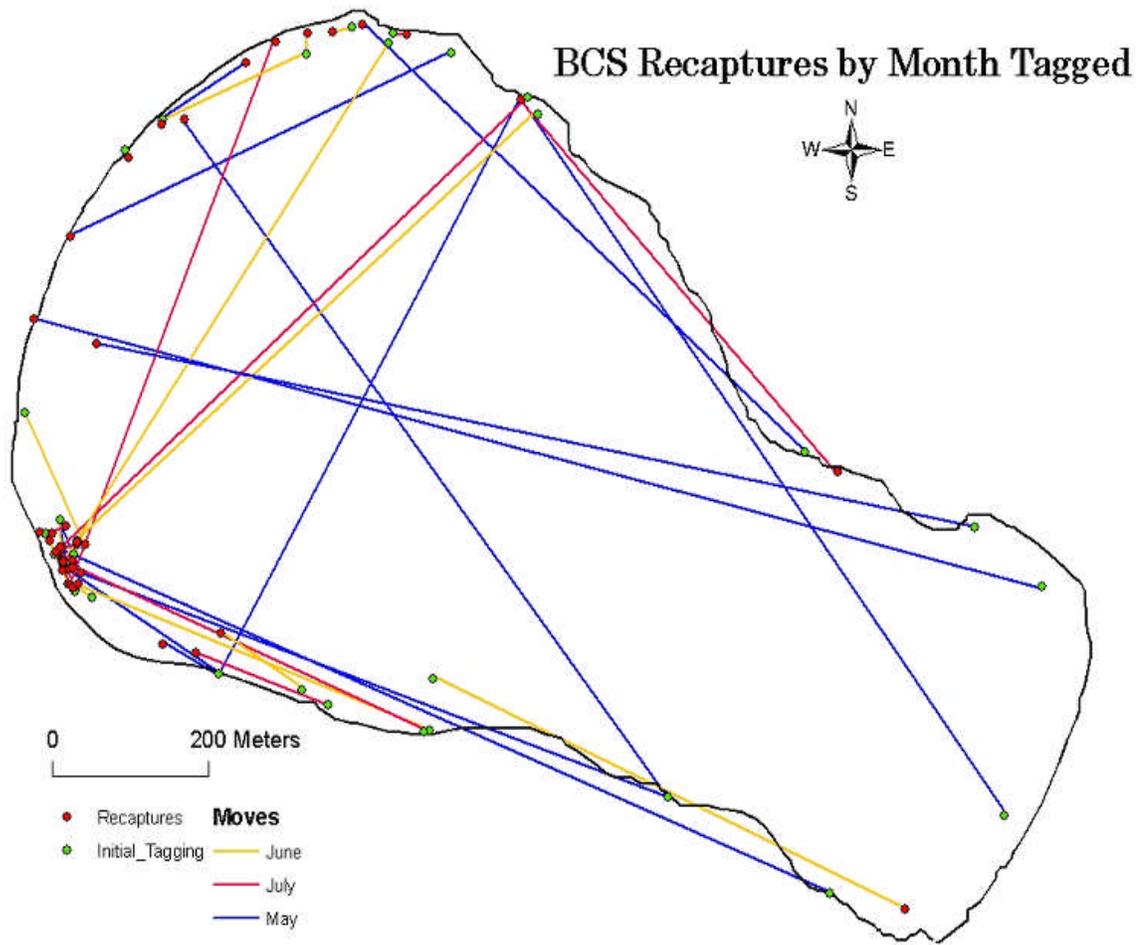


Figure 2.5. Map of recaptured blackchin shiners *Notropis heterodon* from Square Lake, Washington County, Minnesota in 2005 and 2006 by month. “Moves” represent the month in which a fish was tagged and are for illustration only.

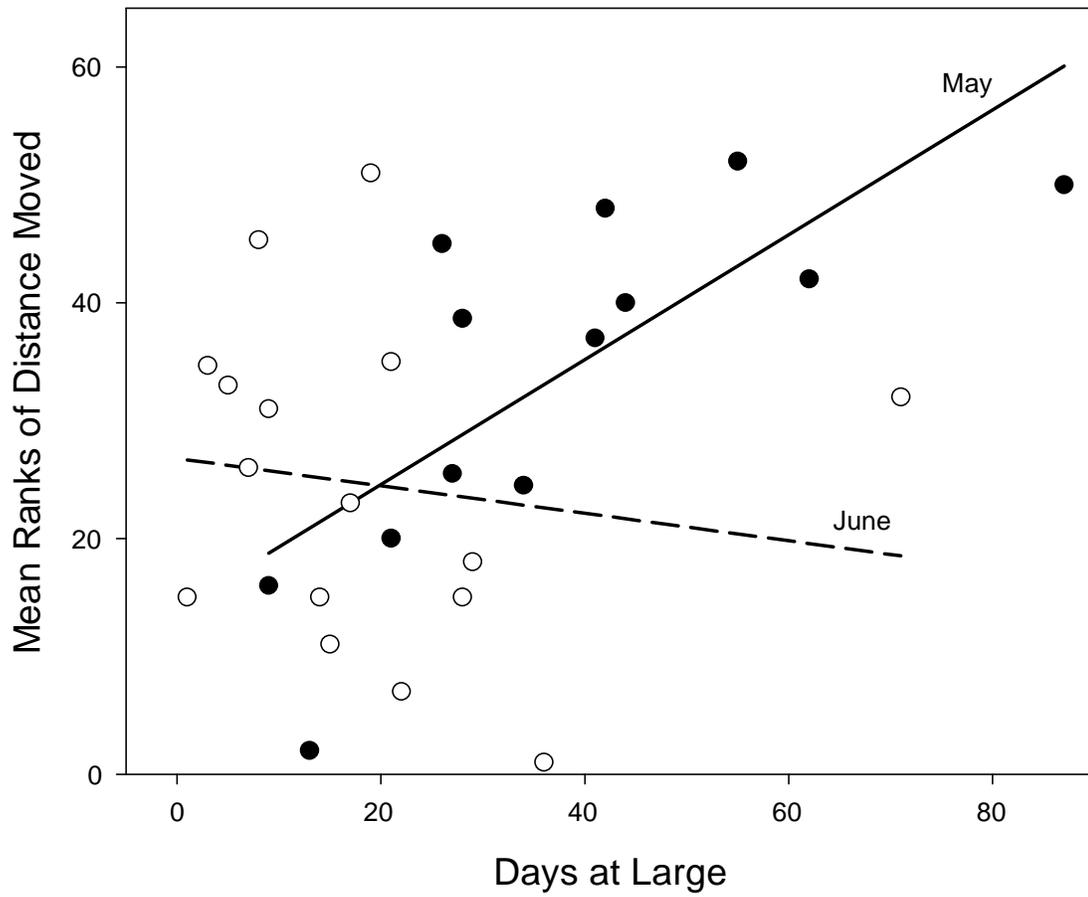


Figure 2.6. Distance traveled and days-at-large for blacknose shiners *Notropis heterolepis* tagged in May and June in Square Lake, Washington County, Minnesota in 2005 and 2006.

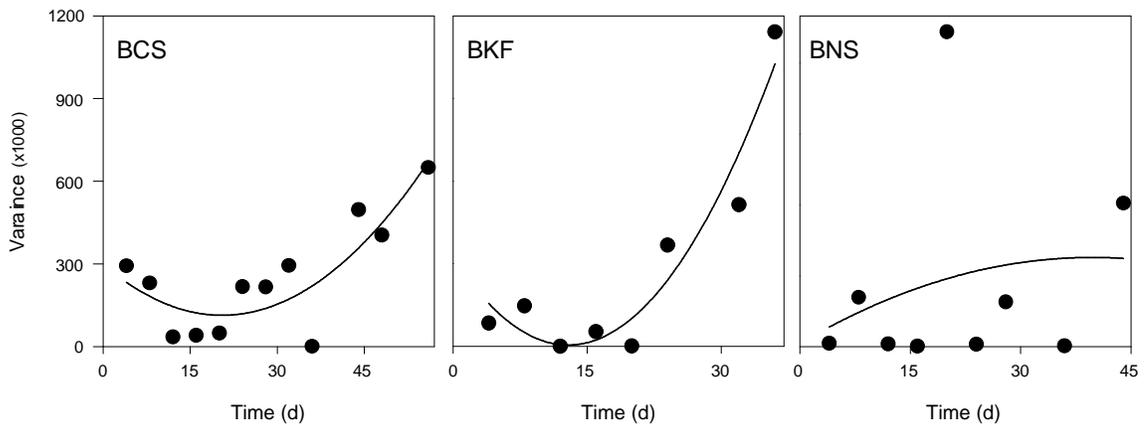


Figure 2.7. Variances of distances moved (m) as they relate to time and the corresponding fitted quadratic model for blackchin shiners (BCS), banded killifish (BKF), and blacknose shiners (BNS) recaptured in Square Lake, Washington County, Minnesota during 2005 and 2006.

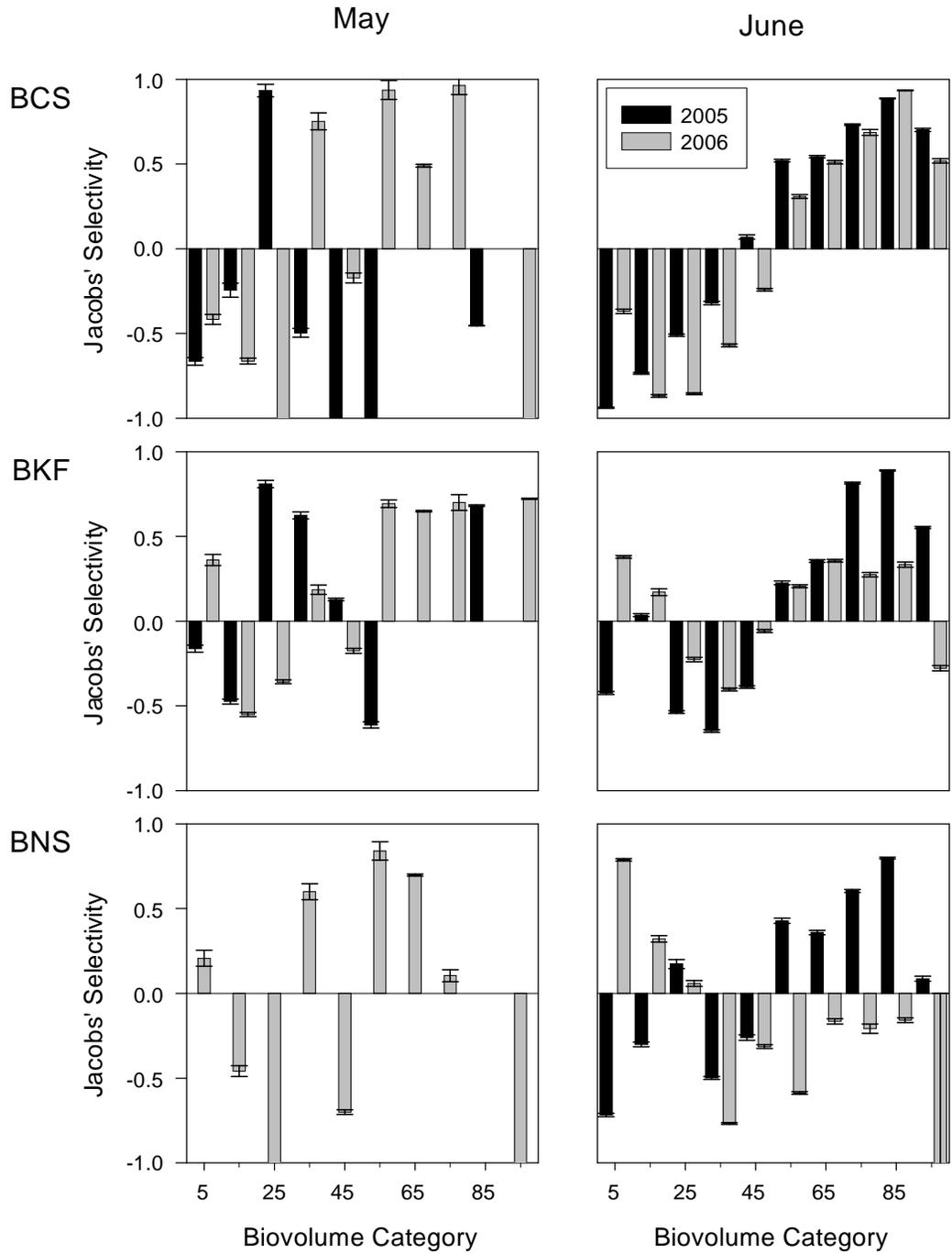


Figure 2.8. Jacobs selectivity values and associated 95% confidence limits for 10% biovolume habitat categories in 2005 and 2006 for blackchin shiners *Notropis heterodon* (BCS), banded killifish *Fundulus diaphanous* (BKF), and blacknose shiners *Notropis heterolepis* (BNS) in Square Lake, Washington County, Minnesota. Selectivity values may not exist in all habitat categories if a species was not sampled in that category. Selectivity values were not calculated for BNS in May 2005.

CHAPTER III
DETERMINENTS FOR HABITAT USE BY THREE SELECTED NON-GAME
FISH SPECIES: MACROPHYTE COMMUNITY AND
PREY AVAILABILITY

Introduction

Fish community composition within aquatic systems is largely determined by the available habitat within that system (Hayes et al. 1999), and aquatic macrophytes are an important component of fish habitat (Dibble et al. 1996). Numerous studies have pointed out the importance of aquatic macrophytes in structuring fish communities within freshwater lakes (Rahel 1984; Poe et al. 1986; Benson and Magnuson 1992; Cross and McNerny 2001; Grenouillet et al. 2002). Particularly, macrophyte abundance and richness have been correlated with fish abundance (Dewey and Jennings 1992; Weaver et al. 1997). Aquatic macrophyte diversity has also been linked to increased fish species richness (Tonn and Magnuson 1982). However, little is known about patterns of fish distribution among different macrophyte types (Chick and McIvor 1997).

Recent increases in lakeshore development have raised concern for fishery managers through the loss of aquatic macrophytes (Beauchamp et al. 1994; Engel and Pederson Jr. 1998; Radomski and Goeman 2001). Knowledge of the relationships between fish communities and aquatic macrophytes aids biologists in managing aquatic

systems, particularly when vegetation removal is used as a management tool (Slade et al. 2005). While fish-macrophyte interactions have been well investigated in many different aquatic systems, these studies have investigated the importance of macrophyte abundance as a whole, ignoring the importance of individual macrophyte species.

Most north temperate fishes use littoral zone vegetated habitats at some point in their lives (Pratt and Smokorowski 2003). Aquatic macrophytes provide numerous benefits to fish communities including: protection from predation (Savino and Stein 1982), spawning habitats (Becker 1983), and foraging sites (Crowder and Cooper 1982). Fish select aquatic habitat patches through a trade-off of physical complexity and foraging returns (Werner et al. 1983; Giannico and Healey 1999). Planktivorous fish prefer to forage in vegetated habitats as the inherent patchiness of macrophyte beds provides cover from predation in proximity to the open spaces utilized by their prey (Weaver et al. 1997).

An understanding of habitat use by threatened fish species is important so critical habitat can be identified and protected (Hess 1996; Hatzenbeler et al. 2000). The Minnesota Department of Natural Resources has identified declines in the numbers of three fish species that are believed to be sensitive to habitat destruction by sedimentation and vegetation removal: blackchin shiner *Notropis heterodon* (BCS), blacknose shiner *Notropis heterolepis* (BNS), and banded killifish *Fundulus diaphanous* (BKF). The objective of this study was to examine how these species relate to available aquatic habitat by assessing: 1) small scale habitat association of fish (abundance and presence) with specific macrophyte communities through underwater observation, and 2) whether

local fish abundance was related to prey density using zooplankton tows at seine sample sites.

Methods

Sampling Design

Habitat Use

Fish presence and abundance within individual macrophyte species was investigated through underwater observations using snorkel gear and a modification of the straight line transect method and instantaneous counts (Thresher and Gunn 1986). Underwater observations were made starting July 24 2006 through July 28 2006, with 2 to 4 transects surveyed each day for a total of 14 transects. The location of each transect was stratified in water depths of ≤ 2.0 m within the west basin of Square Lake (see Chapter II of this document for site description). The observations were stratified to this area because the higher abundance of fish within that area (unpublished data) allowed examination of the selection of specific macrophyte species by fish. Transect starting points and directions of travel were randomly assigned, but limited to areas that I had not previously sampled. All underwater observations began approximately 10 m from the boat and I waited two minutes for the area to settle before recording data. Preliminary observations indicated fish in the immediate area returned to normal activity after this time. Underwater observations consisted of an instantaneous count of all fish seen in a 1 m² area. Thresher and Gunn (1986) found instantaneous counts to be an effective method

for determining fish density via underwater observation. The 1 m² sample area was used to measure the abundance of BCS, BNS, and BKF in the available plant types present. The presence/absence (p/a) of aquatic macrophytes within this area was recorded at the underwater observation site. Data were recorded on a Dive Writes (Lamartek, Inc., Lake City, Florida) underwater notebook. Subsequent observations were recorded along the transect at intervals of 7-10 m; a distance corresponding to the maximum lateral underwater visibility. I continued to the next sample area if fish were observed “spooking” from an area I planned to count. The length of transects was not recorded, but was estimated at an average length of 150 m. The number of instantaneous counts per transect ranged from 15 to 35. These methods allowed me to attain a large number of counts of small scale habitat associations with little effort as compared to other methods (e.g. Popnets, throw traps, minnow traps).

Prey Density

BCS, BNS, and BKF feed primarily on zooplankton (Scott and Crossman 1973), and chydorid cladocerans have been found to be the preferred prey of BCS (Becker 1983) and BNS (Roberts et al. 2006). Fish were sampled using 5 m long, 3.175 mm nylon mesh bag seines pulled parallel to shore for 5 m in water depths < 1 m. Zooplankton were sampled using a standard style plankton tow net with an opening diameter of 20 cm and 80 µm mesh. The plankton net was tossed in the center of the area sampled with the seine and retrieved perpendicular to shore for 5 m so that the area sampled with the net intercepted the site previously seined. Fish were sampled with the seine first to avoid spooking them from the area with the plankton net. The potential disturbance caused by

the seine to the plankton within the sample area was recognized, but I assumed the potential for disturbing fish within the sampling area was greater and restricted me to this sequence. Assumably, the mixing of the water column caused by the seine could have distributed the zooplankton evenly within the sample area resulting in a more accurate representation of the zooplankton in the sample. In vegetated areas the net was retrieved in the water column immediately above the top of the plants, and the net was retrieved mid-depth where plants were absent. Fish and zooplankton sample points were chosen at random locations within the west basin of Square Lake and at random times between 8:00 a.m. and 3:00 p.m. Nineteen samples were taken in two consecutive days in May and 22 samples were taken in two consecutive days in June and July each, for a total of 63 samples. Plankton samples were preserved in a 10% buffered sugar-formalin solution and dyed with a Rose Bengal solution.

Zooplankton samples were rinsed in a 64 μm sieve prior to processing. All samples were diluted to 50 ml and sub-sampled using a disposable pipette until the entire sample had been processed or at least 100 organisms were counted upon the completion of a sub-sample. Zooplankton density was then calculated as number of organisms per liter. Zooplankters were identified into the following groups: *Daphnia spp.*, Ostracoda, Copepoda, chydorid cladocerans, and Cladocera. The cladoceran family Chydoridae was classified as a separate group from the cladocerans as they have been shown to be a principal diet contributor to BCS and BNS (Becker 1983; Roberts et al. 2006).

Data Analysis

Habitat Use

Abundances of BKF, BCS, and BNS were used in data analyses. Due to difficulty in differentiating BCS and BNS at all underwater observations sites, I grouped these species together and further refer to them as BS. Although data on individual species would be preferred for the scope of this thesis, the BS are known to share similar characteristics in habitat and foraging (Eddy and Underhill 1974; Becker 1983). BKF and BS were analyzed separately. Due to a large number of zero counts for fish during my observations and a desire for a large sample size, all instantaneous counts were assumed to be independent and all observations were merged into one data set. Independence of samples was ensured by using sites that were separated by the distance of maximum underwater visibility, and as stated earlier, a sample site was not used if fish were seen moving to or from the intended observation site. All significance tests were deemed significant at $\alpha = 0.05$.

Spearman rank correlations were used to determine if BS and BKF abundance was correlated with macrophyte species richness at 1) all observation sites and 2) at sites only where fish were present (Conover 1999; O'Rourke et al. 2005). Logistic regression was used to determine if a relationship existed between fish p/a and macrophyte species richness. Fish abundances were transformed into binary data so that if at least one individual of a species was present in a sample area that species was coded "1"; contrarily, that species was coded "0" if no individuals were counted. Fish p/a was used as the dependent variable and macrophyte species richness was used as the predictor

variable. I assumed a linear relationship between the independent variables and the log odds (logit) of the dependent variables. A Hosmer and Lemeshow Goodness-of-Fit Test was performed on each regression to ensure that the fitted model was adequate (SAS 2000).

Nonmetric multidimensional scaling (NMS) was used to determine if any correlations existed between fish abundance and macrophyte species composition. NMS is a nonparametric ordination method best suited for ecological data (McCune and Grace 2002). Ordination techniques such as NMS, can reveal interpretable patterns that appear obscure when examining only the raw data from a study (Hair et al. 1995) by condensing the raw information into a smaller set of dimensions with minimal loss of information (McGarigal et al. 2000). NMS uses an iterative approach to locate the best positions of n entities on k dimensions (McCune and Grace 2002). In other words, NMS reduces a complex matrix of species abundances across many sample units to an n -dimensional space which creates a simplified graphical view of how the species abundances relate to one another, i.e. the species community. NMS assigns a unique score to each sample unit (observation) for each dimension (axis). The resulting site scores are then used to place each sample unit along the according axis. The computer software PCORD for Windows (McCune and Medford 1999) also provides Pearson correlations of each species for each axis enabling the user to identify which species contribute the most information to each axis. The procedure described by McCune and Grace (2002) using the Sorenson (Bray-Curtis) distance measure was used to run NMS. The “step down in dimensionality” option and an examination of the scree plots were used to determine which axes to include for final data analysis. Each final solution inherently has a unique stress value for

that dimensionality, and the scree plot plots this reduction in stress with each addition of an axis. The number of axes is then chosen at the point when the plot begins to level off (elbow appearance) and a minimal reduction in stress is gained each additional axis. This selection process is analogous to that of model selection for multiple regression using R^2 . Stress is a measure of the amount of the variance of the discrepancies not accounted for in the model (Hair et al. 1995), and most ecological data sets contain final stress values between 10 and 20 (McCune and Grace 2002). Stress tends to increase with sample size and Clarke (1993) cautions against over-reliance of stress values.

PCORD was used to perform NMS upon the aquatic macrophyte p/a obtained during snorkeling. The site scores obtained through NMS for each axis were then correlated with fish abundance at each site using Spearman correlations. Separate tests were run for each species and axes. This procedure reduced the macrophyte community of Square Lake into smaller unique dimensions to examine if any correlations existed between fish abundance and the macrophyte community axes.

The site scores obtained through NMS for each axis were also used in logistic regression to determine if a relationship existed with the p/a of fish and the reduced macrophyte communities. The NMS site scores represented the predictor variable and fish p/a the dependent variable. Again, I assumed a linear relationship between the independent variables and the log odds (logit) of the dependent variables, and performed a Hosmer and Lemeshow Goodness-of-Fit Test to ensure model fit. Separate tests were run for each fish species and axis.

Prey Density

Spearman correlations were used to determine if BCS, BNS, and BKF abundance was related to prey density at the sample sites in Square Lake. Separate correlations were run for each fish species and each plankton group in May, June, and July. Correlations were deemed significant at $\alpha = 0.05$.

Results

Habitat Use

In all, 380 instantaneous counts were recorded from 14 transects in Square Lake. A total of 21 macrophyte species, 292 BS, and 13 BKF were observed in the 1m² count areas. Fish abundance was negatively correlated with macrophyte richness for all observations; however, the correlations were weak and insignificant (BS: $r = -0.067$, $p = 0.2015$; BKF: $r = -0.028$, $p = 0.5688$). Macrophyte species richness was also not significantly correlated to BS ($r = 0.185$, $p = 0.0975$) and BKF ($r = 0.518$, $p = 0.0845$) abundance at only those observation sites where fish were observed (reduced data set). Logistic regression also failed to detect any significant effects of macrophyte richness upon p/a of BS ($\chi^2 = 2.4664$, $p = 0.1163$) and BKF ($\chi^2 = 0.1806$, $p = 0.6708$).

A three dimensional solution (3 axes) was chosen for the final NMS analysis (Figure 3.1). Figure 3.2 illustrates the final NMS run reached a stable solution where stress in the model levels near the 20th iteration (McCune and Grace 2002). NMS axis 2 accounted for the greatest amount of variation (40.6%) in the ordination followed by axes 3 (25.9%) and 1 (18.4%). Spearman correlations for BS abundance and NMS axes 1 and

3 were insignificant while the correlation for BS abundance and axis 2 was ($r = 0.1175$, $p = 0.0219$; Table 3.1). Examining the macrophyte species correlations to the NMS axes (Table 3.2) it can then be seen observed BS abundance was higher in macrophyte communities composed of *Potamogeton Zosteriformis*, and *Myriophyllum sibiricum*, and lowest in communities composed of *Chara Spp.* and *P. amplifolius*. No significant correlations for BKF abundance and the NMS axes were found (Table 3.1). Logistic regression revealed BS presence was affected by NMS axis 1 ($\chi^2 = 4.0462$, $p = 0.0443$), but not by NMS axes 2 or 3 (Table 3.3). Therefore, BS presence was associated with macrophyte communities composed of *P. amplifolius* and *Stuckenia pectinata* and BS absence was associated with macrophyte communities composed of *P. illinoensis* and *Vallisneria Americana*.

Prey Density

No clear trends were found for fish abundance and zooplankton density across the sample period, and very few of the correlation tests were significant (Table 3.4). Interestingly, all but one of the six significant correlations were negative; the exception being BKF and *Daphnia spp.* in July ($r = 0.469$; $P = 0.028$). In other words, fish abundance at the sample sites was lower in areas of increased plankton density. Chydorid cladocerans were significantly correlated with BCS and BNS abundance in May only, ($r = -0.553$; $P = 0.014$, $r = -0.463$; $P = 0.046$, respectively) and as stated earlier this relationship was negative.

Discussion

Previous studies on the habitat associations of BCS, BNS, and BKF are informative but vague; descriptions include clean, clear, vegetated waters with clean bottoms and an associated reduction in population with increased turbidity (Trautman 1957; Scott and Crossman 1973; Becker 1983; Houston 1996; Roberts et al. 2006). Roberts et al. (2006) described a diverse vegetation community of pondweeds *Potamogeton spp.*, naiads *Najas spp.*, water milfoils *Myriophyllum spp.*, American elodea (*Elodea canadensis*), coontail (*Ceratophyllum demersum*), and sago pondweed (*Stuckenia pectinatus*) to be vital to the persistence of BNS in Illinois lakes, but did not investigate the importance of specific species of macrophytes on BNS. Many of the macrophyte species observed by Roberts et al. (2006) were also present and observed underwater in Square Lake. However, similar to previous studies, I found few significant associations between BS and BKF and specific macrophyte species or communities.

Results from previous investigations of fish abundance and macrophyte richness are variable. Brazner and Beals (1997) and Weaver et al. (1997) found a correlation between fish abundance and macrophyte richness, but not all of the species they investigated followed this trend. Slade et al. (2005) found no relationships between fish abundance and macrophyte richness at the same sampling scale as this study (1 m²) in urban Minnesota lakes. BCS, BNS, and BKF were also present in these urban Minnesota lakes, but no relationships of these fish species with the existing macrophyte community were found (personal communication).

Information regarding BS and BKF associations with particular macrophyte species is limited. However, Poe et al. (1986) found significant associations of BKF with

Najas flexis and *P. richardsonii* in littoral habitats of Lake St, Clair. Similarly, I noted these plants were important in Square Lake as axis 3 of the NMS ordination I performed was correlated with these two plant species, however the axis was weakly related ($r = 0.023$) to observed BKF abundance. NMS axis 2 was nearly significantly related to BKF abundance ($P = 0.0502$) and *N. flexis* and *P. richardsonii* were correlated with the axis, but only *N. flexis* was associated with increasing BKF abundance. These macrophyte and BKF relationships may have been significant in Square Lake, but the sample size may have prevented a detection of this relationship.

BS abundance was negatively correlated with axis 2, which was highly and inversely correlated to *P. amplifolius*, and BS presence was significantly related to axis 1, which was highly correlated to *P. amplifolius*. In other words, BS presence was correlated to macrophyte assemblages containing *P. amplifolius*, but higher abundances of BS were correlated to assemblages not containing *P. amplifolius*. This statement however, can be taken only as a generalization as the remaining macrophyte species that make up NMS axes 1 and 2 are dissimilar, and ordination axes should not be totally interpreted by one highly correlated species (McCune and Grace 2002). The negative relationship of BS abundance to *Chara* (NMS axis 2) was particularly interesting because while investigating the movements of BS their abundance appeared to be greater in areas of Square Lake composed of expansive, dense beds of *Chara*. These observations were made at a much larger scale (lake-wide) than that investigated in this study, so it is likely the relationship between BS abundance and *Chara* is scale dependent. *Chara* was present in 85% of the observation samples (Table 3.5), so it is also likely that the dominance of *Chara* throughout the sample sites affected my results whereby *Chara* was

present equally at the observations sites where BS were counted and at observation sites where BS were absent. Therefore, *Chara* would have been equally correlated to higher fish abundance and lower fish abundance essentially, i.e. no correlation was statistically detectable. Pratt and Smokorowski (2003) suggest the difficulty they observed in differentiating vegetated habitat types with multivariate analyses was due to dominance of a few, highly abundant macrophyte species. This may explain the seemingly arbitrary results of my NMS ordinations as my sample sites were dominated by a few macrophyte species (*Chara*, *P. richardsoni*, *P. amplifolius*).

The relationship between fish and aquatic macrophytes is often complex, and simple correlations of fish and macrophytes may be inconclusive (Weaver et al. 1996). Abiotic factors such as pH (Rahel 1984), winter dissolved oxygen (Tonn and Magnuson 1982), and depth gradient (Benson and Magnuson 1992) have been shown to be important factors in structuring fish assemblages in north temperate lakes. However, water chemistry parameters within Square Lake were similar between sample points (unpublished data) and water depth was held constant throughout the underwater observations. Romare et al. (2003) found that large scale lake-wide patterns in fish distribution affected their interpretations of small scale data. In a controlled experiment, Chick and McIvor (1997) found habitat selection of small fish in three different vegetation types was random in the absence of an introduced predator. Therefore, BKF and BS may have been choosing habitats at random in the absence of predators. Although I did not specifically address this theory, I did not observe predators within the immediate area of my observation sites. Habitat patches adjacent to those used in the observations may have also influenced habitat use (Pulliam 1988). Therefore, the lack of

significant fish-macrophyte associations may be the result of: 1) fish associated with macrophytes at a scale different than that used in this study, 2) fish used macrophyte habitats at random, 3) other factors not examined in this study contributed to fish habitat selection, or 4) some combination of 1-3.

Available prey density did not explain habitat use by BCS, BNS, and BKF, and where relationships existed, they were negative. This is converse to previous studies that suggest cladocerans are a preferred prey of these fish species. Romare et al. (2003) found a negative correlation between the abundance of fish and abundance of cladocerans, and suggested predation by fish and zooplankton behavior as reasons for shaping the distribution patterns of cladocerans in vegetated edge habitats. However, copepod behavior or food distribution tends to have a greater effect on copepod distributions, than predation by fishes (Romare et al. 2003). Hülsmann et al. (1999) and Burks et al. (2001) have found that high densities of planktivorous fish can affect the biomass of zooplankton. Thus it is possible that direct predation mediated the negative relationship between fish and prey abundances I observed in Square Lake. Although highly speculative, it is possible that fish were using habitats with higher prey abundances, but when we sampled the fish had already reduced prey densities. The only significant positive correlation found in this study was for BKF and *Daphnia* in July. Hülsmann et al. (1999) found fish predation upon *Daphnia* greatly increased after the middle of June, so BKF may have started to select habitats in July as the *Daphnia* population in Square Lake increased.

Several other explanations exist for the few significant correlations between fish and prey abundances. Plankton densities vary temporally and spatially (Hall et al. 1979),

which can cause problems when relating them to fish foraging models (Werner et al. 1983). Zooplankton have been shown to aggregate in patches (Whiteside 1974; Williams 1982), so it is possible that fish were relating to habitat in response to zooplankton aggregations. This may particularly be the case if fish were using vegetated habitats as protection from predation and moving to feed on zooplankton just outside the vegetation edge (Weaver et al. 1997). However, I could not validate this in my study since I did not investigate zooplankton aggregations. I collected zooplankton and fish samples at random locations and from different habitat types (vegetated and non-vegetated) and zooplankton abundances and behavior differ between these habitats (Williams 1982; Romare et al. 2003). Therefore it is possible that fish sampled in these different habitats were using these habitats for different reasons. For example, fish may have been using vegetated habitats for feeding and using un-vegetated habitats for spawning (Scott and Crossman 1973). A reduction in the variability of habitats sampled may help further investigations elucidate fish-prey relationships.

Results from this study indicate habitat use of BCS, BNS, and BKF was not correlated with macrophyte species richness or prey abundance, but habitat use by these fish species was correlated with several individual macrophyte species. There is no doubt vegetated habitats are important to BCS, BNS, and BKF, and preservation of these fish species should focus on protecting macrophyte habitats against degradation and fragmentation and maintaining clear waters (Trautman 1957; Scott and Crossman 1973; Roberts et al. 2006). The conservation of these species will be a continual process as anthropogenic alterations to aquatic systems increase. Future investigations into these fish species' habitat use should include additional habitat variables not used in this study

and at different spatial scales. Experimental manipulations of macrophyte communities and prey availability will offer further insight into how these fish species' habitat use responds to changes in the environment.

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Table 3.1. Spearman correlations (r) and associated P-values for the first three NMS axis site scores of aquatic macrophyte presence/absence and abundance of blackchin shiners *Notropis heterodon* and blacknose shiners *Notropis heterolepis* (Shiner Group) and banded killifish *Fundulus diaphanous* (BKF) from underwater observations in Square Lake, Washington County, Minnesota in July 2006.

	Axis 1		Axis 2		Axis 3	
	r	P	r	P	r	P
Shiner Group	0.093	0.0707	0.118	0.0219	0.074	0.1505
Banded killifish	-0.011	0.8338	-0.101	0.0502	0.0230	0.6545

Table 3.2. Pearson correlations for the macrophyte species and three axes (dimensions) calculated by nonmetric multidimensional scaling (NMS) from macrophyte presence/absence data recorded during underwater observation in July 2006 from Square Lake, Washington County, Minnesota.

Macrophyte Species	Axis 1	Axis 2	Axis 3
<i>Ceratophyllum demersum</i>	0.167	0.339	-0.108
<i>Chara Spp.</i>	0.060	-0.618	0.671
<i>Elodea canadensis</i>	0.047	0.215	-0.024
<i>Myriophyllum sibiricum</i>	-0.017	0.525	-0.133
<i>Najas flexilis</i>	-0.279	-0.219	-0.309
<i>Nuphar Spp.</i>	0.030	0.133	0.008
<i>Potamogeton amplifolius</i>	0.394	-0.517	-0.109
<i>P. gramineus</i>	0.009	-0.179	-0.106
<i>P. illinoensis</i>	-0.620	-0.040	0.113
<i>P. praelongus</i>	0.048	0.142	-0.029
<i>P. pusillus</i>	-0.159	-0.074	-0.113
<i>P. richardsonii</i>	0.316	0.368	-0.557
<i>P. robinsii</i>	0.020	0.158	-0.149
<i>P. zosteriformis</i>	0.001	0.542	-0.496
<i>Ranunculus longirostris</i>	-0.034	0.155	0.147
<i>Scirpus pectinatus</i>	0.373	0.059	0.074
<i>Vallisneria americana</i>	-0.511	-0.212	-0.463
<i>Zosterella dubia</i>	-0.062	0.024	0.013

Table 3.3. Wald chi-square and associated p-values from logistic regression of the first three NMS axis site scores of aquatic macrophyte presence/absence (independent variable) and presence/absence of blackchin shiners *Notropis heterodon* and blacknose shiners *Notropis heterolepis* (Shiner Group) and banded killifish *Fundulus diaphanous* (BKF; dependent variable) from underwater observations in Square Lake, Washington County, Minnesota in July 2006.

	Axis 1		Axis 2		Axis 3	
	Wald χ^2	<i>P</i>	Wald χ^2	<i>P</i>	Wald χ^2	<i>P</i>
Shiner Group	0.0904	0.7637	3.2768	0.0703	0.6244	0.4294
Banded killifish	4.0462	0.0443	3.6339	0.0566	0.5578	0.4551

Table 3.4. Spearman rank correlations for abundance of blackchin shiners *Notropis heterodon*, blacknose shiners *Notropis heterolepis* and banded killifish *Fundulus diaphanous* and zooplankton group density from sampling sites in May, June, and July 2006 from Square Lake, Washington County, Minnesota. Bolded values indicate a significant relationship at $\alpha = 0.05$. N indicates number of samples during each sampling period.

Month	N	Plankton Group	Fish		
			Blackchin shiner	Blacknose shiner	Banded killifish
May	19	Chydoridae	-0.553	-0.463	-0.435
		Daphnia	0.209	0.062	-0.012
		Other Cladocera	0.284	0.041	0.019
		Copepoda	-0.490	-0.300	-0.354
		Ostracoda	-0.064	-0.144	-0.103
		Groups Combined	-0.362	-0.204	-0.416
June	22	Chydoridae	0.268	-0.165	-0.014
		Daphnia	-0.008	-0.441	-0.211
		Other Cladocera	-0.090	-0.047	0.311
		Copepoda	-0.220	-0.460	-0.237
		Ostracoda	0.127	-0.150	-0.369
		Groups Combined	0.220	-0.372	-0.130
July	22	Chydoridae	0.071	0.181	0.248
		Daphnia	0.338	-0.153	0.469
		Other Cladocera	0.134	-0.072	0.099
		Copepoda	0.003	-0.237	-0.145
		Ostracoda	-0.204	-0.135	0.246
		Groups Combined	0.003	-0.064	0.190

Table 3.5. Macrophyte species occurrences within the entire littoral zone (lake-wide), and macrophyte species occurrences from 380 underwater observations in Square Lake, Washington County, Minnesota in 2006.

Macrophyte Species	Lake-Wide	Observation Sites
<i>Chara Spp.</i>	87%	85%
<i>Ceratophyllum demersum</i>	24%	4%
<i>Myriophyllum sibiricum</i>	23%	18%
<i>Potamogeton richardsonii</i>	22%	25%
<i>P. illinoensis</i>	20%	18%
<i>P. zosteriformis</i>	17%	15%
<i>Nitella Spp.</i>	16%	-
<i>Elodea canadensis</i>	10%	2%
<i>Vallisneria americana</i>	9%	18%
<i>Scirpus pectinatus</i>	8%	13%
<i>Ranunculus longirostris</i>	6%	2%
<i>Nuphar Spp.</i>	5%	1%
<i>P. gramineus</i>	4%	4%
<i>P. friesii</i>	4%	-
<i>Najas flexilis</i>	4%	8%
Filamentous algae	3%	1%
<i>P. praelongus</i>	3%	2%
<i>Zosterella dubia</i>	3%	2%
<i>P. robinsii</i>	3%	1%
<i>P. amplifolius</i>	2%	23%
<i>P. pusillus</i>	1%	5%
<i>P. natans</i>	1%	1%
<i>P. strictifolius</i>	1%	-
<i>S. validus</i>	1%	-
<i>S. americanus</i>	1%	-

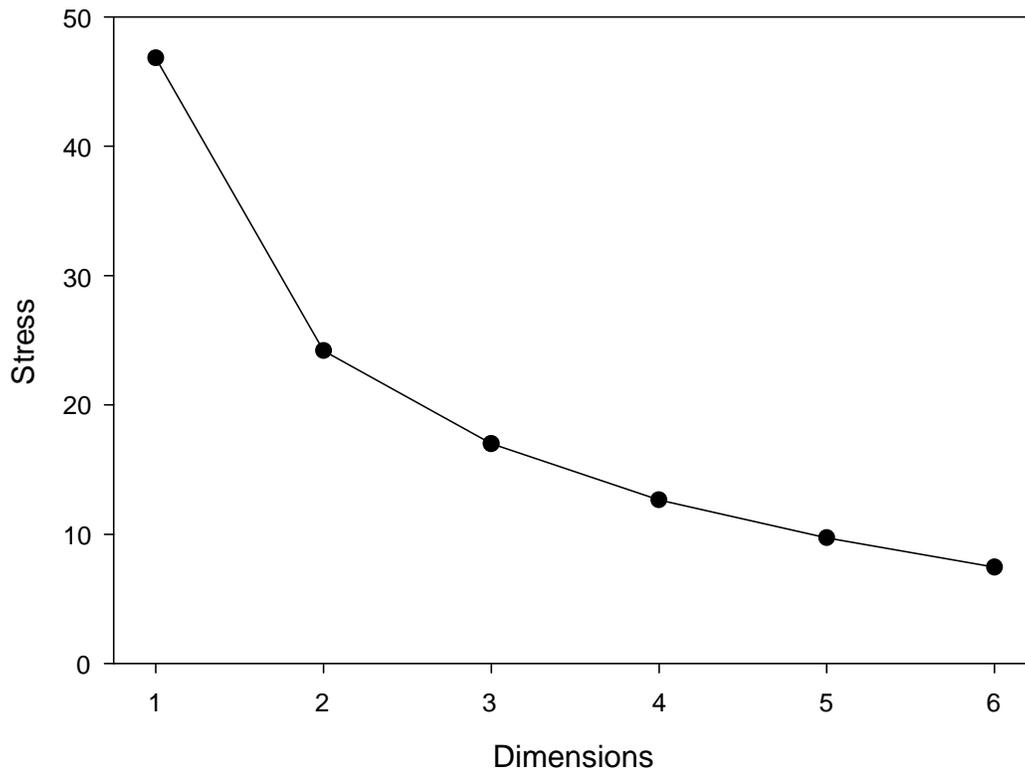


Figure 3.1. Scree plot for the initial 6-dimensional nonmetric multidimensional scaling (NMS) run for aquatic macrophyte presence/absence data from underwater observations in Square Lake, Washington County, Minnesota in July 2006. Note the minimal reduction in stress beyond 3 dimensions.

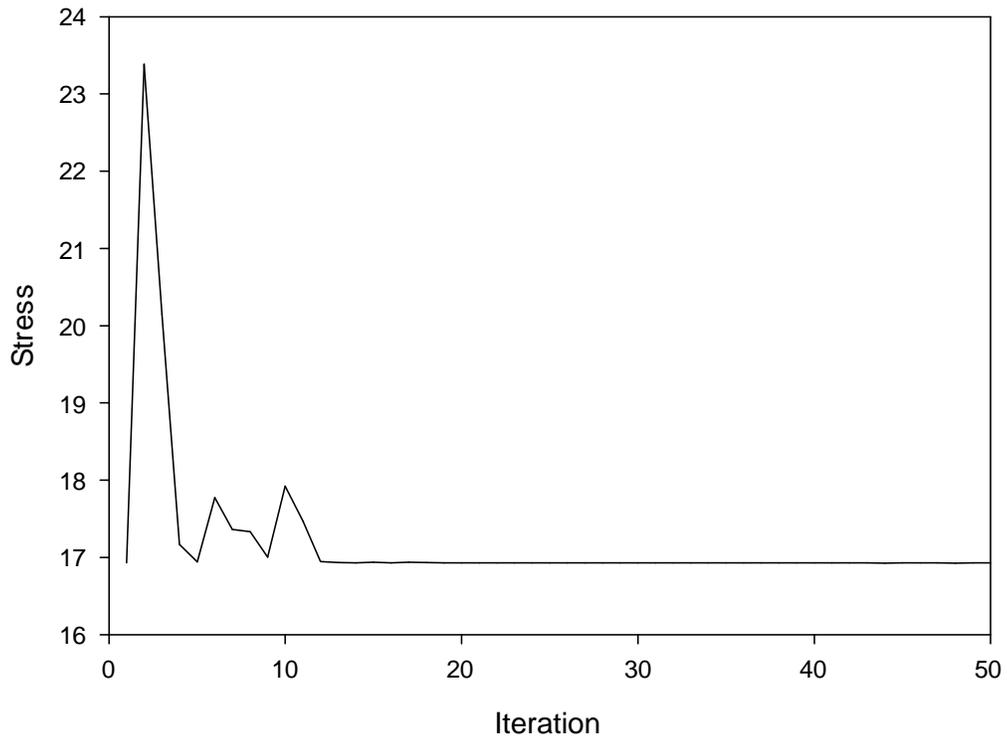


Figure 3.2. Stress vs. iteration number for the final 3-dimensional nonmetric multidimensional scaling (NMS) run for aquatic macrophyte presence/absence from underwater observations in Square Lake, Washington County, Minnesota in July 2006. Note the final solution reached stability near the 20th iteration.

CHAPTER IV

CONCLUSIONS

Anthropogenic alterations to aquatic ecosystems have recently come to the forefront in fisheries management, where they will remain well into the future as the global human population continues to grow. Shoreline development has negatively impacted fish habitats through loss of aquatic macrophytes (Beauchamp et al. 1994; Engel and Pederson Jr. 1998; Radomski and Goeman 2001) and reduced water quality (Jennings et al. 1999). Fish species sensitive to such perturbations will continue to be threatened as human use of and reliance upon aquatic systems grow. To effectively manage and protect habitat-sensitive non-game fishes, adequate information regarding life history, behavior, and habitat needs and use is necessary.

I observed seasonal differences in the movement of blackchin shiners *Notropis heterodon* (BCS) and blacknose shiners *Notropis heterolepis* (BNS) in Square Lake. These species moved farther on average during spring (May) than summer (June and July), and used different habitats during these seasons. The plants in the vegetated habitat within Square Lake did not reach their highest abundance and diversity until mid-summer. However, I found no significant effect of habitat selection on distances traveled for these species so it is conceivable these fish species may have been acting as habitat generalists with respect to habitat use and did not demonstrate habitat preferences until

vegetated habitats became available. Even though I observed selectivity for habitats with greater BV (> 60%), it is highly likely that BCS and BNS did not move to the vegetated habitats solely based on available biovolume (BV) within those habitats as other biotic and abiotic factors may be as influencing for habitat selection by fish. Abiotic factors such as pH (Rahel 1984), dissolved oxygen (Tonn and Magnuson 1982), and depth gradient (Benson and Magnuson 1992) have been shown to be important factors in structuring fish assemblages, and Hall and Werner (1977) observed bluegills dispersed throughout a Michigan lake as water temperatures warmed through seasonal progression. Biotic factors such as presence of a predator (Chick and McIvor 1997), food availability (Crowder and Cooper 1982), and conspecific interactions (Fretwell and Lucas 1970) can also influence habitat selection and movements.

My investigation into the fine scale habitat use of BCS, BNS, and banded killifish *Fundulus diaphanous* (BKF) revealed abundance of these fish species was not related to macrophyte richness. Although not significant, the association of BKF with *Najas flexis* in Square Lake corroborates with the findings of Poe et al. (1986). Although no other studies have examined BCS and BNS relationships with individual macrophyte species, I found the presence of BCS and BNS was related to macrophyte assemblages containing *Potamogeton amplifolius*. Although I did not directly investigate the mechanisms responsible for movements by these fish species as they relate to *N. flexis* and *P. amplifolius*, additional field study and/or experimentation may provide new insight into use of these habitats.

The greater fish movement observed in spring may also have been related to the availability of preferred prey. Chydorid cladocerans have been shown to be the preferred prey of these fish species (Becker 1983; Roberts et al. 2006), and abundance of this zooplankton peaks in summer (Williams 1982). Chydorid abundance has been related to the abundance and species composition of aquatic macrophytes (Quade 1969) and to the extent of the littoral zone (Harmsworth and Whiteside 1968). Since the vegetated littoral zone of Square Lake peaked in size in mid-summer, and although I did not specifically measure for changes in prey abundance, it is conceivable that the abundance of the preferred prey of BCS, BNS, and BKF followed accordingly. Conceivably, the greater fish movement observed in spring was due to the fish moving to areas of increasing prey density as the vegetated habitats developed as most of the movements observed in May were directed towards the west end of Square Lake where the littoral zone is more diverse and expansive. However, results from my investigation of prey availability and fish abundances revealed BCS, BNS, and BKF abundances were not higher in areas of increased prey density; particularly, BCS and BNS were negatively correlated with chydorid cladocerans abundance in spring. Two possible explanations for this finding are: 1) the fish were not selecting habitat areas based upon chydorid abundance because chydorids were not abundant during this time (Werner et al. 1983), or 2) the fish were selecting habitats based upon chydorid abundance but predated upon the chydorids sufficiently enough to reduce the present abundance of chydorids, thereby masking any positive relationship (Williams 1982; Romare et al. 2003).

The primary objective of this study was to describe the movements and habitat use of BCS, BNS, and BKF in a lake not yet drastically affected by anthropogenic

alterations. This study was designed to gain knowledge about the populations of these sensitive fish species and how they might respond to habitat degradation and fragmentation. Conclusions from Chapter II give insight into the movement capabilities of BCS, BNS, and BKF. At the time of this study, these fish species existed as one continuous population through unrestricted dispersal as they were capable of reaching all habitats within Square Lake. However, the potential impact upon this dispersal from any alteration to the aquatic habitat (i.e. shoreline development) I investigated is unknown. Fragmentation of the existing habitat corridors in Square Lake may prevent these fish species from continuing as one continuous population (Johnston 2000).

My observations suggest these fish species move and use habitat throughout the range of BV available in Square Lake, and that even minor removal of some isolated habitat may adversely affect these populations as a whole. Since I did not make any comparisons to other lakes with higher disturbances to littoral habitat, I suggest future experimental designs incorporate different levels of lake development and shoreline fragmentation to address these impacts. Results from Chapter III indicate several macrophyte species found in Square Lake were important in determining abundances of BCS and BNS at a small scale. Despite the overall lack of significant relationships for BCS, BNS, and BKF and aquatic macrophytes, my results along with others, indicate that these fish species require vegetated habitats. This study represents the first attempt at quantifying the movement of BCS, BNS, and BKF in lacustrine habitats, and the marking methods employed during this study can easily be applied to other fish species and aquatic systems. The disturbances to lacustrine systems are cumulative and hierarchical, and developments within the watershed of lakes can affect aquatic communities arguably

as equally as direct impacts such as aquatic vegetation removal. Proactive management for the conservation of these sensitive fish species in Square Lake should focus on protecting vegetated habitats and preserving water quality.

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