EVALUATION OF HATCHERY-REARED LARGEMOUTH BASS USING RADIO TELEMETRY

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
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To my patient and loving wife whose support was critical in the writing of my thesis

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The use of supplemental stocking of largemouth bass is a common management practice to augment poor natural recruitment. We have a very poor understanding about how changes in behavior from living in an artificial culture environment at the hatchery affects stocked fish's behavior after being released into a natural lake environment. Miniaturization of radio transmitters have recently allowed researchers to study stocked juvenile fish and make inferences about the entire stocked population based on tagged individuals. The short-term effects of surgically inserting dummy microradio transmitters (1.2 to 2.7% of the fishes' body weight) in hatchery-reared largemouth bass was investigated by evaluating growth and predator avoidance differences with untagged control fish. Some mortality of tagged fish and slight impairment of growth was observed for experiments run to 21 and 30 d. At the conclusion of each growth experiment, almost all fish shed their suture, incisions were completely healed, and no transmitters were expelled. During predation trials, tagged fish were eaten at the same rate as controls, suggesting that vulnerability to predation was not affected by the presence of radio tags. Although some mortality and growth

impairment can be expected, this study suggests that surgical implantation of radio transmitters can be a valid tagging technique to examine the behavior of juvenile largemouth bass in short-term field studies. A laboratory study was conducted to determine how radio transmitters (0.3 g) implanted in juvenile largemouth bass that were eaten by adult largemouth bass were evacuated relative to predator size, prey size and water temperature. All transmitters ingested by the predators were evacuated within 84 h. The tags passed through the intestine independently of the size of predators or prey, whereas water temperature was directly related to the timing of evacuation. A field study using radio telemetry investigated the post stocking survival and behavior of hatchery-reared advanced fingerling (90-120 mmTL) largemouth bass in Lake Carlton, Florida. We also tested the hypothesis that behavior and survival of domesticated hatchery stocked fish differs from wild fish. In 2009, we inserted radio transmitters in 50 hatchery stocked fish and in 2010, 30 hatchery fish were tagged along with 20 wild-reared fish. When compared directly to tagged wild fish (n = 20), tagged hatchery stocked fish (n = 30) exhibited lower survival, higher movement, higher proportion of offshore use, and slower growth. All mortality observed for hatchery fish resulted from fish and bird predation and the majority occurred in the first seven days. Results of this study suggest that domestication affects from the hatchery rearing process resulted in reduced fitness of hatchery largemouth bass immediately following their release into the lake. I recommend acclimating advanced fingerling largemouth bass to predators and live prey prior to release to reduce initial morality after they are stocked.

CHAPTER 1
INTRODUCTION

Largemouth bass *Micropterus salmoides* are one of the most popular and most intensively managed sport fish in North America. Use of supplemental stocking of largemouth bass is a common management practice to augment poor natural recruitment (Boxrucker 1986; Buynak and Mitchell 1999; Mesing et al. 2008). To provide a benefit to the recipient lake, stocked fish must survive and contribute to the natural population. When stocking evaluations have been conducted for largemouth bass, contribution to the natural population has commonly been low (Boxrucker 1986; Crawford and Wicker 1987; Buynak and Mitchell 1999; Buckmeier and Betsill 2002; Hoxmeier and Wahl 2002; Porak et al. 2002; Hoffman and Bettoli 2005). Several studies have investigated factors influencing stocking success and a variety of factors such as predation, starvation and health issues have contributed to low survival (Hoxmeier and Wahl 2002; Porak et al. 2002; Diana and Wahl 2009). Hoxmeier and Wahl (2002) found that predation rates from adult largemouth bass were an important source of mortality on stocked advanced-fingerling bass. Mesing et al. (2008) also found that advanced fingerling bass reared on natural prey had contributions up to 40% the following fall in Lake Talquin, Florida. Stocking advanced-size fingerlings has been shown to improve stocking success (Buynak and Mitchell 1999; Colvin et al. 2008; Mesing et al. 2008; Diana and Wahl 2009) as larger fish are less vulnerable to predation (Loska 1982).

Hatchery-reared fish exhibit deficits in survival behaviors, such as predator avoidance, foraging, and migration, because of the unnatural conditions in which they are reared (Brown and Laland 2001). Domestication effects of pellet-reared hatchery

largemouth bass raised at the Florida Bass Conservation Center (FBCC) have included altered feeding kinematics and strike modes; difficulty transitioning from artificial diets to live prey; excessive vulnerability to angling; health and fitness issues; and poor post-release survival (Porak et al. 2002, Patel-Wintzer 2004, Pouder et al. 2010). Improvements in the culture of advanced fingerlings prior to release include transition to live prey, improved formulated diet (Cardeilhac et al. 2008), and early spawned fish to expedite the timing of release (2010 stocking). These improvements could potentially improve survival and anticipation of a more successful stocking program and has warranted additional research.

Limited studies have focused on advanced fingerling survival immediately after release (Pouder et al. 2010) and we have a very poor understanding about how changes in behavior from living in an artificial culture environment at the hatchery affects their behavior after being released into a natural lake environment. Technological advances in radio telemetry equipment have made it possible to study the behavior of very small animals due to development and production of very small transmitters. For the first time ever, we had the opportunity to study the initial dispersal, movement, habitat use, and survival of hatchery largemouth bass after their release into a lake using radio telemetry techniques. We were also able to compare their behavior and survival to that of wild fish. This information will help scientists, fishery managers, and hatchery personnel understand some of the mechanisms that affect post-stocking survival of hatchery fish, which may lead to improvements in fish culture, handling, and release protocol.

Studies conducted within this thesis are divided into three chapters (Chapter 2-Chapter 4). In Chapter 2, we determined the effects of surgically implanting transmitters on growth, survival, and predator avoidance of small bass to validate whether or not tagged animals adequately represent untagged animals in field telemetry studies. In Chapter 3, we developed an evacuation model which can be used to predict the probability of a tag remaining in a predator's gut over time; after the predator had eaten the radio-tagged juvenile bass. This information was useful during field telemetry studies for determining whether a fish location was a small radio-tagged bass or a predator that had eaten the radio-tagged. In Chapter 4, our study objectives were to compare the behavior and survival of hatchery-reared largemouth bass to similar size wild juvenile bass after being stocked into a lake.

CHAPTER 2
TAG EFFECTS ON HATCHERY LARGEMOUTH BASS

Intro

A major concern in any biotelemetry study is the assumption that results obtained using tagged animals accurately represents untagged animals, which allows inferences to the entire population. The presence of the tag or the surgical process could potentially affect the movement, behavior, or the chance of predation, which would bias results. Before accepting this assumption, the effects of transmitters on a fish's behavior should be evaluated.

With the miniaturization of radio transmitters, extensive research has been conducted on juveniles to test the effects of transmitter presence on fish. Prior to implantation of tags, consideration of the target species' physiological constraints should be assessed. A widely accepted "2% rule" recommends that fish should not be equipped with transmitters that weigh more than 2% of the fish's weight in air (Winter 1996). Some recent studies, however, have successfully tagged fish outside this range, up to 10% in some species, without adverse affects (Brown et al. 1999; Jepsen et al. 2002).

Swimming performance, growth rates, and predator avoidance are commonly used to evaluate the effects of radio and acoustic transmitters on fish behavior and biology. Many studies have shown no significant differences in swimming performance of fish implanted with transmitters and controls (Cote et al. 1999; Robertson 2003; Anglea et al. 2004). Transmitter effect on juvenile fish growth rates has yielded variable results. After 30 days, Frost et al. (2010) found that growth rates in Chinook salmon *Oncorhynchus tshawytscha* were significantly less in surgery fish than in controls when

transmitters were used that weighed 2.6-5.9% of the fish's body weight. Conversely, growth was not significantly different between controls and tagged rainbow trout *Oncorhynchus mykiss* (Lucas 1989), subyearling Chinook salmon (Martinelli et al. 1998), and juvenile Atlantic cod *Gadus morhua* (Cote et al. 1999). Anglea et al. (2004) found that that juvenile Chinook salmon tagged with transmitters did not result in greater predation susceptibility than untagged fish in a laboratory experiment.

Despite the importance of studying the early life history of hatchery and wild largemouth bass *Micropterus salmoides*, the majority of the research associated with tag effects on juvenile fish has concentrated on salmonids. Differences in physiological characteristics in largemouth bass and the environment in which they inhabit could give significantly different results than that found for other species. Largemouth bass occupy much warmer systems, and elevated water temperature has been shown to have increased incision healing time which poses a greater risk of infection when fish were tagged in relatively warm water (Knight and Lasee 1996; Walsh et al. 2006). Cooke et al. (2003) studied the effects of suture material on incision healing of juvenile largemouth bass (140 ± 0.8 mm total length [TL]), and found that successful implantation of micro-transmitters can be achieved.

I investigated tagging advanced-fingerling largemouth bass produced at the Florida Bass Conservation Center (FBCC), which are defined as fish reared to a mean of 100 mm (TL) before stocking. These fish represent some of the smallest largemouth bass to be implanted with radio transmitters. My objective was to determine the effects of surgically implanting radio transmitters on the growth and predator avoidance of advanced fingerling largemouth bass.

Methods

Fish acquisition and surgical procedures. We conducted two independent studies starting July 24, 2009 and March 28, 2010 to evaluate tagged bass at the FBCC during both study years. In order to avoid excessive tag-to-body mass ratio for surgery fish, largemouth bass greater than 90 mm were chosen at random from hatchery raceways for each experiment. In 2009 and 2010, test fish were held in tanks where a constant flow of water was supplied at 22-25˚C. Fish were fed a maintenance diet prior to each trial by feeding to satiation three times per week. Feed was withheld 48 hours prior to and 24 hours after surgeries.

Fish tagged in this study were similar in size to fish that would typically be stocked as advanced fingerlings. Dummy tags used were exact replicates (but no working parts) of the A2414 transmitter (Advanced Telemetry Systems, Isanti, Minnesota). The tag weight was 0.24 g in air, measured 5 x 12 mm and represented between 1.2% and 2.7% (mean = 1.7%) of the body weight of the fish. The trailing antenna wire length was 10 cm and consisted of a flexible braided metal.

Surgical procedures were similar to Adams et al. (1998b), although incision and antenna placement were modified for largemouth bass (Cooke et al. 2003). Prior to surgeries, each fish was anesthetized in a 70 mg L^{-1} tricaine methanesulfonate (MS 222) bath, measured (mm TL), and weighed (0.1 gram). The fish was then transferred ventral side up to a surgical foam pad customized to fit the form of an advanced fingerling bass, where a rubber tube was inserted in the fish's mouth; providing a continuous water flow of 30 mg L^{-1} MS 222. A 9-mm long incision was made just off the mid-ventral line posterior to the pelvic girdle using a miniature, 3-mm blade scalpel. A modified shielded needle technique (Ross and Kleiner 1982) provided a guide for the

transmitter antenna to pass through the body wall. Once the antenna was threaded through the catheter, the catheter was removed and the transmitter (or dummy tag) was gently inserted into the body cavity. Due to the small incision site, the incision was closed with one Ethicon (5-0 taper RB-1 needle) coated vicryl absorbable suture. Tagged fish were placed in a recovery bath until equilibrium was restored. Surgery time averaged 149 seconds from when the fish was removed from the anesthetic bath until transferred to the recovery bath.

Growth. A 21-day growth experiment was initiated on July 24, 2009 and similar experiment was conducted for 30 days on March 28, 2010. Fish were randomly selected for the surgery and control treatment groups and all fish were anaesthetized in a 70 mg L^{-1} MS 222 bath, measured (mm TL), weighed (0.1 g), and placed in research tanks (Table 2-1). Fish in 2009 were uniquely identified by clipping a separate or series of dorsal spines as a marker to identify individual fish so that growth for these individuals could be calculated during the study period. In the 2010 growth study, a mean growth rate was calculated for the control and treatment groups in each tank using the mean size (TL, mm) of all fish at the beginning and the end of the experiment.

I used replicate tanks of fish as the experimental unit, with fish assigned randomly to tanks and growth measured as the mean change in length for each tank. To estimate growth differences between surgery and control fish, and to test for tank effects, 10 tagged and 10 control fish were placed in each of four separate, identical tanks (n = 80 fish per experiment each year). Feeding resumed 24 hours after all surgeries were completed, and then all fish were fed to satiation each day during the experiment each year. In the 2009 experiment, all fish were removed, measured, and

weighed on day 7 and day 21. In the 2010 experiment, all fish were removed, measured, and weighed on day 30. Condition of surgery fish was assessed at the conclusion of the trial by examining the external structures, the incision site, and the exit site of the antenna for redness and swelling. Abnormalities were graded as mild or severe as observed by visual examination. Mortality was recorded for both test groups and tag expulsion was monitored for tagged fish in both years.

Predator avoidance. We tested the effects of surgical procedures and radio transmitter presence on the vulnerability to predators of advanced fingerling largemouth bass. Predation experiments were conducted between June 22, 2010 and August 6, 2010 in an outdoor 9,085-L (3.7-m diameter, 0.84-m depth) circular tank. This tank was supplied with a continuous flow of aerated well water and cooled to a consistent temperature (22-24°C) throughout the experiment. Four rings of artificial plants (80 x 60 cm) were added to the tank, which provided test fish with approximately 15% escapement cover by volume. Six adult largemouth bass averaging 408 mm TL (range = 340-505 mm TL) were captured by electrofishing in Lake Eustis, Florida and were selected as the predators for this experiment. They were acclimated to the tank for one month before the experiment began. Predators were fed juvenile largemouth bass and Seminole killifish *Fundulus seminolis* before and between trials, but they were not fed 48 hours prior to the introduction of prey fish for each trial. Although the same predators were used for each trial, we assumed if any changes if predator efficiency occurred, it would not affect the proportion of each treatment consumed. We also tested the correlation between successive trials and time of trial duration to determine if any learning or increase in feeding efficiency occurred.

For each trial, advanced-fingerling hatchery largemouth bass (n = 20) were randomly assigned one of two treatments, where they were either measured and had radio tags surgically implanted (tagged) or handled only (control). All fish were measured (mm TL) and weighed (g), and then given a 24-hour recovery period. To investigate differences in predator avoidance, we conducted 10 separate trials, each consisting of 10 tagged and 10 control fish to be released into the predator test tank. To begin a trial, predators were crowded to one side of the tank with a fine mesh seine and test fish (n = 20) were then added to the opposite side of the tank and given a 5-minute acclimation period. Direct observations were made as often as necessary to allow near 50% predation of all test fish to occur. After sufficient predation was achieved, test fish were crowded to one end of the tank with a seine and then removed. Survivors from each trial were transferred to an indoor 1060-L tank for 24 hours to be observed for delayed mortality due to predation attempts from the adult largemouth bass predators.

Data analysis. For all analyses in this study, a significance level of $P < 0.05$ was used. Initial lengths and weights between treatments for all experiments were compared using t-test assuming equal variance when appropriate. All analyses were performed using SAS® v 9.2 (Cary, NC) and parametric model assumptions of normality and homogeneity of variance were visually examined to ensure that they were properly met.

Relative growth rates were expressed as percent body weight gained per day (Busacker et al. 1990). Relative growth rates for surviving tagged and control fish were calculated on day 7 and 21 in 2009, and day 30 in 2010. We built linear mixed models assuming a completely randomized block design to account for potential tank effects on

growth in both experiments. When tank effects were estimated at 0, data were pooled and we used a t-test to evaluate differences between tagged and control fish. In 2010, because control fish were not individually marked, mean relative growth rate was calculated for each tank and treatments were compared using a linear mixed model with treatment (tag, control) as the fixed factor and tank as the random factor (to account for both treatments being contained in the same tank).

Differences in the proportion of fish consumed between both test groups for predation trials were compared using a generalized linear mixed model assuming a binary distribution and a random effect for trial. We tested for differences in the proportion of fish eaten based on tag treatment, length, weight, and the interaction of length and weight with tagging on predation of fish.

Results

Growth. During growth experiments in both years, substantial mortality was observed. In 2009, surgically implanted fish experienced 32.5% mortality by day 7, but no additional mortality occurred by day 21. Two control fish died (5%) from day 7 to day 21 and all mortalities were excluded from the growth analysis. In 2010, only 19 of 40 (47.5%) tagged fish and 24 of 40 (60%) control fish survived the 30-day experiment. The majority (89%) of the mortality occurred in the first 7 days of the experiment and 100% occurred in the first 14 days. One of the four replicate tanks suffered 100% mortality of both tagged and untagged fish and therefore, only three tanks were used in the analysis of growth for 2010. Necropsies of all mortalities revealed columnaris *Flexibacter columnaris,* a fish disease common to cultured environments, and damage to the intestines from surgery of tagged fish to be the primary causes of death. All fish surviving their respective experiment visually appeared healthy, robust, and unaffected

by surgery or disease. After 21 days in 2009 and 30 days in 2010, all incisions were completely healed and there was little to no evidence of internal or external infections based on gross examination. All sutures were completely absorbed by the end of each respective experiment and no transmitter expulsion was observed.

Tagged and untagged fish sizes were similar at the onset of each year's growth experiment. Fish ranged from 93 to 118 mm TL and 8.8 to 20.1 g in weight for 2009. In 2010, fish ranged from 96 to 121 mm TL and 8.7 to 19.4 g in weight. The initial average length of tagged and control fish (Table 2-1) that survived each experiment were not significantly different in 2009 ($t_{65} = 0.11$, $P = 0.91$) or 2010 ($t_{47} = 1.7$, $P = 0.28$). There was also no difference in initial weight of tagged and control fish in 2009 ($t_{65} = 0.33$, $P = 0.74$) and 2010 ($t_{47} = 1.5$, $P = 0.28$).

The mean relative growth rate was lower for tagged than untagged fish in both years (Table 2-1). In 2009, relative growth rates for fish that survived to 21 days ranged from 0.05 to 3.42%TL per day for tagged fish and 1.05 to 3.91% for untagged fish. In 2010, relative growth rates at day 30 ranged from 0.80 to 2.09% TL per day for tagged fish and averaged 1.70 to 2.01% for the three remaining tanks of control fish. The mean relative growth rate of tagged fish was significantly lower than controls at day 7 ($t_{65} = 2.57$, $P = 0.012$) and day 21 ($t_{63} = 5.74$, $P < 0.01$) in 2009, and day 30 ($F_{1,2} = 38.35$, $P = 0.025$) in 2010 (Figure 2-1).

Predator avoidance. Fish sizes were similar between tagged and untagged fish in the predation experiments (Table 2-2). Length of tagged advanced fingerling bass ranged from 98 to 121 mm TL (109.5 ± 0.5 mm, mean ± SE) and control fish ranged from 96 to 120 mm TL (109.0 ± 0.5 mm). Comparison of initial size between tagged

and control fish showed no significant difference in lengths ($t_{198} = 0.91$; $P = 0.360$) or weights ($t_{198} = 1.52$; $P = 0.129$). In each of the 10 trials, all fish survived and swam actively following the 24-hour surgical recovery period, showing no apparent stress from tag implantation. After removal of the seine, time to trial completion ranged from 0.5 to 5.0 hours and averaged 2.5 ± 0.5 hours (± SE). Predators did not become more efficient at capturing the prey as the trials were not positively correlated to trial duration ($R^2 = 0.008$). After initial predation attempts, the majority of prey stayed close to artificial vegetation for cover. Predator avoidance behavior observed for tagged fish visually appeared the same as that of control fish. No fish surviving the trial period died within the 24-hour post trial period.

Advanced fingerling bass with surgically implanted tags were consumed in similar proportions to untagged fish (Figure 2-2). Total fish consumed during each trial ranged from 5 to 15 and collectively among the 10 trials, 98 total prey fish were consumed. There were no significant difference in the percent consumed between the two treatment groups ($F_{1,187} = 0.00$; $P = 0.975$) as the survival for each trial (0.51 ± 0.06; mean ± SE) was the same for tagged and control fish. There was also no effect of prey size (TL: $F_{1,187} = 1.50$; $P = 0.222$; wt: $F_{1,187} = 0.72$; $P = 0.398$) or the effect of interaction of size and tagging (TL x tagging: $F_{1,185} = 0.36$; $P = 0.550$; wt x tagging: $F_{1,185} = 0.21$; $P = 0.647$). Thus, tagging the fish did not influence vulnerability of advanced fingerling bass to predators.

Discussion

This study represents some of the smallest Centrarchids to be tested with radio transmitters. Although advanced fingerling largemouth bass experienced tank mortality and slowed growth from surgery in the laboratory, tagging did not hinder the fish's ability

to avoid predators. Results indicate that transmitters representing 1.2–2.7% of the fish's body weight would be appropriate for short-term biotelemetry studies of juvenile largemouth bass. However, in field applications, caution should be used by researchers that use these radio telemetry techniques when interpreting initial growth data and separating tagging mortality from predation mortality after tagged fish have been released into a lake or river system.

Observed mortality during the growth experiments was higher than expected in laboratory studies. When investigating survival of tagged juvenile largemouth bass, Cooke et al. (2003) observed similar mortality to this study with up to 33% mortality in the first five days after implantation. High tagging-associated mortality in 2009 could have resulted from inexperience of surgeon conducting surgical procedures (Cooke et al. 2003) and high water (25° C) and air (32° C) temperatures during surgeries (Knight and Lasee 1996). High mortality in 2010 for control and tagged treatments along with analysis of necropsies indicated columnaris to be the primary cause of death, although the stress from the surgical procedure could have suppressed the immune system and increased the susceptibility of tagged fish to columnaris. Further study of the effects of tagging on the mortality of advanced fingerling largemouth bass in the laboratory and field should be assessed for future use of these radio tags on juvenile bass.

During both years' growth experiments, fish did not expel their radio tags. Although many short-term laboratory studies show that radio and acoustic transmitters are not commonly expelled, Frost et al. (2010) studied growth and survival of subyearling Chinook salmon and partial to complete tag expulsion was observed in 37% of the tagged fish after 30 d. Tags used in their experiment were a prototype acoustic

tags which consisted of 2.6% to 5.9% body weight ratio which could have affected tag expulsion. In field applications, an expelled tag can result in misinterpreted data and tag expulsion should be evaluated prior to field implementation.

Both tagged and untagged individuals grew substantially in length and weight (mean >1% of their body weight per day) over the course of each respective experiment with tagged fish showing some impairment of growth when compared to untagged fish. Differences in growth may not be explained by inefficient feeding. Robertson et al. (2003) found negative results on growth up to day 36 for tagged Atlantic salmon parr, however, consumption rates were similar between tagged and control fish. Differences in growth were consistent with several other studies tagging juvenile fish with transmitters (Adams et al. 1998a; Martinelli et al. 1998; Robertson et al. 2003; Frost et al. 2010). Adams et al. (1998a) found that growth rates of juvenile Chinook salmon (114-159 mm fork length) tagged with radio transmitters were slightly impaired at day 21, but by day 54, they were growing at rates comparable to control fish. Although tagged fish exhibited slower growth than untagged fish, observed statistical differences may not be biologically pertinent for meeting the objectives of our field study based on the following rationale discussed by Johnson (1999). We argue that the slight observed growth differences will not alter the survival or behavior of radio-tagged, advanced fingerling largemouth bass in the field during the short battery life of the tag. In a longer field trial, however, slowed growth may influence survival by size-dependent mortality of smaller individuals (Ludsin and DeVries 1997; Post et al. 1998; Pine et al. 2000).

This study showed no difference in predation rates in laboratory tanks between tagged and control fish. Many factors influence the vulnerability of prey to avoid

predators such as fast-start performance, inability to school effectively, failure to detect predators, and prey conspicuousness (Mesa et al. 1994). No significant differences between treatments or prey size supports our hypothesis that implantation of radio transmitters in advanced fingerling largemouth bass does not increase vulnerability to predation. Anglea et al. (2004) studied the predator avoidance and swimming performance of tagged juvenile Chinook salmon (122-198 mm fork length) and found that surgical implantation did not significantly affect swimming performance or result in greater predation susceptibility. Although we did not test the affect of tagging on swimming performance, a relationship has been demonstrated between swimming performance and vulnerability to predation (Bams 1967). Studies that have tested both predator avoidance and swimming performance on juvenile fish have either shown that both were either affected or both unaffected (Adams et al. 1998b; Anglea et al. 2004).

Results indicate that short-term mortality was higher in tagged fish, but elevated temperatures that induces stress and high frequency of bacterial infections during lab experiments made it difficult to assess the extent that surgically implanting tags contributed to mortality. Transmitter slowed growth rates of advanced fingerling largemouth bass, but did not affect their ability to avoid predators. We assume that some impairment of growth should not significantly affect the movement and behavior of tagged individuals, therefore inferences made from tagged largemouth bass should be adequately representative of untagged stocked fish in the field. Further research that evaluates a modified tag weight to fish ratio and surgeon experience should be investigated to assess the potential to reduce the effects of radio tags on growth or mortality of juvenile bass.

Conclusions from this study show that successful implantation of radio transmitters in juvenile warm water fish (90-120 mm TL) such as largemouth bass is possible. This information could have useful application for researchers studying the recruitment and early life history of juvenile Centrarchids and other warm water fish. As transmitter size continues to decrease with advances in technology, the ability to study the behavior of previously unstudied small fish will be possible. Future researchers should evaluate the impacts of implanted transmitters in the laboratory prior to using radio tags in the field to avoid misinterpreting data due to their effect on the fish.

Table 2-1. Summary of initial fish length (mm TL), weight (g), sample size (N), day of growth measurement, and percent length and weight gained per day of tagged and untagged (control) advanced fingerling largemouth bass for growth and mortality experiments. In 2009, (N) represents individual fish, whereas in 2010, (N) represents tanks. All measurements are mean ± SE.

Group	Day	N	Initial TL (mm)	Weight (g)	% TL gain per day	% weight gain per day
2009 Tagged	7	27	106.7±1.35	14.7±0.58	0.21±0.03	1.70±0.19
	21	27			0.37±0.03	1.44±0.15
2009 Control	7	40	106.9±1.19	14.4±0.47	0.44±0.02	2.18±0.09
	21	38			0.58±0.02	2.63±0.13
2010 Tagged	30	3	103.4±1.40	11.87±0.56	0.28±0.07	1.49±0.11
2010 Control	30	3	105.1±1.13	12.56±0.40	0.46±0.07	1.97±0.11

Table 2-2. Mean total length (mm), weight (g), ± standard error, and number of fish eaten by adult predators per trial of juvenile largemouth bass by treatment group in predator avoidance tests.

	Control				Tagged			
	Mean TL (mm)	Weight (g)	Prey eaten	n	Mean TL (mm)	Weight (g)	Prey eaten	n
Trial 1	111 ± 1.2	13.3 ± 0.6	7	10	112 ± 1.4	13.3 ± 0.4	7	10
Trial 2	112 ± 1.5	16.4 ± 0.7	3	10	113 ± 0.9	16.8 ± 0.6	5	10
Trail 3	107 ± 1.1	13.5 ± 0.4	3	10	109 ± 1.0	13.8 ± 0.3	6	10
Trial 4	107 ± 1.4	12.8 ± 0.6	8	10	107 ± 0.6	13.2 ± 0.3	7	10
Trial 5	108 ± 1.6	12.9 ± 0.6	4	10	110 ± 2.1	13.9 ± 0.9	1	10
Trial 6	105 ± 1.1	12.1 ± 0.4	5	10	106 ± 0.9	12.7 ± 0.3	6	10
Trial 7	111 ± 1.7	13.3 ± 0.6	4	10	110 ± 1.5	13.2 ± 0.5	5	10
Trial 8	110 ± 1.1	13.0 ± 0.5	5	10	109 ± 1.9	13.3 ± 0.7	3	10
Trial 9	104 ± 1.6	11.5 ± 0.5	5	10	107 ± 1.7	12.8 ± 0.6	3	10
Trial 10	112 ± 0.9	13.8 ± 0.4	5	10	112 ± 0.8	13.7 ± 0.4	6	10
Overall	109 ± 0.5	13.2 ± 0.2	49		110 ± 0.5	13.7 ± 0.2	49	

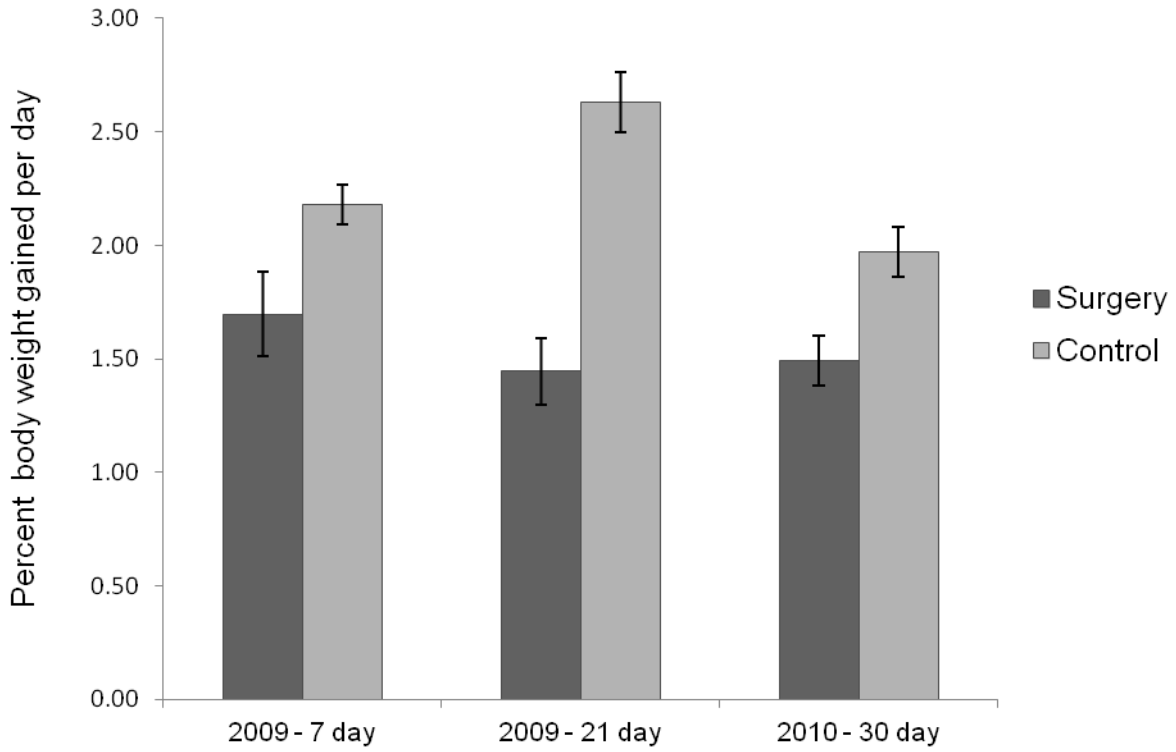

Figure 2-1. Relative growth rate (percent body weight gained per day) at 7 and 21 days in 2009 and 30 days in 2010 for surgically implanted radio tagged fish and control (untagged) hatchery largemouth bass.

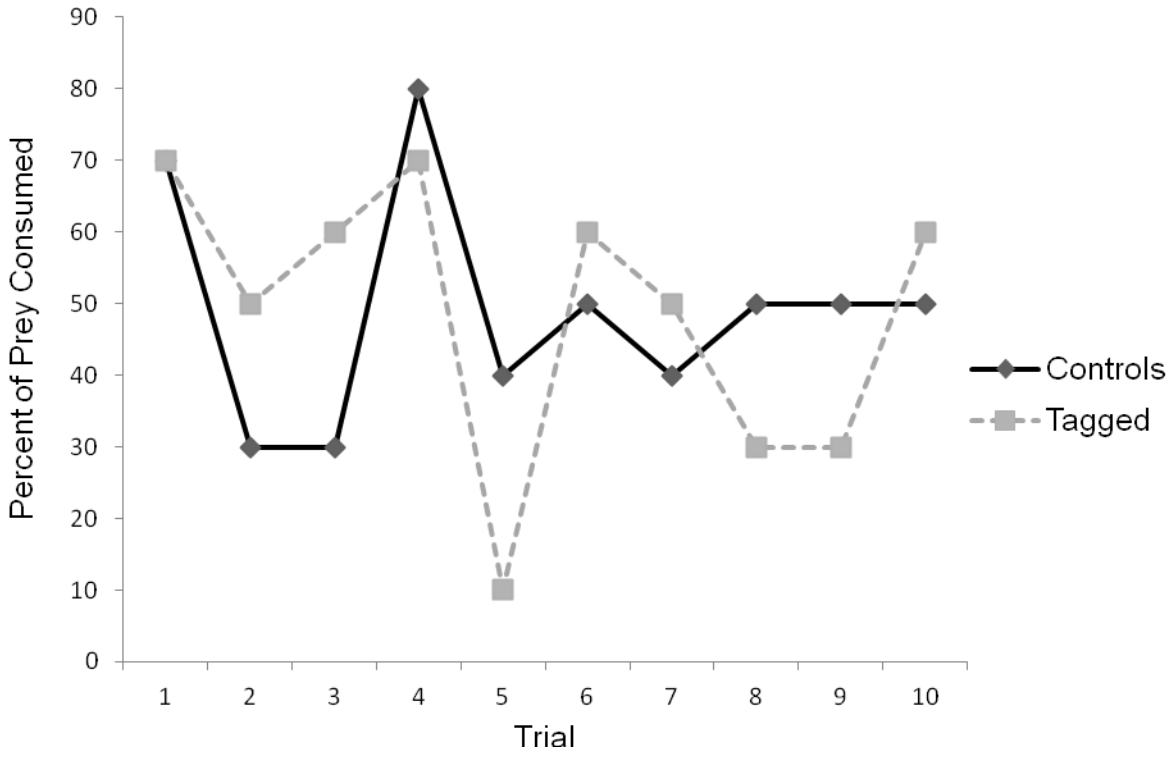

Figure 2-2. Proportion of tagged and control (untagged) advanced fingerling hatchery largemouth bass that were consumed by adult largemouth bass in each of 10 predator avoidance trials.

CHAPTER 3
EVACUATION RATES OF RADIO TRANSMITTERS BY PREDATORS

Intro

Radio and acoustic transmitters can provide valuable information about the survival, movement, and habitat use of fish. Micro-transmitters have expanded this research to include many species of juvenile fish (Bolland et al. 2008; Jepsen et al. 1998; Koed et al. 2002). Tracking juvenile fish with radio telemetry allows inferences about their early life history characteristics based on the movement of the tagged individual. An assumption made by scientists conducting radio telemetry studies is that the information obtained about locations and movements of a study animal is from the study animal itself and not from a predator that consumed the tagged study animal.

Juvenile wild and hatchery-reared largemouth bass are highly susceptible to predation during their early life stages. For example, stocked largemouth bass in Texas experienced losses to predation as high as 27.5% within 12 hours of stocking (Buckmeier et al. 2005). Thus, predators that eat radio-tagged individuals can impact the results of a telemetry study, if the investigators are unable to distinguish between the movement and behavior of radio-tagged fish or a predator that might have eaten the tagged fish.

Estimates of predator evacuation rates may be critical when conducting predation studies of wild or hatchery reared fish. Knowing how long different types of tags will remain in a predator may allow researchers to estimate the time when the study animal was consumed. Evacuation rates have been estimated for coded wire tags (Niva and Hyvarinen 2001) and passive integrated transponder (PIT) tags (Petersen and Barfoot 2003) to quantify predation of stocked fish. For example,

predation by double-crested cormorants *Phalacrocorax auritus* on stocked cutthroat trout *Oncorhynchus clarkii* and rainbow trout *Oncorhynchus mykiss* has been successfully estimated utilizing coded wire tags (Lovvorn et al. 1999). Although body size and food size may play a role, research suggests that temperature is the most important factor affecting gastric evacuation rate (He and Wurtsbaugh 1993; Henson and Newman 2000; Petersen and Barfoot 2003).

Researchers have studied the evacuation rates of coded wire tags (Niva and Hyvarinen 2001) and passive integrated transponder (PIT) tags (Petersen and Barfoot 2003), but little is known about the evacuation rates of radio transmitters. The increased size and mass of radio transmitters could slow or impede transit through the intestines of a predator and bias results assumed to be from tagged individuals. With the increased use of micro transmitters to estimate the survival and behavior of stocked fish, this information becomes critical.

Previous studies in Florida indicated low survival within one year after stocking advanced fingerling largemouth bass (Porak et al. 2002), suggesting that losses due to predation could be significant. To make accurate inferences about the mortality of the stocked population, we must know if radio transmitters accumulate in the intestine of adult largemouth bass and estimate when evacuation is likely to occur after predation. Using short-lived batteries that are common in micro transmitters, a final fate for each tagged fish cannot be accurately determined without knowledge of evacuation time by predators. I conducted a laboratory experiment to determine evacuation rates of radio transmitters in relation to three variables; water temperature, predator size, and prey size.

Methods

Adult largemouth bass were used as the predator in our evacuation trials, because they are the primary predator in Lake Carlton, Florida (study site for radio telemetry). Thirteen adult largemouth bass (mean = 381 mm TL ± 6.1) were captured by electrofishing from Lake Eustis, Florida and were divided (six in one tank and seven in the other) between two 1,987-L (1.82-m diameter, 69-cm deep) circular tanks at the Eustis Fisheries Research Laboratory (EFRL), where an inflow of aerated well water was supplied at a constant temperature of 22˚C. Adult largemouth bass were measured (mm TL), weighed (g), and individually marked with a PIT tag (Table 3-1). Predators were acclimated to the tanks for one month and fed a maintenance diet of juvenile largemouth bass and Seminole killifish *Fundulus seminolis* prior to initiation of the trials.

Advanced fingerling hatchery largemouth bass to be used as radio tagged prey in this study were acquired from the Florida Bass Conservation Center (FBCC) and transported to the EFRL. These hatchery fish were acclimated and held in 1,060-L (1.24-m diameter, 69-cm deep) circular tanks until evacuation trials began. All holding tanks were supplied with aerated flow through well water at approximately 22˚C and hatchery largemouth bass were fed to satiation three times per week with a commercially produced petted feed. A surgical procedure similar to Adams et al. (1998) was used to implant radio transmitters (ATS, model A2414), weighing 0.24 g in air, into the peritoneal cavity of advanced fingerling largemouth bass. Tagged bass were measured (mm TL), weighed (0.1 g; Table 3-1) and allowed to recover from anesthesia.

Six 1,060-L test tanks were simultaneously used for individual trials and included three heated, insulated tanks, and three unheated tanks at ambient temperature. Evacuation rates for each predator (n = 13) were evaluated at two water temperatures;

heated (30˚C) and ambient (~ 22˚C). These temperatures represent the normal range of water temperatures at Lake Carlton, Florida during the time of hatchery bass stocking. Two predators from our experiments died and one became diseased after trials were completed in the ambient temperature tanks; resulting in three fewer trials (n = 10) for heated tanks.

Evacuation experiments were conducted between December 2, 2009 and January 18, 2010. Predators were starved for 48 hours prior to the introduction of radio tagged fish to ensure empty stomachs at time of feeding. Predators were moved from the 1,987-L holding tanks into individual test tanks (1,060 L) seven days prior to testing. The three heated tanks were at ambient temperature when the predators were moved into them. After the introduction of the predators, a heating element was used to slowly increase the water temperature of the heated tanks, allowing the predators to acclimate while the unheated tanks remained at ambient temperatures. During trials, each tank contained one predator and one introduced tagged prey fish. The first four trials consisted of six tanks, except where predators died (one tank in two different trials), became diseased, or did not consume tagged fish (two fish in two different trials). A fifth trial was conducted to complete the testing of four individual fish (using four tanks; two heated and two unheated) that had only been tested in one water temperature. Tagged juvenile bass were placed in each of the test tanks five minutes after tagging and in all trials, prey fish were not force fed to predators. Tanks were checked every three hours to determine when the prey was eaten. The time to evacuation was calculated from the time the prey fish was eaten until the radio tag was observed on the bottom of the tank.

The effects of water temperature, predator size, and prey size on evacuation rates were evaluated using an analysis of covariance (ANCOVA) with Proc MIXED in SAS® v 9.2 (Cary, NC). Water temperature was included as a categorical fixed effect, while predator size and prey size were included as continuous fixed effects. In addition, a random fish effect was included because each predatory fish was used during both the ambient and heated temperature trials. Model assumptions of normality and homogeneity of variance were examined visually to ensure validity of the analyses. To predict the evacuation time of radio tags, we modeled the percent of tags evacuated at a given time after consumption for each water temperature using the power function described in Peterson and Barfoot (2003):

$$Ptag_t = 2^{-\left(\frac{t}{H}\right)^s},$$

where $Ptag_t$ is the percent of tags evacuated at time t, S is a parameter that controls the shape of the function, and H is a measure of the half-life of the prey item being evacuated. We parameterized the gastric evacuation process power function using non-linear modeling (Proc NLIN) in SAS® v 9.2 (Cary, NC) for both water temperature treatments. Significance for all statistical analyses was set at $P < 0.05$.

Results and Discussion

The predators in this experiment (Table 3-1) ranged from 343 to 455 mm TL and 571 to 1,325 g in weight. Advanced fingerling largemouth bass used as prey ranged in length from 96 to 137 mm TL and 8 to 23 g in weight. Radio transmitters implanted in juvenile bass did not accumulate in the intestine of adult largemouth bass and evacuation times ranged from 15 to 84 hours (Table 3-1). The median evacuation time of radio transmitters for adult bass was 51 h in non-heated tanks and 34 h in heated

tanks (Table 3-1). The effect due to repeated observations from using the same predator fish in multiple trials was estimated at zero in the ANCOVA. Results of the ANCOVA modeling showed no significant differences in evacuation time due to predator total length ($F_{1,17} = 0.02$, $P = 0.88$) or prey total length ($F_{1,17} = 0.04$, $P = 0.85$). However, evacuation rates increased significantly with water temperature ($F_{1, 21} = 5.33$, $P = 0.03$).

Using the power function described in the methods, the model was a significant predictor of evacuation ($F_{4, 54} = 997.8$, $P < 0.001$), which can be seen by the tight fit of the model predictions to the observed evacuation times (pseudo-R2 = 0.97; Figure 3-1). The half-life for tag evacuation was estimated at 31 hours at 30˚C and 48 hours at 22˚C (Table 3-2). From this model, we are able to predict the percent of tags remaining in a predator's gut at any time after ingestion for both water temperatures (Figure 3-1).

In this study, all adult largemouth bass evacuated radio transmitters that were implanted in juvenile bass in less than 84 h. Our results were similar to Niva and Hyvarinen (2001) who found northern pike had evacuated coded wire tags within 3 d. Petersen and Barfoot (2003) studied the rates of northern pikeminnow evacuating PIT tags after ingesting juvenile Chinook salmon and found that median evacuation times were 40 h at 14°C and 22 h at 18°C. I anticipated faster evacuation rates for largemouth bass in high water temperatures compared to other studies that were conducted in cooler water temperatures. Results could be explained by differences in species evacuation rates or the larger mass of our transmitter slowing transit through the intestine. Hunt (1960) studied the digestion rate of largemouth bass (n = 18) at water temperatures between 23°C and 26°C and found that a mean of 99% of the food was digested within 27 hours. Beamish (1971) found similar results, studying

largemouth bass held in tanks at 25°C that evacuated lake emerald shiners *Notropis atherinoides* in less than 24 h. In our study, at 24 hours, only 30% of the transmitters were evacuated at 30°C and only one of 13 at 22°C. This gives support to speculate that transmitters could have been temporarily impeded from evacuating, although, additional consumption of prey, which typically would occur in the wild, could have increased this evacuation time.

Variation of evacuated transmitters was largely explained by water temperature and not significantly affected by predator or prey size. These results were consistent with other studies documenting significantly faster evacuation times with higher water temperatures, owing to higher metabolic processes (Petersen and Barfoot 2003; Henson and Newman 2000; He and Wurtsbaugh 1993). Petersen and Barfoot (2003) saw significantly higher evacuation rates of PIT tags by northern pikeminnow at 18°C compared to 14°C. Cochran and Adelman (1982) used an exponential decay model to quantify gastric evacuation of largemouth bass in and found that the exponent of gastric evacuation increased exponentially with temperature.

In radio telemetry field applications, this model of evacuation probabilities could help predict if a located tag is from a juvenile radio tagged fish or is within a predator that has consumed a radio tagged fish. Once the transmitter stops movement, the estimated timing of predation can be calculated. As a result, once a tag stops moving, investigators could exclude the location information from the three days prior to the cessation of movement, because those locations likely resulted from predator movement.

Evacuation rates of radio or acoustic transmitters have not been previously studied to determine if and when radio transmitters will be evacuated from a predators stomach. Transmitters are much heavier and rigid than previously studied coded wire tags or PIT tags and often possess a trailing antenna (10 cm in this study). In this study, we were able to confirm that miniature radio transmitters did not accumulate in a predator's stomach. Upon evacuation, all transmitters exhibited a distinct curling of the antenna (Figure 3-2), which can be used to distinguish natural mortality and predation in field applications when radio tags are recovered. If evacuation rates are unknown by scientists using radio transmitters to study juvenile bass, then significant movement and habitat information could be erroneously assigned to the radio tagged juvenile fish instead of the predator that had consumed a radio tagged bass (i.e., prior to evacuating the transmitter). This knowledge of the timing of evacuation will reduce bias for any type of radio telemetry experiment that studies the survival or behavior of juvenile fish or any small species of fish that has a high predation risk. Evaluation of evacuation rates and tag accumulation should be assessed with other predator species and when using larger transmitters.

Table 3-1. Comparison of Prey Size, Predator size, and Evacuation times (mean ± SE) at two temperatures for adult largemouth bass (predator) consuming advanced-fingerling largemouth bass with surgically implanted radio tags (prey).

Temp	Prey size		Predator size		Evacuation times			
(^{o}C)	TL (mm)	Weight (g)	TL (mm)	Weight (g)	Average Time (h)	Min (h)	Max (h)	n
22	114.8±3.2	14.2±1.3	383±8.5	836±56.8	51.0±5.4	27	84	13
30	116.6±3.3	15.1±1.4	379±9.0	802±60.5	33.6±5.0	15	54	10
Overall	115.6±2.3	14.6±1.0	381±6.1	820±40.7	45.4±4.4	15	84	23

Table 3-2. Parameter estimates for a model that predicts the probability of a predator evacuating a tag at a given time after consumption; using the power function described in Peterson and Barfoot (2003): $Ptag_t = 2^{-\left(\frac{t}{H}\right)^s}$, where Ptagt is the probability a tag is evacuated at time t. The parameter estimates (and 95% confidence intervals) in the table are for S (parameter that controls the shape function) and H (a measure of the half-life of the prey item being evacuated) used in predicting the proportion of radio transmitters evacuated by adult largemouth bass at time t after consumption.

	Parameters	
Treatment	*S*	*H*
22˚C	2.56 (2.19, 2.93)	47.72 (45.62, 49.82)
30˚C	2.02 (1.70, 2.34)	30.73 (28.80, 32.65)

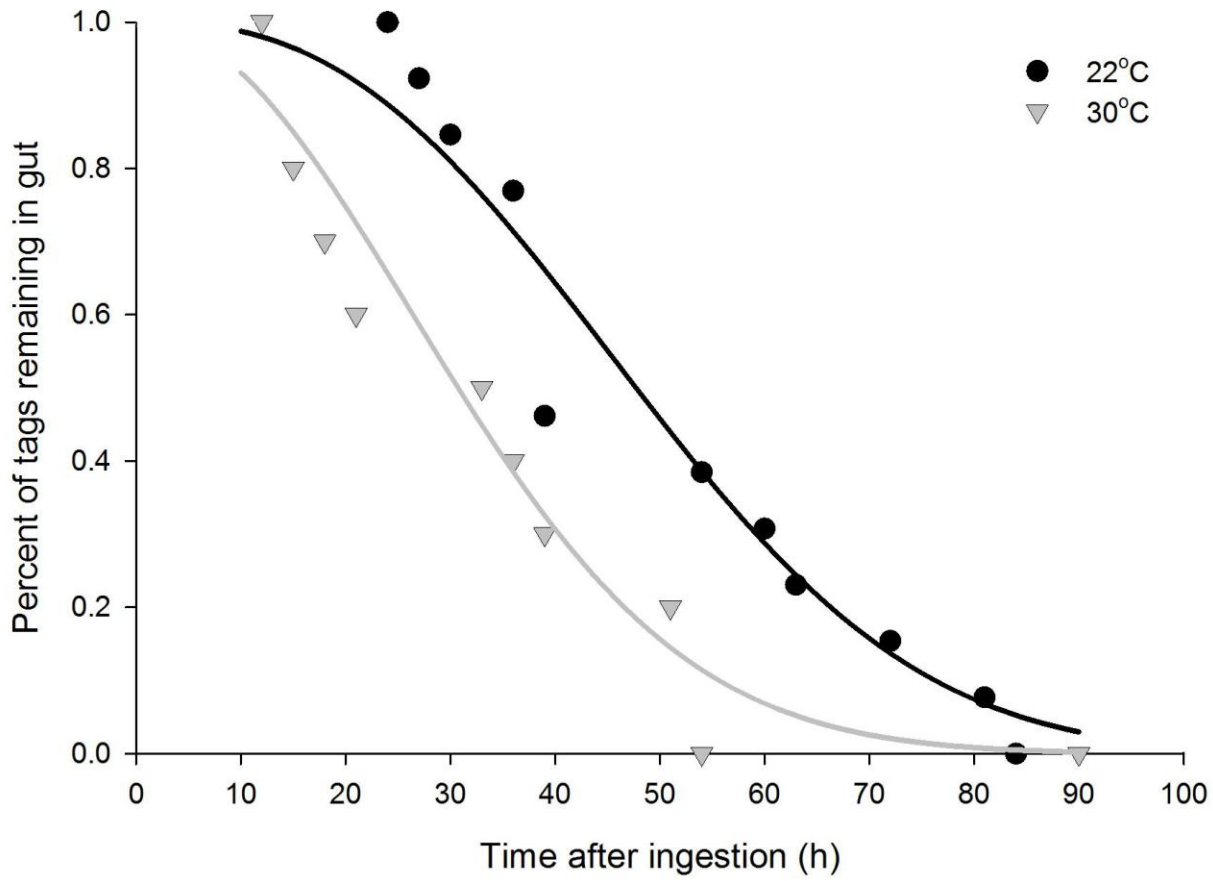

Figure 3-1. Percent of radio tags remaining in the gut of a predator (i.e., adult largemouth bass) after consumption of radio tagged prey at 22°C and 30°C; observed during laboratory experiments.

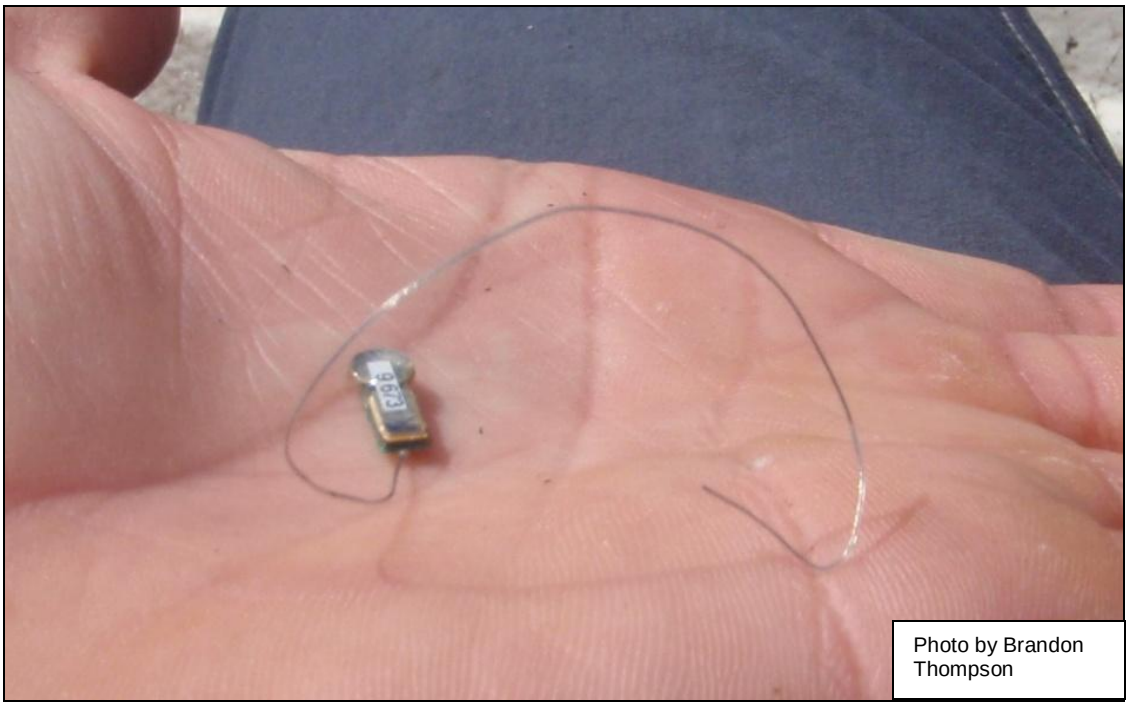

Figure 3-2. A photo illustrating the curling effect on the antennae of a radio transmitter after ingestion of tagged fish by a predator (i.e., largemouth bass) and evacuation of radio tag after the tagged bass was digested.

CHAPTER 4
MOVEMENT, SURVIVAL, AND HABITAT USE OF HATCHERY-REARED VERSUS WILD JUVENILE LARGEMOUTH BASS

Intro

Studies have been conducted to determine the contribution and long-term survival of stocked largemouth bass (Buynak and Mitchell 1999; Porak 2002), but little information exists on their immediate survival and behavior after release. For example, Porak et al. (2002) found less than three percent survival for stocked largemouth bass in Florida lakes one-year post stocking, but mechanisms that cause the mortality were largely undetermined. It also remains unclear if low contribution of hatchery fish tot the population results from higher mortality in stocked fish compared to wild bass or if we simply cannot stock high enough numbers of hatchery fish to gain sufficient contribution.

Behavior is an important mechanism of survival. Hatchery-reared fish stocked in the wild must go through a critical post-stocking period when feeding and anti-predatory skills must be developed (Heggberget et al. 1992; Brown and Laland 2001). Pouder et al. (2010) found that stocked advanced-fingerling largemouth bass had difficulty transitioning to natural prey within seven days post stocking; possibly contributing to high initial mortality. Buckmeier et al. (2005) showed high predation of stocked largemouth bass immediately after release. I am particularly interested in determining how the behavior of stocked hatchery largemouth bass compares to that of wild bass. Behavioral differences might lead to high predation and/or an inability to locate and consume prey during this critical period. Intensive research has focused on hatchery-reared salmonids and has identified domestication issues, including behavioral deficits such as naivety to predation (Huntingford 2004). Understanding how movement and habitat use influence the fate of stocked largemouth bass during the initial period

following release can help improve the effectiveness of stock enhancement programs for largemouth bass.

Biotelemetry has been used extensively to provide researchers with valuable information about adult largemouth bass mortality (Hightower et al. 2001), movements (Hanson et al. 2007), and habitat use (Mesing and Wicker 1986). Micro transmitters now allow scientists to research post-stocking survival and behavior of juvenile fish (Jepsen et al. 1998; Dieperink et al. 2001; Bolland et al. 2008). Results from these studies have identified differences in survival (Dieperink et al. 2001) and behavior (Bolland et al. 2008) between hatchery-reared and wild fish. However, to this point, micro transmitter biotelemetry has not been utilized to evaluate survival and/or behavior of hatchery-reared largemouth bass during the critical period following release into the wild.

The population of largemouth bass on the Oklawaha Chain of Lakes (Figure 4-1) has been identified as recruitment limited due to loss of nursery habitat (Benton et al. 1991, Benton 1999, Wicker and Johnson 1987). Stocking of advanced-fingerling hatchery largemouth bass has been attempted to increase bass abundance in this system and to improve angler catch rates. The objectives of this chapter were (1) to assess the behavior, movements, habitat use, and survival of hatchery-reared advanced-fingerling largemouth bass after being stocked into the wild and (2) to examine differences in survival, movements, habitat use, and growth between hatchery-reared and wild juvenile largemouth bass. Comparison of wild fish and hatchery-reared stocked fish should help inform managers if survival rates are affected by lake conditions and/or the fitness of hatchery fish.

Methods

Study Area. Lake Carlton (157 ha) is a part of the Oklawaha Chain of Lakes (30,756 ha), located in central Florida (Figure 4-2). These lakes are characterized as hypereutrophic, they have high densities of phytoplankton, and they have a narrow band of emergent vegetation that exists around the shoreline. Lake Carlton was selected for this study, because its habitat was representative of the entire chain of lakes and its small size allowed for efficient tracking of radio tagged fish. The primary aquatic macrophytes include panic grasses *Panicum* spp., bulrush *Scirpus californicus*, cattails *Typha latifolia*, and pickerelweed *Pontederia* spp., along with sparse patches of spatterdock *Nuphar luteum*. Limited submersed aquatic vegetation existed in the lake and consists primarily of eelgrass *Vallisneria americana*. The perimeter of Lake Carlton is 4.49 km and two separate navigable canals (Figure 4-2) connect to Horseshoe lake (13 ha) and Lake Beauclair (459 ha).

Habitat mapping. To evaluate habitat use of radio tagged largemouth bass, an extensive evaluation of the littoral aquatic habitat was conducted in Lake Carlton. We measured the lake edge (practical shoreline) and all emergent aquatic vegetation in the littoral zone of Lake Carlton during spring 2009. We completed the habitat survey in spring 2010, when we measured submersed species of aquatic plants (e.g., *Vallisneria* spp) using transects spaced at 50-m intervals around the lake and created polygons from those data. Primary plant communities were measured to sub-meter resolution with a Trimble® GeoXT GPS unit, imported into ESRI® ArcMAP™ v.9.2, and ground-truthed to verify community polygons. We redefined the boundary of Lake Carlton in spring 2010 to account for a rise in water levels, although due to time restraints, the vegetation community polygons were not redrawn. Many species encountered between

the former lake edge and the new shoreline were terrestrial plants that grew during the low-water periods prior to and during 2009. As such, this creates an unknown source of bias and inaccuracy of habitat use when radio tagged largemouth bass utilized shallow water areas during 2010. A 3-m buffer was added in Arc/View to quantify offshore locations by tagged fish; assuming that fish within this buffer were relating to inshore vegetation. A small section of the Lake Carlton vegetation map is shown in Figure 4-3 to illustrate the types of aquatic plant polygons and largemouth bass locations that were created using the ArcMAP™ mapping software.

Radio tags and surgical procedure. We used model A2414 radio transmitters (Advanced Telemetry Systems; Isanti, MN, 148-151 MHz), which were the smallest commercially available tags. These transmitters weighed 0.24 g in air (5 x 12 mm) and were equipped with a 10-cm flexible braided wire antenna. These radio tags had a pulse rate of 60 pulses per minute in 2009 and had an expected battery life of 24 days. Transmitter pulse rates were modified to 30 pulses per minute in 2010 in an attempt to achieve a 30-day tag life.

Tag range and accuracy were assessed for the water depth and conductivity at Lake Carlton. To assess range, live transmitters were suspended under a float at differing water depths in Lake Carlton from a shallow depth (<1 m) to the maximum depth of the lake (4 m). Distance from the radio transmitter was recorded where the transmitter pulse could no longer be heard. To assess transmitter range in vegetated areas, we placed transmitters at mid-water depth in varying vegetation densities and near artificial structures. High accuracy (±3 m) of locating tags in vegetated areas was essential for estimating habitat use. Accuracy of detection was assessed by hiding

transmitters from the tracking biologist in dense vegetation, and having that biologist throw a buoy where they thought the transmitter was located. Accuracy was measured by the distance (m) from the buoy to the known location of the transmitter.

Tag failure rates were assessed in 2010 due to higher than expected tag failure rates in 2009. We were assured by the manufacturer in 2009 that tag failure would be negligible, so we did not assess tag failure during the first year of the study. However, high tag failure rates in 2009 made it difficult to differentiate whether the radio tagged bass died via avian predators or if the tags had failed. Therefore, in 2010, we studied tag failure rates in the laboratory. We allowed 30 live transmitters to expire in the lab to estimate expected tag failure in the field. Tags were submersed in water in tanks at the Eustis Fisheries Research Laboratory (EFRL) in water temperatures similar to those in Lake Carlton. The same model transmitters were used in the field and lab studies. The 30 control tags were selected systematically (every 2-3 tags) from a batch of 80 tags and failure rates were analyzed by frequency (149-151 MHz).

A ‘shielded needle’ surgical procedure (Ross and Kleiner 1982) similar to the technique described in Adams et al. (1998) was used to implant transmitters into the peritoneal cavity of largemouth bass and provide a lateral wall exit for the antennae. Each individual fish was anesthetized in 70 mg/L tricaine methanesulfonate (MS-222), then placed ventral side up on a molded foam pad soaked in Stress Coat ® where water with 30 mg/L MS-222 was continually pumped over the gills (Figure 4-4). Each transmitter was inserted through a 9-mm incision 3 mm away from and parallel to the mid-ventral line anterior to the pelvic girdle. The incision was closed with a single individual coated vicryl absorbable suture (5-0). The surgery took between two and

three minutes to complete. When a surgically tagged fish recovered, it was placed in a recovery tub (wild fish tagged at the lake) or transferred to a raceway (hatchery fish tagged at FBCC).

Hatchery bass stocking 2009. Radio transmitters were inserted into 50 hatchery-reared advanced fingerling largemouth bass (105 ± 0.8 mm; mean TL ± SE) at the FBCC on May 19, 2009 (Table 4-1). Hatchery bass were selected for tagging from a group of fish raised using standard FWC culture and stocking protocol for bass that were to be stocked in Lake Carlton. After surgeries, fish were given a 24-hour recovery period before being transported to Lake Carlton. On May 20, 2009, we stocked the 50 radio tagged fish evenly around the shoreline of Lake Carlton simultaneously with untagged fish that were being stocked. Each radio tagged fish was released with a group of hatchery fish (~100-200) to avoid biasing the post-stocking experience of the radio tagged hatchery largemouth bass. Individual stocking locations of the 50 radio tagged fish were recorded for dispersal and movement analysis. At the time of stocking, four plastic mesh cages were deployed in Lake Carlton with tagged and untagged hatchery fish. Each cage contained 25 control stocked hatchery fish and 12 tagged fish and were distributed evenly among the four cages.

Hatchery and wild bass stocking 2010. A total of 30 hatchery-reared largemouth bass (106.2 ± 1.62 mm; mean TL ± SE) and 20 wild bass (110.7 ± 1.53 mm) were surgically implanted with radio transmitters to compare their behavior and survival (Table 4-1). Hatchery bass were selected for tagging from a group of fish that were raised at the FBCC for stocking in Lake Carlton. A minimum size of 90 mm TL (~9.0 g) was chosen to stay close to the “2% rule” of a fish accepting a 0.3-g tag (Winter 1996;

Table 4-1). Attempts were made to keep both the hatchery and wild bass sizes between 90 and 120 mm TL. Surgeries were conducted on juvenile hatchery bass on March 29, 2010 at the FBCC and fish recovered for 24 hours before they were transported and stocked into Lake Carlton the following day.

Wild juvenile largemouth bass were collected from Starke Lake (Orange County, Florida) via electrofishing on March 30, 2010, transported to Lake Carlton, and surgically implanted with radio transmitters. Wild radio tagged fish recovered for 1 hour before they were stocked into Lake Carlton. Fish were stocked at 10 random, evenly spaced locations around the lake, with each stocking site receiving 5 radio tagged fish (3 hatchery and 2 wild fish) and approximately 1,100 microwire-tagged hatchery bass.

Mortality caused by stocking and tag implantation used in the survival analysis was determined by field observations of tag recoveries and movement. At the time of stocking, six plastic mesh cages were deployed in Lake Carlton with tagged and untagged hatchery and wild radio tagged fish to identify differences that may have resulted from stocking or surgical implantation. Eleven dummy tagged hatchery fish (105.6 ± 2.2 mm; mean TL ± SE) were divided among three cages and 10 dummy tagged wild fish (113.7 ± 1.9 mm; mean TL ± SE) were divided into three separate cages. Non-tagged hatchery fish (n = 75) were spread evenly among three cages as controls for the dummy tagged hatchery fish. Thirty wild non-tagged wild bass collected from Starke Lake were spread evenly among three cages as controls for the dummy tagged wild fish. Cages were checked at 24, 48 and 72 hours post stocking for missing or dead fish.

Tracking. Radio tagged fish were manually tracked daily by boat with an ATS R410 scanning receiver and a three-element yagi antennae until their signals were lost. Due to the high numbers of tagged fish at large and time required tracking them in 2009, one half of the tagged fish were tracked one day and the other half were tracked the next day until a reduction in the remaining fish numbers facilitated daily tracking of all remaining fish. Similar to 2009, daily tracking was done in 2010 with the exception that two tracking boats and a search boat (utilizing an omni-directional dipole antennae) were deployed each day to ensure that all fish were located each day. The small size of Horseshoe Lake allowed us to manually search for fish that might have emigrated from Lake Carlton through the canal (Figure 4-2). However, the large size of Lake Beauclair made searching difficult and we experienced a relatively high number of fish moving into Lake Beauclair during 2009. Therefore, in 2010, to detect fish movement out of the lake, two ATS R4500S stationary data-logging receivers and antennas were deployed at the narrow canal connecting Lake Carlton and Lake Beauclair (Figure 4-2). Each of these loggers scanned 25 frequencies sequentially for six seconds (5-minute cycle), allowing two pulses to be recorded. Information was uploaded daily from data-loggers and extensive searches were conducted in Lake Beauclair for a frequency that was identified passing through the canal.

When a fish was located, coordinates were recorded with a TRIMBLE® GPS unit along with temperature, depth, and habitat data. Tracking began each day at a randomly chosen radio-tagged bass location from the previous day. Trackers flipped a coin to determine a random direction, that is, whether to go clockwise or counter clockwise around the lake. When a signal was not quickly heard from the previous

day's location, the tracking biologist moved on to the next frequency. In 2009, searches for missing fish were conducted at the end of the day. However, in 2010, the search boat was immediately notified. The biologist in the search boat entered the frequency into their receiver and began searching the lake to determine if each missing fish had moved extensive distances or if the signal had disappeared from the lake. Additional frequencies were added to the scan throughout the day and trackers were notified when a signal was identified. Extensive searches were conducted daily for previously missing frequencies.

Daily tracking was critical to make fate determinations, because we were using radio transmitters that had a very short battery life. We assumed that if a transmitter had moved from its previous day's location, the tag had been in a live fish on that previous location. We also assumed from laboratory studies (Chapter 3) that 95% of transmitters would be evacuated by a predator (adult largemouth bass) within 72 hours of consuming a radio tagged fish. When fish movement ceased for two consecutive days, attempts were made to "spook" the fish by disturbing the immediate area as an attempt to coerce the fish to move. If no movement was observed by the third day, electrofishing was conducted to determine the final fate of the individual. When the targeted fish was not captured via electrofishing, we assumed the fish had died and attempted to recover the radio tag from the lake bottom utilizing a high-strength magnet (2.4 x 28 cm) attached to a 3-m aluminum pole or end of a throw rope when deep water recovery was necessary. When a tag was recovered with the magnet, we assumed that predation had occurered when the distinct "curling" of the recovered transmitter antenna was observed (Chapter 3). If the transmitter antenna did not display curling, we

concluded that the fish had died from tagging effects or natural causes. Predation was also assumed (but unconfirmed) if the tag could not be recovered or, when abnormal movements were followed by no movement two to three days later. For example, if a fish spent 10 days in shallow, dense vegetation with little movement and then moved 0.5 km per day offshore for two days followed by no movement offshore for 15 more days, we assumed this fish was eaten by a predator three days prior to cessation of movement. I assumed that 100% of the live transmitters in the lake were detected and that "lost" transmitters were tag failures. As such, this does not account for lost transmitters due to unobserved avian predation or undetected emigration from the lake.

Once transmitters began to expire near the end of the study (28-30 days post stocking), we attempted to collect all remaining fish via electrofishing, after which they were measured to compare growth between groups. Relative growth rates for wild and hatchery fish at the conclusion of the study were expressed in percent body weight and length gained per day (Busacker et al. 1990).

Data analysis. Survival was calculated as the number of days that hatchery or wild largemouth bass remained alive in the system. For survival analysis, fish that became permanently lost from the system were censored because we couldn't differentiate losses due to tag failures or avian predation. In 2009, we assessed the initial hatchery survival for the first 14 days post stocking due to limited tag life. In 2010, we compared daily survival rates between hatchery and wild released fish to 30 days post stocking using a parametric accelerated failure time regression in PROC LIFEREG (SAS® v 9.2, Cary NC; Allison 2010) assuming a Weibull hazard function. We then compared survival rates of hatchery and wild fish using Wald's chi-square test. We also

compared daily survival of the three tag frequencies in a controlled laboratory setting (PROC LIFEREG).

Dispersal and movement of hatchery and wild fish were calculated to the nearest meter using Arc/View. Dispersal was defined as the distance traveled from individual stocking sites by a fish that was alive and had a live transmitter at days 7 and 14. Dispersal was assessed in 2009 for hatchery fish and mean dispersal was compared between hatchery and wild fish in 2010 at days 7 and 14 using a non-parametric Mann-Whitney U test because the data was not normally distributed. Movement was defined as the sum of each distance traveled for all locations of an individual fish. Only locations where the fish was determined to be alive were included in movement analysis. We assessed the movement per day for hatchery fish in 2009 and compared the movement of fish that were eaten by predators versus fish that survived the experiment using a Mann-Whitney U test. We also compared the average movement per day between hatchery fish in 2009 and 2010, and movement of hatchery versus wild fish in 2010 using Mann-Whitney U tests.

Habitat use by hatchery and wild fish was described in 2009 and 2010 by taking the mean proportion of locations of individual fish found in each vegetation layer. Water level changes affecting our habitat map along with limited locations in each habitat made it difficult to compare specific habitat use for hatchery fish between years and hatchery to wild fish in 2010. The offshore edge of the vegetated zone was largely unaffected by water level changes and the number of locations in either offshore or inshore zones was sufficient to test for differences. Therefore, comparisons of offshore

use were made for hatchery fish between years and between hatchery and wild fish in 2010 using a Mann-Whitney U test.

Only live locations (where transmitter moved from previous location) were used in the analysis of movement and habitat use. We also excluded locations 2-4 days (Chapter 3) prior to when a tag stopped moving if predation was the assigned fate, because movement and habitat use during that time period could have resulted from a predator. Growth in percent body weight gained per day was compared between hatchery and wild fish surviving the experiment using a two-tailed t-test assuming equal variance. All tests in this study were considered statistically significant at $P < 0.05$.

Results

Range and accuracy of radio tag locations. Range and accuracy was first determined to learn the distances required to track each radio-tagged fish and the accuracy of each location at various depths and habitats. Transmitter range detection was negatively related to water depth. When the transmitter was placed in a known location at a 1-m depth (average depth of littoral zone), range varied from 300 to 600 m. When placed in a 2.5-m depth (average depth of offshore zone), range decreased and averaged 150 to 300 m. When a transmitter was dropped to the bottom of the lake where it was 4 m deep (the deepest known location in the study lake), the range to detect sound from the tag further decreased to an average of 100 meters. The type and density of vegetation or artificial structures did not appear to affect the range and accuracy of the transmitters. Locations of transmitters in the vegetated zone (±1 m) were more accurate than locations in the open water (±3 m), presumably due to shallower water depths and references (eg., plants and shoreline) useful to help

triangulate the location. This information was applied to the logistics, protocols, and strategies used for tracking radio-tagged largemouth bass each day on Lake Carlton.

Tag failure rates. A portion of the radio transmitters expired early in both years of tracking. In 2009, some missing transmitters gave abnormal pulses 1-2 days prior to disappearing, indicating that tag failure was occurring. Although assured by the manufacturer that the high rate of tag failure experienced in 2009 was rectified, early tag failure persisted in 2010, based on observations of control tags monitored in the lab. Two control tags failed to produce any sound as early as two days after they were activated in the lab and 36% failed by day 21. Field tags resulted in similar failure over the 30-day study period, although field tags were missing in a higher proportion from day 20 to day 30 (Figure 4-5). After analyzing the persistence of control tags in the lab by frequency, we found a significantly higher failure rate of tags with the 151 MHz frequency when compared to 149 MHz and 150 MHz (Figure 4-6). At day 21, control tags in the lab had low failure rates for frequencies of 149 MHz (10%) and 150 MHz (11%), whereas 82% of the 151 MHz frequency had failed. Transmitters with frequency 151 MHz comprised 36% (11 of 30) of the control tags and 18% (9 of 50) of the field tags in 2010 and thus, field tags should have resulted in an overall lower failure rate than observed in the lab. All of the 151-MHz tags during field studies had coincidentally been implanted into wild largemouth bass. The manufacturer (ATS) suggested that high failure rates of transmitters with frequency 151 MHz may have resulted from a flaw in the construction of these particular tags.

Hatchery bass stocking 2009. All fish implanted with radio transmitters (n = 50) survived the 24-hour recovery period following surgery and appeared healthy and active upon stocking. In four test cages, only one of the 12 radio tagged bass and four of 100 control hatchery fish died during the 3-day trial. We recorded 379 locations radio tagged of fish determined to be alive during this study segment. About one half of the hatchery fish survived the 14-day period and the other one half were confirmed deaths or disappeared (Table 4-2). Throughout the study period, predation was determined to be the cause of death for eight of the 50 tagged fish. Bird predation was confirmed for three of the eight mortalities. One transmitter had been eaten by a great blue heron Ardea Herodias. Two other radio transmitters were found evacuated with a distinct curling effect on the antenna and on dry ground within 10 m of the lakeshore under trees commonly used by double-crested cormorants Phalacrocorax auritus for roosting. Of the remaining five observed mortalities, one tag was found on the lake bottom and displayed distinct curling of the antenna. The other four deaths were deduced to be a result of predation based on movement and behavior prior to cessation of movement. Eighteen tagged fish disappeared from the study area; resulting from either tag failure or bird predation (i.e., birds carrying radio tagged bass outside of study area). The remaining fish (n = 24) survived at least 14 days post stocking and all tags expired by June 7, 2009 (19 days post stocking).

Losses due to mortality and missing fish were highest during the first seven days post stocking (Figure 4-7). Of the eight observed mortalities during this study, seven of the radio tagged largemouth bass died in the first week. Based on the survival curve and excluding missing fish, survival of radio-tagged hatchery fish in 2009 was estimated

to be 83% by 14 days (Figure 4-8). Because tag failure was not assessed in 2009, a range of survival to 14 days post-stocking was estimated from 48–84% depending on how missing fish were interpreted. If we assume that all missing fish were due to bird predation (i.e., tags were evacuated outside our study area), then only 48% (24 of 50) of hatchery fish survived the first 14 days post stocking. However, if we assume that all missing tags resulted from tag failures, hatchery survival could be nearly 83% at day 14 (Figure 4-8).

Hatchery and wild bass stocking 2010. All radio tagged hatchery largemouth bass (n = 30) and hatchery dummy tagged fish (n = 11) held in mortality test cages survived the 24-hour recovery period and appeared to be in excellent condition when stocked into Lake Carlton. Only one of the 21 (4.8%) dummy tagged bass distributed throughout six mortality test cages died during the 3-day trial. This dead fish was one of the 11 dummy tagged hatchery fish and was found dead during the 48-hour check. It is likely that the cause of death for this fish was related to stress associated with the tag implantation procedure, based on our lab studies on tag effects (Chapter 2). All of the dummy tagged wild fish were alive after three days. Among the 75 controls (i.e., non-radio tagged hatchery fish that had been spread evenly among three cages), no fish died. There were no mortalities among the 30 non-tagged wild fish that were spread evenly among three cages and served as controls for the radio tagged wild largemouth bass. Thus, we assumed handling mortality from surgical procedures and stocking to be negligible, and did not influence results. All transmitters expired within 34 days of stocking.

Hatchery-reared and wild bass ranged in total length from 93 to 121 mm and 97 to 122 mm, respectively (Table 4-1). Hatchery-reared largemouth bass were slightly shorter than wild fish (t_{48} = 1.93; P = 0.031) but weighed the same as wild fish (t_{48} = 0.31; P = 0.76), because wild fish were thinner than the pellet-fed hatchery fish. We recorded a total of 678 (390 hatchery and 288 wild) locations for radio tagged fish that were determined to be alive.

Half of the wild fish and only about a third of the hatchery fish survived the 30-day experiment (Table 4-2). Of the 50 radio tagged individuals, we determined that 16 fish died: 14 hatchery fish (47% mortality) and two wild fish (10% mortality). Survival through 30 days appeared to be much lower for hatchery bass than wild bass (Figure 4-10). There were marginal differences in survival rates between wild and hatchery fish (Wald x^2 = 3.54, P = 0.0599). Survival rates at 14 days post stocking were 91% for wild fish and 64% for hatchery bass. Survival rates at 30 days post stocking for wild and hatchery bass was 82% and 39%, respectively. We deduced that two of the radio tagged wild bass died from tagging stressors, because both fish stopped moving within three days post release and neither antenna was curled when their transmitters were later recovered. If both wild fish mortalities due to tagging were removed from this analysis, survival of wild fish would be 100% through 30 days. In that case, survival differences between wild and hatchery bass become much more significant. Sixteen tagged fish disappeared from our study area, which could have been due to either tag failure or avian predation. All mortality for hatchery and wild fish occurred within 14 days of stocking and 69% (11 of 16) took place in the first week.

Predation was determined to be the cause of all mortality of radio tagged hatchery largemouth bass (n = 14), because eight tags (27%) were found on the lake bottom with a distinct “curling” effect and the other six displayed radical movements 2-3 days prior to cessation of movement. The other six transmitters were not found because they were evacuated in deep water where tag recovery was virtually impossible and predation was determined based on movement and behavior prior to cessation of movment. Similar to 2009, bird predation was suspected in three (10%) of the 14 predation events. One radio tagged largemouth bass was tracked in a live cormorant in flight. Another transmitter was found on land with a curled antenna. A third was found evacuated on shore near a cormorant roost adjacent to Lake Beauclair, but had not been detected by the stationary receiver that had been placed to detect fish moving through the Carlton-Beauclair canal. Tagging mortality was determined to be the cause of death for both of the wild fish that died. We came to this conclusion because both fish ceased movement within three days of being stocked and upon tag recovery, antennas were straight and did not display the signs of having been consumed and evacuated by predators. Despite numerous attempts to retrieve the tags with the high strength magnet and electrofish the radio tagged fish if it was still alive, neither of the tags could be found until 10 days after movement stopped; suggesting that the tag was still inside of a fish until decomposition had fully taken place.

Hatchery fish suffered high predation (14 of 30) in the first 30 days post stocking (Table 4-2). We assumed that all missing radio tagged fish (n = 8) were tag failures, but lab studies indicate that only six should have failed (Figure 4-5). Thus, undetected bird

predation may have removed more transmitters from our study area, which would have made the estimates of predation mortality even higher for radio tagged hatchery largemouth bass. No predation was observed for radio tagged wild fish and it appeared that tags in the field failed in proportion to control tags in the laboratory. Two of 11 wild fish that were tagged from frequency 150 MHz became missing and this frequency had an expected failure rate of 20%. Nine wild fish were tagged with frequency 151 MHz (80% failure rate) and six of an expected seven became missing.

Dispersal. Dispersal was highly variable for individual fish in both years. The distance that hatchery radio tagged largemouth bass dispersed from their original stocking sites in 2009 ranged from 12 to 1,340 m after 7 days and 11 to 2,010 m at day 14 (Table 4-3). Hatchery largemouth bass dispersed rapidly; 17 of 27 fish (63%) dispersed more than 400 meters from their stock site within the first week (Figure 4-11). Hatchery tagged fish appeared to disperse much greater distances than wild tagged fish (Table 4-3) but again, there were no significant differences at day 7 (U = 174; P = 0.140) or day 14 (U = 54; P = 0.488).

Dispersal was commonly limited by the size of the lake and in both years, substantial numbers of tagged fish emigrated from the lake. Considering the diameter of Lake Carlton is only 1,400 m, some individuals circled around the entire lake and ended near their stocking location. In 2009, four of the remaining 27 (15%) fish had dispersed into Lake Beauclair by day 7 and by day 14, five of the remaining 23 (22%) fish had moved into Lake Beauclair. In 2010, seven of the 50 (14%) fish were observed emigrating from Lake Carlton, all of which were hatchery-reared fish (23%).

Movement. Average individual movement of hatchery fish was variable among individuals and similar between years. Total movement for hatchery fish locations in 2009 ranged from 11 m for a radio tagged largemouth bass that only persisted four days (3 m per day) to 6,005 m for a fish that persisted 18 days (334 m per day). Distances moved per day during the study in 2009 were not found to be statistically significant (U = 78; *P* = 0.795) between fish that survived (mean = 113 m) and fish that died (mean = 155 m). Movement per day for radio tagged hatchery bass that were located at least once (n = 47) ranged from 3 m to 500 m in 2009 and 1 m to 456 m in 2010 (Table 4-3). Average movement per day was not significantly different (U = 830; P = 0.114) in 2010 between hatchery fish in 2009 (124 m) and 2010 (75 m). In 2009, 49% of radio-tagged fish moved over 100 m compared to only 21% in 2010 (Figure 4-12).

In 2010, hatchery fish displayed a high range of movement and wild fish consistently moved very little (Figure 4-12). Hatchery fish had significantly higher (U = 444; *P* = 0.002) movements per day than wild fish (Table 4-3). Only one wild fish (5%) in our study moved over 50 m per day on average compared to 52% of the hatchery fish (Figure 4-12). The maximum distance traveled by a hatchery fish was 3.03 km in a 24-hour period in 2009, which included swimming the distance of Lake Carlton. We confirmed this was a live hatchery bass (and not a tag being carried by a predator) by capturing it with an electrofishing boat.

Habitat use. Habitat use varied by individual hatchery fish in 2009 and 2010 commonly using offshore habitats whereas wild fish mostly used vegetation (Figure 4-13). The offshore zone (excluding buffer) comprised 146.0 ha (92.7%) and the inshore vegetated zone (including buffer) contained 11.4 ha (7.3%). Some fish were

consistently located in open water, while others were consistently located in dense littoral zone vegetation. Average percent of offshore locations were not significantly different (U = 837; *P* = 0.063) between hatchery fish in 2009 (34.7% SE = 4.8) and 2010 (23.0% SE = 5.1). A Mann-Whitney U test revealed a significant difference (U = 370; *P* = 0.036) in offshore use between hatchery (23.0% SE = 5.1) and wild fish (7.9% SE = 4.1). In a direct comparison, 11 of 30 (37%) hatchery fish were located offshore on at least 30% of their locations, whereas only 1 of 20 (5%) wild fish displayed this behavior.

Although the exact depth tagged fish were suspended at was unknown, the mean depth and water temperature were determined from locations where the fish was alive. In 2009, mean depth was 1.02 (SE = 0.08) and mean water temperature was 26.7°C (SE = 0.12; range 22.9 – 31.7˚C). In 2010, mean depth used by hatchery fish (1.37 m; SE = 0.10) was significantly deeper (t_{47} = 2.47; *P* = 0.026) than that used by wild fish (1.04 m; SE = 0.10). Water temperature in 2010 ranged from 19.8°C to 25.2°C and averaged 23.7°C during the 30-day experiment.

Lake Carlton's littoral zone consists of a relatively homogenous band of vegetation and thus, we have little ability to detect differences in habitat selection with a small number of fish and short battery life, which limited the number of locations. Although we only tracked fish for 30 days post stocking, we did observe some fish begin to make habitat choices. For example, one fish resided for seven days within 50 m of its stock site and then proceeded to travel from 300-800 m per day for four days to an area of complex habitat (eelgrass adjacent to emergent vegetation) in Lake Beauclair where it spent the final 19 days (all within 50 m) prior to being captured by electrofishing.

Growth. Radio tagged hatchery and wild bass surviving the study in 2010 differed in growth rate (Figure 4-14). Surviving radio tagged wild fish used in this analysis had similar (t_{12} = 1.5; P = 0.16) initial total lengths as surviving hatchery fish. Wild radio tagged fish grew an average of 1.73% of their body weight per day, which was significantly higher (t_{12} = 3.83; P = 0.002) than hatchery fish (0.41%; Figure 4.14). Although tag presence was shown to impair growth of tagged individuals in 30-day laboratory trials (Chapter 2), wild fish growth rates in Lake Carlton were comparable to untagged fish growth seen in the lab that were fed pellets. This high rate of growth indicates that tagged wild fish were able to forage efficiently soon after the surgical implantation of radio tags.

Discussion

This study represents the first attempt to compare the behavior and survival of stocked hatchery and wild largemouth bass, and it demonstrated the benefits of using radio telemetry. My findings suggest that domestication effects of hatchery-reared bass can have a negative influence on behavior and survival after release into the wild. Post stocking survival was lower for hatchery-reared fish than wild bass during the first 14-days and predation was largely responsible for the mortality of hatchery bass. Further, we noted behavioral differences between wild and hatchery fish that could have contributed to high mortality of hatchery fish. After the initial high mortality, survival of hatchery fish equaled that of wild fish through 30 days. If domesticated hatchery fish could be acclimated to wild conditions to modify their behavior closer to that of wild fish prior to their release, improvements in survival could be expected.

Survival rates of juvenile fish surgically implanted with radio tags should be interpreted with caution; they should be considered the minimum survival rate of non-

tagged individuals (i.e., due to the possibility of mortality related to tag implantation). However, stress due to tagging was nearly equal between treatments, and we demonstrated significant differences in survival between hatchery and wild fish due to predation. Similar to our results in this study, Ebner and Thiem (2009) used radio telemetry to quantify large differences in survival for hatchery (9%) and wild (95%) trout cod *Maccullochella macquariensis* at 13 months post release. Dieperink et al. (2001) radio tagged 50 wild and 50 hatchery sea trout and found that hatchery-reared fish had significantly higher mortality than wild fish. Although our mortality at 30 d of 62% for radio tagged hatchery fish in 2010 seems high, Pouder et al. (2010) who studied found 95% mortality of advanced fingerling hatchery largemouth bass 90 d post stocking. Hatchery largemouth bass stocked in Lake Carlton (73 fish/ha) simultaneously with our radio tagged fish in 2010 had an estimated 98% mortality one year post stocking (FWC unpublished data).

Predation by fish and avian predators was determined to be the sole source of mortality experienced by hatchery fish both years while wild fish appeared to avoid predation. Evidence suggests that antipredator behavior in hatchery-reared fish is not developed (Olla and Davis 1989). In 2010, fish predation was deduced to be responsible for 50% mortality and avian predators were responsible for 14%. This estimate of avian predation may have been higher if the rate of transmitter failure was not so high. The transmitter located in a live great blue heron was not observed again, suggesting that the bird evacuated the tag outside the study area. Jepsen et al. (1998) researched the survival of radio-tagged Atlantic salmon and found pike to be responsible for 56% of the observed mortality and avian predators 31%. Avian

predation was shown to be extremely high by Dieperink et al. (2001) when they observed 65% mortality by birds in hatchery-reared sea trout. Stein et al. (1981) also found largemouth bass to be significant predators on stocked tiger muskellunge (*Esox masquinongy* x *E. lucius*), accounting for 26% and 45% mortality of muskellunge stocked in two lakes. In my study, the majority of the observed predation of hatchery fish occurred during the first 7 days post stocking in both years and all predation occurred during the first 14 days. This suggests that vulnerability to predation is high immediately after release and then survival may reach levels near wild fish. We did not observe natural mortality of hatchery fish due to starvation, but starvation could influence vulnerability to predation. Pellet-reared hatchery largemouth bass often have exceptional fat reserves upon stocking. If predation due to starvation had occurred, we expect we would have likely seen higher predation after 14 days post-stocking when low reserves and fatigue would have rendered hatchery fish more vulnerable. Because initial predation was high and wild fish avoided predation, we suggest that naivety to predators was the primary cause for the observed high predation.

This study demonstrates that advanced fingerling hatchery largemouth bass are capable of large-scale dispersal. In 2009, we found that 37% of stocked hatchery bass had dispersed over 700 m by 7 days post stocking. These results are similar to fingerling largemouth bass stocked in Tennessee (Hoffman and Bettoli 2005) where 31% had dispersed over 600 m in one embayment after 7 days. In our study, we also didn't see large dispersal increases from 7 to 14 days post stocking, which agrees with previous research suggesting that high initial dispersal will stabilize (Buckmeier and Betsill 2002; Hoffman and Bettoli 2005). Although dispersal for both hatchery and wild

fish was highly variable and differences were not significant, only one wild fish (7%) dispersed over 300 m at 7 days post stocking compared to 26% of hatchery fish. Copeland and Noble (1994) found that age-0 wild largemouth bass in North Carolina tagged with coded wire tags rarely moved from coves where they were tagged. Other research studying wild and hatchery fish movement have shown higher dispersal for hatchery stocked fish in trout cod *Maccullochella macquariensis* (Ebner and Thiem 2009) and rainbow trout *Oncorhynchus mykiss* (Bettinger and Bettoli 2002). Our radio telemetry study indicates that hatchery largemouth bass are capable of traveling long distances from their stocking site within one or two weeks and stocking evaluations for largemouth bass should consider this in their sampling design.

We observed marked differences between the post-stocking movements made by hatchery largemouth bass and wild bass. The significantly greater daily movements of the hatchery fish in our study was similar to patterns demonstrated by stocked and wild juvenile chubs (Bolland et al. 2008). In 2010 of my study, higher movement was observed, although not significant due to small sample size, for hatchery fish that died (155 m) compared to fish that survived the study (113 m). This, combined with the lower movement observed for wild fish that avoided predation would suggest that higher movement results in higher mortality. Aarestrup et al. (2005) used radio telemetry to investigate the movement and mortality of stocked brown trout and found surviving fish had significantly lower mean movement per day than fish that died. I suspect that domestication in hatchery raceways, inability to capture prey, and naivety to predators could contribute to higher rates of movement of hatchery bass than wild bass. These raceway cultured hatchery fish have absolutely no experience living in a natural lake

environment, they very likely become disoriented, and they may instinctively attempt to find surroundings that they had become familiar with in the hatchery.

Differences in habitat use were detected between wild and hatchery-reared largemouth bass during this initial post stocking period. Hatchery fish tended to occupy areas in open water more often than wild fish. Wild fish selected areas of high complexity along the shoreline in shallower water; possibly to utilize cover to avoid predators, which would also have reduced energy expenditure associated with open water. Bolland et al. (2008) found similar results for juvenile chubs, where hatchery-reared fish spent significantly more time offshore than wild fish (68.2% vs. 24.1%, respectively). Because stocked fish were often located in open water, and most evaluations of stocked hatchery largemouth bass rely on electrofishing in littoral zones, electrofishing could underestimate the contribution or survival of hatchery fish.

The offshore habitat use and high movement exhibited by hatchery-reared bass could result from the complete lack of habitat complexity that these fish experience while developing in hatchery raceways and/or other domestication issues. The foraging arena theory (Walters and Juanes 1993) describe the continual decisions a fish must make with inherent trade-offs between preferred foraging areas and predation risk (Walters and Martell 2004). Fish using offshore habitats may increase vulnerability to predation with reduced available cover along with reduced capture efficiency of prey compared to the vegetated littoral zone. Savino and Stein (1982) found that largemouth bass were unable to capture bluegills *Lepomis macroc* in uniform stem densities of 250 or 100 stems m^{-2} and were eaten more when venturing into open water (Svino and Stein 1989). Hatchery fish's naivety to predators and lack of efficiency capturing prey

(Pouder et al. 2010) likely produced extremely risky “foraging arenas”; resulting in the observed high predation mortality.

Radio tagged hatchery largemouth bass exhibited significantly slower growth rates compared to wild fish during the course of this experiment. Hatchery largemouth bass reared on artificial diets have been shown to have significantly reduced foraging efficiency (Colgan et al. 1986). This could hinder growth by reducing the total prey consumed during the study period and increasing the energy expended on foraging due to a high proportion of failed predation attempts. In a study comparing diets of advanced fingerling largemouth bass to wild bass, Pouder et al. (2010) observed differences in diet similarity and a significantly greater proportion of hatchery fish had empty stomachs than wild fish at 7 d post stocking, suggesting an inability of the hatchery fish to transition to live prey. In a direct comparison, we observed slow growth for hatchery fish which could suggest foraging inefficiencies. Behavioral differences observed in this study such as high rates of movement and open water habitat used by hatchery bass could have conceivably furthered energetic loses and contributed to slower growth. Wintzer and Motta (2005) demonstrated differences in skull morphology for largemouth bass raised on artificial versus live feed which could also influence successful predation in the initial stocking period. Predation appeared to be the primary cause of mortality during this study, however fish weakened by hunger may be more likely to fall victim to predation (Brown and Laland 2001). Thus, an inability to transition to natural prey or lack of recognition of where prey was located may have indirectly contributed to predation. By day 14, 30 and 60, Pouder et al. (2010) saw similar diets

between hatchery and wild fish, again suggesting after 14 days, surviving hatchery fish behave and survive similar to wild fish.

This study revealed survival and behavioral differences for hatchery largemouth bass compared to wild fish, suggesting domestication effects continue to affect stocked hatchery largemouth bass post release. Factors such as high rates of movement, use of open water habitats, reduced growth, and poor foraging efficiency could cause excessive energy expenditure and increased vulnerability to predation. Conversely, wild fish had low rates of movement, utilized complex habitats more consistently, and displayed high growth rates; resulting in no observed predation. The results of the wild bass stocking demonstrated that advanced fingerling-sized largemouth bass can have high short-term survival after being stocked into a lake with high densities of predators. This suggests that low stocking survival of hatchery-reared fish was due to domestication issues and not degraded lake conditions. If hatchery-stocked fish survive as well as wild fish in this study, the effectiveness of largemouth bass stocking would likely be improved. We suspect that the experiences that wild fish had during the first year of life (awareness of predators, use of complex habitats to avoid predation, foraging efficiency, energy conservation) were responsible for their high survival relative to hatchery fish when stocked into Lake Carlton. Schlechte and Buckmeier (2006) found that habituation 60 min before releasing stocked largemouth bass into research ponds significantly reduced predation. Although it's difficult to draw accurate inferences from ponds studies to natural lakes, Brennan et al. (2006) found that acclimation (3 d) at release sites has the potential to significantly improve post-release survival of juvenile hatchery-reared common snook. Opportunities to provide hatchery-reared largemouth

bass with similar learning experiences should be investigated and could result in higher initial survival (Suboski and Templeton 1989; Brown and Laland 2001).

Table 4-1. Number of fish radio tagged, total length (mm), weight (g) and tag to body weight ratio (%) for hatchery-reared fish (2009 and 2010) and wild fish collected from Starke Lake in 2010.

	Number tagged	Total Length (mm) [mean ± SD (range)]	Weight (g) [mean ± SD (range)]	Tag ratio [mean (range)]
Hatchery 2009	50	105.0±5.6 (97–120)	13.6±2.9 (9.4–21.1)	1.76 (1.14-2.55)
Hatchery 2010	30	105.8±8.1 (93–121)	13.7±3.5 (8.4–22.2)	1.76 (1.08–2.86)
Wild 2010	20	110.7±6.8 (97-122)	13.4±2.8 (8.6-19.8)	1.80 (1.21-2.79)

Table 4-2. Fate of radio tagged hatchery and wild stocked juvenile largemouth bass at the end of the study period in 2009 and 2010. Actual numbers are presented in parentheses.

	Percentage of total released		
	Disappeared	Died	Survived
2009 Hatchery (14 d)	36% (18)	16% (8)	48% (24)
2010 Hatchery (30 d)	27% (8)	47% (14)	27% (8)
2010 Wild (30 d)	40% (8)	10% (2)	50% (10)

Table 4-3. Dispersal and movement for radio tagged hatchery (2009 and 2010) and wild (2010) advanced-fingerling largemouth bass. Dispersal (7 and 14 day) and movement per day are presented as mean ± SE (m) with the range in parentheses.

	Dispersal		Movement
	7 day	14 day	Per day
Hatchery 2009	582±88 (12–1340)	661±121 (11–2010)	124±18.1 (3–500)
Hatchery 2010	272±68 (7–1110)	400±172 (7–1528)	75±16 (1-456)
Wild 2010	153±51 (12–703)	184±68 (12–685)	25±10 (1–211)

Table 4-4. Initial and ending total lengths (mm) and weights (g) for radio tagged hatchery and wild largemouth bass stocked into Lake Carlton on 30 March 2010 and collected via electrofishing. Two fish had no weights (Unk).

Fish origin	Date Collected	Initial Measure		Ending Measure	
		TL (mm)	Weight (g)	TL (mm)	Weight (g)
Hatchery	28 April	110	14.2	116	16.1
Hatchery	17 April	115	17.5	118	16.0
Hatchery	17 April	114	15.5	117	18.7
Hatchery	28 April	98	10.2	105	11.6
Hatchery	28 April	112	16.5	122	20.0
Hatchery	28 April	111	15.0	116	17.4
Hatchery	28 April	109	15.0	114	15.2
Wild	13 April	121	18.0	127	Unk
Wild	28 April	108	13.3	116	17.8
Wild	28 April	115	13.5	131	23.4
Wild	17 April	112	14.2	120	16.7
Wild	28 April	110	11.6	117	17.7
Wild	27 April	122	19.8	141	31.8
Wild	16 April	112	14.3	120	Unk

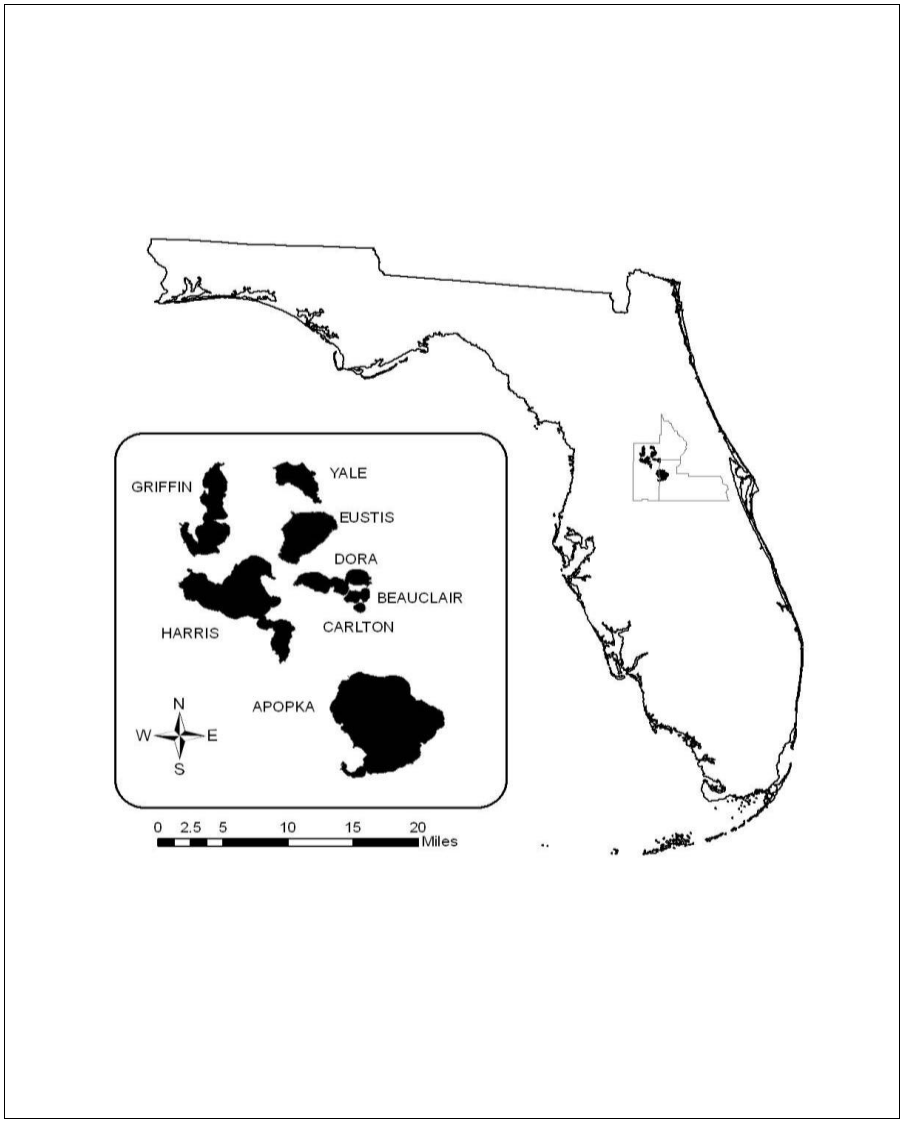

Figure 4-1. Illustration of primary study area depicting the geographical location and schematic of the water bodies that comprise the Oklawaha Chain of Lakes. Lake Carlton (157 hectares) in Lake County, Florida was used as the study lake for radio telemetry.

Figure 4-2. Satellite image showing the canals connecting Lake Carlton to Lake Beauclair (located at the top of Lake Carlton in this image) and Horseshoe Lake (located at the bottom of Lake Carlton). Points on the map also show the locations from 50 stocked advanced-fingerling bass as determined by radio telemetry in 2009.

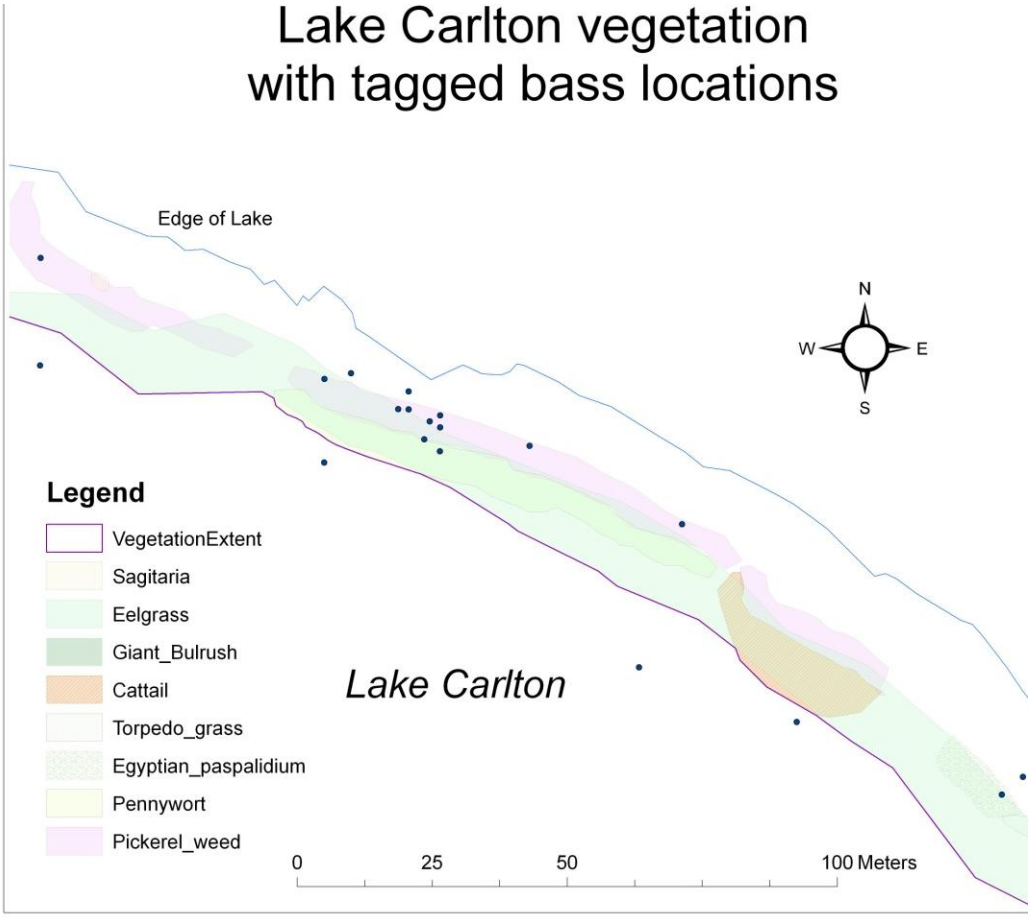

Figure 4-3. Example of the detailed Lake Carlton vegetation map developed using ArcMAP™ software. Telemetry locations of radio tagged bass are illustrated by black dots in the littoral zone. The vegetation map does not extend to the lake edge during 2010, because the vegetation community was mapped during low-water conditions of 2009. The vegetation between the mapped communities and the lake edge, if any, were primarily terrestrial species.

Figure 4-4. Photograph of the surgical procedure used to implant a radio transmitter in an advanced-fingerling largemouth bass.

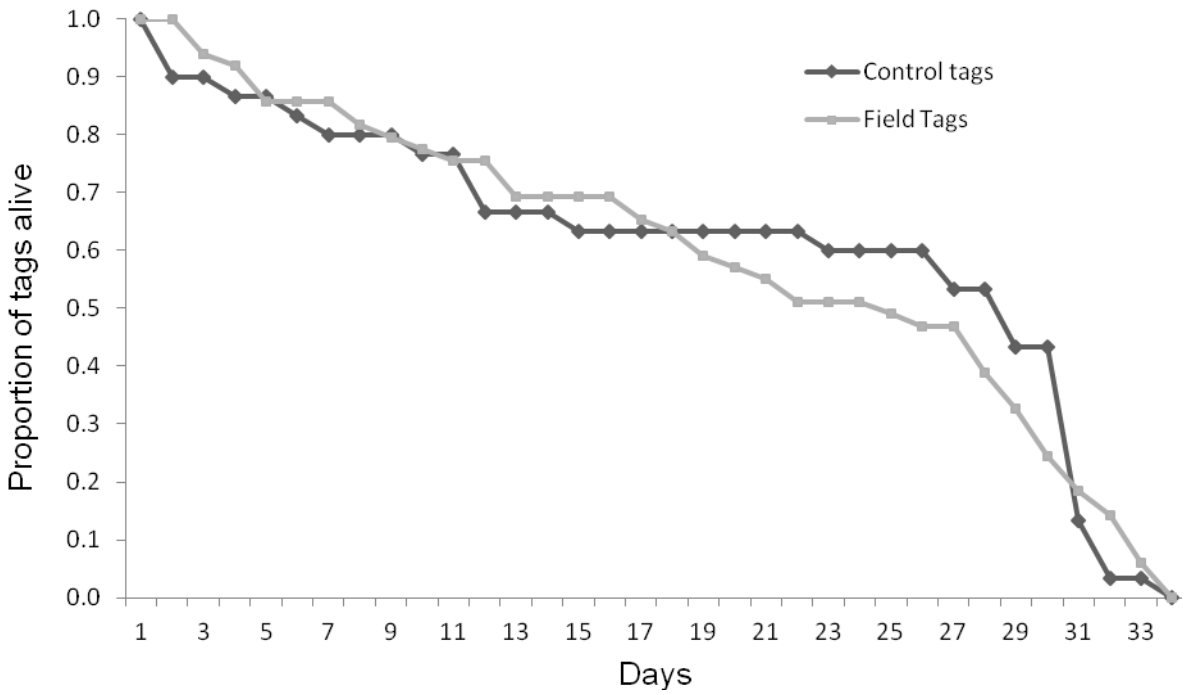

Figure 4-5. Proportion of tags that remained operational over a 34-day period as measured by live control tags that were allowed to expire in the laboratory and the field tags used in Lake Carlton in 2010.

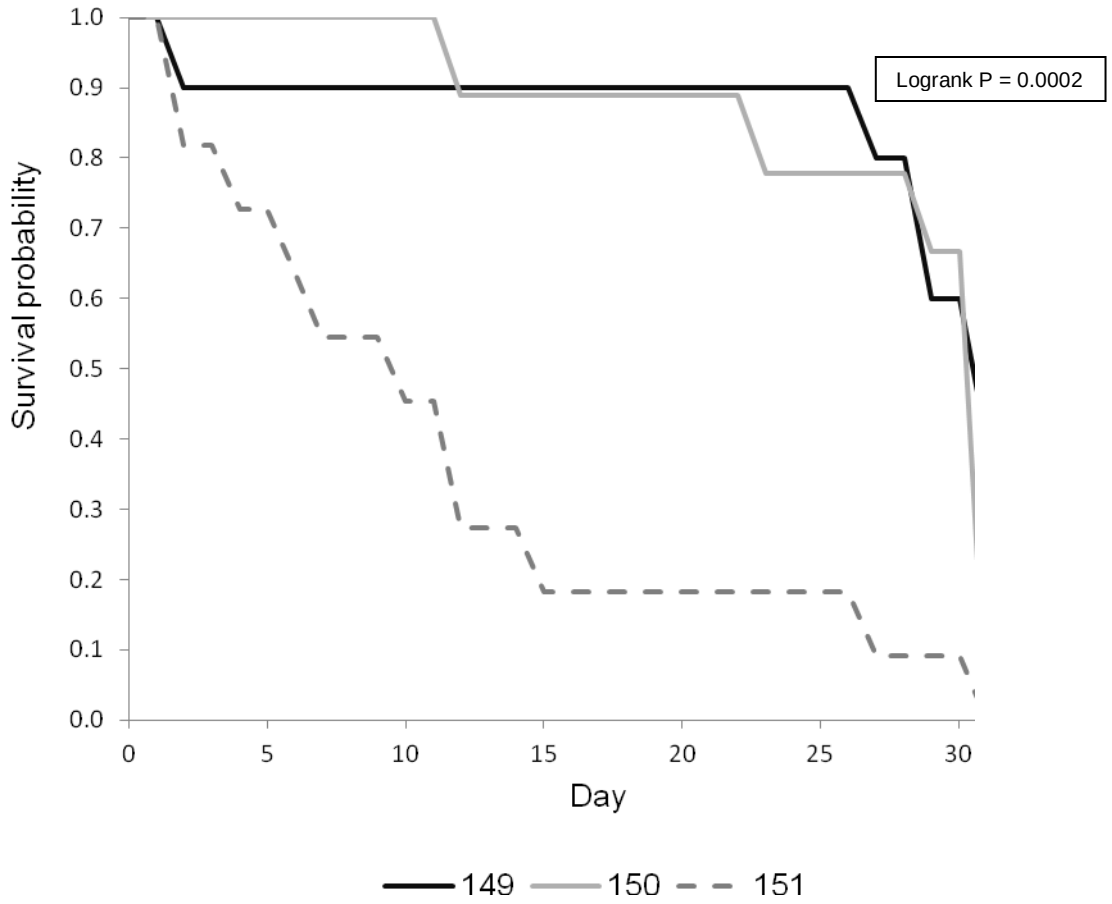

Figure 4-6. Persistence of 30 control radio tags allowed to expire in the lab. The y-axis is the probability that a transmitter frequency will continue to emit a signal each day after the battery was started. Transmitter frequencies (MHz) tested were 149 MHz (n = 10), 150 MHz (n = 9), and 151 MHz (n = 11).

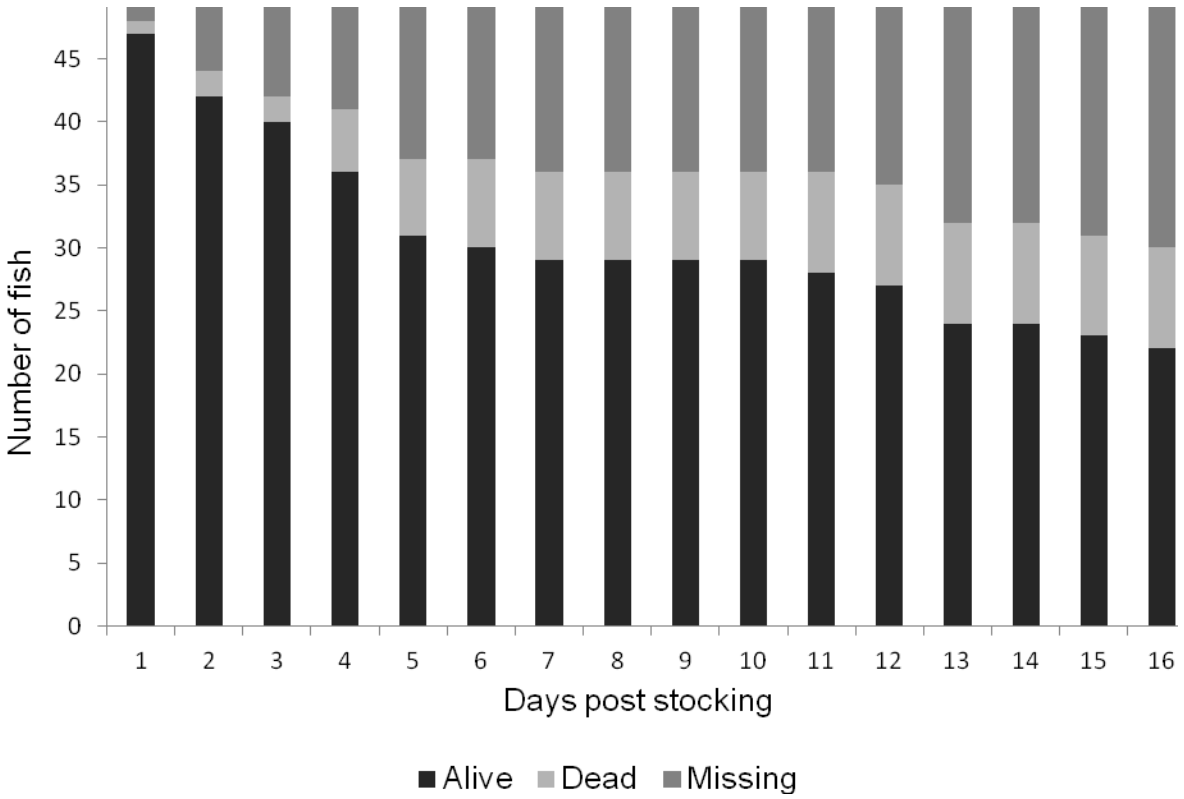

Figure 4-7. Fate of radio tagged hatchery largemouth bass during the 2009 study period. The reporting period began the first day after fish were stocked into Lake Carlton.

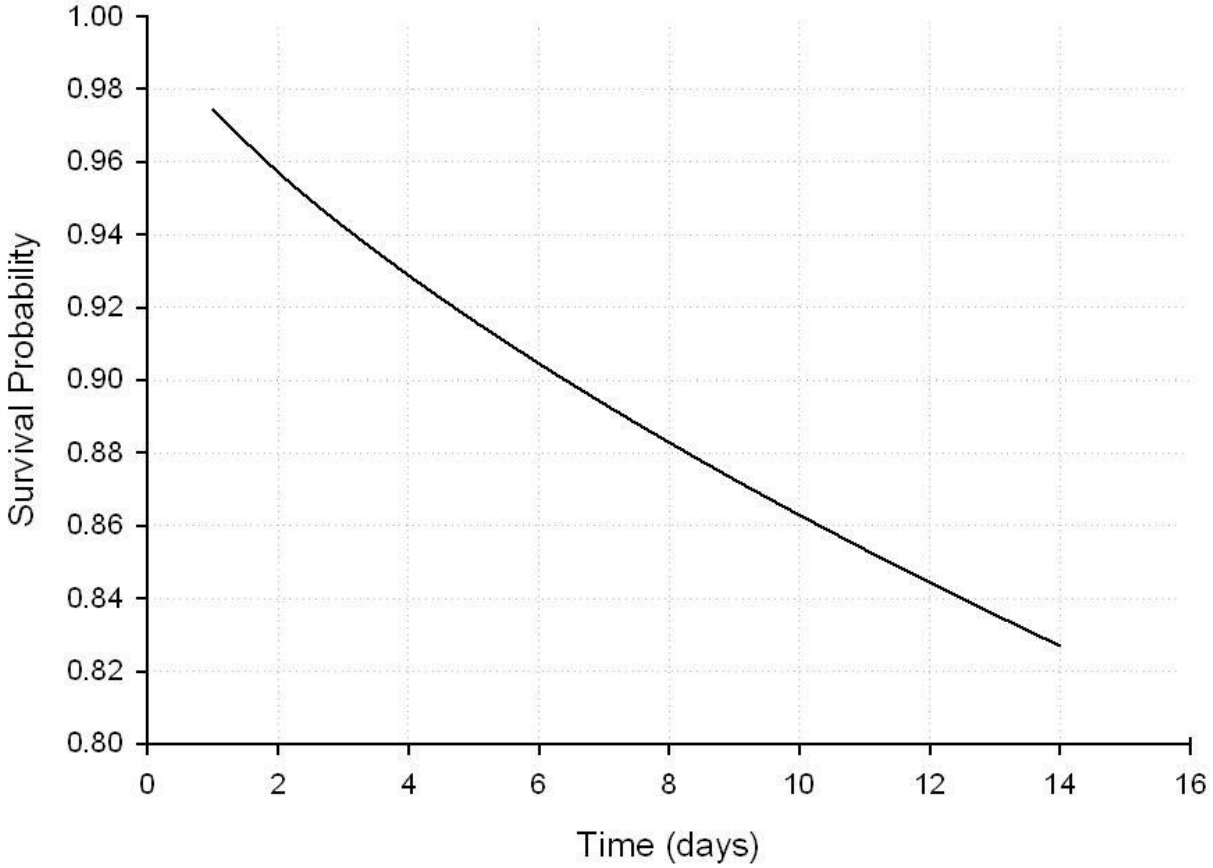

Figure 4-8. Survival propabilities over 14 days for radio tagged hatchery largemouth bass stocked in Lake Carlton in 2009.

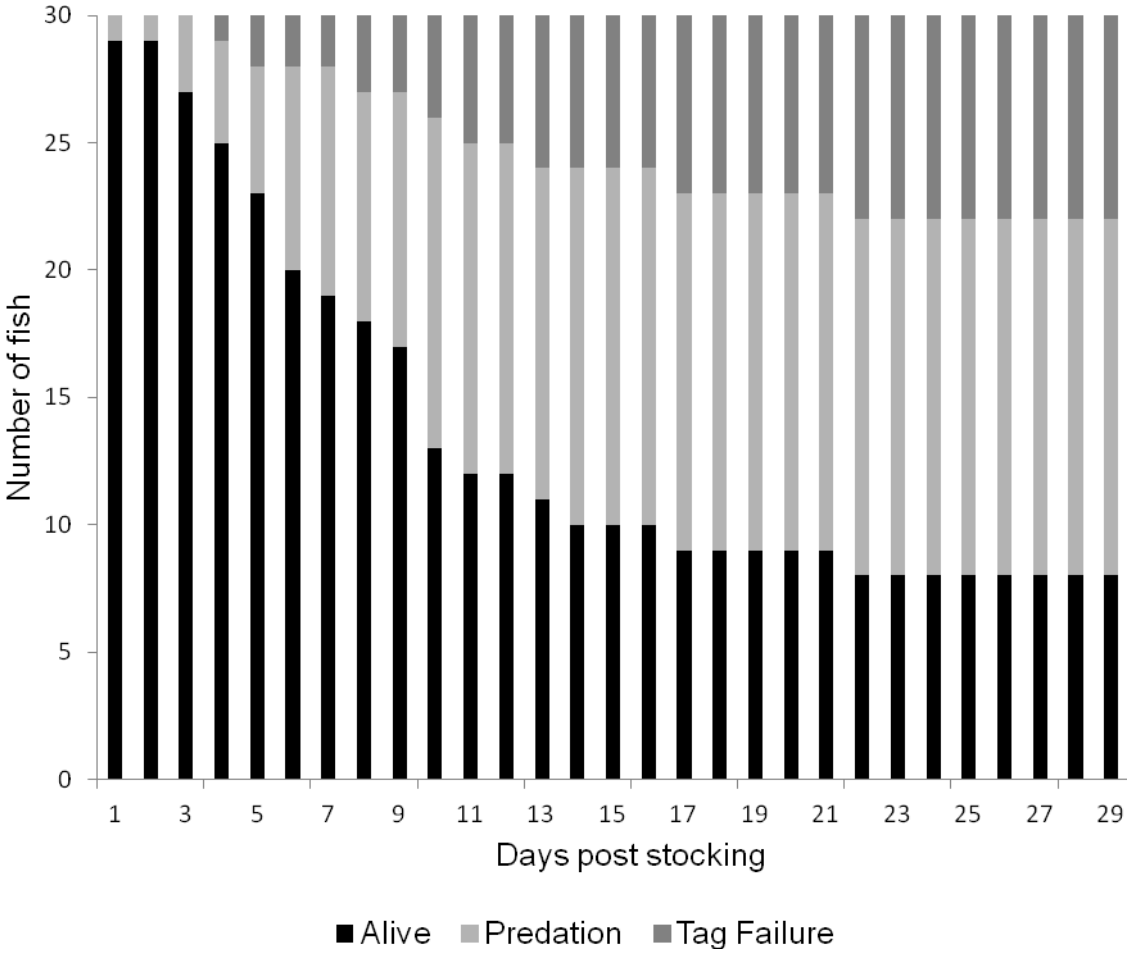

Figure 4-9. Fate of radio tagged hatchery largemouth bass throughout the 2010 study period after being stocked into Lake Carlton.

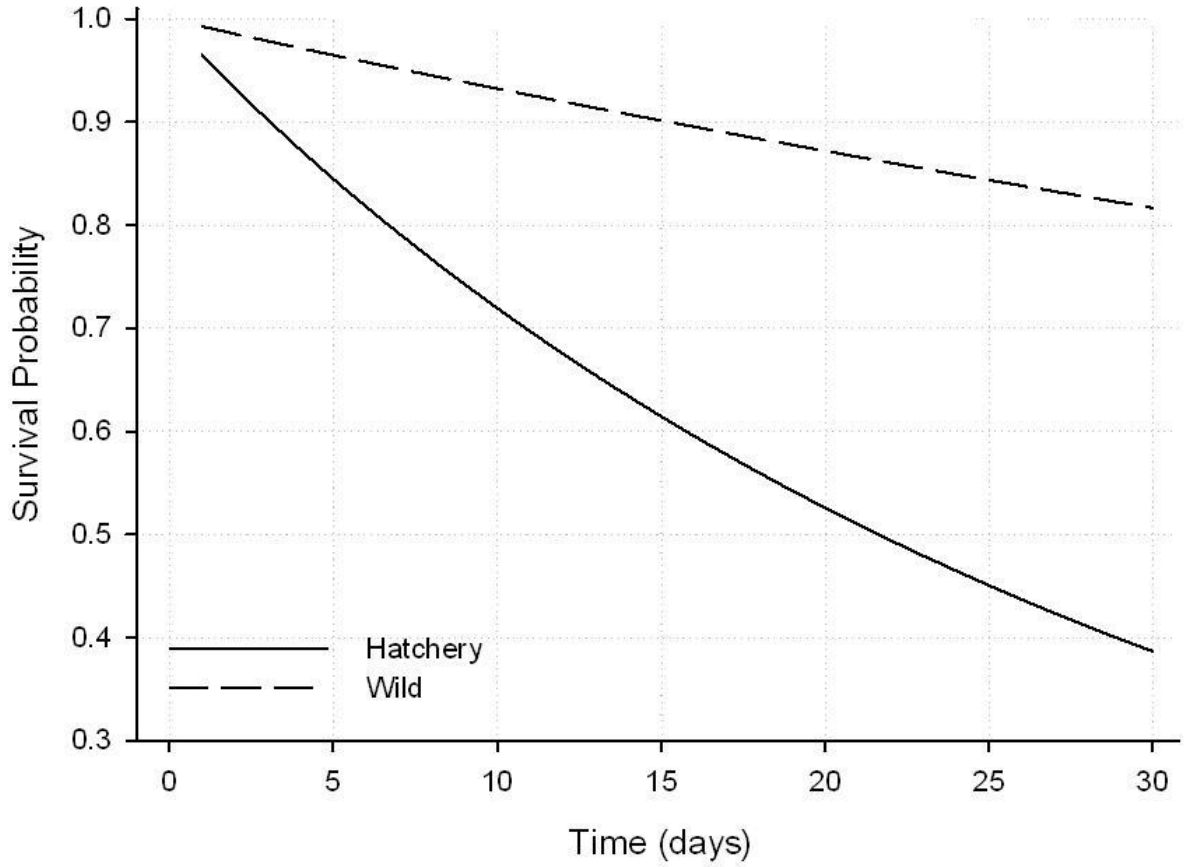

Figure 4-10. Survival propabilities over 30 days for radio tagged hatchery and wild largemouth bass stocked in Lake Carlton in 2010.

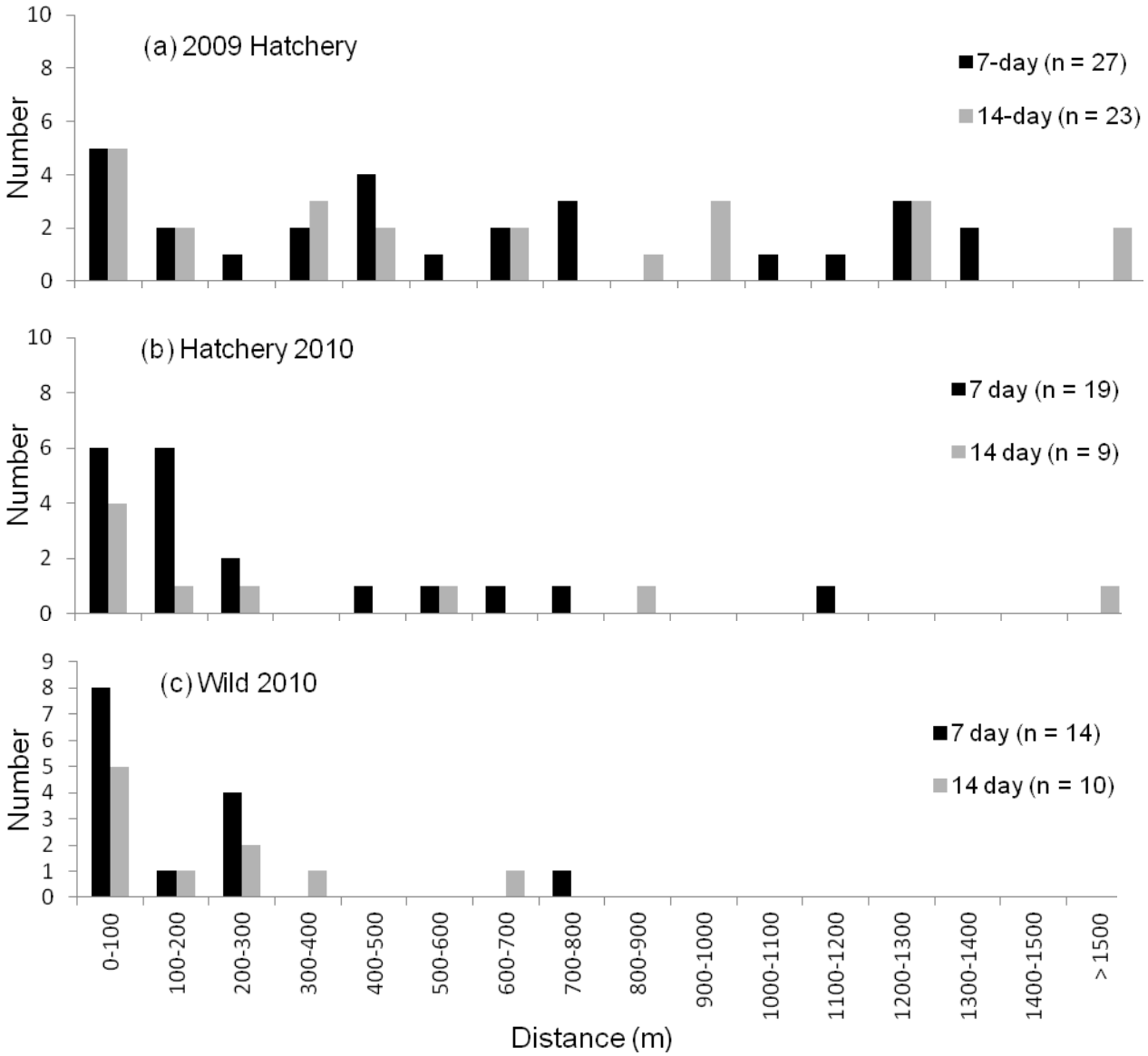

Figure 4-11. Dispersal of radio tagged hatchery and wild largemouth bass (number of individuals) at 7 and 14 days post stocking for: (a) hatchery 2009; (b) hatchery 2010; and (c) wild 2010 fish.

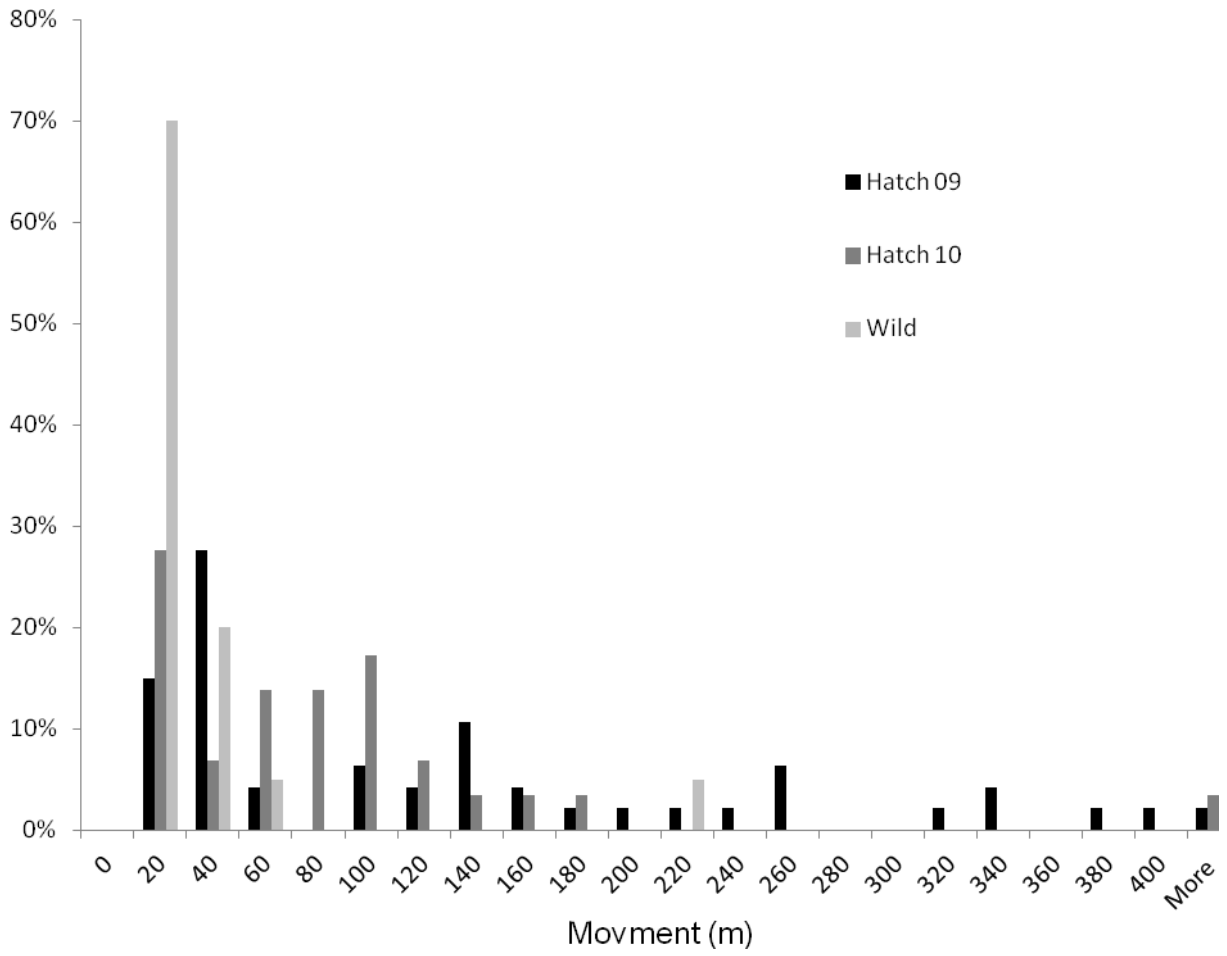

Figure 4-12. Average movement per day of radio tagged hatchery and wild largemouth bass for live locations that survived greater than two days after stocking.

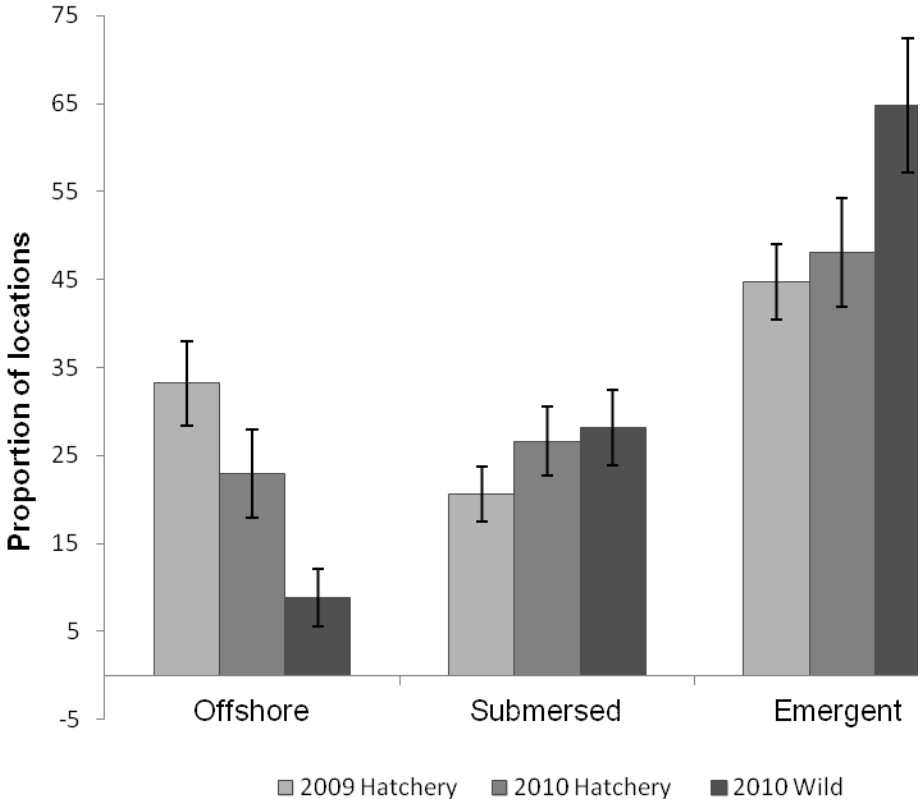

Figure 4-13. Habitat use of hatchery and wild radio tagged largemouth bass stocked in Lake Carlton in 2009 and 2010, reported as the mean proportion of observations that occurred in each habitat type.

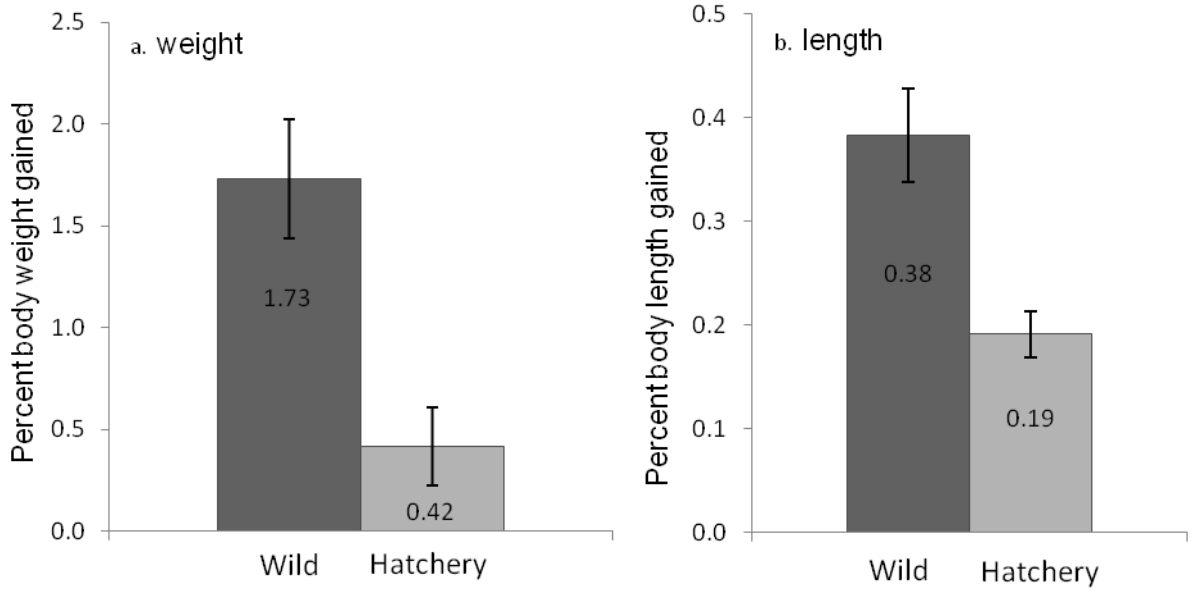

Figure 4-14. Comparison of percent body weight (a) and length (b) gained per day between radio tagged wild (n = 7) and hatchery bass (n = 7) upon recapture near the conclusion of telemetry study in 2010.

CHAPTER 5
CONCLUSION

The objectives of this study were three-fold: (1) to determine the effects of surgically implanting transmitters on growth, survival, and predator avoidance of small bass to validate whether or not tagged animals adequately represent untagged animals; (2) to determine if radio transmitters evacuate through predators after consumption and develop an evacuation model which can be used to predict evacuation rates of tags; (3) to compare the behavior and survival of hatchery- reared largemouth bass to similar size wild juvenile bass after being stocked into a lake.

This study successfully radio tagged some of the small juvenile fish to date. Prior to field application, we were able to document the effects of the surgical process and transmitter presence on the growth and predator avoidance for juvenile largemouth bass. This study confirmed that warm water fish such as largemouth bass could potentially be radio tagged with little effect of their behavior. Using radio tags to estimate predation on juvenile largemouth bass would be appropriate because we saw no difference in predator avoidance between tagged and untagged fish. Depending on the objectives of the researcher, transmitters representing 1.2 to 2.7% of the fish's body weight may not be appropriate for estimating growth. Study design for field application should recognize the potential and limitations for this technology.

As the number of telemetry studies increase for juvenile fish that estimate survival from tagged individuals, evacuation of transmitters by potential predators becomes a factor when inferring survival and behavior information. In this study, we were able to document that microtransmitters did not accumulate in the intestine of adult largemouth bass that consumed juvenile largemouth bass and that evacuation rates could be

estimated relatively accurately. This allowed more accurate fate determination for largemouth bass that died in the field and allowed accurate behavior data to be applied to the tagged individual.

This study documented behavioral and survival differences between wild and hatchery-reared largemouth bass. Significant differences in predation, movement, habitat use, and growth suggests that fish raised in domestication are less fit to survive initially in the wild. Unless these deficiencies can be reversed by conditioning hatchery fish to the wild, stocking pellet-reared advanced fingerling largemouth bass has limited application to supplement existing populations.

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BIOGRAPHICAL SKETCH

Brandon Charles Thompson was born in Wausau, Wisconsin in 1979. His love of the outdoors, passion for fishing, and fear of an office job led him to seek a career in fisheries science. In 2003, he earned a BS. in Fish and Wildlife Sciences at the University of Wisconsin–Stevens Point. He reinforced his drive to succeed in fisheries while working fisheries internships through college for the Wisconsin DNR and US Forest Service. After graduating from Stevens Point, Brandon put off graduate school to gain experience and ensure this was the field he wanted to make a career. He took a temporary job with the Wisconsin DNR restoring trout habitat and he realized that he could not only work in a job he loved, but also make a positive difference in the natural resources around him. He then accepted a position with the U.S. Fish and Wildlife Service in California monitoring salmon and steelhead populations. He finally moved to Florida to work for the Florida Fish and Wildlife Conservation Commission as a fisheries biologist. After working three years, Brandon decided that fisheries career was indeed what he desired and a graduate degree would help him conduct his work at a higher level. He was able to start taking courses in the fall of 2009 under Dr. Mike Allen while working for the FWC and started his master's research working on radiotelemetry with hatchery largemouth bass. Brandon completed his courses and research in 2012.