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Hybrid striped bass: culture and use in Midwestern waters

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Hybrid striped bass: Culture and use in Midwestern waters

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DEDICATION

The following work is dedicated to my parents John and Judy Wamboldt for helping me get to where I am and for the accomplishments I have made. It has only been possible through their dedication and sacrifice.

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CHAPTER 1. GENERAL INTRODUCTION

Background

In recent years, increasing demand for hybrid striped bass *Morone saxatilis* × *M. chrysops* has peaked interest for many natural resource agencies beyond the Atlantic seaboard to begin culturing them for introductions into state fisheries (Mauldin 2002; Ludwig 2004; Woods 2005). Hybrid striped bass have become increasingly popular for both the commercial production of food fish as well as for management stocking of sport fisheries (Harrell et al. 1992; Weirich et al. 1992; Bergerhouse 1993). Aside from enhancing a fishery with the addition of a large, sought-after predator, hybrid striped bass are increasingly being stocked for biological management of over-abundant or underutilized prey species (Jenkins and Smith 1985; Jahn et al. 1987; Dettmers et al. 1998; Neal et al. 1999a; Ostrand et al. 2001; Olson et al 2007).

The original hybrid striped bass, known as the palmetto bass, was first produced by Robert Stevens in 1965 by crossing a female striped bass *Morone saxatilis*, with a male white bass *Morone chrysops* (Stevens 1966; Kohler 2004). Due to limitations associated with maintaining female striped bass for propagation in states outside of the Atlantic seaboard, the majority of hybrid striped bass cultured in the United States are the reciprocal cross, or sunshine bass; herein referred to as two different taxa (Morris 1999; Kohler 2004). Both hybrids are considered to be ideal culture candidates because they have an increased growth rate during their first 2 years (hybrid vigor), adapt to formulated feeds, and are more resistant to diseases than other *Morone* species (Morris 1999; Kohler 2004). Kohler (2004) considered the hybrid striped bass to be an aquaculture species of high priority because of its importance as a cultured foodfish as well as its potential to be used for enhancing many sport fisheries.

Hybrid striped bass culture is divided into three distinct phases based upon specific life stages and production needs of the fish. Phase I in the culture period consists of larval fish to 2.5-cm fingerlings and typically lasts 30-45 days (Hodson and Hayes 1989; Harrell et al. 1992; Morris et al. 1999). Because phase I fish rely on live food items (copepods, cladoceran, and *Artemia* sp.) for their initial feeding (Baragi and Lovell 1986; Webster and Lovell 1990; Woods 2005), the majority are produced though extensive culture in ponds (Hodson 1995; Morris et al. 1999; Ludwig et al. 2009). Phase II production includes fingerlings to 15-cm juveniles ending with the first year of life. Phase III production includes 15-cm juveniles to market size for food (Kohler 2004) in the second year of production.

The purpose for most stocking programs of hybrid striped bass is to increase and diversify recreational fishing opportunities. It was common in past years to stock large numbers of fry with the assumption that some will survive and add to the fishery (Trushenski et al. 2010). However, in recent years, it has become more common to raise fish to a larger size in the hopes that greater numbers will add to the fishery. Larger fish tend to have greater survival because they are less affected by stress from transport and stocking as well as possible predation (Pitman and Gutreuter 1993).

In response to increased demand from fisheries managers, the Iowa Department of Natural Resources (IDNR) state hatcheries have been tasked to produce greater numbers of fingerling hybrid striped bass to stock local waters. However, recent years of trial and error during phase I production has met with limited success and inconsistent results at Iowa hatcheries. In part, poor survival is perceived to be related to high transport induced stress as these fry are transported from Oklahoma as well as a lack of site-specific fertilization and feeding regimes for Iowa hatcheries. A cooperative effort between Mount Ayr Fish Hatchery, Rathbun Hatchery/Research Facility, and Iowa State University was developed in 2010 to produce a standard protocol for culturing phase I hybrid striped bass in an effort to increase consistent production and improved annual survival.

Production

Production facilities will ship 1-2 days old sac fry during May or June depending on broodstock collection during the spring spawning migration (Morris et al. 1999; McGinty & Hodson 2008). Typically, polyethylene bags with rounded corners are used with 7.6-L of highly oxygenated water; approximately 75% of the bag volume should be oxygen (Harrell 1997). When transporting hybrid striped bass fry in a closed system container, shipping densities range from 5,000-13,000/L, with lower rates for extended transit (Harrell 1997; Morris et al. 1999). Survival of sac fry during the transport process to rearing ponds is dependent on suitable water quality, quick transit time, and proper acclimation procedures (Pitman and Gutreuter 1993).

Stocking time is variable between taxa and is related to fry size, mouth gape, and zooplankton populations present within the culture pond (Ludwig 1999). Proper stocking time can be estimated using mean water temperature to approximate desirable zooplankton abundance (Ludwig 2003) but will also depend on water source (Parmley and Geiger 1985; Morris 1999; Ludwig 2012). White bass and the sunshine bass should be stocked 2-7 days after pond flooding due to their reliance on rotifer populations (Ludwig 1999; Morris et al 1999; Lane & Kohler 2007). In contrast, the palmetto bass rely almost entirely on larger copepods and small cladoceran (Woods et al. 1985) which is the reason for their longer pre-stocking period (1-2 weeks) after pond flooding to allow for peak cladoceran and copepod populations to become established (Parmley and Geiger 1985; Morris et al 1999).

The dynamic characteristics of zooplankton populations have led some researchers to use particular fertilization techniques and species-specific zooplankton inoculations in culture ponds (Colura and Matlock 1983; Geiger 1983a; Farquhar 1984; Turner 1984; Geiger et al. 1985). The intent of these management techniques is to maintain high densities of specific zooplankton species in pond throughout the approximate 45-d rearing period of phase I fingerlings. Current recommendations are to use a combination inorganic and organic fertilizers during phase I production of hybrid striped bass to promote primary and secondary production in the ponds (Ludwig et al. 1998; Ludwig 2012).

In addition to pond fertilization, some culturists will use a commercial fish feed during phase I culture of striped bass and its hybrids. However, it is unclear why larval *Morone* do not appear to utilize the prepared diets effectively for growth (Baragi and Lovell 1986). Fitzmayer et al. (1986) obtained 53% survival when 15-day striped bass fry were fed twice per hour and 35% when fish were fed twice per day. Although in both treatments, most fry examined contained feed in their guts, they suggested that the artificial diet enhance rather than replaced the natural food supply for phase I fry.

During the approximate 45-day period in which 6-9 mm fry (Houde and Lubbers 1986; Ludwig 2004) grow to 25-50 mm (Morris et al. 1999), survival is expected to be between 30-50% for the original cross (Hodson et al. 1987; Hodson and Hayes 1989a; Morris et al. 1999). Lower survival (25-40 %) is expected for experienced culturists who raise the reciprocal cross hybrid (Ludwig 2004). Although reciprocal cross larvae are initially about half the size of the original cross (Houde and Lubbers 1986; Ludwig 2004), it is unclear whether growth or production are affected by this.

Management

Hybrid striped bass have been considered as an ideal candidate for reservoir introductions in the southeastern United States because they can establish a fishery for a large, open water game fish and can be used for predatory control of undesired prey species (Ott and Malvestuto 1981; Neal et al. 1999a). They have the ability to inhabit the highly underutilized pelagic zones typical of most large reservoirs (Fernando and Holoik 1991; Miranda and Bettoli 2010). However, the most common motivation for hybrid striped bass introductions throughout the United States is the potential for the species to regulate overabundant gizzard shad *Dorosoma cepedianum* populations (Ott andMalvestuto 1981; Dettmers et al. 1998; Neal et al. 1999b; Ostrand et al 2001). While management programs continue to utilize hybrid striped bass stocking in much of the Southeast, its use as a biological control is not as widespread throughout the Midwest.

The first hybrid striped bass fry introduced into Iowa waters were released into Saylorville Reservoir in 1981 by the IDNR (Mayhew 1987). Since then, a yearly stocking program of fry and fingerlings has resulted in the taxa's dispersal throughout many areas of southern Iowa. A growing interest for use of hybrid striped bass as a biological control by fisheries managers has focused resources of state hatcheries to increase future production.

When hybrid striped bass are introduced into systems that contain abundant gizzard and threadfin shad *D. petenense* populations, consumption rates have been reportedly between 60-95% of their total diets (Ott and Malvestuto 1981; Borkowski and Snyder 1982; Gilliland and Clady 1984; Jahn et al. 1987; Dettmers et al. 1998). Dettmers et al. (1996) reviewed 26 *Morone* introductions and found that 15 resulted in some type of gizzard shad control. However, only 22% of hybrid striped bass introductions resulted in apparent success, based on management objectives (Dettmers et al. 1996). Although total eradication was never cited, reduced abundance and altered size structure of shad populations were observed after many *Morone* introductions. Since it may be possible for hybrid striped bass to switch their diet in the absence of shad (Gilliland and Clady 1984; Neal et al. 1999b), the effects that introductions have on other game fish populations have been investigated.

During the last 4 years, IDNR fisheries biologists have struggled to understand the diets of a newly introduced population of hybrid striped bass in a southern Iowa reservoir. Gut content analysis during that time has shed little light on what the fish are consuming because of a high incidence of empty stomachs, difficulty identifying fish from stomach contents, and a large gastric presence of zooplankton (R. Schultz, IDNR Fisheries Biologist, personal communication).

The initial goal of the 2004 introduction attempted to establish a predatory species that would depress a probable future population of yellow bass. However, it is unclear how hybrid striped bass predation habits would evolve in a large Midwestern impoundment that has an established predator-prey fish assemblage present that is also void of a shad prey-base.

In recent years, the use of stable isotope analysis in conjunction with gut content analysis has increased in popularity amongst ecologists for measuring many food web dynamics and trophic level interactions. However, its use as a management tool is not commonly practiced. Techniques for using stables isotopes to trace dietary items rely on the assumption that a consumers muscle tissue is a product of its diet, and that this is reflected in the isotopic composition of the individual (Fry 2006). Using a combination of carbon and nitrogen stable isotopes, we attempted to describe the diets of hybrid striped bass along with the trophic dynamics of fish assemblages present in Three Mile Lake, Iowa.

Thesis Organization

The following thesis is organized into six chapters. Included are a general introduction, general conclusion, and four individual studies. Chapter two is formatted according to the World Aquaculture Society (WAS) magazine. All other chapters are formatted according to the American Fisheries Society (AFS) North American Journal of Aquaculture and North American Journal of Fisheries Management. Each chapter is followed by a list of tables and figures, as well as a reference section specific to individual chapters.

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CHAPTER 2. COMPARISON OF AQUACULTURE OPERATIONS WITH PUBLISHED GUIDELINES FOR PHASE-I HYBRID STRIPED PRODUCTION

(In prep)

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Introduction

The original hybrid striped bass *Morone saxatilis* × *M. chrysops*, known as the palmetto bass, was first produced by Robert Stevens in 1965 by crossing a female striped bass Morone *saxatilis*, with a male white bass *Morone chrysops.* The reciprocal cross *M. chrysops* x *M. saxatilis*, known as the sunshine bass, was developed later as an alternative (Kerby and Harrell 1990). For purposes of discussion both crosses will be referred to as separate taxa when appropriate. Many natural resource agencies that have access to the brood striped bass females continue to use the original cross although production can be somewhat unreliable due to environmental conditions, e.g., drought conditions and temperature swings that limit access (Ludwig 2012). In contrast, the private sector is almost entirely comprised of the reciprocal cross as the private aquaculture industry either does not have access to wild female striped bass from public water ways or choose not to maintain the large female striped bass in captivity. Both hybrids are considered to be ideal culture candidates because they have an increased growth rate during their first two years, adapt to formulated feeds, and are more resistant to diseases than other Morone species (Morris et al. 1999; Kohler 2004).

Hybrid striped bass culture is divided into three distinct phases based upon specific life stages and production needs of the fish. Phase I consists of larval fish to 2.5-cm fingerlings (30- to 60-d post-hatch) and is highly dependent on the availability of adequate numbers of quality zooplankton within nursery ponds. Phase II production includes feed trained fingerlings to 15 cm juveniles, ending with the first year of life. Phase III fish are age-1 that are cultured in a second year to market size foodfish (Harrell et al. 1992). For management stocking, it is common for many state agencies to immediately release fry into lakes and reservoirs although fishery managers typically prefer larger individuals because survival is greater and more consistent.

In response to increased management demand for hybrid striped bass in the state of Iowa, the Iowa Department of Natural Resources (IDNR) has obtained newly hatched fry from other state agencies to either stock directly into public waters or into established culture ponds to produce phase I fingerlings for management stocking purposes. However, in spite of agency noted success in producing other fish species, e.g., walleye *Sander vitreus*, the IDNR has had limited success and inconsistent results. In preparation for a detailed research project on hybrid striped bass culture in Iowa IDNR and Iowa State University (ISU) biologists developed and distributed a questionnaire among various state agencies and selected universities. The survey focused on current hybrid striped bass culture techniques and annual mean production variables. The goal of this study is to compare actual culture practices with published guidelines for phase I hybrid striped bass production.

Methods

With cooperation from the IDNR, we developed a self-administered e-mail based questionnaire to assess current expectations and production techniques used that yield good survival and size of phase I hybrid striped bass. The survey consisted of 42 questions concerning annual production, fry transport, phase I culture, water quality, feeding practices, harvest, and phase I performance data. All questions were an open ended, fill-in-the-blank format with some specification concerning units or parameters. The survey was sent to 25 state agencies and 2 universities, consisting of 21 separate states.

Results

We received 26 completed surveys from 20 different states (93% reply rate). Of the hatcheries which responded, 58% reported production of hybrid striped bass fry while 77% reported production of fingerlings. Median annual production was 1,500,000 fry and 300,000 fingerlings. Fifty eight percent of the hatcheries produced only the original cross hybrid, 30% produced only the reciprocal cross, and 12 % reported production of both taxa.

Fry Transport

The 10 hatcheries that imported fish from out-of-state reported the mean maximum transit time was 5.13 hours. Length of transit did not appear to affect total acclimation time (median = 30 min). No indication of fry stocking rate during transport was reported in our survey; however, 1-2 day old sac fry in closed system containers should be shipped at a rate of 5,000-13,000/L, with lower rates during extended transit times(Morris et al. 1999). Shipping 1-2 day old sac fry is recommended over older fry to reduced transport mortality (McGinty and Hodson 2008).

During fry transport, pH values can decrease greatly due to an accumulation of CO_2 during longer durations of transport (Amend et al. 1982). Reduced pH within transport bags, along with the relatively high pH of typical culture ponds, increases the importance of a long-term tempering process. To help maintain appropriate pH levels, it is recommended to have source water with high total alkalinity (>100 mg/L) and hardness (>100 mg/L). Mean hardness and alkalinity of surveyed hatcheries was 162 and 90 mg/L respectively.

Baking soda ($NaHCO_3$) is commonly used to stabilize pH by increasing the alkalinity of transport water for many species of juvenile and adult fish (Swann 1993). Increasing water salinity is another popular technique used to reduce osmoregulatory stress during transport of striped bass and its hybrids (Mazik et al. 1991; Weirich et al 1992). However, our survey indicated that only two hatcheries (8%) used sodium chloride as an additive during transport of fry. One facility indicated that they increased water hardness of their culture water through injecting $CaCl_2$, and subsequently shipped fry to other hatcheries. Another facility indicated that a buffering agent was used when fry were shipped from Byron State Fish Hatchery, Oklahoma, a hatchery historically known to have soft culture water. Sodium chloride was also used for the transport of fingerlings at two separate hatcheries.

Phase I Pond Stocking

Stocking time is variable between crosses and is related to fry size, mouth gape, and zooplankton populations present within the culture pond (Ludwig 1999). White bass and the reciprocal cross hybrid should be stocked 2-7 days after pond flooding due to their reliance on initial rotifer populations. Because the original cross relies almost entirely on larger copepods and small cladoceran, it is recommended that they be stocked 1-2 weeks after pond flooding to allow these zooplankton taxa to establish (Morris et al. 1999). We found that hatcheries that cultured the original cross flooded their ponds a mean of 6-9 days prior to stocking compared to those which cultured the reciprocal flooded a mean of 5-6 days prior to stocking. Facilities that allowed ponds to be flooded for a longer period prior to stocking original fry had greater fish biomass (Figure 1.1). However, this relationship was not evident for the reciprocal cross.

The appropriate stocking density for phase I hybrid striped bass is highly dependent on the culture pond and its zooplankton production potential (Woods 2005); recommended stocking rates are typically between 250,000 and 500,000 fry/ha. Because survival of the reciprocal cross is expected to be low, it is recommended to stock at a higher rate. Overall, lower stocking rates can result in larger fish while higher rates can reduce growth and survival. It is this conflict in production strategies that are problematic to fishery managers that want both high numbers of fish per culture pond that are also robust.

From our survey, we found the mean stocking rate among all hatcheries was roughly 400,000 fry/ha with a range between 200,000-741,000/ha. There was no difference in stocking rates between the original and reciprocal cross. However the actual numbers of fish stocked are suspect since the majority (92%) of facilities staff indicated that fry were visually or volumetrically enumerated and not counted with a fry counter. Mean annual survival appeared to be negatively affected by stocking rate of the original cross but not the reciprocal (Figure 1.2). Total final length and weight were also negatively affected by stocking rate of both crosses (Figure 1.3).

Phase I Pond Fertilization and Feeding

Current recommendations are to use a combination inorganic and organic fertilizers during phase I production of hybrid striped bass (Ludwig 2012). Although application rates vary depending on the type of organic and inorganic fertilizers used and water quality, an initial application of 224-560 kg/ha of organic fertilizer should be applied 1-2 weeks before stocking.

Weekly applications of 28-168 kg/ha have also been recommended (Geiger and Turner 1990; Morris et al. 1999; Woods 2005). For the reciprocal cross, Ludwig (2004) recommended an initial application of 140 kg/ha of rice bran in combination with 7.5-L of a 10-30-0 NPK liquid fertilizer in combination with weekly applications of 28 kg/ha, provided good water quality.

Our survey indicated that all hatcheries used some type of fertilizer during phase I culture. All but one facility used an organic fertilizer; 26% of the hatcheries reported using an additional inorganic fertilizer. Alfalfa and cottonseed meal were the most commonly used organic fertilizers (52% and 44%, respectively). Sixteen percent of the hatcheries indicated the use of soybean meal with another 12% using other products (brewing bi-products and old fish feed). Because 32% of the responses indicated use of more than one type of organic fertilizer, overlap in their percent usage occurred. It was unclear whether those facilities used a combination of products in their fertilization regime or there were yearly variations in fertilizer choice. The variation in organic fertilizers makes it difficult to determine the actual amount of nutrients, i.e., nitrogen and phosphorus, being added to the ponds. For many state agencies, organic fertilizers are typically chosen based on availability and cost over actual production performance.

All but one hatchery that used an organic fertilizer implemented an initial application prior to fry stocking. Initial application rates of organic fertilizers in earthen and plastic-lined ponds ranged from 112-673 kg/ha and 0-336 kg/ha respectively. Hatcheries that used earthen ponds applied significantly greater amounts of organic fertilizer during their initial application (Figure 1.4); secondary applications were common at 70% of the facilities. Although not statistically significant, secondary applications in earthen ponds appeared to be greater as well. However, plastic-lined ponds restrict nutrient influx, and organic fertilization may be more critical when culturing fish that rely on both zooplankton and benthic forage (Rogge et al. 2003).

In addition to pond fertilization, some culturists will use a commercial fish diet during phase I culture of striped bass and its hybrids. Whether the feed is actually being used by the fish or preforming as another form of organic fertilizer is a question for many fish culturists. Baragi and Lovell 1986 observed that it is often unclear why some larval fish do not often appear to utilize the prepared diets effectively for growth. Supplemental feeding is typically recommended to begin as natural zooplankton populations begin to decline or around 14-21 days posthatch (Hodson and Hayes 1989). The original cross will begin accepting feed around 21-28 days posthatch (Morris et al. 1999); there is no literature specific to reciprocal cross fish use of feed.

The use of a supplemental commercial diet during phase I culture occurred at 58% of the hatcheries we surveyed. Only two facilities raising the reciprocal cross indicated the use of a supplemental diet. Hatcheries that used a commercial diet began feeding fish a mean of 10 days post stock (~15 days post hatch) with a range between 2-18 days post stock. No relationship was detected between the hybrid cross raised and the number of days before the initial day of feed application. Daily feeding rates had a positive effect on fish biomass (Figure 5).

Phase I Production

Typically, good survival for the original cross is considered to be 40-50% and 15-20% for the reciprocal during phase 1 production (Morris et al. 1999). Our survey indicated that median annual survival was 44% for the originals and 37% for the reciprocal, assuming the initial stocking densities are correct. There were no detectable differences for harvest lengths, weights, or growth between the original and reciprocal crosses.

Conclusion

The way in which our survey was conducted made it difficult to concisely analyze the data collected. The greater importance of this survey is in the new research questions that arose. First, the culture differences between the two hybrid striped bass taxa need to be more fully considered by fish culturists. Because stocking density and feeding rate appear to have an effect on production, related field production protocols need to be adopted by culturists. High variability in initial stocking rates and subsequent annual survival (0-110%) caused additional concerns as to which practice(s) is/are best for hybrid striped bass production. Furthermore, the wide range of techniques being implemented makes it clear that actual practices are not following tested procedures from the literature. It is unclear whether this disconnect is caused by limited resources within agencies, poor communication between researchers and managers, or perhaps operational constraints placed on the hatcheries, e.g., availability of fry and multiple species being cultured at a specific hatchery.

Discontinuity was most apparent in the number of days that ponds were filled prior to stocking. It appears that production may improve if culturists use published guidelines as well as collect and assess field samples of zooplankton to assure the establishment of adequate populations (size and species). Our survey showed that the majority (62%) of facilities typically collect zooplankton samples; however staff at only two hatcheries indicated that zooplankton populations dictated the time of stocking. Although stocking time can be highly dependent on fry suppliers as well as logistic restraints at individual hatcheries, distinct culture requirements of the two hybrid taxa need to be considered.

Organic fertilization rates also appear to have distinct differences between earthen and plastic-lined ponds along with an overall under usage of inorganic fertilizers. Further research is essential to determine proper fertilization regimes, amounts, and types of fertilizers, for both taxa within the two different pond types. Although current production of the reciprocal cross appears to have improved over the last 20 years, production of the original cross seems to be stagnant. More than likely, this difference may be the product of a greater use within the private sector. Continued research, along with an examination of private production techniques is necessary for future success.

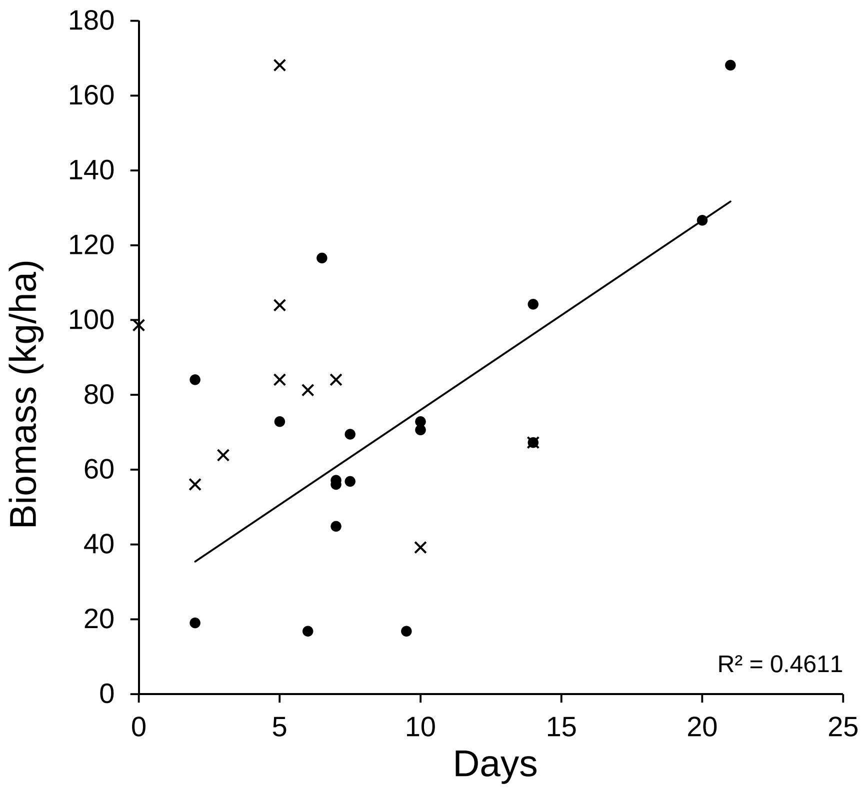
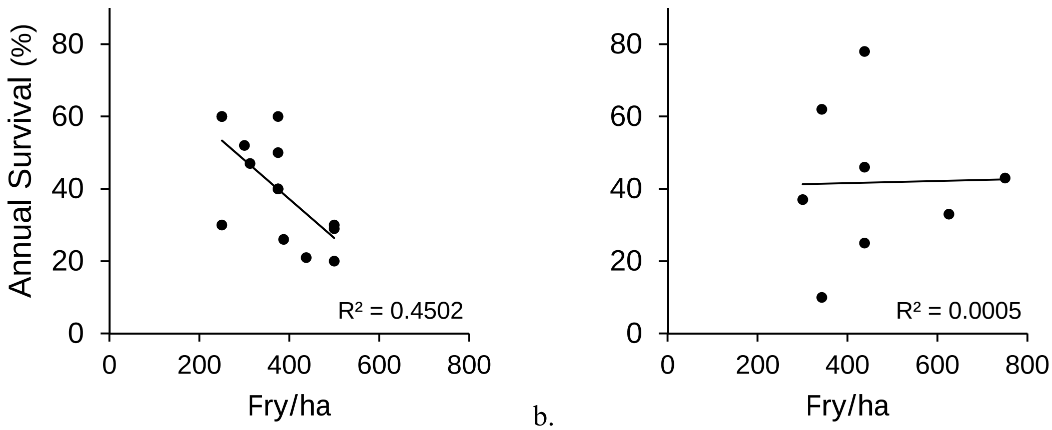


FIGURE 1.1. Mean annual fish biomass (kg/ha) of original (•) and reciprocal (×) cross hybrid striped bass and maximum number of days culture ponds filled prior to stocking fry. Regression line includes only the original cross hybrids (p=0.0027). Three responses were estimated using multivariate statistics. 2010 survey of natural resource agencies.



a. b.

FIGURE 1.2. Median annual survival (%) from 21 different hybrid striped bass hatcheries against phase I stocking rate (100,000) (a.) original (b.) reciprocal (P = 0.008 and 0.959 respectively). 2010 survey of natural resource agencies.

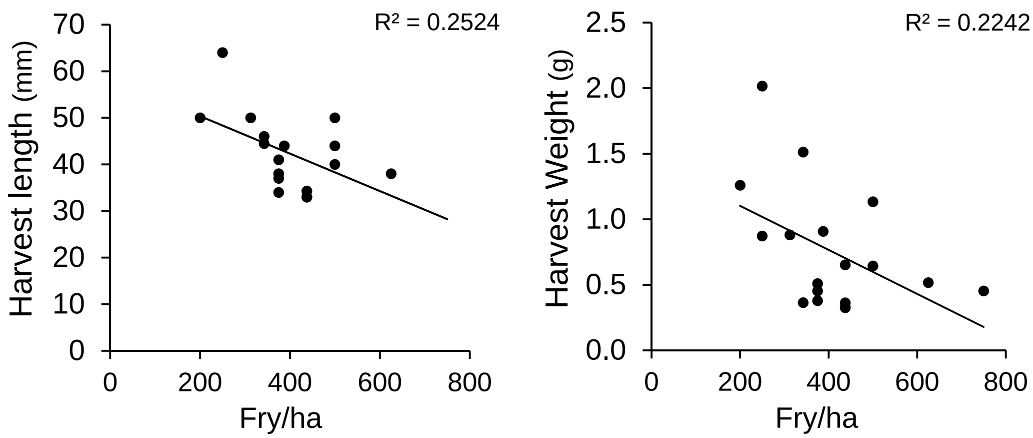


FIGURE 1.3. (a.) Mean annual harvest length (mm) and (b.) mean annual harvest weight from 21 different hybrid striped bass hatcheries against phase I stocking rate (100,000; P = 0.040 and 0.048 respectively). 2010 survey of natural resource agencies.

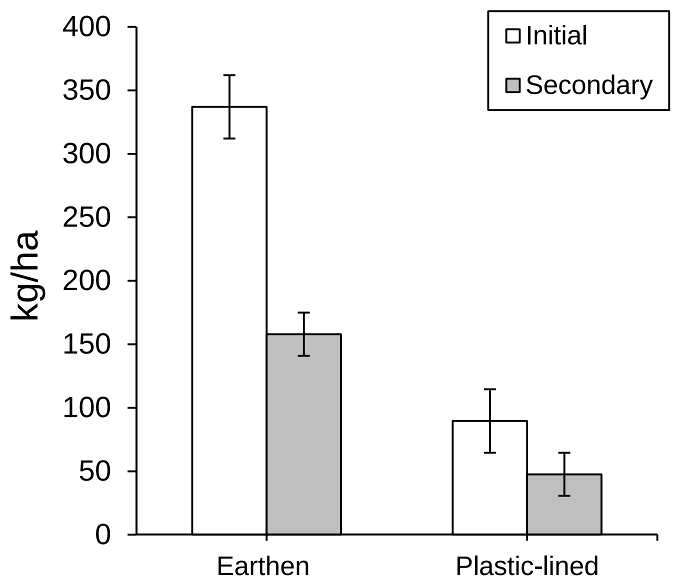


FIGURE 1.4. Mean (±SE) initial (pre-stocking) and secondary (weekly/biweekly) application rate (kg/ha) of organic fertilizers from 17 U.S. hybrid striped bass hatcheries. 2010 survey of natural resource agencies.

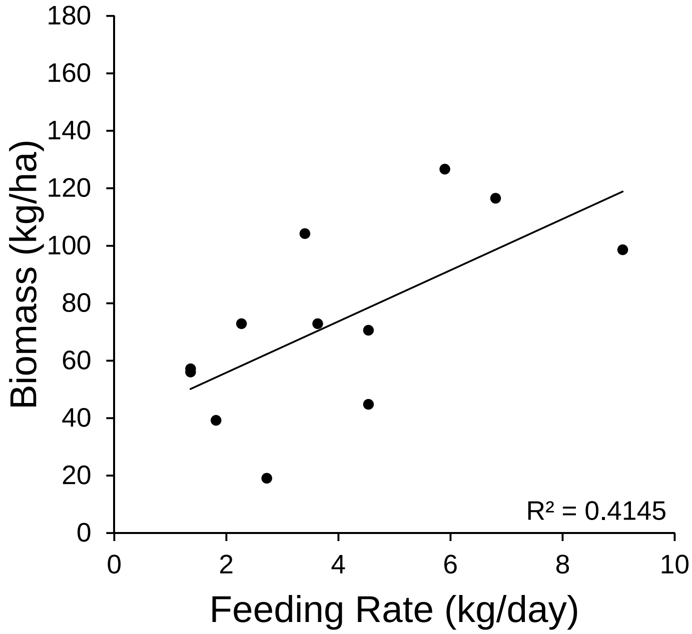


FIGURE 1.5. Mean annual fish biomass (kg/ha) and daily feeding rate of hybrid striped bass fry from 12 different hatcheries. 2010 survey of natural resource agencies.

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CHAPTER 3. EFFECTS OF POND FERTILIZATION ON HYBRID STRIPED BASS PRODUCTION IN EARTHEN CULTURE PONDS

(In Prep)

James J. Wamboldt and Joseph E. Morris.

Abstract

The role of an organic fertilization regime was evaluated for production of phase I hybrid striped bass (Palmetto) *Morone saxatilis* × *M. chrysops* in nutrient-enriched earthen culture ponds. Our objectives were to determine the effects of an organic fertilizer on fish production, water quality, and numbers of desired zooplankton taxa in highly eutrophic culture ponds in 2011. Multiple production parameters were compared between the two treatments at the Mount Ayr Fish Hatchery, Iowa. Palmetto bass were stocked into six earthen ponds of various sizes (range 0.2-ha to 0.5-ha) at a rate of 116,000/ha. During the 29-36 days of phase I, three pond replicates received weekly applications of alfalfa pellets at a rate of 112-kg/ha/week; three control ponds received no fertilizer application. One control pond was removed from further analysis due to stocking error. Mean hybrid striped bass total length and weight at the time of harvest were similar between the two treatments. However, final mean (±SE) fish biomass was significantly greater within the fertilized ponds (66 kg/ha ±6.6) than in the non-fertilized (38 kg/ha ±12.4) ponds, suggesting that survival may have benefited from the additional organic fertilizer. Except for mean NO_3: N:P ratios being significantly greater within ponds that did not receive fertilizer, no other differences in water quality were detected between treatments. Although there were no significant differences in mean zooplankton populations, greater peak densities of preferred prey items (Cyclopoida, *Daphnia*, and nauplii) within the fertilized treatment suggest a potential for better fish production during phase I. Results suggest minimal

benefits of organic fertilizers in earthen culture ponds with existing high nutrient levels. Further research should focus on the possible benefits of nutrient ratio manipulations to foster the development of desirable zooplankton for larval hybrid striped bass.

Introduction

Pond fertilization has become a popular technique for larval fish culture, including that of striped bass *Morone saxatilis* and hybrid striped bass (palmetto) *Morone saxatilis* × *M. chrysops* (Farquhar 1974; Barkoh 1996; Buurma et al. 1996). The goal of any pond fertilization regime is to stimulate production of suitable-size food organisms, which are essential for the growth and survival of many larval fish taxa (Ludwig 1999). During their transformation from endogenous to exogenous feeding behaviors, cultured hybrid striped bass fry require natural prey (zooplankton) for their initial feeding (Geiger 1983a; Geiger and Turner 1990; Ludwig 1999). Although older fry and fingerlings readably accept commercial feed pellets, live food items must be available during their critical period (Ludwig 1994; Ludwig et al. 2008; Ludwig and Lochmann 2009).

Numerous field studies of striped bass fry have noted the role of specific prey taxa needed for successful phase I culture in culture ponds (Harper et al. 1969; Humphries and Cumming 1973; Harrell et al. 1977; Geiger et al. 1985; Fitzmayer et al. 1986). Woods et al. (1985) found that the diet of palmetto bass fry, 6-9 mm total length (TL), consisted entirely of cladoceran and copepod taxa and that a discernible shift towards insect larvae (chironomidae) occurs with increased fish size (about 20-dph). Once fry reached 15-20 mm TL, the frequency of occurrence of chironomid larvae in the stomach samples was 100% (Woods et al. 1985). Their reliance on copepods, cladoceran, and Chironomidae larvae during phase I make pond fertilization essential for adequate growth and survival (Parmley and Geiger 1985; Geiger and Turner 1990).

The dynamic characteristics of zooplankton populations have led some researchers to use particular fertilization techniques and species-specific zooplankton inoculations in culture ponds (Colura and Matlock 1983; Geiger 1983a; Farquhar 1974; Turner 1984; Geiger et al. 1985). The intent of these management strategies is to maintain high densities of specific zooplankton species in pond throughout the approximate 45-d rearing period of phase I fingerlings. Having the shortest generation time, rotifers will be the first to appear in a culture pond within 2-3 days (Allan 1976; Geiger 1983a). The next taxa of zooplankton to establish within fertilized ponds are copepod nauplii (Parmley and Geiger 1985; Ludwig 1999). Cladocerans reach peak reproductive capacity within pond by day 14, (Geiger 1983a) which is the justification behind stocking palmetto bass at this time (Parmley and Geiger 1985; Brewer and Rees 1992; Morris et al 1999).

It is recommended that a combination of organic and inorganic fertilizers be used for phase I culture (Geiger 1983a; Geiger 1983b; Geiger et al. 1985; Farquhar 1987; Ludwig 2004). However, there are limited peer-reviewed publications that deal directly with phase I palmetto bass and mixed organic/inorganic fertilization regimes. Many of the guidelines that culturists follow come from a combination of striped bass (Geiger 1983a; Geiger et al. 1985; Farquhar 1987; Barkoh 1996), and sunshine bass *Morone chrysops* × *M. saxatilis* research (Ludwig 2004; Ludwig 2012). Typically, managers are not willing to sacrifice production by having control ponds that do not receive an application of fertilizer. It may be for this reason that there has not been a side-by-side comparison between non-fertilized ponds and fertilized ponds during phase I culture of palmetto bass.

Fertilization.—Through decompositional processes, inorganic nutrients are released from organic fertilizers, which in turn, stimulate phytoplankton growth (Geiger and Turner 1990). Organic fertilizers also help establish beneficial bacteria and protozoa, which in combination with phytoplankton, constitute the primary diet of many zooplankton species (Parmley and Geiger 1985; Boyd 1990; Geiger and Turner 1990; Soderberg et al. 1997). In addition to zooplankton production, organic fertilizers are an important source of carbon which can be essential for establishing benthic invertebrates (Rogge et al. 2003). Although culturists typically select fertilizers on the basis of availability and cost, it is recommended to use an organic fertilizer with a low carbon:nitrogen (C:N) ratio (Geiger and Turner 1990).

Inorganic fertilizers encourage phytoplankton growth faster than organic fertilizers because nitrogen and phosphorus are present in the soluble form; making them immediately available to primary producers (Swingle and Smith 1938; Boyd 1990). Phosphorus is considered as the limiting nutrient for phytoplankton growth within freshwater ponds; phosphorus-only fertilizer application has shown to increase fish yield (Boyd 1990). However, Geiger and Turner (1990) recommended using a liquid inorganic fertilizer containing nitrogen in addition to phosphorus to promote bacterial generation and increase the decomposition of organic fertilizers. Depending on seasonal variability and geographic locality, the application of an inorganic phosphorus fertilizer within nutrient rich waters may be unnecessary (Rogge et al. 2003) and alfalfa meal alone may be an adequate source of phosphorus for striped bass production (Barkoh 1996). Some evidence showed that ponds with excess phosphorus can have nitrogen as the limiting nutrient (Rogge et al. 2003; Mischke and Zimba 2001).

Risks associated with organic fertilizers include dissolved oxygen (DO) depletion due to high aerobic decompositional demands (Schroeder 1978; Qin and Culver 1992; Ludwig 2012) and increased growth of undesired filamentous algae (Swingle and Smith 1938; Myers et al. 1996). Conversely, inorganic fertilizers that have elevated levels of nitrogen, in the form of urea, can hydrolyze to ammonia and become toxic to fish at high levels of pH (Soderberg et al. 1997;

Soderberg and Marcinko 1999). These unintended consequences of pond fertilization programs are important to remember since adequate water quality is especially important during phase I culture of striped bass and its hybrids because they are substantially less tolerant of elevated pH and ammonia than other cultured fish species (Bergerhouse 1993; Pitman and Gutreuter 1993).

The goal of this study was to determine if the use of an organic fertilizer can improve phase I production of hybrid striped bass in earthen ponds that are considered to be highly eutrophic due to the high nutrient water source. Our objectives were to evaluate the effects that organic fertilization had on: (1) fish growth, survival and production; (2) water quality parameters; and (3) copepod and cladoceran populations, in earth culture ponds.

Methods

Study Site.—The Mount Ayr Fish Hatchery is located 3-km north of Mount Ayr, Iowa. The facility consists of six earthen ponds of various sizes (range 0.2 to 0.5-ha). Three ponds are located directly below the Loch Ayr Reservoir. The other three ponds are separate from the rest by a gravel road (State Hwy. 344). Water is supplied via the reservoir and filtered through a 300-µm mesh sock. Elevated levels of cyanobacteria are typical in the Loch Ayr Reservoir due to high nutrient loading (Naidenko et al. 2012); excessive cyanobacteria presence is indicative of eutrophic waters (Taranu et al. 2012).

Study Design.—Sac fry, 5-days post hatch (DPH), were transported by plane to Iowa from the Milford Fish Hatchery, Kansas. To approximate the mortality due to transport stress, fry were placed into submerged barrels for 24 h. Barrels were placed 5-10 cm below the water's surface to allow live fish to escape into the culture pond. Fry were protected from ultraviolet radiation with Styrofoam insolation placed above the barrels. After 24 h, dead sac fry present at the bottom of the barrels were enumerated and subtracted from our stocking estimates. The number of fish stocked was estimated using a fry counter (Jensorter™ FCM Jensorter Incorporated, Bend, Oregon). Six separate bags from the same shipment were enumerated at another facility using the fry counter. A correction factor (42%) was then applied to all bags (50,000/bag = 29,000/bag) used to stock ponds at the Mount Ayr facility.

Pond filling began 7 days pre-stock on 16 May, 2011; filling was interrupted on the day of stocking (23-May) and resumed on 24 May to allow for enumeration of dead fry in submerged barrels. All attempts were made to fill each of the six ponds at an equal rate once filling resumed. The initial goal was to stock all ponds with 200,000 fish/ha, but given the final number of fry available for stocking, our actual stocking rate was estimated to be 116,000 fish/ha.

Treatments included fertilized and not-fertilized, with three replicate ponds per treatment. Treatment application was randomly assigned taking into account initial terrestrial vegetation cover within each pond prior to filling. Ponds in the fertilized treatment received an initial application of 112-kg/ha of alfalfa pellets around the perimeter of each pond; limited to a depth of 0.6-0.9 meters. Alfalfa pellets were applied twice a week thereafter at a rate of 56-kg/ha. Although current agency culture protocols dictate the initial minimal total phosphorus (TP) levels to be 0.10 mg/L, supplemental inorganic fertilizers were not applied since the TP levels were well in excess of 0.10 mg/L.

Sampling methods.—Water samples were collected mid-morning from the kettle of each pond using a 5.0-cm PVC tube sampler (Graves and Morrow 1988) twice weekly. One additional random sample was collected for validation. Samples were stored in acid-washed polyethylene bottles and kept on ice (< 8 h) until tested for TAN, NO_2-N, and NO_3-N using a HACH ™ DR/3000 spectrophotomer. Total phosphorus (TP) was tested at the beginning, middle, and end of the culture season. Additional water chemistry analysis conducted weekly included total hardness, and alkalinity.

On the same days that water samples were collected, pond-side water quality measurements were taken mid-morning and early-afternoon using a HACH™ HQ 40d meter (Hach, Loveland, Colorado). Temperature and dissolved oxygen (DO) were measured at the bottom, middle and top of each kettle. Additionally, pH was taken at mid-water within the kettle. Turbidity was analyzed using a HACH™ 2100P turbidimeter (Hach, Loveland, Colorado) pond-side.

Zooplankton were collected twice weekly from three locations in each pond using an 80-µm Wisconsin plankton net. Due to pond-shape irregularity, standard sampling locations were set at the kettle and midpoints of the two longest sides. Three parallel tows were 2 m in depth, 8 m long and filtered 255-L of water. One additional random sample was collected for validation. Zooplankton samples were preserved with a chilled formalin/sucrose solution (Haney and Hall 1972) for future identification and enumeration.

Harvest began on 20-May and continued a total of 8 days, for a grow-out period of 29-36 days. Ponds were drained overnight and seined from the kettle mid-morning the following day. To account for an immense tadpole *Rana catesbeiana* population within all ponds, total yield was calculated using a bulk weight at the time of harvest. As fish were transferred from the raceway to transport tanks, sub-samples of fish and tadpoles were enumerated and weighed. Using the percent by weight of the tadpoles within each sub-sample, we were able to discount their additional biomass in the bulk weight.

Total length (0.1-mm) and weight (0.01-g) was collected from 100 randomly sampled fish from each pond. Instantaneous growth was converted to a specific growth rate (SPG):

$$SGR = \left[\frac{Ln(w_t) - Ln(w_i)}{t}\right] \times 100$$

where w_t is the weight at time t and w_i is the initial weight (Hopkins 1992). Specific growth rate was reported as G (Hopkins 1992). Due to the small initial fry size, an initial weight of 0.0069-g was estimated using a linear regression of pooled weekly fish samples (Figure 3.1).

Statistical Analyses.—Prior to analysis, data from pond 7 was removed from the control treatment because it was apparent that the initial stocking density within the shipment bag was inaccurate since estimated final survival exceeded 100%. Additionally, our shipping mortality estimates during the barrel experiment indicated that mortality within the bag that was used to stock pond 7 was 75% greater than any other bag.

Statistical Analysis Systems version 9.3 (SAS Institute 2011) was used for all statistical analyses. Repeated measures analyses on water quality and zooplankton parameters were done using the mixed model. The error term used in treatment comparisons was pond (treatment); the error term for daily comparisons was day × pond (treatment). Tukey's honestly significant difference was used for pairwise comparisons with statistical significance set at $P < 0.10$. Final fish production variables were evaluated using pairwise comparisons (two-sample *t*-test).

Results

Production.—Mean hybrid striped bass total length and weight at the time of harvest were the same within both treatments: 48-mm and 1.4-g respectively. Mean (±SE) fingerling survival was 54% ±18 within the fertilized and 30% ±11 within the non-fertilized treatments. Though not significant, fingerling survival was greater for all three replicates of the fertilized treatment compared to the unfertilized treatment (Figure 3.2). Final mean (±SE) fish biomass was significantly greater within the fertilized ponds (66 kg/ha ±6.6) than in the non-fertilized (38 kg/ha ±12.4) ponds ($t = 3.49$; df = 3; $P = 0.04$). Mean (±SE) fingerling specific growth rates were 14.9 ±0.5 and 15.8 ±0.6 for fertilized and non-fertilized ponds, respectively.There were no significant differences between fertilization treatments for fish length, weight, or specific growth at the time of harvest (Table 3.1). Although not statistically significant, survival was greater within the fertilized ponds.

Water Quality.—Prior to the original application of fertilizer and stocking of fish, initial mean (±SE) TP within fertilized and not-fertilized ponds was 0.36 ± 0.15 mg/L and 0.17 ± 0.01 mg/L respectively. At the time of stocking mean (±SE) TP dropped to 0.08 ± 0.00 mg/L and 0.08 ± 0.01 mg/L for fertilized and non-fertilized ponds, respectively. Throughout the 29-36 days of culture, NO_3-N: TP was significantly higher within the non-fertilized ponds (Figure 3.3; P = 0.04); values were closer to the desired 7:1 ratio described by Mischke (1999). No other differences in water chemistry were detected between treatments (Table 3.2) but there were a significant interaction between treatment and time with UIA. However, this interaction is only noted in the final day of culture (Figure 3.5), and was a result of elevated pH within non-fertilized ponds (Figure 3.4). Morning and afternoon mean pH values were not significantly different between the two fertilization treatments (Table 3.3, Figure 3.4). Temperature and DO profiles were fairly uniform between treatments throughout the culture period (Table 3.3). However, morning surface temperatures were significantly warmer within fertilized ponds (P = 0.01). Although not significant, turbidity was greater within the non-fertilized ponds every sampling period after 10-days post stocking (Figure 3.6).

Total ammonia nitrogen remained below the lethal level of 2.5 mg/L as suggested by Hargreaves and Kucuk (2001) within all but one pond throughout the culture period. A pond within the non-fertilized treatment had an initial TAN spike during the first week of the study but subsequently remained below harmful levels (Figure 3.5). Elevated afternoon pH levels caused

UIA concentrations to exceed detrimental levels (0.05 mg/L; Ludwig et al. 2007) multiple times within both treatments (Figure 3.5). Fertilizer treatment did not affect the number of cases in which UIA exceeded 0.05 mg/L (20% in the fertilized and 25% in the non-fertilized treatments). Afternoon pH exceeded the recommended range of 6.7-8.5 (Hodson and Hayes 1989; Morris et al. 1999) within both treatments an equal number of days. No ponds had pH levels above the hybrid striped bass mortality threshold of 9.8 (Bergerhouse 1993). There was no significant difference in pH between the two treatments.

*Zooplankton.—*Zooplankton concentrations were not significantly different between the two treatments (Table 3.4). Although not significant, cladoceran and copepod abundances were greater in ponds fertilized with alfalfa pellets (Table 3.4; Figure 3.7; Figure 3.8) However, there was a significant interaction between treatment and time with Cyclopoida, nauplius, and *Daphnia* concentrations (Table 3.6; P = 0.07, 0.08, and 0.02 respectively). Significant interactions between treatment and time for Cyclopoida, nauplius, and *Daphnia* populations suggest that greater peak populations of preferred zooplankton taxa occurred earlier in the culture period for fertilized ponds (Figures 3.8 and 3.9).

Rotifer populations peaked higher within non-fertilized ponds around 11-dps. Near the end of the culture season, rotifer populations declined within both treatments. Mean (±SE) copepod populations peaked 11-dps within the fertilized ponds (285/L ±54) and 15 dps in the non-fertilized (164/L ±42) ponds (Figure 3.7). Adult copepod samples predominantly consisted of (~ 80%) Cyclopoida and (~ 20%) Calanoida. Mean (±SE) cladoceran populations peaked at 163/L ± 32 around 15-dps within fertilized ponds. Thereafter, peak cladoceran concentrations within non-fertilized ponds reached 59/L ±13 at 18-dps (Figure 3.8). Cladoceran samples primarily consisted of (~ 78%) *Daphnia*, (~ 11%) C*eriodaphnia*, (~ 10%) *Bosmina*, and (< 1%)

Scapholeberis. Although not significant, fertilized ponds had greater concentrations of all copepod and cladoceran taxa (Table 3.6). Copepod and cladoceran species compositions within pond were not affected by treatments (Table 3.6).

Discussion

The use of pond fertilization has long been used to increase fish production within earthen ponds (Swingle and Smith 1938; Boyd 1981; Geiger 1983a; Murad and Boyd 1987; Boyd 1990). Although survival within our ponds was not significantly different between treatments, all fertilized ponds did have numerically greater survival rates than non-fertilized ponds. While the difference in final fish biomass may be related to the inherent difficulties of comparing production between ponds with unequal survival (Morris and Muncy 1989), we did not find a difference between fish length, weight, or growth, as would be expected with differential survival (Brewer and Rees 1992).

Although we had anticipated that density-dependent growth would occur within ponds with greater survival (Barkoh 1996), it was not detected because our ponds contained a relatively low stocking rate due to inaccurate shipping densities that resulted in fewer fry stocked. Given that our actual stocking rate was well below what is typically recommended (Brewer and Rees 1992; Morris et al. 1999; Woods 2005), differences between treatments may have been more pronounced if higher stocking rates had allowed for greater competitive interactions within pond. Since all ponds were initially stocked equally, the difference in final fish biomass may have also been a good indication that survival was affected by the application of fertilizer. More than likely, the inability to determine a statistically significant difference in survival was an effect of low replication.

Within all ponds the major water quality concern was relatively high afternoon pH values, causing elevated UIA. However, similar DO, pH, and ammonia concentrations between fertilized and non-fertilized treatments suggest that the addition of alfalfa pellets did not adversely affect conditions for culturing phase I hybrid striped bass. Our results agree with Barkoh (1996) and Ludwig et al. (2007) in that organic fertilization alone does not increase pH levels in ponds. It was unclear why water surface temperatures were higher within fertilized ponds, but differences in primary production and turbidity may have contributed to this effect.

Low NO_3-N:P ratios within our fertilized ponds suggest that the phytoplankton base, most likely, was dominated by filamentous blue-green cyanobacteria (Smith 1986; Culver et al. 1992). Typical high levels of cyanobacteria within our source water (Naidenko et al. 2012) and initially high TP levels seen in our ponds may also have been an indication that nitrogen was the limiting nutrient (Rogge et al. 2003). Nitrogen limitation is possible in ponds with excessive phosphorus loads (Mischke 1999; Stone et al. 2012). Helal (1990) and Culver et al. (1992) showed that low N:P ratios will cause a greater dominance of non-edible phytoplankton taxa (cyanobacteria), causing poor zooplankton production. Since zooplankton populations are negatively affected by the presence of cyanobacteria (Gliwicz and Lampert 1990; DeMott et al. 1991), copepod and cladoceran populations within our fertilized ponds may have not responded as effectively as would be expected through fertilization.

Although there were not significant differences between the two treatments in either cladoceran or copepod populations, there were significant differences in both the timing of the population pulses and the actual number of preferred prey. Our analysis indicates that the fertilization treatment increased both copepod and cladoceran taxa during the second week of culture; as typically observed (Geiger 1983a, b; Parmley and Geiger 1985; Johnson and

Schlosser 1991; Ludwig1999). Given little differences in fish production between treatments, variations seen between peak magnitudes suggest that an early pulse in preferred prey may be more beneficial during phase I. Appropriate stocking time may be more overbearing than overall number of preferred prey.

Through optimal foraging behaviors, fish will benefit from having the ability to utilize larger, more energy rich prey (Mittelbach 1981). As fish grow larger, a greater selectivity towards larger prey items is common (Zaret 1980; Mittelbach 1981, 1984; Geiger 1983a). It appears that final fish biomass may have been affected by having greater peak densities of larger copepod (Cyclopoida) and cladoceran (*Daphnia*) taxa present within fertilized ponds. In relation to handling expenditure and consumption, a diet consisting of large-bodied cladocerans, e.g., *Daphnia* are beneficial to hybrid striped bass (Morris 1988). It appears that the energetic benefit to fish production from the presence of larger zooplankters was enhanced through fertilization of our ponds.

Conclusion.—Although this study showed marginal benefits from the addition of an organic fertilizer during phase I culture of hybrid striped bass, i.e., increased concentrations of copepods and cladocerans during the second week of culture, there was still improved survival and production in the fertilized ponds. Information garnered from our study indicate that zooplankton populations did increase 2 weeks post pond filling compliments recommendations from published literature that palmetto bass should be stocked at this time. Although we saw declining prey populations within all ponds during the final days of culture, greater stocking densities may be possible if zooplankton had been at their peak production prior to stocking fish.

Although initial phosphorus levels should continue to be maintained to a target level of 0.10-mg/L, it is unlikely that additional inorganic phosphorus application will be necessary given the highly nutrient rich source water. Instead, consideration should be given to managing the actual nutrient ratios. Zooplankton populations and thus fish production may also benefit from the addition of an organic fertilizer with a higher nitrogen content or an inorganic nitrogen fertilizer to ensure that N:P ratios remain within the range suitable for green alga production.

Acknowledgements

We would like to thank the Iowa Department of Natural Resources, Mount Ayr Fish Hatchery personnel, Milford Fish Hatchery personnel, and Iowa State University NREM staff. Funding provided by Fish Restoration Project F-160-R for funding of this project.

TABLE 3.1. Mean ± SE final length, final weight, survival, specific growth, and fish biomass of phase I hybrid striped bass cultured in earthen ponds with two treatments of organic fertilization at Mount Ayr Fish Hatchery, Iowa. Fertilized ponds received applications of 112-kg/ha/week of alfalfa pellets. Parameters marked with an asterisk (*) were significantly different, $P < 0.10$.

Treatment	Final Length (mm)	Final Weight (g)	Survival (%)	Specific Growth (G)	Biomass * kg/ha
Fertilized	47.7 (1.6)	1.4 (0.2)	54 (10)	14.9 (0.3)	66.6 (3.8)
Not-Fertilized	48.1 (0.3)	1.4 (0.1)	30 (8)	15.8 (0.4)	38.0 (8.8)

TABLE 3.2. Least-square means (SEM) of water chemistry variables (mg/L) in fertilized and not-fertilized earthen ponds during the 2011 hybrid striped bass culture season at Mount Ayr Fish Hatchery, Iowa. Fertilized ponds received applications of 112-kg/ha/week of alfalfa pellets.

Analysis (mg/L)	Fertilized	Not-fertilized	Treatment *P*-value	Treatment x date *P*-value
Total Ammonia Nitrogen	0.46 (0.10)	0.77 (0.12)	0.14	0.31
Unionized Ammonia	0.03 (0.01)	0.05 (0.01)	0.25	0.01
Nitrate	0.80 (0.09)	1.08 (0.11)	0.16	0.64
Nitrite	0.04 (0.01)	0.07 (0.01)	0.25	0.72
Total Phosphorus (TP)	0.16 (0.02)	0.15 (0.03)	0.59	0.43
NO_3: T:P	4.8 (0.48)	7.34 (0.58)	0.04	0.66
Alkalinity	110 (5.1)	125 (6.2)	0.14	0.69
Hardness	136 (6.7)	139 (8.0)	0.78	0.34
Turbidity	22.2 (3.73)	26.7 (4.57)	0.50	0.75

Repeated measures mixed model, $N = 3$.

TABLE 3.3. Least-square means (SEM) of morning (AM) and afternoon (PM) pH and dissolved oxygen (DO) profiles in fertilized and not-fertilized earthen ponds during the 2011 hybrid striped bass culture season at Mount Ayr Fish Hatchery, Iowa. Fertilized ponds received applications of 112-kg/ha/week of alfalfa pellets.

			Treatment	Treatment x date
Analysis	Fertilized	Not-fertilized	*P*-value	*P*-value
AM pH	7.56 (0.12)	7.4 (0.15)	0.49	0.25
AM DO Top	6.62 (0.35)	6.30 (0.43)	0.61	0.64
AM DO Middle	5.12 (0.85)	5.11 (1.05)	0.99	0.09
AM DO Bottom	2.88 (0.37)	2.71 (0.46)	0.80	0.88
PM pH	8.18 (0.12)	8.14 (0.15)	0.88	0.02
PM DO Top	8.04 (0.37)	8.44 (0.47)	0.56	0.11
PM DO Middle	6.26 (0.63)	5.76 (0.77)	0.65	0.47
PM DO Bottom	4.07 (0.81)	3.86 (1.02)	0.88	0.57

Repeated measures mixed model, $N = 3$.

TABLE 3.4. Least-square means (SEM) of zooplankton (organisms/L) in fertilized and not-fertilized earthen ponds during the 2011 hybrid striped bass culture season at Mount Ayr Fish Hatchery, Iowa. Fertilized ponds received applications of 112-kg/ha/week of alfalfa pellets.

	Fertilized	Not-fertilized	Treatment *P*-value	Treatment x date *P*-value
Rotifera	**65.67 (15.64)**	**61.70 (19.58)**	**0.88**	**0.40**
Asplanchna	11.85 (5.07)	1.67 (6.34)	0.30	0.51
Brachionus	10.67 (3.39)	13.17 (4.26)	0.68	0.29
Filinia	0.26 (0.09)	0.06 (0.12)	0.26	0.86
Keratella	30.22 (10.41)	33.53 (13.05)	0.86	0.27
Polyarthra	5.33 (1.69)	10.93 (2.13)	0.13	0.29
Testudinella	7.33 (1.91)	2.39 (2.52)	0.22	0.48
Copepoda	**31.82 (4.09)**	**27.58 (5.18)**	**0.57**	**0.07**
Calanoida	4.74 (0.94)	4.50 (1.20)	0.88	0.99
Cyclopoida	27.19 (4.34)	23.38 (5.50)	0.62	0.07
Nauplius	54.93 (4.97)	52.27 (6.50)	0.77	0.08
Cladocera	**55.18 (6.59)**	**32.78 (8.48)**	**0.13**	**0.09**
Bosmina	7.11 (2.09)	1.48 (2.58)	0.19	0.10
Ceriodaphnia	6.41 (1.88)	3.57 (2.46)	0.43	0.91
Daphnia	41.19 (5.58)	27.24 (7.14)	0.22	0.02
Scapholeberis	0.41 (0.34)	0.17 (0.44)	0.69	0.99

Repeated measures mixed model, $N = 3$.

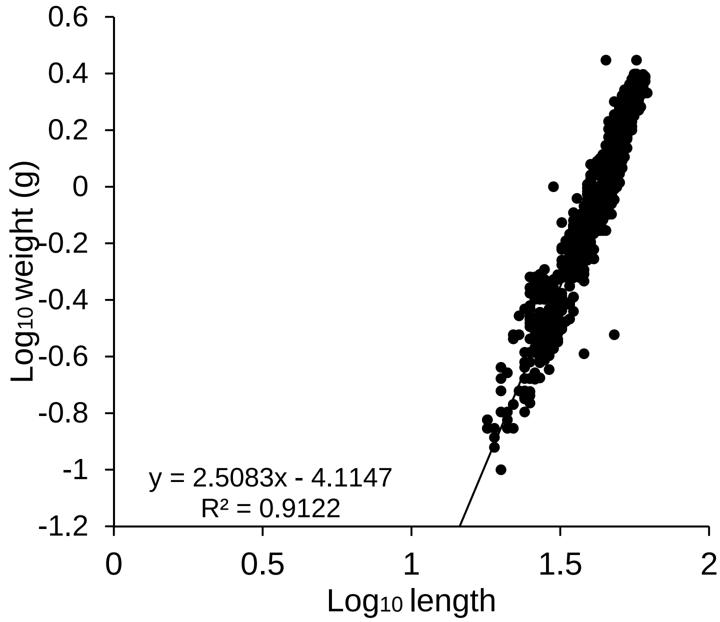


FIGURE 3.1. Log transformed total length and weight of 1,670 hybrid striped bass sampled weekly from 9-May 2011 to 28-May 2011 at Mount Ayr and Rathbun Fish Hatcheries, Iowa. Weight of fry was estimated using the following equation: $y = 2.5083x - 4.1147$. Initial total lengths of 30 fish were used to estimate a mean fry length for x.

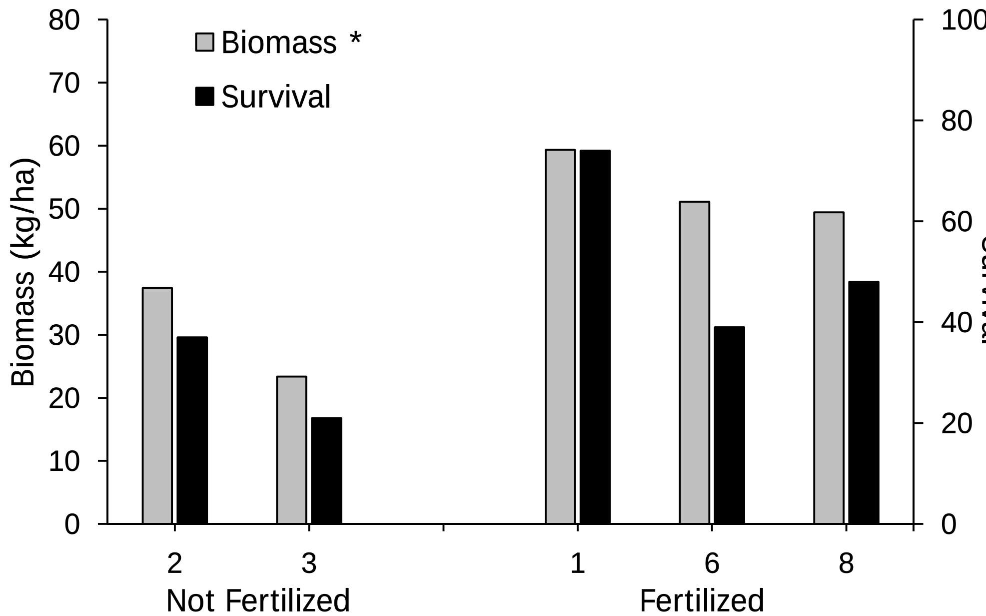


FIGURE 3.2. Survival (%) and biomass (kg/ha) of hybrid striped bass fingerlings from earthen ponds during the 2011 culture season at Mount Ayr Fish Hatchery, Iowa. Fertilized ponds received applications of 112-kg/ha/week of alfalfa pellets. Parameters marked with an asterisk (*) were significantly different, $P < 0.10$.

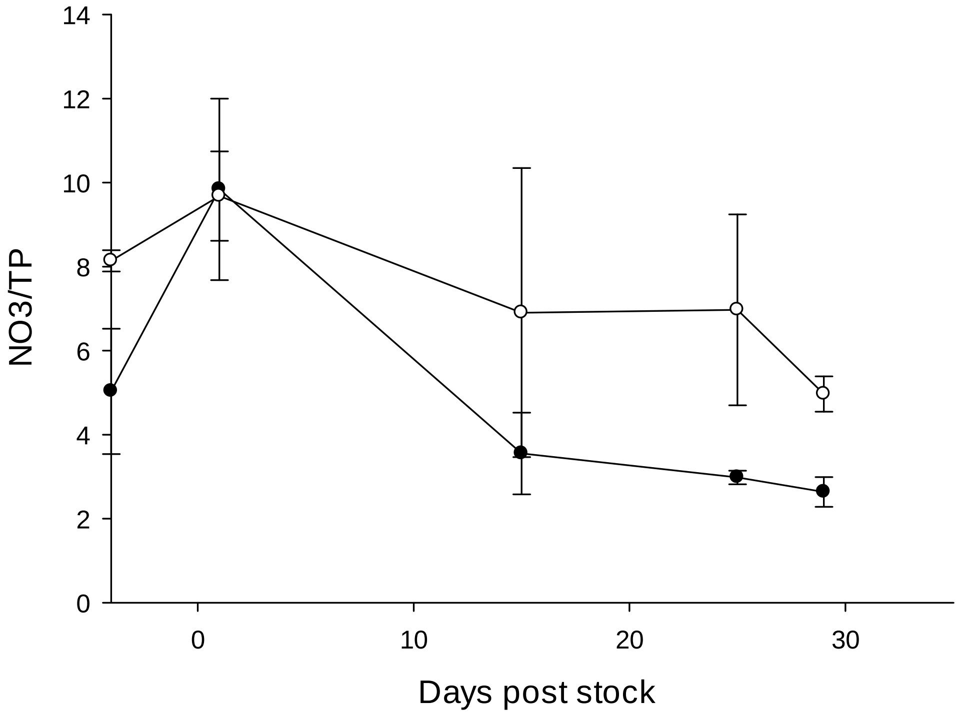


FIGURE 3.3. Mean ± SE ratios of NO_3-N:TP in earthen ponds during the 2011 hybrid striped bass culture season at Mount Ayr Fish Hatchery, Iowa. Fertilized ponds (closed circle) received weekly applications of 112-kg/ha/week of alfalfa pellets. Open circles represent control ponds which did not receive any fertilizer.

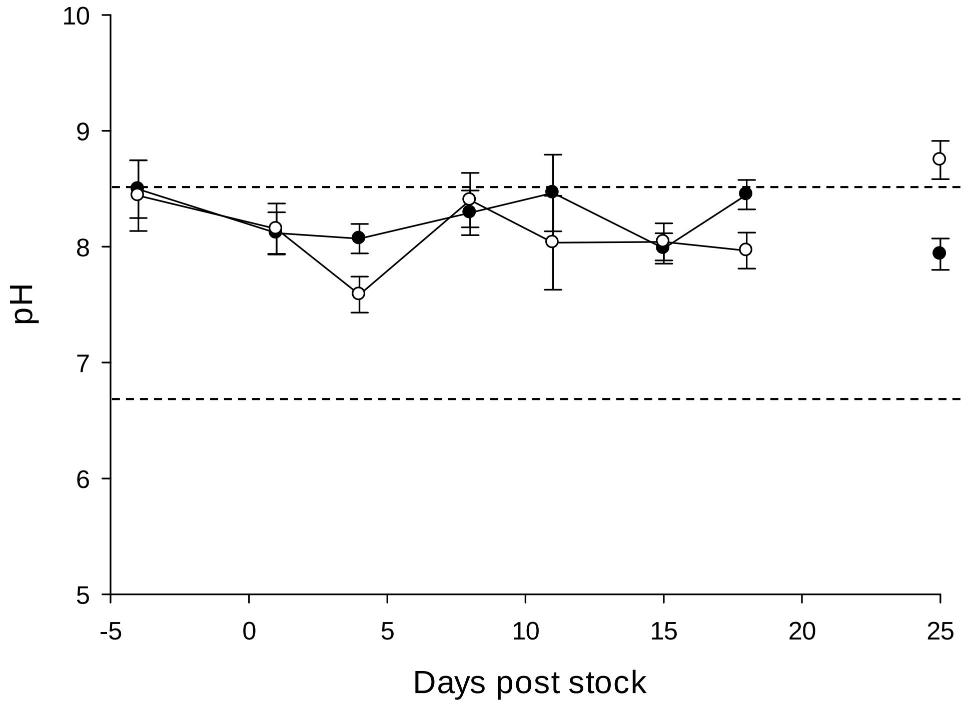


FIGURE 3.4. Mean ± SE afternoon pH values in earthen ponds during the 2011 hybrid striped bass culture season at Mount Ayr Fish Hatchery, Iowa. Fertilized ponds (closed circles) received weekly applications of 112-kg/ha/week of alfalfa pellets. Open circles represent control ponds which did not receive any fertilizer. Dashed lines represent the recommended range (Hodson and Hayes 1989; Morris et al. 1999).

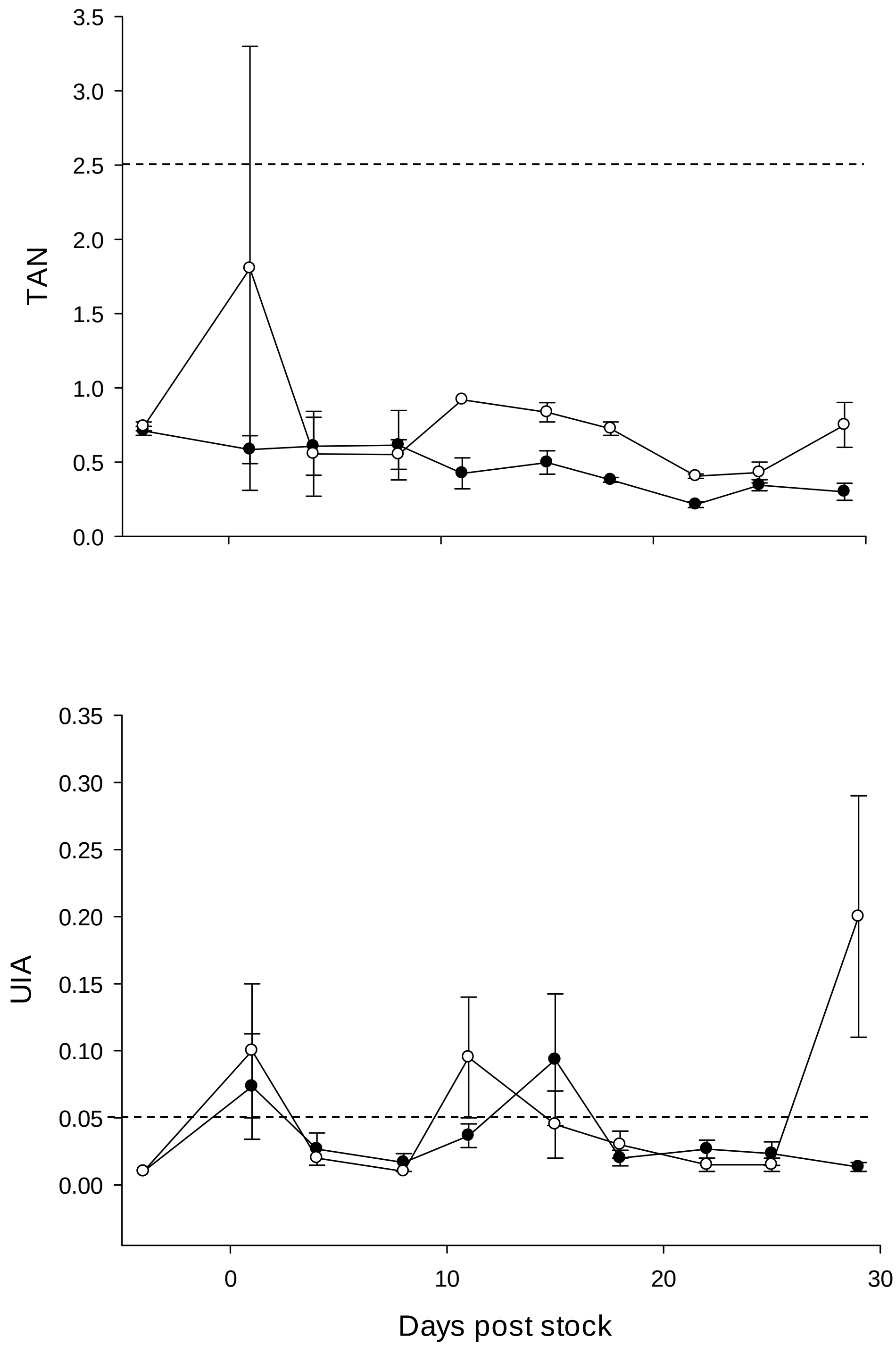


FIGURE 3.5. Mean ± SE total ammonia nitrogen (TAN) and unionized ammonia (UIA) concentrations in earthen ponds during the 2011 hybrid striped bass culture season at Mount Ayr Fish Hatchery, Iowa. Fertilized ponds (closed circles) received weekly applications of 112-kg/ha/week of alfalfa pellets. Open circles represent control ponds which did not receive any fertilizer. Dashed lines represent lethal TAN and detrimental UIA concentrations (Ludwig 2007).

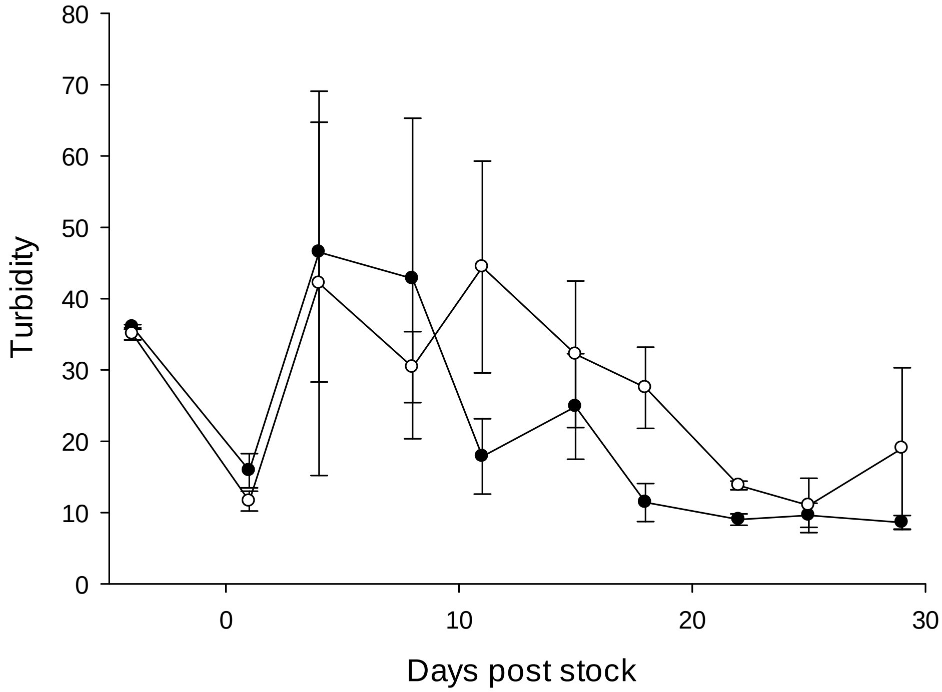


FIGURE 3.6. Mean ± SE turbidity in earthen ponds during the 2011 hybrid striped bass culture season at Mount Ayr Fish Hatchery, Iowa. Fertilized ponds (closed circles) received weekly applications of 112-kg/ha/week of alfalfa pellets. Open circles represent control ponds which did not receive any fertilizer.

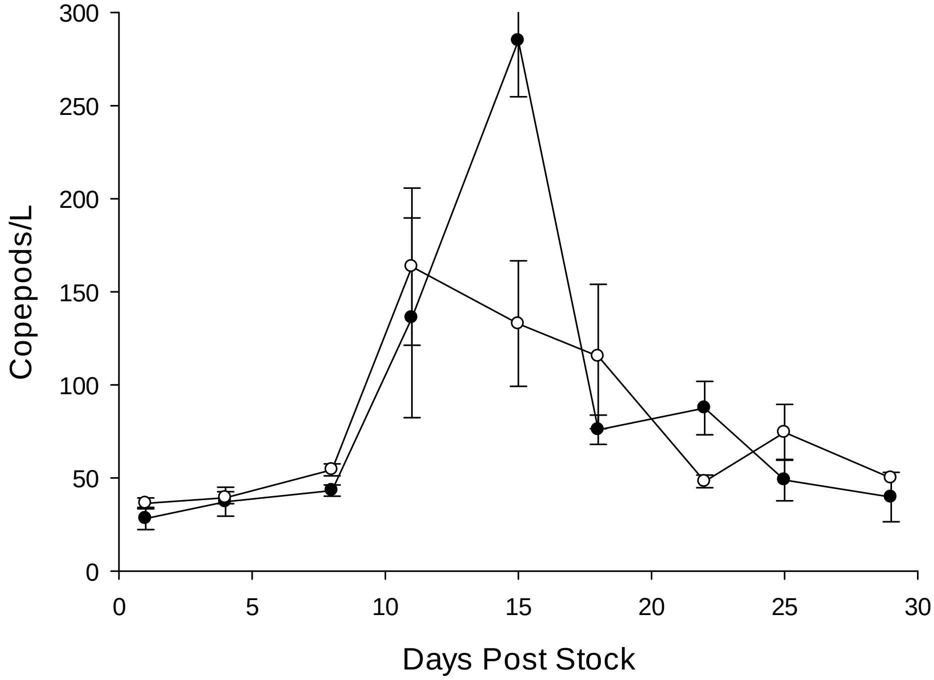


FIGURE 3.7. Mean ± SE concentrations (#/L) of copepod populations in earthen ponds during the 2011 hybrid striped bass culture season at Mount Ayr Fish Hatchery, Iowa. Fertilized ponds received applications of 112-kg/ha/week of alfalfa pellets. Open circles represent control ponds which did not receive any fertilizer.

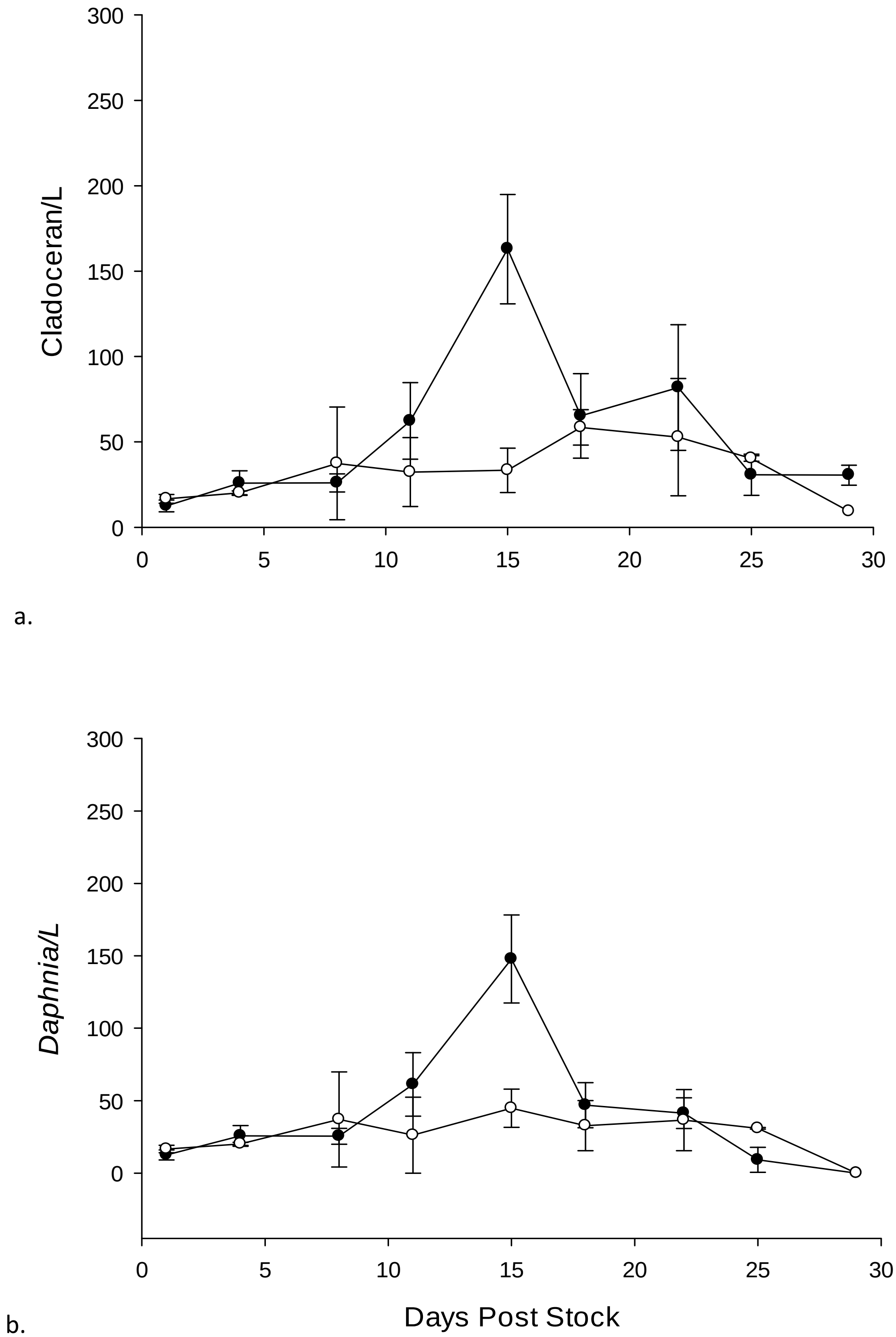
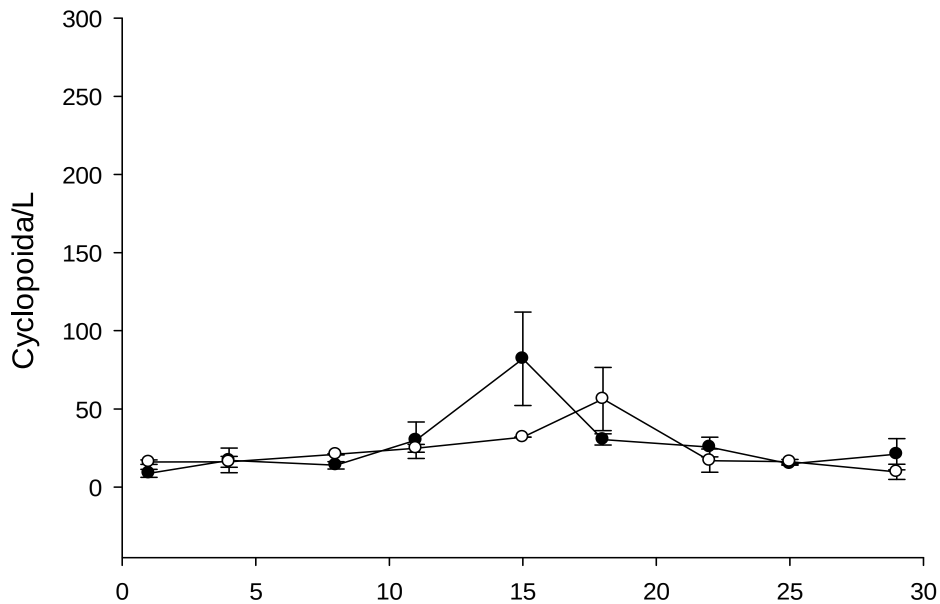
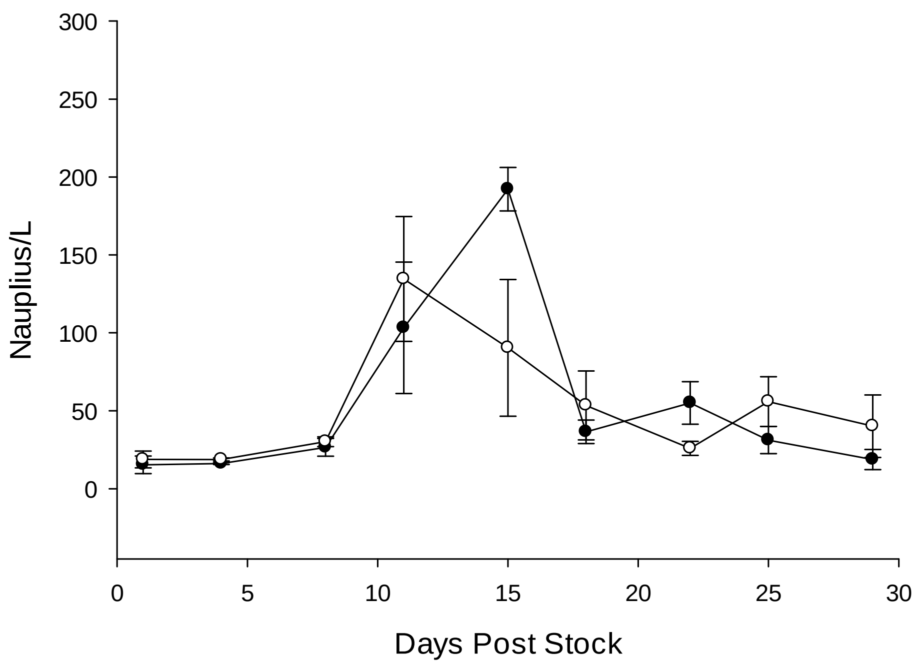


FIGURE 3.8. Mean ± SE concentrations (#/L) of cladoceran (a.) and *Daphnia* (b.) populations in earthen ponds during the 2011 hybrid striped bass culture season at Mount Ayr Fish Hatchery, Iowa. Fertilized ponds received applications of 112-kg/ha/week of alfalfa pellets. Open circles represent control ponds which did not receive any fertilizer.



a.



b.

FIGURE 3.9. Mean ± SE concentrations (#/L) of Cyclopoida (a.) and nauplius (b.) populations in earthen ponds during the 2011 hybrid striped bass culture season at Mount Ayr Fish Hatchery, Iowa. Fertilized ponds received applications of 112-kg/ha/week of alfalfa pellets. Open circles represent control ponds which did not receive any fertilizer.

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CHAPTER 4. ROLE OF SUPPLEMENTAL FEEDING ON PHASE I PALMETTO BASS PRODUCTION IN PLASTIC-LINED PONDS

(In Prep)

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Abstract

The role of offering a commercial pelleted diet has been characterized as both an expensive organic fertilizer and as a selected food item for larval hybrid striped bass (palmetto) *Morone saxatilis* × *M. chrysops* culture operations. Our study investigated the effect of a commercial diet on palmetto bass production and zooplankton densities during phase I culture in plastic-lined ponds. We also sought to estimate relative dietary contribution of a commercial fish feed relative to natural biota using stable isotope analysis. In 2011, palmetto bass were stocked into six 0.04-ha plastic-lined ponds at a rate of 125,000/ha. During the 31-d culture period, all ponds received weekly applications of alfalfa pellets until 14 days post stock (dps) at a rate of 112-kg/ha/week. At 14-dps, the application of organic fertilizer was discontinued within fed ponds and Silver Cup™ Trout Fry diet was offered at a rate of 13.6-kg/ha daily until harvest; ponds in the unfed (control) treatment continued to received weekly applications of alfalfa meal (three ponds per treatment). Mean final fish length was significantly greater within the fed ponds; fish weight, survival, growth, and biomass were not significantly different between treatments ($P < 0.1$). Although there were no significant differences in overall zooplankton population densities, copepod taxa concentrations in the fed ponds were greater once feed was applied. We found a significant enrichment in both $\delta^{15}N$ and $\delta^{13}C$ values of fish, zooplankton, and Chironomidae larvae within fed ponds ($P < 0.10$). Using a three-source mixing model, the mean (±SE) percent composition of feed in the fish's isotopic signature increased from 5% ±2 to 20% ±6 within 16 days. Although fish production was not greatly impacted through the addition

of a commercial fish feed, enriched $\delta^{15}N$ and $\delta^{13}C$ values of fish tissue indicated that palmetto bass fingerlings did utilize prepared diets. However, natural pond biota continued to account for ≥ 80% of the fish's isotopic signature, based on tissue analysis. Enrichment of both zooplankton and Chironomidae larvae also indicate that the feed acted as a pond fertilizer through enhancing lower trophic organisms.

Introduction

The culture of striped bass (*Morone saxatillis)* and its hybrids is divided into three distinct phases based upon specific life history stages and production needs of the fish. Phase-I consists of larval fish to 2.5-cm fingerlings (30-to 60-d post-hatch) and is highly dependent on the availability of zooplankton. Phase-II production includes feed trained fingerlings to 15-cm juveniles cultured their first year of life. Phase-III fish are those intended to be raised to market size foodfish in the second year (Brewer and Rees 1992). Kohler (2004) considered the hybrid striped bass to be a species of high priority because of its importance as a cultured foodfish as well as its use by many agencies for improving or enhancing many sport fisheries. Although hybrid striped bass culture does not appear to be gaining in popularity within the Midwest, it continues to be an economically important fish cultured in the Southeastern United States (Turano 2012).

As with most cultured fish, hybrid striped bass have tested production techniques and specific environmental requirements for pond culture (Harrell et al. 1992). Acceptable water quality includes pH levels of 6.7-8.5 (Hodson and Hayes 1989; Morris et al. 1999) with acute mortality occurring in 2-d post hatch (dph) fry when pH exceeds 9.8 for even minimal exposure times (Bergerhouse 1993). In exceedingly productive culture ponds, high pH will result in elevated levels of un-ionized ammonia (UIA), which is proven harmful to the sunshine bass

Morone chrysops x M. saxatilis at a concentration of 0.05 mg/L (Ludwig et al. 2007). Total ammonia-nitrogen (TAN) levels higher than 2.5 mg/L are lethal to hybrid striped bass (Hargreaves and Kucuk 2001).

Many cultured fish, including the hybrid striped bass (palmetto) *Morone saxatilis* × *chrysops*, require natural prey during their transformation from endogenous to exogenous feeding (Geiger 1983a.; Ludwig 1999). Endogenous use of yolk sac reserves occurs during the first 4 to 6-dph (Gatlin 1997), at which point adequate populations of appropriately sized prey items, including zooplankton, are required for their initial feeding (Morris et al. 1999). Survival of hybrid striped bass sac fry is highly dependent on having the proper size and taxa of zooplankton present at the time of stocking (Geiger 1983a; Ludwig 1999; Morris et al. 1999).

Production of desirable zooplankton is often stimulated using a combination of organic and inorganic fertilizers within larval culture ponds (Geiger 1983a, b; Geiger et al. 1985; Farquhar 1987; Ludwig 2004). Dietary requirements during early development coupled with sensitivity to elevated pH levels (associated with pond fertilization) require a balance between high productivity and adequate water quality.

Numerous field studies of striped bass fry have noted the role of specific prey taxa needed for successful phase I culture in ponds (Harper et al. 1969; Humphries and Cumming 1973; Harrell et al. 1977; Geiger et al. 1985; Fitzmayer et al. 1986). Although striped bass fry are a good surrogate for the palmetto bass because of similar size and production requirements, diet studies for the palmetto bass are not common (Ludwig 1999). Woods et al. (1985) found that the diet of palmetto bass fry, 6-9 mm total length (TL), consisted entirely of cladoceran and copepod taxa and that a discernible shift towards insect larvae (Chironomidae) occurs with increased fish size (about 20-dph). Once fry reached 15-20 mm TL, the frequency of occurrence of chironomid larvae in the stomach samples was 100% (Woods et al. 1985). Their reliance on copepods, cladoceran, and Chironomidae larvae during phase I make pond fertilization essential for adequate production (Parmley and Geiger 1985; Geiger and Turner 1990).

In addition to boosting natural production through fertilizer applications, culturists will sometimes use commercial diets during phase-I culture of hybrid striped bass (Ludwig 2004; Wamboldt 2013). Studies involving other species of cultured fish, e.g. *chondrosteans*, indicate the importance of offering artificial diets prior to natural food depletion in order for fish to accept it as food (Buddington and Christofferson 1985). However, increased biomass and yield has warranted offering a commercial diet immediately upon stocking hybrid striped bass fry (Morris and Muncy 1989). With feed costs being the primary expenditure of most culturists, it is essential to minimize waste and to better understand the advantages of using a commercial feed during phase I culture.

Although phase I fish readily accept a commercial diet, it is typically considered to be supplemental to natural prey in ponds (Fitzmayer et al. 1986; Morris 1988). It is also unclear why larval *Morone* do not appear to utilize prepared diets effectively for growth (Baragi and Lovell 1986). Fitzmayer et al. (1986) was able to obtain greater survival of striped bass fry fed at higher rates but noted that natural pond biota contributed a high percentage the fish's diet under all feeding regimes. Similarly, Morris (1989) was able to increase growth of palmetto bass fingerlings through the use of a commercial feed but also indicated that consumption of copepods, cladoceran, and chironomid larvae persisted throughout phase I. Although the fact that phase I production appears to benefit from the use of a formulated fish feed is important to the fish culturists, there remains the question of how the feed is actually contributing to the culture environment.

Stable Isotopes.—Typical diet studies that rely on gut content analysis (GCA) are both labor and time intensive because they require large sample sizes and extensive identification techniques for stomach contents (Clarke et al. 2005; Christensen and Moore 2009). Furthermore, ontogenetic diet shifts and temporal variability of consumption limits GCA to relatively recent feeding activity (Power and Dietrich 2002; Clarke et al. 2005; Christensen and Moore 2009). Other methods that rely on the addition of a pigment to formulated feeds (Levine and Sulkin 1984; Walsh et al. 1987; Morris et al. 1990) are short term, only determine ingestion, and do not explain digestibility and assimilation for tissue growth. All of these issues are compounded when working with larval fish because of their small size and fast growth.

A relatively new approach using a combination of stable carbon and nitrogen isotopes has become a popular technique to examine many ecological community interactions (Peterson and Fry 1987; Kling et al. 1992; Post 2002; Fry 2006). The benefit of using stable isotope analysis over stomach content analysis is that it can give researchers a temporally integrative, long term description of a fish's consumption in addition to a quantitative measure of trophic interactions within a food web (Vander Zanden et al. 1998; Post 2002; Clarke et al. 2005). Difficulties associated with larval fish culture and the assessment of consumption for extremely small, fast growing entities make stable isotope analysis ideal for measuring the direct incorporation of dietary material (Vay and Gamboa-Delgado 2011). Stable isotope values are denoted as a difference measurement made relative to standards during analysis and relate values as a ratio of the heavy isotope to the light isotope of an element (Fry 2006). Values are reported as a permil (‰) relative to the standards in a standard delta notation:

$$\delta = \left[\left(\frac{R_{SAMPLE}}{R_{STANDARD}} - 1\right)\right] \times 1{,}000$$

in which R = either $^{15}N/^{14}N$ or $^{13}C/^{12}C$ (Fry 2006).

The technique of using stables isotopes to trace dietary items relies on the assumption that a consumers muscle tissue is a product of its diet, and that this is reflected in the isotopic composition of the individual (Fry 2006). As nitrogen isotopes are transferred to higher trophic positions within a food chain, via consumption, ^{15}N is enriched by roughly 3.4‰ through a process known as trophic fractionation (Vander Zanden and Rasmussen 2001; Vander Zanden et al.1999; Fry 2006). Trophic fractionation is the result of heavier isotopes being preferentially selected at the cellular level within the consumer during many metabolic processes; producing isotopic enrichment within higher trophic organisms (Fry 2006; Gamboa-Delgado et al. 2008).

During photosynthesis, ^{13}C fractionates distinctly between plants with different photosynthetic pathways, such as C3 or C4 plants (O'Leary 1988). Differences in these photosynthetic pathways and relative fractionation allow $\delta^{13}C$ to be used to trace energetic inputs within many aquatic ecosystems (Hecky and Hesslein 1995; Hobson 1999; Schmidt et al. 2007). Furthermore, with little trophic fraction of ^{13}C (difference > 1‰) between consumers and prey (Gearing 1991; Van Zanden et al. 1998), carbon inputs within aquatic ecosystems can be traced through subsequent consumers based on sources of primary production (Clarke 2003; Hecky and Hesslein 1995). The relative dietary contribution of isotopically distinct food items can be estimated by incorporating $\delta^{15}N$ and $\delta^{13}C$ signatures into many mixing models (Fry 2006; Gamboa-Delgado et al. 2008). An overview of such isotope mixing models is provided by Layman et al. (2012).

Our goal for this study was to understand the effects of offering a commercial fish feed during phase I too palmetto bass in plastic-lined ponds. The question we sought to answer with this research was to determine how much of the commercial diet contributed directly to fish growth. By estimating the contribution of natural pond biota and feed that is incorporated into the fish's muscle tissue, we attempted to determine whether feed was directly utilized by the fish or indirectly affecting growth and survival by performing as an expensive pond fertilizer. The objectives of this study were to (1) determine if feed application affected production of phase I palmetto bass or zooplankton populations, and (2) determine an estimate of the relative dietary contribution of a commercial fish feed relative to natural pond biota for palmetto bass cultured in fertilized plastic-lined ponds. During the 2011 culture season, a 6-week experiment was conducted within six experimental plastic-lined ponds located at the Rathbun Fish Culture Research Facility, Iowa.

Methods

Study Site.—The Rathbun Fish Culture Research Facility is located 11.3 km north of Centerville, Iowa. Six 0.04-hectare plastic-lined ponds served as the experimental unit. Water was supplied via the Rathbun reservoir and filter through a 300- um drum filter.

Study Design.— Sac fry, 5-dph, were transported by plane to Iowa from the Milford Hatchery, Kansas. To quantify mortality due to transport induced stress, fry were placed into submerged barrels for approximately 24 h. Barrels were placed 5-10 cm below the water's surface to allow living fish to escape into the pond. Fry within the barrels were protected from ultraviolet radiation with Styrofoam insolation placed above the barrels. After 24 h, dead sac fry present at the bottom of the barrels were enumerated and subtracted from our stocking estimates. The number of fish stocked was estimated using a fry counter (Jensorter™ FCM Sweet Home, Oregon).

Pond filling began 5 days pre-stock on 19 May, 2011. Filling was interrupted on the day of stocking (23 May) and resumed on 24 May to allow for enumeration of dead fry in submerged barrels. All attempts were made to fill each of the six ponds at an equal rate once filling resumed. The initial goal was to stock all ponds with 200,000 fish/ha. However, due to the inherent inaccuracies of density estimates (Matlock 2011) of larval fish within shipping containers, our actual stocking rate was estimated to be 125,000 fish/ha.

All ponds received an initial application of 112 kg/ha of alfalfa pellets around the perimeter of each pond; applications limited to a depth of 0.6-0.9 meters. Alfalfa pellets were then applied twice a week thereafter at a rate of 56-kg/ha/week. In accordance with current agency culture protocols, individual ponds were adjusted to have an initial minimal total phosphorus (TP) level of 0.10 mg/L using Scotts Pond Fertilizer.

Treatments included fed and not-fed with three replicate ponds randomly assigned per treatment. At 14-dps, fish within fed treatment ponds were offered Silver Cup™ trout diet (Purina Mills, LLC, Gray Summit, Missouri) at a rate of 13.6-kg/ha on a daily basis until harvest. Once feeding began, fertilizer application was discontinued within the fed ponds. Non-fed treatment (control) ponds continued to receive weekly applications of alfalfa pellets but were not offered a commercial diet.

Fish were harvested on 23-June, 2011 for a grow-out period of 32 days. Ponds were drained overnight and seined from the kettle mid-morning the following day. Total fish harvested was estimated using multiple subsample check-weights pond-side.

Sampling methods.—Twice weekly, water samples were collected mid-morning from the kettle of each pond using a 5.0-cm PVC tube sampler (Graves and Morrow 1988). One additional random sample was collected for validation. Water samples were stored in acid-washed polyethylene bottles and kept on ice until tested for total ammonia nitrogen (TAN-N), nitrate (NO_3-N), and nitrite (NO_2-N) using a Hach ™ DR/2800 spectrophotomer (Hach, Loveland, Colorado). Additional water chemistry analysis conducted weekly included total phosphorus, total hardness, and alkalinity. On the same days which water was collected and analyzed, pond-side water quality measurements were taken mid-morning and early-afternoon using a Hach™ HQ 40d meter (Hach, Loveland, Colorado). Temperature, pH, and dissolved oxygen (DO) were measured at the bottom, middle and top of each kettle.

We attempted to collect 30 fish from each pond twice a week to monitor growth. Fish were sampled using a modified combination of multiple Quatrefoil light-trap designs (Floyd et al. 1984; Secor et al. 1992; Summerfelt et al.1996) during the first 2 weeks of culture. A 1-cm mesh seine was used for collection once fish no longer appeared to be attracted to the light traps (week 2 of culture). During each collection period, a random subsample of five fish was stored at -20° C to be used for stable isotope analysis at a later date.

Thawed fish smaller than 20 mm were decapitated and removed of dorsal and caudle fins while larger individuals were filleted and skinned. All prepared samples were freeze-dried using a LABCONCO™ 4.5 freeze dry system (Kansas City, MO) for at least 24 h. Once dry, samples were ground into a fine powder and homogenized using a mortar and pestle. Fish that were not large enough to be ground individually were combined with multiple samples from the same pond and treated as a single replicate.

Zooplankton was collected twice weekly from three standard locations around the parameter of each pond using an 80-ųm Wisconsin plankton net. Oblique plankton tows were two meters in depth and filtered 68-L of water. One additional random sample was collected for validation. Zooplankton samples intended for identification and enumeration were preserved with a chilled formalin/sucrose solution (Haney and Hall 1972).

Additional zooplankton samples were collected in the same manor described above and stored frozen at -20^{0} C for stable isotope analysis. Prior to freezing, zooplankton samples were filtered through a 270-um mesh screen and rinsed with nano-pure in an attempt to remove rotifers from the sample. Chironomidae larvae and other debris were removed manually from each filtered sample and inspected under a dissecting microscope to contain predominantly copepods and cladocerans. Filtered samples were freeze-dried for at least 24-h and ground into a fine powder as described above. Stable isotope signatures for zooplankton represent the aggregate of several zooplankters within the sample from individual ponds.

Benthic invertebrates were collected once a week from three random locations in each pond using a benthic pump sampler (Morris and Clayton 2007). Benthic samples were stored at -20^{o} C for stable isotope analysis at a later date. An aggregate of ~30 chironomids from each pond were freeze-dried for at least 24-hr and ground into a fine powder as described above.

Stable Isotopes. — A 500-ug sub-sample of the resulting zooplankton, chironomidae, and fish powder from each pond was weighed into a 0.2 x 0.2-cm aluminum weigh boat and measured with a Finnigan MAT Delta Plus XL mass spectrometer in continuous flow mode connected to a Costech Elemental Analyzer. Twelve reference standards (2 Caffeine [IAEA-600], 3 Cellulose [IAEA-CH-3], 2 Ammonium Sulfate [IAEA-N-1], 2 Ammonium Sulfate [IAEA-N-2], and 3 Acetanilide [laboratory standards]) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale. The typical analytical uncertainty for $\delta^{13}C$ and $\delta^{15}N$ was ±0.18 and ±0.12 respectively.

To estimate consumption based on relative percent contribution of sources to isotopic signature of the consumer, we used a dual-isotope, three-source mixing model. Trophic fractionation was accounted for by applying + 0.8‰ $\delta^{13}C$ and +3.4‰ $\delta^{15}N$ to consumer delta values. Isotopic values of individual fish were applied to the following formula:

$$\%\,X\ in\ diet = \left(1 - \frac{(DA' + DB' + DC')}{DA' + DB' + DC'}\right) \times 100\%$$

where *X* = zooplankton (*A*), Chironomidae (*B*), or feed (*C*) and *DX′* corresponds to the Euclidean distance between *D* and *X′* (Kline et al. 1993).

Euclidean distances between isotopic source values and isotopic consumer values + trophic fractionation represent the relative contribution (%) of source *X* to hybrid striped bass muscle tissue values. Two collection periods in which there were missing zooplankton and chironomidae samples, delta values were estimated using either regression analysis or mean values from congruent sampling days. Percent contribution was calculated six separate days using individual fish signatures with corresponding zooplankton and Chironomidae from individual ponds. Isotopic values of feed used in the mixing model also corresponded with the type of feed used at the time fish were sampled. An assumption of this model is that the closer a source is to a consumer within a dual-isotopic plane, the greater that source contributed to the consumers' isotopic signature.

Analyses. — All statistical analyses were performed with Statistical Analysis Systems version 9.3 (SAS Institute 2011) with statistical significance set at $P < 0.10$. Repeated measures analyses on water quality and zooplankton parameters were done using the mixed model. The error term used in treatment comparisons was pond (treatment); the error term for daily comparisons was day × pond (treatment). Final fish production variables and isotope delta values were evaluated using pairwise comparisons (two-sample *t*-test). Tukey's honestly significant difference was used for pairwise comparisons. Simple linear regression was used to evaluate relationships in isotopic values and time throughout the culture period.

Results

Production.—Mean (±SE) final TL of hybrid striped bass within the fed treatment (51.9 mm ± 2.3) was significantly longer than fish that were not offered a pelleted diet (45.9 mm ± 1.6; $P = 0.09$). No significant differences were detected between treatments for final weight, survival, or growth (Table 4.1). Though not statistically significant, mean (±SE) final fish biomass was numerically greater in the fed (71.4 kg/ha ± 6.1) than in the non-fed ponds (54.6 kg/ha ± 6.4).

Water quality.—Mean water chemistry parameters and water temperature profiles were not significantly different between the two treatments (Tables 4.2 and 4.3). Morning and afternoon pH values were typically above 8 within all ponds and remained above the recommended range of 6.7-8.5 (Hodson and Hayes 1989; Morris et al. 1999) within all ponds. Although late season pH values were relatively high within both treatments, no ponds exceeded the mortality threshold of 9.8 (Bergerhouse 1993). Surface and mid-depth DO concentrations were similar within the fed and non-fed ponds. However, once the initiation of feeding began, mean morning and afternoon DO concentrations were significantly lower at the bottom of the ponds within the fed treatment (Figure 4.2; Table 4.3).

Zooplankton.— Zooplankton concentrations were not significantly different between the two treatments (Table 4.4). However, there was a significant interaction between treatment and time with rotifers (*Branchionus, Filinia*, *and Testudinella),* nauplius, and *Bosmina* (Table 4.4).

Although rotifer populations did not appear to be affected by the application of feed, there were greater densities of *Branchionus* and *Testudinella* during the final week of culture within the non-fed ponds (Table 4.4; Figure 4.3). Numerically greater densities of copepods within the fed treatment is reflected in a significant interaction between treatment and time for nauplius ($P = 0.04$; Figure 4.4). Because no ostracods were identified throughout the duration of the study, nauplii were assumed to be Copepoda. Mean (±SE) copepod population densities peaked 10-dps at similar concentrations within both treatments (403/L ± 26 in fed ponds and 372/L ± 35 in non-fed ponds). A second spike in copepod (primarily nauplii) densities occurred at 24-dps within both treatments, but was significantly greater in fed ponds ($t = 5.68$; $df = 3$; $P = 0.005$). Adult copepod samples predominantly consisted of (~ 96%) Cyclopoida and (~ 4%) Calanoida. Cladoceran concentrations tended to increase throughout the culture period and appeared to peak near the final days of culture (Figure 4.5). Cladoceran concentrations also appeared to be more sporadic within the fed than in non-fed ponds. *Bosmina* densities were significantly greater within the non-fed ponds near the end of the culture season ($P = 0.01$; Figure 4.5b). Cladoceran samples primarily consisted of (~ 57%) *Bosmina*, (~ 41%) *Daphnia*, and (~ 2%) C*eriodaphnia*.

Stable Isotopes.—Ten days post stock, initial mean (±SD) $\delta^{15}N$ value of hybrid striped bass in fed ponds was 7.69‰ ± 0.34 and 8.10‰ ± 0.03 in non-fed ponds. Initial mean (±SD) $\delta^{13}C$ values of hybrid striped bass were -24.10‰ ± 1.09 in the fed ponds and -24.70‰ ± 0.48 in the non-fed ponds. Prior to feeding, there were no significant differences in both $\delta^{15}N$ and $\delta^{13}C$ between treatments ($P = 0.23$ and 0.55 respectively). Eight days after the initial feeding began (22-dps), the mean (±SD) $\delta^{15}N$ value of hybrid striped bass within fed ponds were enriched to 8.86‰ ± 0.44. Twenty-two days post stock, and thereafter, mean $\delta^{15}N$ values were significantly more positive in the fed treatment ($P = 0.016$, $P = 0.026$, $P = 0.037$, $P = <0.001$). In contrast to the $\delta^{15}N$ values there were no significant differences in mean hybrid striped bass $\delta^{13}C$ values between treatments ($P > 0.10$). A strong positive relationship between mean hybrid striped bass $\delta^{15}N$ values and time was evident within fed ponds (Figure 4.6; $P = 0.008$). Though not statistically significant, a trend indicated that the mean hybrid striped bass $\delta^{15}N$ values did increase over time in the non-fed ponds as well (Figure 4.6; $P = 0.064$).

Initially, mean (±SD) zooplankton $\delta^{15}N$ values dropped from 4.54‰ ± 0.34 to 1.20‰ ± 0.44 with no difference between treatment ponds. Three days after the initial feed application, mean (±SD) zooplankton ^{15}N values was enriched (4.16‰ ± 0.19) within fed ponds, and 3.41‰ ± 0.38 in the non-fed ponds. From this time forward (17-dps+), mean zooplankton $\delta^{15}N$ values were significantly more positive within the fed ponds (Figure 4.7; $P = 0.04$ and 0.008). By 24-dps mean (±SD) zooplankton $\delta^{13}C$ values were significantly less negative within the fed ponds (-23.48‰ ± 0.97) than in the non-fed ponds (-25.42‰ ± 1.55; $P = 0.03$). Prior to this time, mean zooplankton $\delta^{13}C$ values were similar between treatments ($P > 0.1$).

Before to the initial day of feed application, chironomid larvae had similar mean (±SD) $\delta^{15}N$ values in the fed (4.28‰ ± 0.42) and non-fed ponds (4.12‰ ± 0.15). Ten days after the first feeding, mean (±SD) chironomid larvae ^{15}N values were enriched (8.43‰ ± 0.77) within fed ponds. Mean (±SD) $\delta^{15}N$ values of chironomid larvae (3.87‰ ± 0.31) did not change over time in non-fed ponds (Figure 4.8; $P = 0.86$). By 24-dps mean (±SD) chironomid larvae $\delta^{13}C$ values were significantly less negative within the fed ponds (-21.46‰ ± 0.31) than in the non-fed ponds (-25.12‰ ± 0.92; $P = 0.003$). Prior to this time, mean chironomid larvae $\delta^{13}C$ values were similar between treatments ($P > 0.10$).

Proximate analysis of the different pellet sizes of the commercial diet show that the protein and lipid concentrations of the #1 crumble and #2 crumble were similar. Likewise, $\delta^{15}N$ and $\delta^{13}C$ values were comparable. Conversely, the #3 crumble diet had lower protein and lipid concentrations, as well as, lower $\delta^{15}N$ and $\delta^{13}C$ values than the first two feeds (Table 4.5).

Mixing Model.—Using the three-source mixing model, 3 days after the initial feeding the estimated mean (±SE) relative contribution in the fish's muscle tissue was 5 % ± 2 within fed ponds. At harvest, mean (±SE) relative contribution of the feed had increased to 20% ± 6 within the fish's muscle tissue within the fed ponds. Estimated mean percent contributions of zooplankton and chironomid larvae were similar between fed and non-fed ponds (Table 4.6). During phase I culture, the mean relative contribution of the feed that was incorporated in the hybrid striped bass muscle tissue had increased while zooplankton contribution decreased within fed ponds (Figure 4.10; P= 0.008; P= 0.037 respectively). Estimated mean percent contribution of zooplankton and chironomid larvae did not significantly change during the culture period within non-fed ponds (Figure 4.11; P= 0.363; P = 0.361 respectively).

Discussion

Production.—Although the majority (~60%) of hybrid striped bass facilities that raise the palmetto cross use a commercial fish feed during phase I culture (Wamboldt et al. 2013), there are few published studies that examined the production benefits of feeding over pond fertilization alone in plastic-lined ponds. Though pond fertilization during phase I production is a common practice among the majority of culturists surveyed in 2010, it appears that the use of commercial diets early in the culture period of these fish is not as accepted. This may be due to a lack of justification through supporting peer-reviewed literature or a perceived notion that the benefits do not offset the high cost of feed.

Similar to previously noted findings of fingerling striped bass (Fitzmayer et al. 1986) and hybrid striped bass production (Morris and Muncy1989), our results suggest that final fish biomass can be enhanced though feeding during phase I culture. Although we were not able to determine a difference in survival between the treatments, Fitzmayer et al. (1986) showed that increased feeding rates positively affected phase I survival of striped bass. Low replication and inherent difficulties in estimating actual stocking densities and total fish harvested are likely reasons why we did not find differences in survival.

Similar water chemistry and quality between the two treatments throughout the culture period suggest that feed application was not detrimental to hybrid striped bass culture in plastic-lined ponds during phase I. However, the naturally high primary productivity of our ponds combined with an alkalinity ≤ 76-mg/L caused pH to reach potentially lethal levels (9.8-10.2; Bergerhouse 1993) near the end of the culture period (highest level reached = 9.78). Alkalinity above 100 mg/L is preferred for hybrid striped bass culture (Hodson and Hayes 1989; Ludwig 2004); alkalinity levels in this study in light of the highly eutrophic waters can result in high afternoon pH levels. Because phase I culture is relatively short (4-6 weeks) and feeding was initiated 2 weeks into the culture period, the effects of feed application on water quality may not have been evident early on.

Water quality during an 18-week grow out of common carp *Cyprinus carpio* and rohu *Labeo rohita* was negatively affected in fed vs. non-fed ponds (Rahman et al. 2008); Fed ponds had significantly lower DO with higher TAN, and NO_3. Similar results were seen in fertilized polyculture ponds with different rates of feed application (Abdel-Tawwab et al. 2007). Reduced bottom DO concentrations within our fed ponds indicate that oxygen demand during decomposition of the feed was greater than that of alfalfa pellets. Turano et al. (2008) found that reducing the amount of feed offered though cyclic feeding protocols during an 18-week trial of phase III hybrid striped bass production improved water quality of hybrid striped bass ponds. In our study the fact that fertilization was discontinued once feeding began likely diminished the deleterious effects of excessive organic loading on water quality.

During extensive culture of hybrid striped bass fingerlings, fertilizer application is essential to produce high densities of copepod and cladoceran taxa for successful phase I production (Parmley and Geiger 1985; Woods et al. 1985; Geiger and Turner 1990; Morris et al. 1999). Overall similarities in zooplankton densities between the fed and non-fed ponds suggest that feed application may not have enhanced zooplankton populations in relation to pond fertilization alone. However, greater copepod (primarily nauplii) densities throughout the culture period within the fed ponds indicate that the feed may have altered the zooplankton community (Abdel-Tawwab et al. 2007). A greater density of copepod populations within fed ponds was also seen in a similar on-site study during fingerling walleye culture (Kaatz 2003).

Higher densities of rotifers and *Bosmina* within our non-fed ponds were indicative of the predictable trends in zooplankton dynamics when fish predation is high. As predation increases, zooplankton communities tend to shift towards a dominance of smaller cladocerans and rotifers (Geiger 1983a). It appears that feed application did promote zooplankton production similar to pond fertilization, but predation seemed to drive zooplankton communities. The slight shift in zooplankton communities between treatments may indicate that increased utilization of feed reduced predation of natural prey items. Furthermore, preferred food availability within the non-fed ponds began diminishing near the end of culture. Given that our actual stocking rate was well below what is typically recommended (Brewer and Rees 1992; Morris et al. 1999; Woods 2005), a more significant decrease in preferred zooplankters may have been apparent in the non-fed ponds if stocking rates were higher.

Stable Isotopes.—Enrichment of hybrid striped bass $\delta^{15}N$ values within fed and non-fed ponds is likely due to ontogenetic diet shifts during growth (Phillips and Eldridge 2006). As hybrid striped bass fingerlings developed, they are known to incorporate larger cladoceran and chironomid larvae into their diets around 20-dph (Woods et al. 1985; Morris 1988). Following an initial isotopic depletion after 10-dph, nitrogen enrichment of fish tissue began between 22-25-dph within non-fed ponds. Given the inherent lag-time for metabolic turnover of existing tissue and the time it takes for consumers to reach isotopic equilibrium with food sources (Fry and Arnold 1982; Phillips and Eldridge 2006), the associated enrichment would be expected for any fingerling stage fish exhibiting fast growth. The initially elevated $\delta^{15}N$ values of fish within both treatments are likely attributed to the parental origin of their nitrogen pool (Vanden Zanden et al. 1998). Depletion in $\delta^{15}N$ sometime after 10-dps suggest that isotopic equilibrium with food sources may occur sometime between 10-16 days at this early stage.

A greater enrichment of carbon and nitrogen isotopes in hybrid striped bass tissues within the fed ponds appear to be associated with the highly enriched values of the Silver Cup™ trout diet. It is typical to find that isotopic values of consumer fish will become analogous to the delta values of offered fish feeds if they are assimilated into fish tissue (Schroeder 1983; Anderson et al. 1987; Lochmann and Phillips 1996). Because nitrogen isotopes become enriched at successive trophic levels (Vander Zanden and Rasmussen 2001; Vander Zanden et al.1999; Fry 2006), it would also be likely for commercial fish feeds to have relatively high $\delta^{15}N$ values due to fish meal content. Nitrogen enrichment of the fish within the non-fed treatment is the product of fish consuming larger diet items as they increase in trophic level (Vander Zanden et al. 1998)..

Variations in the isotopic signatures of zooplankton throughout the culture period may be more difficult to explain because their isotopic values represent an average of multiple specimens and taxa. More than likely, the changes that were noted in $\delta^{15}N$ and $\delta^{13}C$ values of zooplankton were the product of different compositions of taxa within the sample (Matthews and Mazumder 2005). The apparent depletion of ^{15}N for zooplankton samples prior to 10-dps appear to reflect the greater abundance of smaller, lower trophic organisms (nauplius) in pond and thus a greater representative signature within the sample. However, because changes in zooplankton signatures were similar between treatments, prior to feed application, their effects should not alter results since similar variability was seen in all ponds equally.

Since chironomid samples were a mixture of multiple specimens of a single family, we saw less variability in their isotopic signatures throughout the culture period. Similar to zooplankton signatures, Chironomid larvae within the fed ponds became significantly enriched follow the initial day of feeding. Greater enrichment of zooplankton and chironomid $\delta^{15}N$ and $\delta^{13}C$ values within fed ponds following the initial day of feeding suggest that the feed is either being incorporated into the their tissues directly through consumption or secondarily by enrichment of primary producers. This finding gives evidence that feed application influences natural pond biota and does act as a pond fertilizer in some way. However, without baseline isotopic signatures of algae, bacteria, and protozoan taxa, it is unclear how zooplankton and Chironomid larvae are incorporating the feed signatures.

The non-fed ponds served as a good control for understanding the validity of our mixing model results from the fed ponds. Although the trends were not significant, it appeared that zooplankton contribution decreased while Chironomid contribution increased in the isotopic signatures of the fish. These findings seem to agree with the general feeding behaviors described for striped bass and the palmetto bass in previous studies (Harper et al. 1969; Humphries and Cumming 1973; Harrell et al. 1977; Geiger et al. 1985; Woods et al. 1985 Fitzmayer et al. 1986; Morris 1988). However, since our study was relatively short and occurred during the theoretical time which fish were undergoing a shift it diet, it is likely that the fish never reached isotopic equilibrium with prey sources, as is assumed in the model (Fry 2006; Phillips and Eldridge 2006; Vay and

Gamboa-Delgado 2011). Furthermore, $\delta^{15}N$ and $\delta^{13}C$ values for zooplankton and chironomidae larvae within the non-fed ponds were not significantly different during the beginning of the culture period, violating another assumption of our mixing model (Ben-David et al. 1997). As would be expected in such a closed and controlled system, the similarities in zooplankton and chironomidae signatures from non-fed ponds suggest that their diets were quite similar. Since $\delta^{15}N$ signatures clearing indicated that the fish were within one trophic level above both zooplankton and chironomidae larvae, it would be safer to assume that their relative contribution to the fish signatures were similar (1:1) in the non-fed treatment.

Conversely, once feeding began, source signatures within the fed ponds were all significantly different from one another, allowing more precise estimates of relative contribution. However, because the Euclidean distance model is only an index of prey consumption, a combination of the traditional GCA along with stable isotope analysis would have provided greater precision in describing the dietary shift (Gu et al. 1996). Unlike the natural pond biota within the non-fed ponds, the significant difference $\delta^{13}C$ values for zooplankton and chironomid larvae within the fed ponds indicate that their diets were dissimilar. The results from the mixing model used, agree with previous literature in confirming that feed application is supplemental to natural pond biota (Morris 1988; Fitzmayer et al. 1986).

The mixing model which we used was chosen because it involved simple calculations with minimal requirements of knowing the isotopic signatures of a consumer and all possible sources. However, since the model relies solely on the Euclidean distances between isotopic values, it may not accurately identify dietary contributions because multiple combinations of sources could result in the consumer's signature (Layman et al. 2012). Additionally, the model tends to overestimate rare prey items and ignores the possibility that some expected sources are not consumed (Layman et al. 2012). Unlike other possible mixing models, this models simplicity, allowed for the estimate of the relative contribution of a dietary food source (commercial feed) that had a higher $\delta^{15}N$ value than the consumer.

Conclusions.—Although we found that the hybrid striped bass do utilize prepared diets during phase I culture, this did not result in improved fish production compared to pond fertilization alone. However, greater fish length and biomass may warrant the use of a commercial diet. The high percentage (≥ 80%) in which natural pond biota continued to account for in the fishes signatures indicate the importance of keeping a good zooplankton population throughout phase I, whether or not feed is offered. Increasing zooplankton densities at the end of culture suggested that harvest could have been delayed further if elevated pH levels were controlled. If phase I were continued for a longer time period, we most likely would have seen the depletion of zooplankton and subsequently greater differences in final fish production variables.

Supplemental feeding may be of greater importance for fish that are cultured beyond phase I. However, feeding may be less important for fish that are being stocked for management purposes immediately following phase I; difficulty in establishing and maintaining adequate zooplankton populations may necessitate feeding during phase I. Regardless of the actual effect on fish production in culture ponds, enriched carbon and nitrogen isotopic signatures within the zooplankton and chironomid larvae suggest that the natural pond biota is altered though the feed application. However, further research is needed to understand the trophic pathways in which they utilize the fish feed.

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TABLE 4.1. Mean ± SE final length, final weight, survival, specific growth, and fish biomass of phase I hybrid striped bass cultured in plastic-lined ponds at Rathbun Fish Culture Research Facility, Iowa, 2011. Fed treatments was offered Silver Cup™ trout diet at a rate of 13.6-kg/ha daily beginning 14 days post stock. Parameters marked with an asterisk (*) were significantly different, $P < 0.10$.

Treatment	Final Length (mm) *	Final Weight (g)	Survival (%)	Specific Growth (G)	Biomass kg/ha
Fed	51.9 (2.3)	1.6 (0.2)	44 (9)	15.8 (0.5)	71.4 (6.1)
Not-Fed	45.9 (1.55)	1.0 (0.2)	43 (11)	14.6 (0.4)	54.6 (6.4)

TABLE 4.2. Least-square means (SEM) of water chemistry variables (mg/L) in fed and not-fed plastic-lined ponds during the 2011 hybrid striped bass culture season at Rathbun Fish Culture Research Facility, Iowa. Fed treatment was offered Silver Cup™ trout diet at a rate of 13.6-kg/ha daily beginning 14 days post stock.

Analysis (mg/L)	Fed	Not-fed	Treatment *P*-value	Treatment x date *P*-value
Total Ammonia Nitrogen	0.40 (0.02)	0.38 (0.02)	0.66	0.87
Unionized Ammonia	0.07 (0.01)	0.07 (0.01)	0.62	0.98
Nitrate	0.52 (0.03)	0.56 (0.03)	0.41	0.92
Nitrite	0.02 (0.00)	0.02 (0.00)	0.62	0.96
Total Phosphorus (TP)	0.09 (0.01)	0.08 (0.01)	0.85	0.89
NO_3/TP	0.40 (0.05)	0.35 (0.05)	0.53	0.71
Alkalinity	71 (0.8)	70 (0.8)	0.24	0.07
Hardness	68 (7.0)	75 (7.0)	0.54	0.64

Repeated measures mixed model, $N = 3$.

TABLE 4.3. Least-square means (SEM) of morning (AM) and afternoon (PM) pH and dissolved oxygen (DO) profiles in fed and not-fed plastic-lined ponds during the 2011 hybrid striped bass culture season at Rathbun Fish Culture Research Facility, Iowa. Fed treatment was offered Silver Cup™ trout diet at a rate of 13.6-kg/ha daily beginning 14 days post stock.

			Treatment	Treatment x date
Analysis	Fed	Not-fed	*P*-value	*P*-value
AM pH	8.81 (0.04)	8.79 (0.04)	0.76	0.72
AM DO Top	9.16 (0.11)	9.18 (0.11)	0.87	0.36
AM DO Middle	9.10 (0.13)	9.20 (0.13)	0.62	0.44
AM DO Bottom	6.41 (0.19)	7.64 (0.19)	0.01	0.002
PM pH	8.91 (0.08)	8.93 (0.08)	0.92	0.38
PM DO Top	9.70 (0.32)	10.02 (0.32)	0.53	0.76
PM DO Middle	9.81 (0.32)	9.94 (0.32)	0.80	0.33
PM DO Bottom	6.58 (0.38)	7.80 (0.39)	0.09	0.11

Repeated measures mixed model, $N = 3$.

TABLE 4.4. Least-square means (SEM) of zooplankton (organisms/L) in fed and not-fed plastic-lined ponds during the 2011 hybrid striped bass culture season at Rathbun Fish Culture Research Facility, Iowa. Fed treatment was offered Silver Cup™ trout diet at a rate of 13.6-kg/ha daily beginning 14 days post stock.

			Treatment	Treatment x date
Taxa	Fed	Not-fed	*P*-value	*P*-value
Rotifera	**308.85 (108.50)**	**374.19 (108.35)**	**0.69**	**0.01**
Asplanchna	2.94 (0.58)	2.93 (0.57)	0.99	0.60
Brachionus	46.38 (23.40)	55.27 (23.35)	0.80	0.04
Filinia	3.44 (0.55)	2.57 (0.54)	0.32	0.03
Keratella	107.82 (10.81)	75.20 (10.65)	0.12	0.74
Polyarthra	16.74 (6.07)	16.20 (6.03)	0.95	0.78
Testudinella	130.97 (88.68)	222.00 (88.56)	0.51	0.07
Copepoda	**57.71 (10.19)**	**49.63 (10.16)**	**0.60**	**0.86**
Calanoida	2.50 (0.43)	1.57 (0.42)	0.20	0.31
Cyclopoida	55.17 (10.08)	48.10 (10.05)	0.65	0.88
Nauplius	131.38 (18.44)	99.97 (18.39)	0.29	0.04
Cladocera	**202.30 (14.22)**	**208.13 (13.89)**	**0.78**	**0.01**
Bosmina	98.12 (14.85)	120.27 (14.67)	0.35	0.01
Ceriodaphnia	4.60 (0.93)	3.17 (0.90)	0.33	0.23
Daphnia	85.46 (10.17)	71.20 (10.07)	0.38	0.48

Repeated measures mixed model, $N = 3$.

TABLE 4.5. Proximate composition (% of dry matter) and $\delta^{13}C$ and $\delta^{15}N$ values of three different crumble sizes of Silver Cup™ Trout Fry diet used during the 2011 hybrid striped bass culture season at Rathbun Fish Culture Research Facility, Iowa.

Crumble	Dry Matter	Crude protein	Lipid	Ash	$\delta^{13}C$	$\delta^{15}N$
1	92.9	55.1	18.8	11.8	-20.18 ± 0.09	12.06 ± 0.15
2	92.4	55.1	18.6	12.8	-20.58	11.51
3	93.1	53.6	16.7	9.8	-21.34	7.76

TABLE 4.6. Mean ± SE relative percent contribution of potential food sources for hybrid striped bass during phase I culture at the Rathbun Fish Culture Research Facility, Iowa using $\delta^{15}N$ and $\delta^{13}C$ within a linear mixing model. Five fish from each pond were used individually within the mixing model. Parameters marked with an asterisk (*) were estimated using fish from one replicate.

	Fed		Not-fed		
Days Post Stock	Zooplankton	Chironomid	Zooplankton	Chironomid	Silver Cup™
17	62 ± 11	38 ± 11	61 ± 16	34 ± 14	5 ± 2
22	48 ± 11	51 ± 11	76 ± 6	15 ± 4	8 ± 2
24	51 ± 6	49 ± 6	65 ± 5	18 ± 2	17 ± 5
29	64 ± 15	36 ± 15	41 *	31 *	28 *
21	51 ± 9	49 ± 9	43 ± 5	38 ± 6	20 ± 6

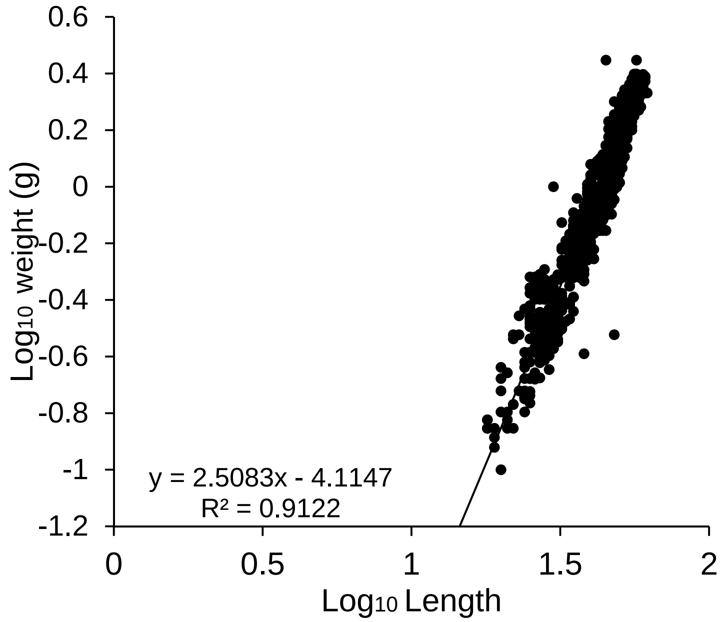


FIGURE 4.1. Log transformed total length and weight of 1,670 hybrid striped bass sampled weekly from 9-May 2011 to 28-May 2011 at Mount Ayr and Rathbun Fish Culture Research Facility, Iowa. Weight of fry was estimated using the following equation: $y = 2.5083x - 4.1147$. Initial total lengths of 30 fish were used to estimate a mean fry length for x.

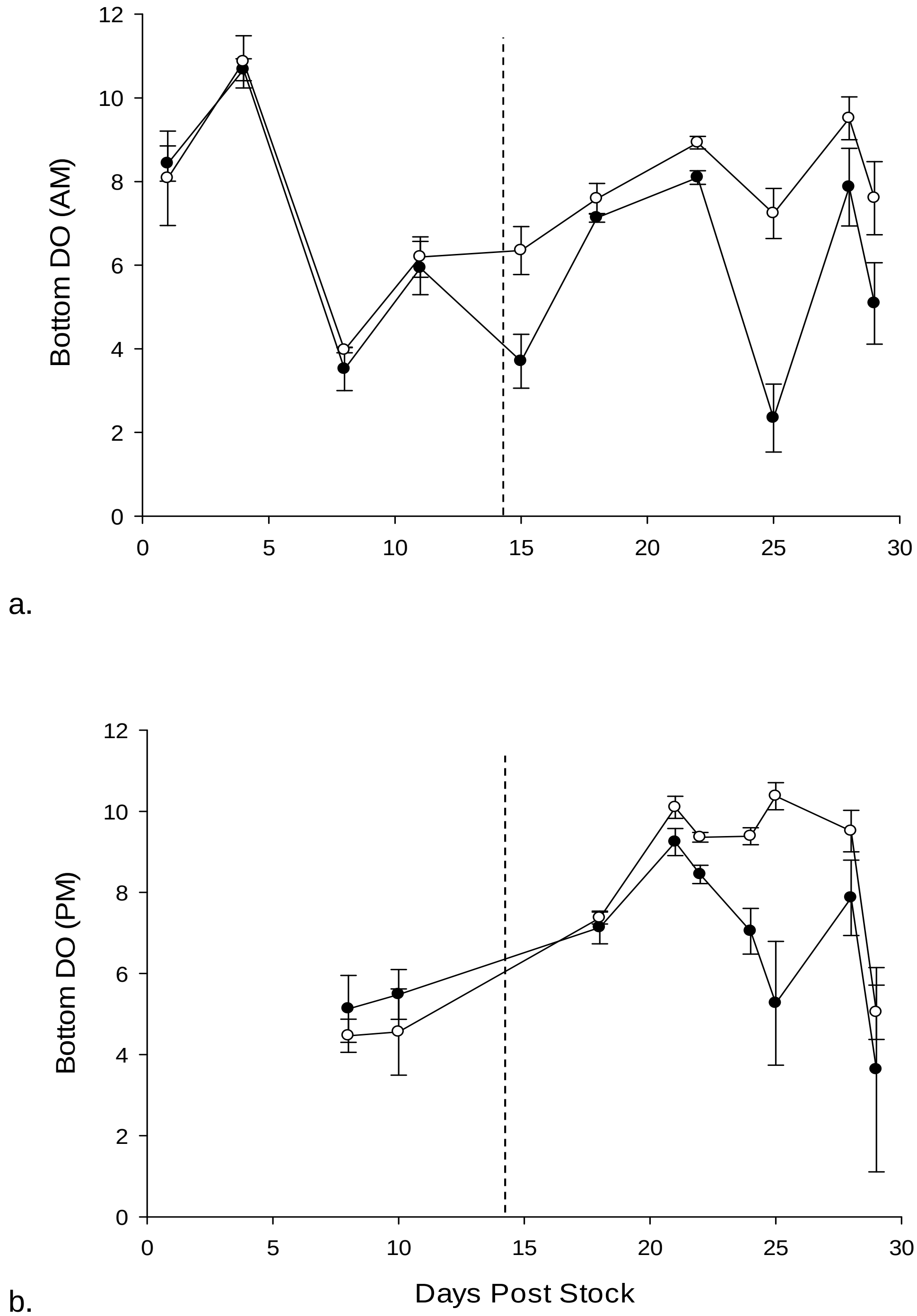
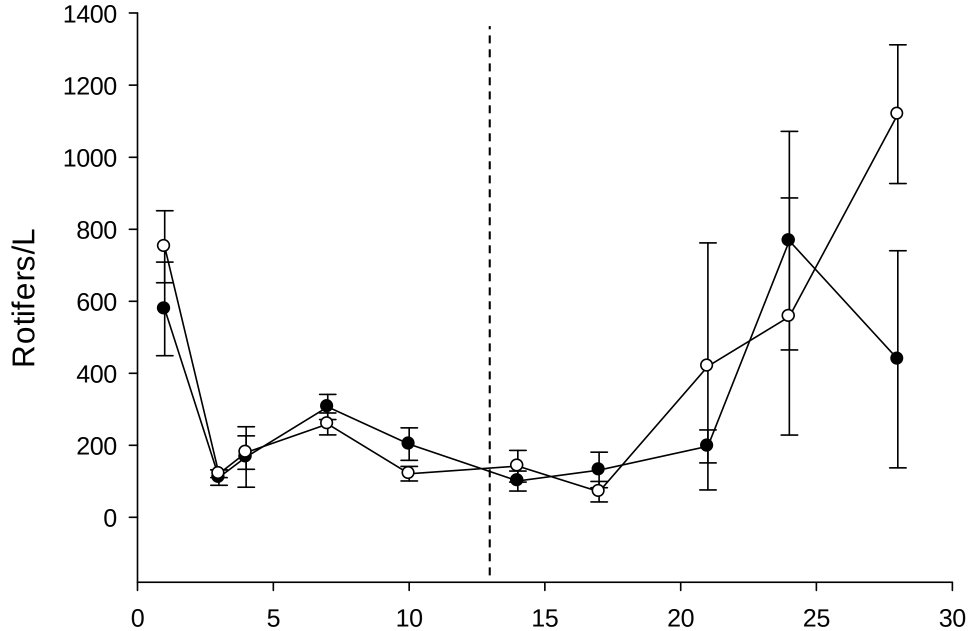
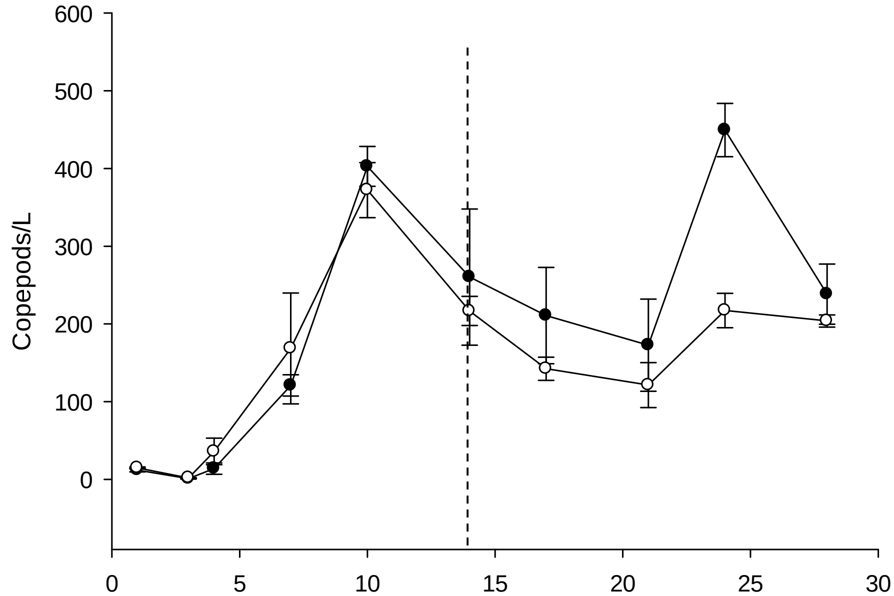


FIGURE 4.2. Mean ± SE morning (a.) and afternoon (b.) bottom dissolved oxygen concentrations (mg/L) in fed (closed circle) and not-fed (open circle) plastic-lined ponds during the 2011 hybrid striped bass culture season at Rathbun Fish Culture Research Facility, Iowa. Fed ponds were offered Silver Cup™ trout diet at a rate of 13.6-kg/ha daily beginning 14 days post stock (represented by dashed line).



a.

FIGURE 4.3. Mean ± SE concentrations (#/L) of rotifer populations in six plastic-lined ponds during the 2011 hybrid striped bass culture season at Rathbun Fish Culture Research Facility, Iowa. Fed ponds (closed circles) were offered Silver Cup™ trout diet at a rate of 13.6-kg/ha daily beginning 14 days post stock (represented by dashed line). No feed was offered to non-fed (open circles) ponds.



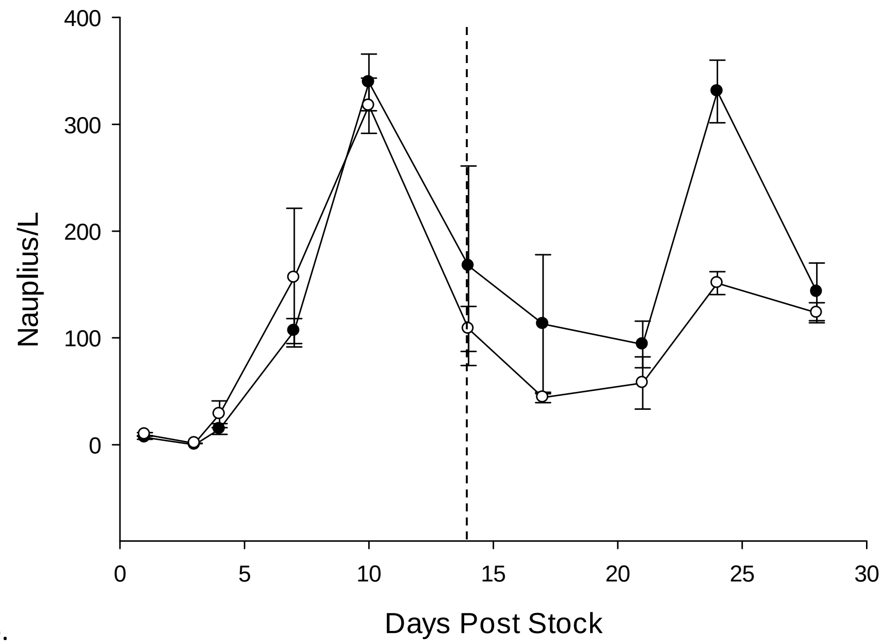


FIGURE 4.4. Mean ± SE concentrations (#/L) of copepod (a.) and nauplius (b.) populations in six plastic-lined ponds during the 2011 hybrid striped bass culture season at Rathbun Fish Culture Research Facility, Iowa. Fed ponds (closed circles) were offered Silver Cup™ trout diet at a rate of 13.6-kg/ha daily beginning 14 days post stock (represented by dashed line). No feed was offered to non-fed (open circles) ponds.

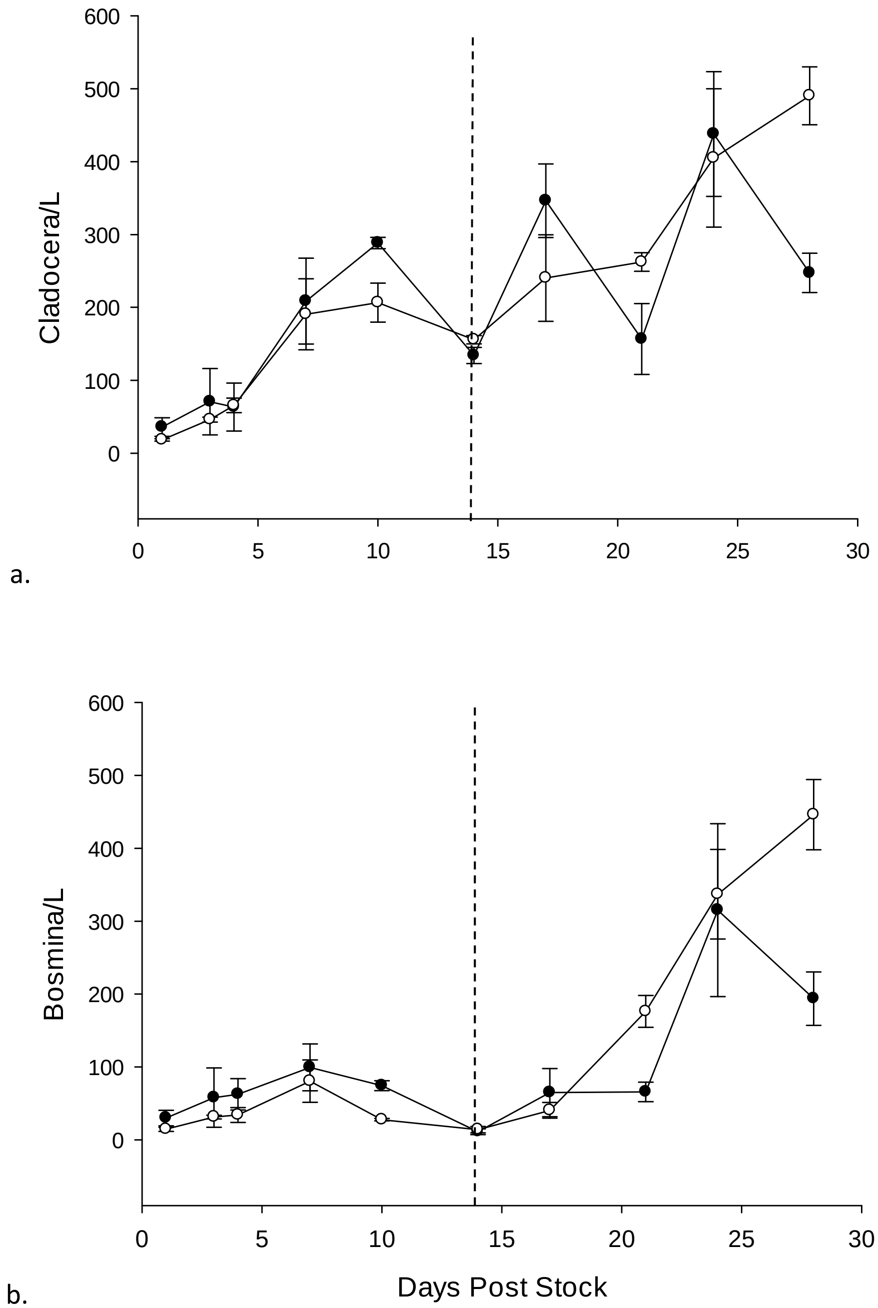


FIGURE 4.5. Mean ± SE concentrations (#/L) of cladoceran (a.) and *Bosmina* (b.) populations in six plastic-lined ponds during the 2011 hybrid striped bass culture season at Rathbun Fish Culture Research Facility, Iowa. Fed ponds (closed circle) were offered Silver Cup^{TM} trout diet at a rate of 13.6-kg/ha daily beginning 14 days post stock (represented by dashed line). No feed was offered to non-fed (open circles) ponds.

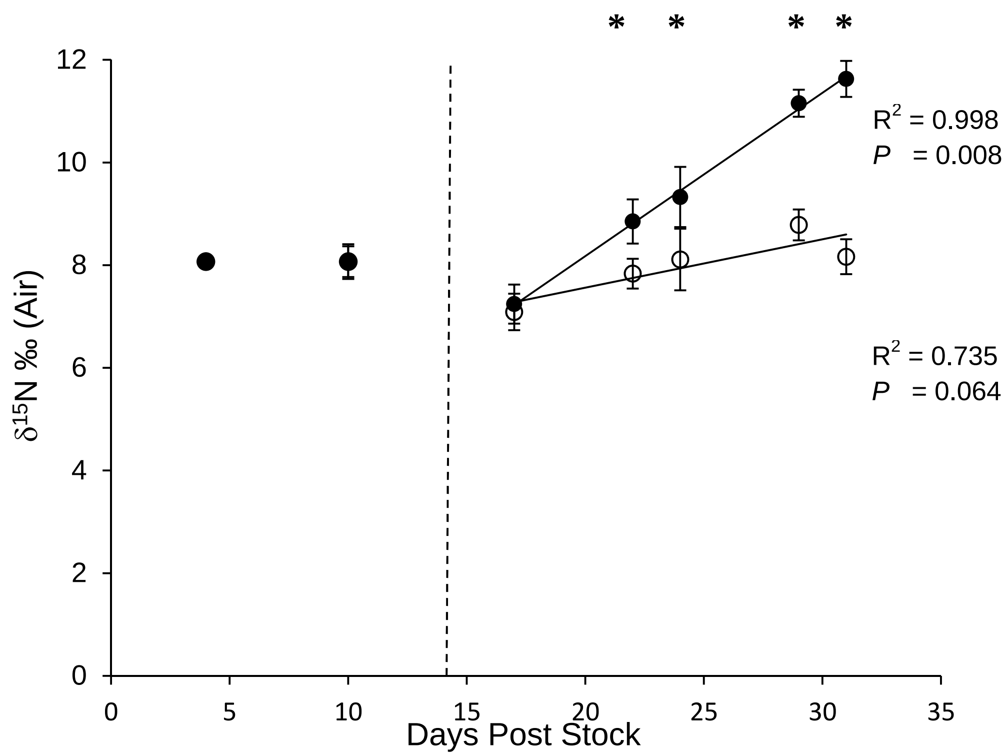


FIGURE 4.6. Mean ± SD $\delta^{15}N$ of hybrid striped bass during the 2011 culture season at Rathbun Fish Culture Research Facility, Iowa. Fed treatment (closed circle) was offered Silver Cup™ trout diet at a rate of 13.6-kg/ha daily beginning 14 days post stock (represented by dashed line). No feed was offered to non-fed (open circles) ponds. Parameters marked with an asterisk (*) were significantly different between treatments, $P < 0.10$.

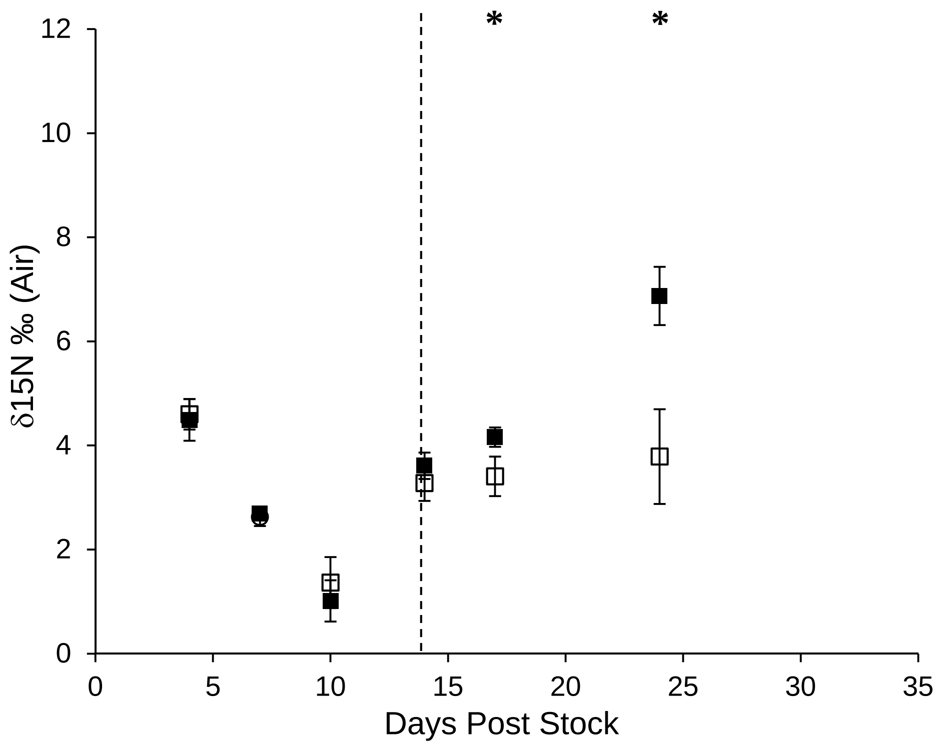


FIGURE 4.7. Mean ± SD $\delta^{15}N$ of zooplankton samples during the 2011 culture season at Rathbun Fish Culture Research Facility, Iowa. Fed treatment (closed square) was offered Silver Cup™ trout diet at a rate of 13.6-kg/ha daily beginning 14 d post stock (represented by dashed line). No feed was offered to non-fed (open squares) ponds. Parameters marked with an asterisk (*) were significantly different between treatments, *P*< 0.10.

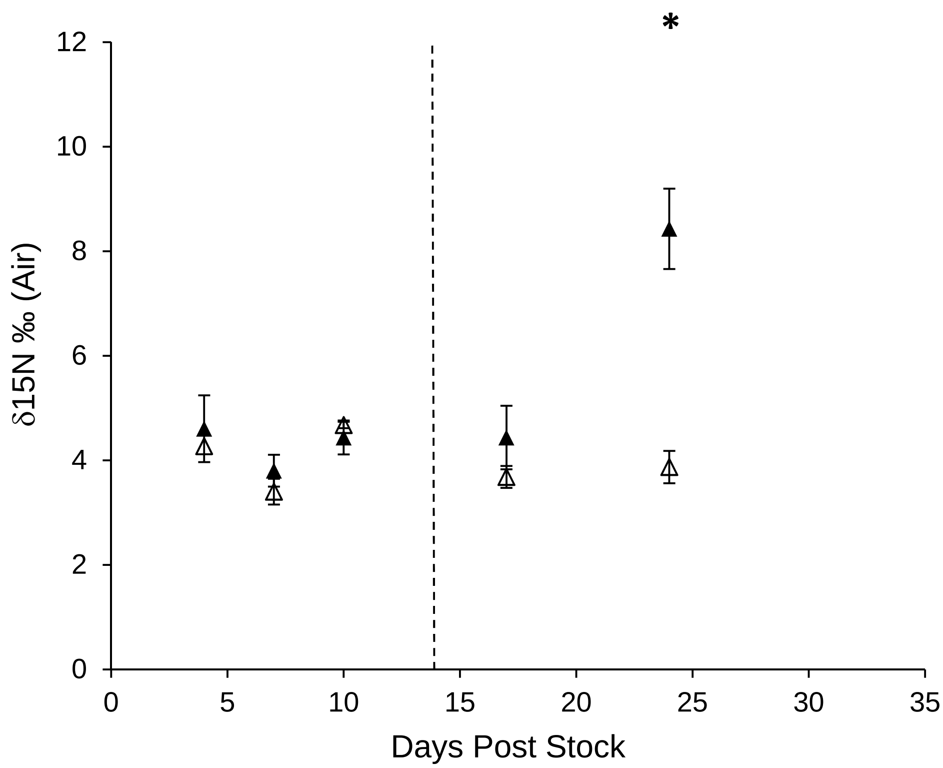


FIGURE 4.8. Mean ± SD $\delta^{15}N$ of chironomid during the 2011 culture season at Rathbun Fish Culture Research Facility, Iowa. Fed treatment (closed triangle) was offered Silver Cup™ trout diet at a rate of 13.6-kg/ha daily beginning 14 d post stock (represented by dashed line). No feed was offered to non-fed (open triangles) ponds. Parameters marked with an asterisk (*) were significantly different between treatments, *P*< 0.10.

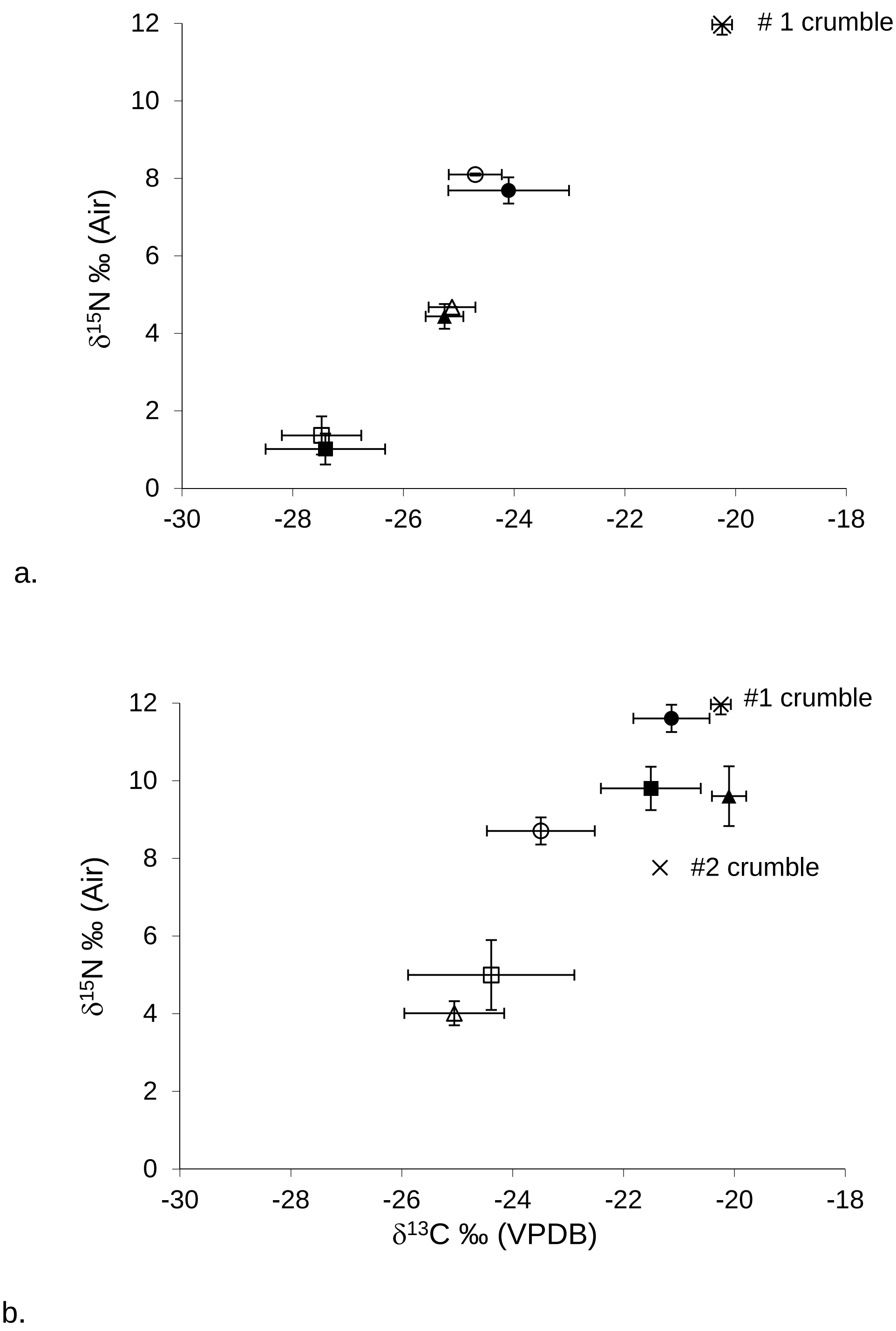


FIGURE 4.9. Mean ± SD $\delta^{15}N$ vs. $\delta^{13}C$ of hybrid striped bass (circles), zooplankton (squares), chironomid larvae (triangles), and Silver Cup™ trout diet (×) (crumble 1 and 2) during the 2011 culture season at Rathbun Fish Culture Research Facility, Iowa. Fed treatment (closed shapes) was offered feed at a rate of 13.6-kg/ha daily beginning 14 days post stock. No feed was offered to non-fed (open shapes) ponds. Figure a. depicts isotopic signatures prior to the initial day of feeding and figure b. depicts signatures 17 days thereafter.

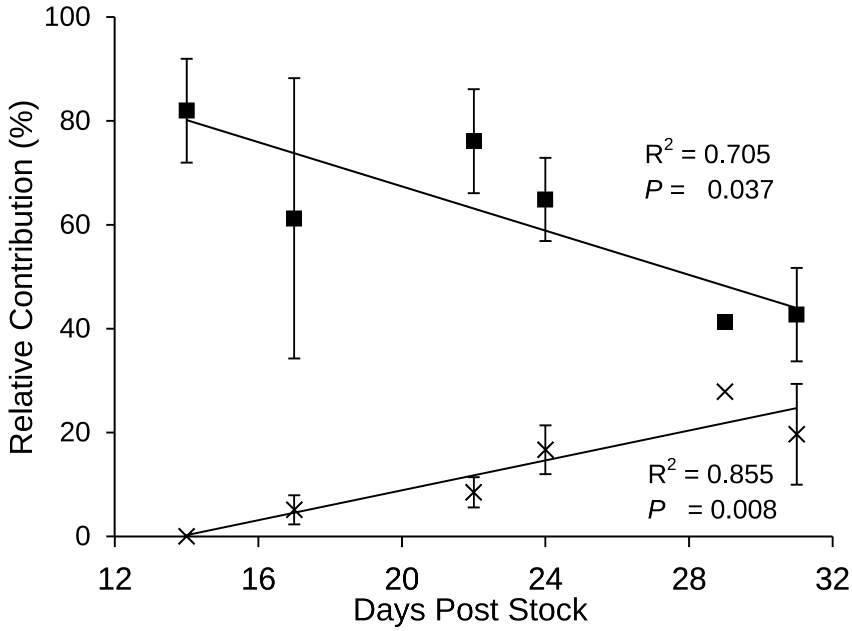


FIGURE 4.10. Mean ± SE relative contribution of Silver Cup™ trout diet (×) and zooplankton (squares) in the isotopic signature of hybrid striped bass in fed ponds during phase I culture at the Rathbun Fish Culture Research Facility, Iowa using $\delta^{15}N$ and $\delta^{13}C$ within a linear mixing model (Kline et al. 1993).

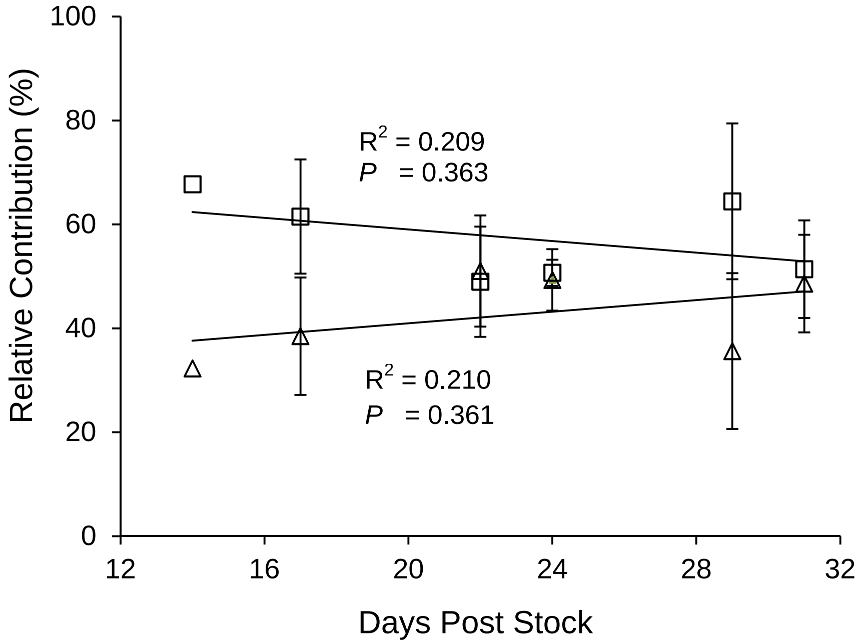


FIGURE 4.11. Mean ± SE relative contribution of chironomid larvae (triangles) and zooplankton (squares) in the isotopic signature of hybrid striped bass in non-fed ponds during phase I culture at the Rathbun Fish Culture Research Facility, Iowa using $\delta^{15}N$ and $\delta^{13}C$ within a linear mixing model (Kline et al. 1993).

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CHAPTER 5. EFFECTS OF AN INTRODUCED TOP PREDATOR ON TROPHIC DYNAMICS OF AN ESTABLISHED RESERVOIR FISHERY: APPLICATIONS OF STABLE ISOTOPE ANALYSIS

(In Prep)

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Abstract

Using stable carbon and nitrogen isotopes, the trophic dynamics and conceptual niche spaces of top piscivores were analyzed following the introduction of hybrid striped bass *Morone saxatilis* × *M. chrysops* into an established reservoir fishery. Prior gut content analysis in 2009-2010 guided our approach for sampling potential prey fish to analyze hybrid striped bass diets using stable isotope analysis in 2012. Combining the current knowledge of carbon and nitrogen stable isotope ecology with multivariate techniques for constructing isotopic niche spaces, we attempted to describe dietary overlap among the top predators in Three Mile Lake, Iowa. From our analysis, we concluded that hybrid striped bass predominately targeted prey fish located in the littoral habitats of the reservoir and did not selectively consume pelagic yellow bass *Morone mississippiensis*. Although hybrid striped bass are typically thought of as open-water pelagic fish, their $\delta^{13}C$ values from Three Mile Lake appear to reflect a more littoral preference. Niche overlap between hybrid striped bass and largemouth bass *Micropterus salmoides* appeared to be the greatest amongst the top predators using both the convex hull and standard ellipse approaches for analysis. Overlap was minimal for walleye *Sander vitreus* and largemouth bass using both methods. Although we could not determine if competition from this conceptual niche overlap has any detrimental effects to either group, continued monitoring of the sport fish population dynamics is highly recommended. Results garnered from this study support the use of stable

isotope analysis as a tool to better understand ecological effects of management decisions on a fishery.

Introduction

With the possibility that newly introduced species can severely alter the behavior of native populations, restructure existing food web dynamics, negatively impact aquatic habitats, and cause complete extirpations of entire native taxa (Taylor et al. 1984; Cucherousset and Olden 2011), current trends within fisheries management have strayed away from new species introductions. Although general consensus within the scientific community appears to conclude that the majority of new fish introductions often lead to negative ecological impacts (Li and Moyle 1981; Townsend 1996; Pimentel et al. 2000; Olden et al. 2004; Vitule et al. 2009; Clarkson et al. 2011), the introduction of economically viable species as well as those with little or no historic ecological risk have historically been considered management options in many fisheries (Gozlan 2008). In highly altered aquatic systems, e.g., large reservoirs, managers often have greater flexibility in manipulating fish assemblages because the relatively recent lacustrine environment is typically considered unsuitable for resident riverine fish (Fernando and Holoik 1991; Miranda and Bettoli 2010). The substantial recreational fisheries that rely on non-native fish and impracticality of removal dictate that large reservoirs will most likely continue to be stocked with favored sport fish (Clarkson et al. 2011).

One major difficulty when managing a reservoir system is that they are not analogous systems to natural lakes with respect to typical predator-prey relationships that have developed over a long time period (Noble 1986). Reservoirs are relatively recent ecological systems, and often do not have the coevolved predator-prey relationships that are traditionally used for understanding and managing most lacustrine fisheries (Raborn et al. 2007). Being a mixture of lentic and lotic ecosystems, reservoirs consist of highly diverse and potentially unstable fish assemblages (Raborn et al. 2007); which typically can support coexisting predatory taxa (Noble 1986; Neal 1999a; Olson et al. 2007). Longitudinal gradients within large reservoirs also allow for a greater diversity in fish habitats (Miranda 1999; Miranda et al. 2008; Miranda and Bettoli 2010) and a higher potential of niche partitioning.

With high recreational demand for largely piscivores species such as largemouth bass *Micropterus salmoides*, striped bass *Mornone saxatilis,* and walleye *Sander vitreus*, there is concern for maintaining the predator-prey balance within reservoirs stocked with little knowledge of system capacity (Raborn et al. 2007). Within the mid-central and south–central regions of the United States, management of large reservoirs primarily focuses on the introduction of native warm water centrarchids, ictalurids, and temperate basses Moronidae (Miranda 1999).

Hybrid striped bass *Morone saxatilis* × *M. chrysops* have been an ideal candidate for reservoir introductions in the southeastern United States because they can establish a fishery for a large, open water game fish and can be used for predatory control of undesired prey species (Ott and Malvestuto 1981; Neal et al. 1999b). They have the ability to inhabit the highly underutilized pelagic zones typical of most large reservoirs (Fernando and Holoik 1991; Miranda and Bettoli 2010). However, the most common motivation for hybrid striped bass introductions, throughout the United States, is the potential for the species to regulate overabundant shad populations (Ott and Malvestuto 1981; Dettmers et al. 1998; Neal et al. 1999b; Ostrand et al 2001). While management programs continue to utilize hybrid striped bass stocking in much of the Southeast, its use as a biological control is not as widespread throughout the Midwest.

When hybrid striped bass are introduced into systems that contain abundant gizzard *Dorosoma cepedianum* and threadfin shad *D. petenense* populations, consumption rates have been reportedly between 60-95% of their total diets (Ott and Malvestuto 1981; Borkowski and Snyder 1982; Gilliland and Clady 1984; Jahn et al. 1987; Dettmers et al. 1998). Dettmers et al. (1996) reviewed 26 *Morone* introductions and found that 15 resulted in some type of gizzard shad control. However, only 22% of hybrid striped bass introductions resulted in apparent success, based on management objectives (Dettmers et al. 1996). Although total eradication was never cited, reduced abundance and altered size structure of shad populations was observed after many *Morone* introductions. Since it may be possible for hybrid striped bass to switch their diet in the absence of shad (Gilliland and Clady 1984; Neal et al. 1999b), the effects that introductions have on other game fish populations have been investigated.

Annual variability of hybrid striped bass diets in Sooner Lake, Oklahoma, showed that they preyed on sunfish *Lepomis* and crappie *Pomoxis* over gizzard shad, but only during summer months (Gilliland and Clady 1984). Lack of predation during summer months is likely related the fact that age-0 gizzard shad can quickly outgrow predation (Bodola 1966; Johnson et al. 1988). However high incidences of empty stomachs (62%) during the Sooner Lake study may have skewed gut content analysis (GCA). Conversely, Jahn et al. (1987) identified no fish other than gizzard shad in the stomachs of hybrid striped bass collected in Spring Lake, Illinois and further concluded that selective predation will occur if sufficient gizzard shad populations are present. Olson et al. (2007) also found gizzard shad to be the primary prey item of hybrid striped bass but also noted that freshwater drum *Aplodinotus grunniens* and white bass *Morone chrysops* were common prey items as well.

In small (2.4-6.5 ha) ponds, void of shad, Neal et al. (1999b) noted increased lengths and relative weights (W_r) of bluegill, *Lepomis macrochirus*, redear sunfish *Lepomis microlophus*, and black crappie *Pomoxis nigromaculatus*, presumably due to elevated predation from newly introduced hybrid striped bass. However, it is unclear how hybrid striped bass predation habits will evolve in a large Midwestern impoundment that has an established predator-prey fish assemblage present that does not include gizzard shad. Although it may be possible for coexisting management of hybrid striped bass and other top predators in highly productive large reservoirs (Olson et al. 2007), a trophic level, niche-based examination of a system that contains a highly diverse assemblage of predatory and prey taxa has not been done.

Since the introduction of hybrid striped bass to Three Mile Lake, Iowa in 2004, fisheries biologists have struggled to determine their dietary behaviors in the absence of a gizzard shad population. The initial goal of their introduction was to create a pelagic piscivore population that could depress a probable future introduction of yellow bass *Morone mississippiensis*. Gut content analysis conducted in 2009-2010 shed little light on hybrid striped bass diets because of a high incidence of empty stomachs, difficulty identifying fish from stomach contents, and a large gastric presence of zooplankton (R. Schultz, Iowa Department of Natural Resources Fisheries Biologist, personal communication). These concerns focused attention towards new techniques, such as stable isotope analysis, for dietary examination.

Traditional gut content analysis requires a large sample size and extensive sampling procedures in order to determine a fish's diet (Clarke et al. 2005; Christensen and Moore 2009). Additionally, ontogenetic diet shifts and temporal variability of consumption limits gut content analysis to relatively recent feeding activity (Power and Dietrich 2002; Clarke et al. 2005 Christensen and Moore 2009). In recent years, the use of stable isotope analysis in conjunction with gut content analysis has increased in popularity among ecologists for measuring many food web dynamics and trophic level interactions. However, its use as a management tool is not commonly practiced.

Stable isotope values are denoted as a difference measurement made relative to standards during analysis and relate values as a ratio of the heavy isotope to the light isotope of an element (Fry 2006). Values are reported as a permil (‰) relative to the standards in a standard delta notation:

$$\delta = \left[\left(\frac{R_{SAMPLE}}{R_{STANDARD}} - 1\right)\right] \times 1{,}000$$

in which R = either $^{15}N/^{14}N$ or $^{13}C/^{12}C$ (Fry 2006). Techniques for using stables isotopes to trace dietary items rely on the assumption that a consumers muscle tissue is a product of its diet, and that this is reflected in the isotopic composition of the individual (Fry 2006). As nitrogen isotopes are transferred to higher trophic positions within a food chain, via consumption, delta ^{15}N is enriched by roughly 3.4‰ through a process known as trophic fractionation (Vander Zanden et al.1999; Vander Zanden and Rasmussen 2001; Fry 2006). Trophic fractionation is the result of heavier isotopes being preferentially selected at the cellular level within the consumer during many metabolic processes; producing isotopic enrichment within higher trophic organisms (Fry 2006; Gamboa-Delgado et al. 2008). Enrichment of ^{15}N at each sequential trophic level allows the defining of trophic positions to consumers within a food web (Vander Zanden et al.1999; Vander Zanden and Rasmussen 2001; Fry 2006). A numeric assignment of trophic position within a community can allow for comparisons over an ecosystem as a whole.

During photosynthesis, ^{13}C fractionates distinctly between plants with different photosynthetic pathways, such as C3 or C4 plants (O'Leary 1988), allowing for the distinction of terrestrial and aquatic sources of carbon (Kline et al. 1993). Within aquatic environments, primary produces utilize aqueous CO_2 during photosynthesis and ultimately, $\delta^{13}C$ values within consumers can reflect CO_2 gradients present in the water column (Hecky and Hesslein 1995). Differences in these photosynthetic pathways and relative fractionation allow $\delta^{13}C$ to be used to trace energetic inputs within many aquatic ecosystems (Kline et al. 1989; Hecky and Hesslein 1995; Hobson 1999; Schmidt et al. 2007).

With little trophic fraction of $\delta^{13}C$ (difference > 1‰; Gearing 1991; Vander Zanden et al. 1998), carbon inputs within aquatic ecosystems can be traced to terrestrial, benthic, littoral, and pelagic origins, based on primary production (France 1995; Clarke 2003; Hecky and Hesslein 1995). Having the ability to discern different primary carbon sources (benthic algae vs. pelagic phytoplankton) may also allow for spatial separation of consumers within aquatic food webs (France 1995; Gu et al. 1996; Vander Zanden and Rasmussen 2001; O'Reilly et al. 2002; Clarke 2003; Fry 2006; Christensen and Moore 2009).

Benefit to using stable isotope analysis over stomach content analysis is that it can give researchers a temporally integrative, long term description of a fish's diet in addition to a quantitative measure of trophic interactions within a food web (Vander Zanden et al. 1998; Clarke et al. 2005; Post 2002). Unlike stomach contents, it allows researchers to focus on assimilated dietary inputs within a food web (Kling et al. 1992). A dual approach using carbon and nitrogen isotopes has become a popular technique to examine many ecological community interactions (Peterson and Fry 1987; Kling et al. 1992; Post 2002; Fry 2006). Recently, quantitative analyses of isotopic systems have given researches the ability to define isotopic niches based on $\delta^{13}C$ and $\delta^{15}N$ bi-plotted data (Bearhop et al. 2004; Layman et al. 2007; Turner et al. 2010; Hammerschlag-Peyer et al. 2011).

The goal of this study was to understand the trophic interactions of recently introduced hybrid striped bass with current fish assemblages in a large Midwestern reservoir. The objectives of this study were to (1) determine if hybrid striped bass will selectively prey on yellow bass; (2) document the feeding behavior and trophic dynamics of hybrid striped bass in a large reservoir that is absent of shad, and (3) determine dietary niche overlap of the top predators within Three Mile Lake, Iowa. Through this project, we attempted to describe the trophic interactions among the major sportfish species present using a combination of gut content analysis and stable isotope analysis.

Methods

Three Mile Lake is a constructed, 327.8 ha reservoir located in Union County, 4.8-km north of Afton, Iowa. It has a mean depth of 5.2 m and a maximum depth of 17.2-m. The shoreline development value is 3.3 with a watershed area ratio of 4.2 (59.9-ha). During typical years, a thermocline is observed at a mean depth of 4.2-m (Iowa Summary Report 2011). As typical for most Iowa reservoirs, the fish assemblage in Three Mile Lake include a wide variety of sport-fish taxa that are supplemented with annual walleye and hybrid striped bass stockings. Advanced fingerling walleye (200-250 mm) are typically stocked in the fall and hybrid striped bass (40-50 mm) are stocked early June.

Hybrid striped bass, largemouth bass, and walleye stomach samples were collected in April and October of 2009-2010 during the Iowa DNR's annual spring and fall surveys. Stomach contents were collected using a gastric lavage, preserved in 10% formalin, and later identified by DNR staff. Fish were collected using a combination of electrofishing and short set gill nets. Electrofishing was conducted from a boat mounted 230 volt Coffelt CCP-15 unit with two booms. Multiple 50-m (5.0-cm bar mesh), 12-m (1.9-cm bar mesh), and 18-m two panel experimental (2.5 and 3.8-cm) gill nets were set on the bottom, perpendicular to the shoreline. Regular gill nets were made from nylon twine and experimental gill nets were monofilament. Electrofishing was done at night in four 15-min runs at randomly selected locations. Gill nets were set for 2-3-h at dusk.

Targeted fish taxa for stable isotope analysis in 2012 included prey items previously detected in lavage samples collected from hybrid striped bass in previous years. Prey species included black crappie, bluegill, largemouth bass, white crappie *Pomoxis annularis*, yellow bass, and yellow perch *Perca flacescens*. Five prey fish from each of the following length classes were targeted: < 40, 40-59, 60-79, 80-99, 100-120, and >120 mm total length (TL). We also attempted to collect five adult hybrid striped bass, largemouth bass, yellow bass, and walleye *Sander vitrsus* from each of four length categories described by Gabelhouse (1984): stock-quality (S-Q), quality-preferred (Q-P), preferred-memorable (P-M), and memorable-trophy (M-T) (Olson et al. 2007).

Fish for stable isotope analysis were collected in April, June, October, and November using similar sampling methods and net locations as described above. Additional modified fyke nets (13 mm mesh) were set overnight to collect smaller fish. A small (~10 g) sample of dorsal white muscle (Pinnegar and Polunin 1999) was removed from the left side of each fish and frozen at -20°C until further processing.

Stable Isotopes.—Stable isotope samples were run at the Stable Isotope Paleoenvironments Research Group (SIPERG) of Iowa State University, Ames Iowa. Fish samples were rinsed with nano-pure and freeze-dried for ≥ 24-hr using a LABCONCO™ 4.5 freeze dry system (Kansas City, MO). Once dry, samples were ground into a fine powder and homogenized using a mortar and pestle. A 500-ug subsample of the resulting fish powder was weighed into a 0.2 x 0.2-cm aluminum weigh boat and measured with a Finnigan MAT Delta Plus XL mass spectrometer in continuous flow mode connected to a Costech Elemental Analyzer (Framingham, MA). Twelve reference standards (2 Caffeine [IAEA-600], 3 Cellulose [IAEA-CH-3], 2 Ammonium Sulfate [IAEA-N-1], 2 Ammonium Sulfate [IAEA-N-2], and 3 Acetanilide [laboratory standards]) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale. The typical analytical uncertainty for $\delta^{13}C$ and $\delta^{15}N$ was ±0.07 and ±0.11 respectively.

Isotope samples were categorized by the time which they were collected: April-June (spring samples) and October-November (fall samples). Seasonal differences in $\delta^{13}C$ and $\delta^{15}N$ of individual fish taxa within the same size class were assessed using pairwise comparisons (two-sample *t*-test). If there were no significant differences in $\delta^{13}C$ and $\delta^{15}N$ between spring and fall samples, delta values of fish within each length class were combined for further analysis. Differences between length classes of individual taxa were tested using a one-way analysis of variance (ANOVA). Length classes of individual taxa were combined if both $\delta^{13}C$ and $\delta^{15}N$ values were not significantly different. Sources were combined to reduce the number of clusters and assign biological groupings rather than arbitrary numeric length classes. Using a general linear model, we assessed whether delta values varied across predator length. All tests were performed in Statistical Analysis Systems version 9.3 (SAS Institute 2011). A significance level of 0.05 was used throughout.

To assess niche overlap between piscivore taxa, we used two recently developed methods of multivariate analysis. Using the convex hull concept discussed by Layman et al. (2007), niche overlap was estimated by determining the number of individuals encompassed by another group's convex hull (Hammerschlag-Peyer et al. 2011; Vaudo and Heithaus 2011). The convex hull of each group was defined as the area of the smallest convex polygon that encompasses all signatures in a $\delta^{13}C$-$\delta^{15}N$-biplot, and represents the total amount of isotopic niche space occupied (Layman 2007; Hammerschlag-Peyer et al. 2011). Because convex hulls can be biased towards sample size, e.g., more individuals sampled produce larger convex hull areas; we also estimated niche overlap by determining the overlap of standard ellipses for each group using the SIBER-ellipse function in R (Jackson et al. 2011). To determine the trophic range and diversity and energetic source variability, we also estimated three other community-wide metrics for each of the top three piscivore groups ($\delta^{15}N$ range, $\delta^{13}C$ range, and mean centroid distance) described by Laymen et al. (2007).

Results

In the initial gut content analysis conducted in 2009-2010, we collected 266 hybrid striped bass (298-626 mm), 156 largemouth bass (205-540 mm), and 125 walleye (269-718) stomach samples. Of the samples collected, 93 hybrid striped bass (35%), 45 largemouth bass (29%), and 71 walleye (56%) had empty stomachs. Although unidentified fish was the major contributing diet item of hybrid striped bass stomach contents in 2010, zooplankton and macroinvertebrates occurred at high frequency in 2009. Yellow bass were the main identified fish present within hybrid striped bass stomach samples in 2010 (Figure 5.1). During both years, crayfish and unidentified fish were the most prevalent diet items in largemouth bass stomach contents, with bluegill being the most common identified fish taxa (Figure 5.2). Although the majority of walleye stomachs were empty during both years, yellow perch were the main identified fish present (Figure 5.3). Hybrid striped bass, largemouth bass, and walleye were not found in any stomach samples of the three predators analyzed.

Stable Isotopes.—During 2012, stable isotope analysis was conducted on 19 black crappie, 24 bluegill, 15 hybrid striped bass, 30 largemouth bass, 17 walleye, 12 white crappie, and 27 yellow bass. Overlap was apparent in both $\delta^{13}C$ and $\delta^{15}N$ of many prey fish (Figure 5.4). There were significant differences in both $\delta^{13}C$ and $\delta^{15}N$ of the first three size-classes (<40, 40-59, and 60-79) of bluegill and no differences detected between any bluegill size-classes greater than 80 mm. All black crappie within size classes smaller than 120 mm had similar signatures. However, larger (stock-size) black crappie had significantly enriched $\delta^{13}C$ values in relation to the smaller size classes ($P < 0.001$). White crappie greater than 100 mm also had enriched $\delta^{13}C$ values. We found that yellow bass had significant enrichment of $\delta^{13}C$ and $\delta^{15}N$ within size classes larger than 80 mm and between sampling periods (Table 5.1).

Although $\delta^{15}N$ values were similar for all hybrid striped bass samples, there was an apparent enrichment in ^{13}C during fall sampling (Figure 1). Hybrid striped bass, largemouth bass, and walleye total lengths were all positively related to their nitrogen signatures (hybrid striped bass, $P = 0.03$; largemouth bass, $P < 0.001$; walleye, $P < 0.001$; Figure 3). There were no relationships detected between hybrid striped bass and largemouth bass total lengths and $\delta^{13}C$. Walleye 200-300 mm were removed from all analysis because they matched the size-range recently stocked and enriched ^{13}C and deplete ^{15}N values were assumed to be artifacts of hatchery conditions. No significant differences were detected in $\delta^{13}C$ and $\delta^{15}N$ values of the three top predators within all size classes' stock-size and larger ($P > 0.05$).

Once trophic fractionation of $\delta^{13}C$ (0.8‰) and $\delta^{15}N$ (3.4‰) was accounted for in the hybrid striped bass signatures, their mean values fall within the prey cluster containing black crappie (40-120 mm), bluegill (<40 mm), largemouth bass (40-79 mm), white crappie (100-120 mm), and yellow bass (40-79 mm). The $\delta^{13}C$ values of hybrid striped bass overlapped with all the previously listed prey fish groups.

Of the top three piscivores analyzed, largemouth bass had the largest convex hull area, $\delta^{15}N$ range, and mean centroid distance. Walleye had the smallest convex hull area, $\delta^{15}N$ range, and mean centroid distance (Table 5.2). Hybrid striped bass had the smallest $\delta^{13}C$ range. Combining spring and fall samples, 18% of the largemouth bass signatures overlapped with the walleye convex hull. Hybrid striped bass signatures overlapped more of the largemouth bass (86%) than the walleye (14%) convex hulls (Figure 5.5). Though not considered to be a top predator within the system, the yellow bass convex hull had zero overlap with any of the other piscivores.

Similar to the convex hulls, largemouth bass had the largest standard ellipse area (1.89) and walleye had the smallest (1.05). Area overlap of the largemouth bass and walleye standard ellipses was 0.48 (25%). The hybrid striped bass standard ellipse (1.27) overlapped the largemouth bass standard ellipse 54% and walleye standard ellipse 43%. No other fish groups (including yellow bass) had overlapping standard ellipses with any of the top three predators.

Discussion

The debate over the utilization of nonnative fish introductions is currently a global issue that is highly polarized within the scientific community and the general public (Gozlan 2008; Vitule et al. 2009; Gozlan et al. 2010). Within the United States, the most common reason for fish introductions are intentional stocking of sport and forage fish (Nico and Fuller 1999). Management though the addition of new fish taxa are reactions to perceived needs for establishing new fisheries, enhancing existing ones, and filling a vacant niche (Gozlan et al.

2010). The possibility of negative ecological impacts of new species introductions make it essential for risk assessment to predict future problems based on past failures and current circumstances. Unfortunately, much of what is known about the impacts of introduced fish taxa is from post hoc correlations made between introductions and subsequent changes in native populations (Taylor et al. 1984).

The introduction of hybrid striped bass to Three Mile Lake represents what is typical for reservoir fisheries management strategies described above. Fish were initially stocked to enhance the sport fishery and fill a perceived vacant niche present within the pelagic zone of the reservoir. The introduction was also a preemptive attempt at biologically suppressing a probable future yellow bass population. All of which was done at with the risk of the established fish assemblages. However, local animosity towards yellow bass within southern Iowa warranted the attempt to biologically control the unwanted taxa (G. Sobotka, IDNR Fisheries Biologist, personal communication) instead of the more typical management option of complete lake renovations with rotenone treatments.

Since the majority of gut contents within hybrid striped bass stomach samples consisted of unidentified fish particulate the assumption was that they primarily utilized fish in their diets. However, high frequencies of occurrence of macro-invertebrates and zooplankton in 2009 but not 2010 may indicate a more generalistic feeding behavior based on food availability. This apparent shift between piscivory and omnivory between years was not seen in other fish taxa. Largemouth bass stomach contents clearly showed a greater reliance on crayfish and bluegill than hybrid striped bass. Unfortunately, not much can be gained concerning walleye diets from the gut content analysis because the majority of individuals had empty stomachs or unidentified fish contents. As typically seen for walleye diets (Parsons 1971; Forney 1974), yellow perch were the most numerous identifiable fish within walleye stomach samples both years. Although diet overlap indices may have been a useful additional parameter, our initial goal was to determine yellow bass consumption, which was reflected in our sampling methods.

Since the potential prey resources sampled had highly overlapping isotopic values, traditional source mixing models to determine percent contribution in the predator's isotopic signature were not possible (Layman et al. 2011). However, using what is currently understood in the scientific literature concerning carbon and nitrogen stable isotopes in aquatic ecosystems, some information related to diets and spatial variation can be inferred from the data we collected. Applying an assumed average trophic fractionation to the hybrid striped bass isotopic values (Vander Zanden et al.1999; Vander Zanden and Rasmussen 2001; Fry 2006), we can conclude that the potential prey-fish consumed by hybrid striped bass included black crappie (40-120 mm), bluegill (<40 mm), largemouth bass (40-79 mm), white crappie (100-120 mm), and yellow bass (40-79 mm) because of overlapping $\delta^{13}C$ values (Olson et al. 2007). Further deduction may imply that it is unlikely that hybrid striped bass consumed white crappie (100-120) and larger black crappie within the 40-120 mm size class because they typically are known to preferentially select smaller prey fish (Ott and Malvestuto 1984; Dettmers et al. 1996; Dettmers et al. 1998). Although we found no largemouth bass in the stomach contents, their contribution to the diets of hybrid striped bass cannot be ruled out using stable isotope analysis.

Unlike the selective feeding behaviors typically documented for hybrid striped bass in reservoirs with a gizzard shad prey-base (Ott and Malvestuto 1981; Borkowski and Snyder 1982; Gilliland and Clady 1984; Jahn et al. 1987; Dettmers et al. 1998), the diets of hybrid striped bass within Three Mile Lake appear to be based more on spatial partitioning and prey size. Crandall (1979) found that hybrid striped bass preferentially selected shad over the predominantly abundant bluegill populations; later researchers concluded that predation on spiny-rayed sport fishes is likely be minimal (Dettmers and Stein 1996). However, the success of establishing a quality hybrid striped bass fishery in the absence of shad, along with $\delta^{13}C$ values that are more reminiscent of littoral fish communities, feeding behaviors within Three Mile Lake do not appear to fit the perception that these fish are the characteristic pelagic predator.

Although hybrid striped bass are typically thought of as open-water pelagic fish, their $\delta^{13}C$ values from Three Mile Lake appear to reflect a more littoral habitat preference. France (1995) demonstrated that $\delta^{13}C$ values are enriched within littoral consumers in relation to pelagic consumers. Because $\delta^{13}C$ values differ among primary producers (France 1995; Hecky and Hesslein 1995; Johnson et al. 2002), we assumed that carbon signatures of consumers reflected spatial separation of feeding habits within Three Mile Lake. Using the $\delta^{13}C$ values of largemouth bass and adult yellow bass as proxies for littoral and pelagic fish, it is apparent that hybrid striped bass δ values resembled those of largemouth bass.

Preferences of largemouth bass for brushy and rocky shorelines (Schlagenhaft and Murphy 1985; Sammons and Bettoli 1999) make them a good representation of expected isotopic signatures for littoral consumers. Although we found a significant relationship between $\delta^{15}N$ and largemouth bass length, indicating an increase in trophic position (Vander Zanden et al. 1998), we found no differences in $\delta^{13}C$ values between any length classes (range = 40-385 mm). Similarities in $\delta^{13}C$ values in all size classes lead us to further conclude that largemouth bass spent the majority of their life within similar zones of the reservoir (littoral).

Conversely, larger yellow bass (100-120 mm and stock-quality size classes) had $\delta^{13}C$ values were much more negative than all other predators sampled suggesting a pelagic consumer (France 1995). Although very little research has focused on the life history of yellow bass, Helm

(1958) describes their behavior to be pelagic in nature. Minimal presence of yellow bass within annual fish surveys conducted by the IDNR suggests a low overall abundance within Three Mile Lake. The lack of pelagic prey for hybrid striped bass is likely the reason for their reliance on young black crappie, bluegill and yellow bass prior to their movement into open water.

Since the carbon and nitrogen signatures of the prey fish only represented what was assimilated from dietary intake (Fry 2006), we can only conclude that the prey fish sampled had fairly similar feeding habits; not that predation from hybrid striped bass definitively took place. We also detected a fairly dramatic shift in both the $\delta^{13}C$ and $\delta^{15}N$ values of bluegill, suggesting a shift in resource utilization (Vander Zanden et al. 1999; Schmidt et al. 2007; Hammerschlag-peyer et al. 2011).

Mittelbach (1981) proposed that larger bluegill will benefit though optimal foraging by moving out of vegetation to forage on larger *Daphnia* present in open water. Mittelbach also suggested that bluegill >100 mm would be most likely to switch habitats in this manor because they are less susceptible to largemouth bass predation at this size. If our analysis concerning the distinction of littoral and pelagic zones using the $\delta^{13}C$ values of largemouth bass and yellow bass is accurate, it could also be suggested that we were able to document the habitat switching behavior of bluegill <40, 40-59, and 60-120 mm as Mittelbach described.

Using two similar concepts with slightly different methods for describing niche widths and overlap, we were able to generalize the partitioning of resources for the top predators within Three Mile Lake. Four community-wide metrics of the trophic structure were applied individually to the different fish taxa (Layman et al. 2007; Hammerschlag-Peyer et al. 2011). Largemouth bass had the largest trophic diversity but smallest niche diversification of food sources, e.g., largest $\delta^{15}N$ range and smallest $\delta^{13}C$ range (Layman et al. 2007). Using the mean centroid distances (CD) as a measure of the average degree of trophic diversity, largemouth bass also was the largest of all fish sampled. However, because stocks of walleye and hybrid striped bass are supported through stocking of advanced fingerlings, the smallest size classes were not represented in the population and thus $\delta^{15}N$ and $\delta^{13}C$ ranges and CD were most likely biased in our samples.

More importantly, using the convex hull overlap of the top predators, we estimated their percent niche overlap based on $\delta^{13}C$ and $\delta^{15}N$ bi-plot analysis (Layman et al. 2007; Hammerschlag-Peyer et al. 2011). The total area of the convex hull for each group was representative of their $\delta^{15}N$ range, e.g., greater trophic diversity would suggest a larger niche space. The convex hull approach is a valuable technique for visually quantifying realized niche space by incorporating multiple stable isotopes because it incorporates each individual sampled as a proxy of the trophic diversity within each group (Layman 2007; Hammerschlag-Peyer et al. 2011). Overlap between hybrid striped bass and largemouth bass was the largest (86% of the hull area) between the top predators. Although there was overlap between hybrid striped bass and walleye (14%), it appeared to be more similar to that of the previously established assemblage of largemouth bass and walleye (18%). A much higher overlap of hybrid striped bass and largemouth bass convex hulls may be hidden by two potential outliers shown in Figure 5.5. However, no biological justification could disprove these values.

To further develop an understanding of the trophic relationships of the piscivore species within Three Mile Lake, we also estimated the percent overlap of the bivariate normal distribution of their standard ellipse area. Since the area of the convex hull can be biased by different sample sizes, the standard ellipse is suggested to be a better proxy of isotopic niche space (Jackson et al. 2012). Unlike the convex hull approach, a standard ellipse of bivariate data represents the variance-covariance of the *x* and *y* data, encompassing only 40% of the data points; making the method insensitive to sample size (Jackson et al. 2012). Similar to the convex hull approach, the area of overlap for hybrid striped bass and largemouth bass was the highest (54%); while smallest for largemouth bass and walleye (25%). However, the standard ellipse technique indicated a greater overlap than the convex hull approach between largemouth bass and hybrid striped bass (43%). Again, our data seems to suggest that although resource overlap does occur, it is less prominent between co-established species.

Many studies have attempted to describe the functional niche of species since Hutchinson's (1957) conceptualization of an n-dimensional hypervolume. Isotope niche space is a novel idea that carbon and nitrogen stable isotopes reflect multiple dimensions of the functional niche space, e.g., diet, trophic position, and spatial locality, into two explanatory variables. Through this concept, it appears that Three Mile Lake was may be a good demonstration for the argument against using species introductions to fill a vacant niche (Herbold and Moyle 1986). Our analysis appears to indicate that the hybrid striped bass have overlapped with the largemouth bass's niche space instead of "filling" the perceived empty pelagic niche of the reservoir. However, the current success of this introduction is most likely due to the highly disturbed nature of reservoirs in general (Herbold and Moyle 1986). Although we were able to describe an apparent overlap in resources between the two piscivores, the current knowledge of competition between hybrid striped bass and other predators suggest that coexistence would be likely (Jahn et al. 1987; Neal et al. 1999b; Olson et al. 2007).

Conclusions

From the previous gut content data and stable isotope analysis, it was apparent that the hybrid striped bass did not selectively feed on the recent, unintended introduction of yellow bass.

Although somewhat surprising to local fishery managers, yellow bass populations in Three Mile Lake continue to be atypical to similar reservoirs. Within the region, typical reservoir fisheries containing yellow bass are diminished in value because the species easily becomes overabundant and a sink for biomass, resulting in dramatic shifts in sport fish populations. Apparent low abundances of yellow bass within Three Mile Lake suggest some means of suppression has occurred. Whether it is directly related to the introduction of hybrid striped bass is unknown. However, highly overlapping niche space, as indicated via stable isotope analysis, suggest that continued monitoring of gamefish populations will be necessary. Assessment of the sport fish population dynamics and individual condition should be the next stage in determining any detrimental effects of continued hybrid striped bass stocking. Results garnered from this study support the use of stable isotope analysis as a tool to better understand ecological effects of management decisions on a fishery. It is recommended that fisheries managers attempt to incorporate stable isotope analysis as a tool for assessing management decisions based on local conditions.

Acknowledgements

We would like to thank the Iowa Department of Natural Resources staff, the Stable Isotope Paleoenvironments Research Group (SIPERG) staff, and Iowa State University NREM staff.

TABLE 5.1. Mean ± SD $\delta^{13}C$ and $\delta^{15}N$ values of black crappie (BLC), bluegill (BLG), hybrid striped bass (HSB), largemouth bass (LMB), walleye (WAE) yellow bass (YEB), and yellow perch (YEP) muscle tissue during 2012 spring and fall collection periods at Three Mile Lake, Iowa.

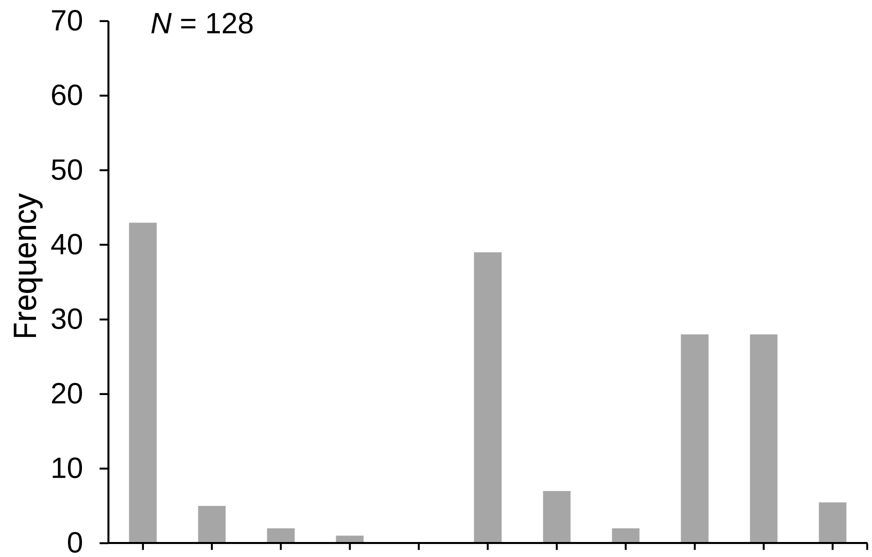
	Spring		Fall	
Species (length class)	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
BLC (40-59)	-25.65 (0.33)a	12.41 (0.16)b		
BLC (60-79)			-27.30	13.51
BLC (80-99)			-26.41 (0.87)a	13.04 (0.56)b
BLC (100-120)			-25.75(1.11)a	12.48 (0.06)b
BLG (<40)	-25.78 (0.81)a	12.69 (0.47)b		
BLG (40-59)	-27.05 (0.59)c	15.26 (0.18)d		
BLG (60-79)	-28.30 (0.63)e	14.62 (0.41)f		
BLG (80-99)	-27.94 (0.03)g	14.86 (0.54)h		
BLG (100-120)	-28.01 (0.89)g	14.50 (0.48)h		
HSB (S-Q)	-26.42	16.36	-25.13	13.92
HSB (Q-P)	-28.59	14.50	-25.25 (0.59)a	16.59 (0.32)b
HSB (P-M)	-26.40 (0.11)c	16.79 (0.18)b	-25.76 (0.09)a	16.77 (0.20)b
HSB (M-T)	-26.64	16.24	-25.83	16.22
LMB (40-59)	-25.93 (0.26)a	13.22 (0.21)b		
LMB (60-79)	-25.97 (0.62)a	13.16 (0.28)b		
LMB (80-99)	-26.06 (0.37)a	13.89 (0.50)c		
LMB (100-120)	-26.47 (0.45)a	15.25 (1.47)d	-25.28 (0.36)a	13.66 (1.34)c
LMB (S-Q)	-26.20 (0.75)a	15.99 (1.37)d		
LMB (Q-P)	-25.79 (0.85)a	16.52 (0.55)d		
LMB (P-M)	-25.80 (0.85)a	16.98 (0.29)d		
WAE (S-Q)	-21.99 (2.77)a	12.78 (1.84)b		
WAE (Q-P)	-26.36 (0.10)c	17.19 (0.38)d		
WAE (P-M)	-26.60 (0.18)c	17.25 (0.14)d		
WAE (M-T)	-26.41	17.09		
WHC (60-79)			-26.92 (0.30)a	13.50 (0.33)b
WHC (80-99)			-26.97 (0.27)a	12.99 (0.61)b
WHC (100-120)			-25.84 (0.35)c	12.82 (0.23)b
YEB (40-59)	-25.79 (0.48)a	12.60 (0.11)b		
YEB (60-79)	-25.64 (0.28)a	12.70 (0.31)b		
YEB (100-120)	-28.84 (0.17)c	14.48 (0.18)d		
YEB (S-Q)	-28.67 (0.52)c	14.53 (0.25)d	-26.38 (0.89)e	12.42 (0.46)b
YEB (Q-P)	-28.08	15.40		

Comparisons made between collection periods (spring and fall) and among size classes (mm) of similar taxa for both $\delta^{13}C$ and $\delta^{15}N$. Corresponding letters represent no significant difference between groups or collection periods (P <0.05).

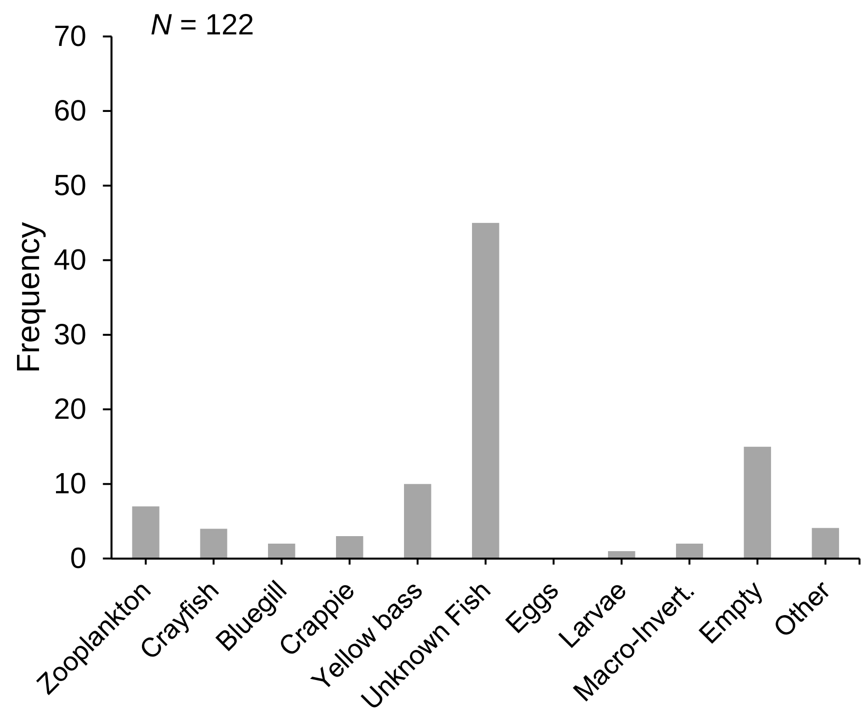
TABLE 5.2. Convex hull area, $\delta^{15}N$ range (i.e., maximum $\delta^{15}N$ – minimum $\delta^{15}N$), $\delta^{13}C$ range (i.e., maximum $\delta^{13}C$ – minimum $\delta^{13}C$), and mean distance to centroid (CD) calculated from stable isotope analysis of the fish community in Three Mile Lake during 2012.

Taxa	Hull	$\delta^{15}N$ range	$\delta^{13}C$ range	CD
HSB	2.99	3.09	3.76	0.65
LMB	3.04	4.53	2.40	1.57
WAE	1.55	2.01	3.40	0.79

The mean distance to centroid represents the mean Euclidean distance of each taxon to the $\delta^{13}C$-$\delta^{15}N$ centroid (mean $\delta^{13}C$ and $\delta^{15}N$ values for all taxa within the food web). Community-wide metrics were calculated using all fish sampled for stable isotope analysis (N = 151). Hull areas were calculated using hybrid stripe bass (HSB), largemouth bass (LMB), walleye (WAE) and yellow bass (YEB) from stock-quality, quality-preferred, preferred-memorable, and memorable-trophy size classes.

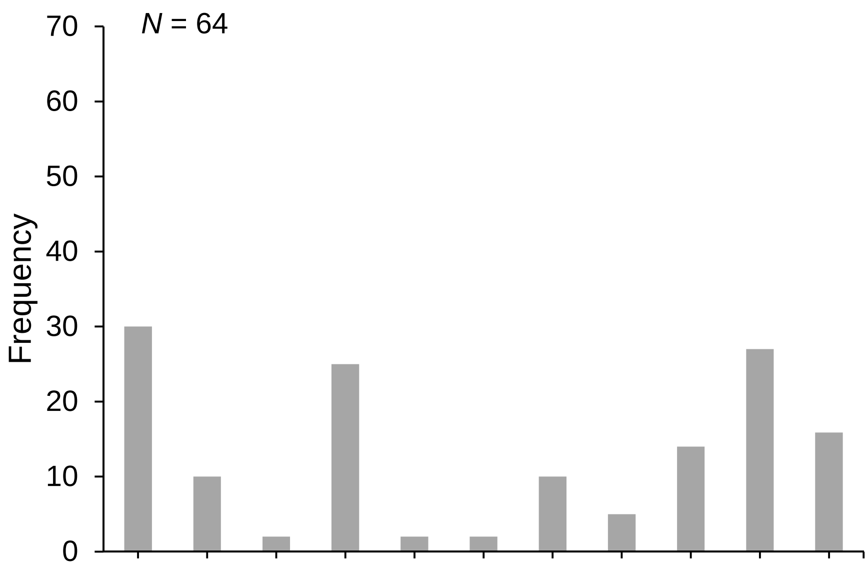


a.

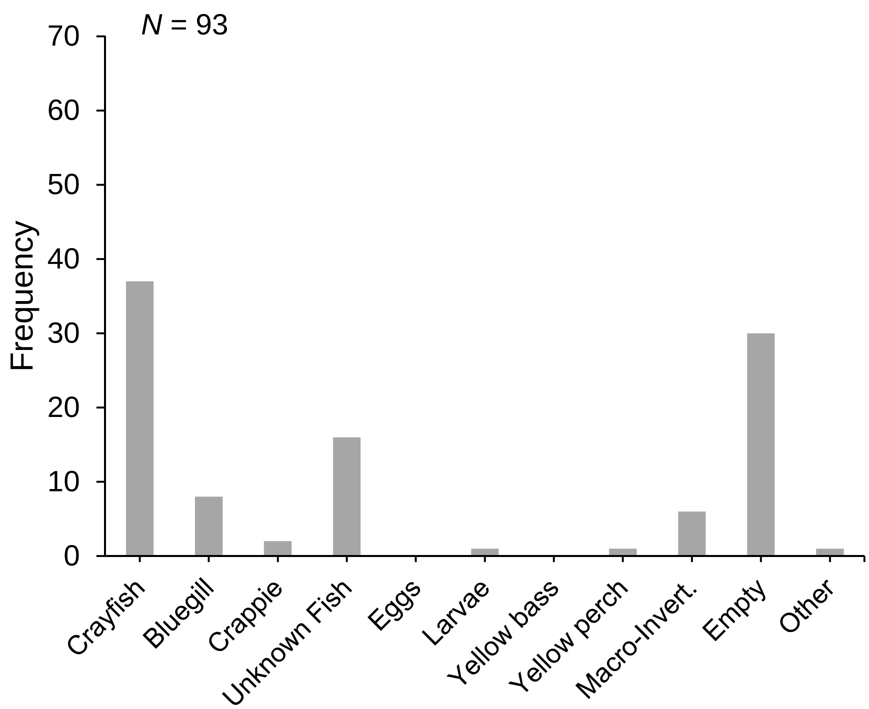


b.

FIGURE 5.1. Dietary frequency of occurrence of hybrid striped bass *Morone saxatilis* × *M. chrysops* from gastric lavage stomach samples collected April-May of 2009 (a.) and 2010 (b.) from Three Mile Lake, Iowa. Identified items that occurred in less than 1% of sampled fish were grouped into the other category.

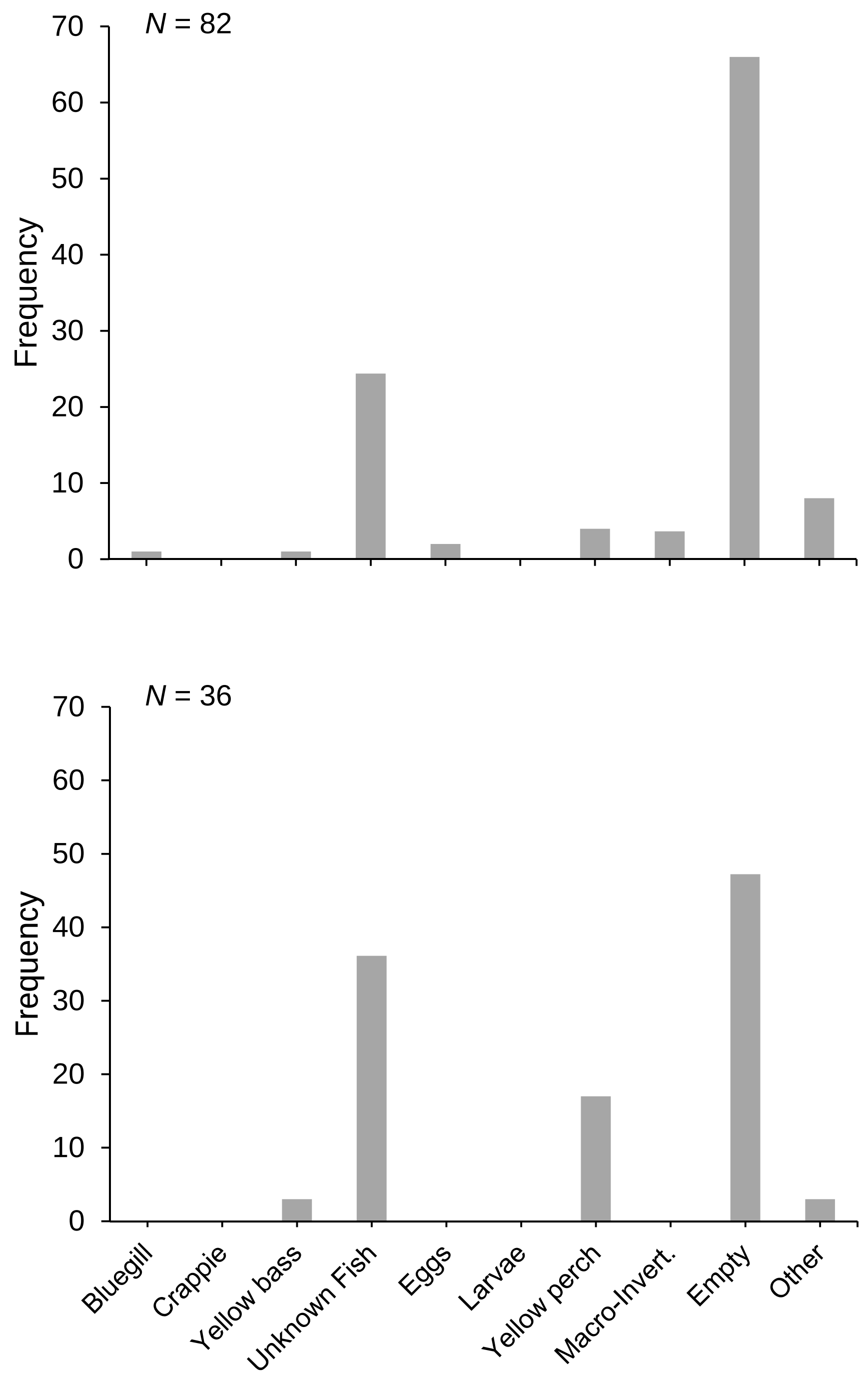


a.



b.

FIGURE 5.2. Dietary frequency of occurrence of largemouth bass *Micropterus salmoides* from gastric lavage stomach samples collected April-May of 2009 (a.) and 2010 (b.) from Three Mile Lake, Iowa. Identified items that occurred in less than 1% of sampled fish were grouped into the other category.



a.

b.

FIGURE 5.3. Dietary frequency of occurrence of walleye *Sander vitreus* from gastric lavage stomach samples collected April-May of 2009 (a.) and 2010 (b.) from Three Mile Lake, Iowa. Identified items that occurred in less than 1% of sampled fish were grouped into the other category.

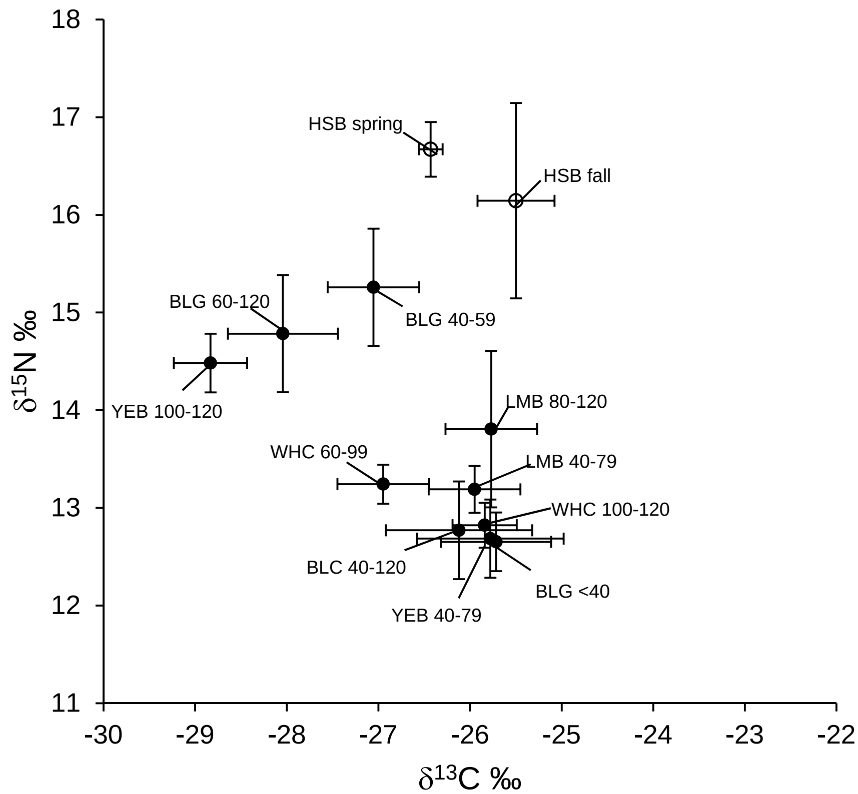


FIGURE 5.4. Mean ± SD $\delta^{13}C$ and $\delta^{15}N$ values of hybrid striped bass (open circles) and potential prey (closed circles) collected from Three Mile Lake, Iowa during April-October 2012. Clusters were formed using a one-way analysis of variance (ANOVA) for both $\delta^{13}C$ and $\delta^{15}N$ between seasons (spring and fall) and size classes (<40, 40-59, 60-79, 80-99, and 100-120 TL). If no differences were detected, groups were combined. Hybrid striped bass signatures (HSB) were separated by season and all length categories (stock-trophy) were combined. Prey species collected include black crappie (BLC), bluegill (BLG), largemouth bass (LMB), white crappie (WHC), and yellow bass (YEB).

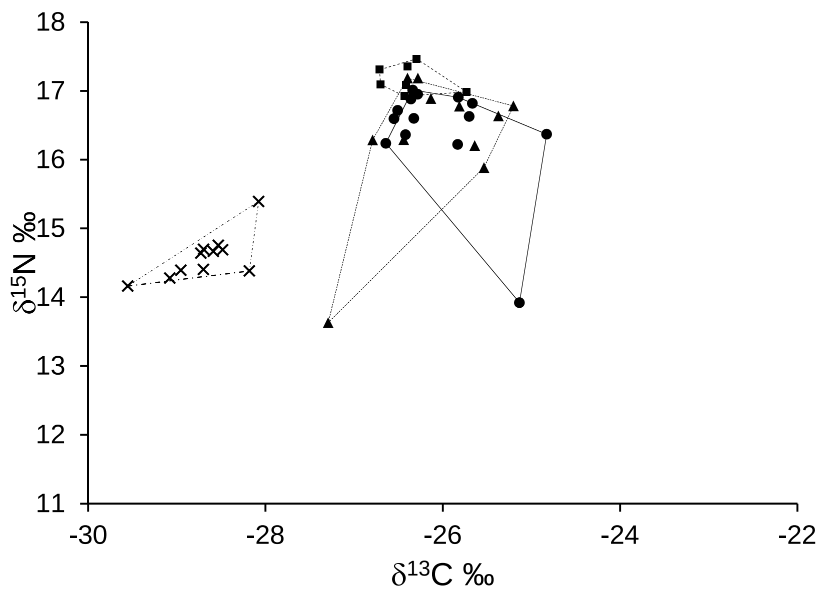


FIGURE 5.5. Stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$) of hybrid striped bass (circles), largemouth bass (triangles), walleye (diamonds) and yellow bass (×) collected from Three Mile Lake, Iowa during April-October 2012. Isotopic niche space is represented for each group by their convex hull polygons.

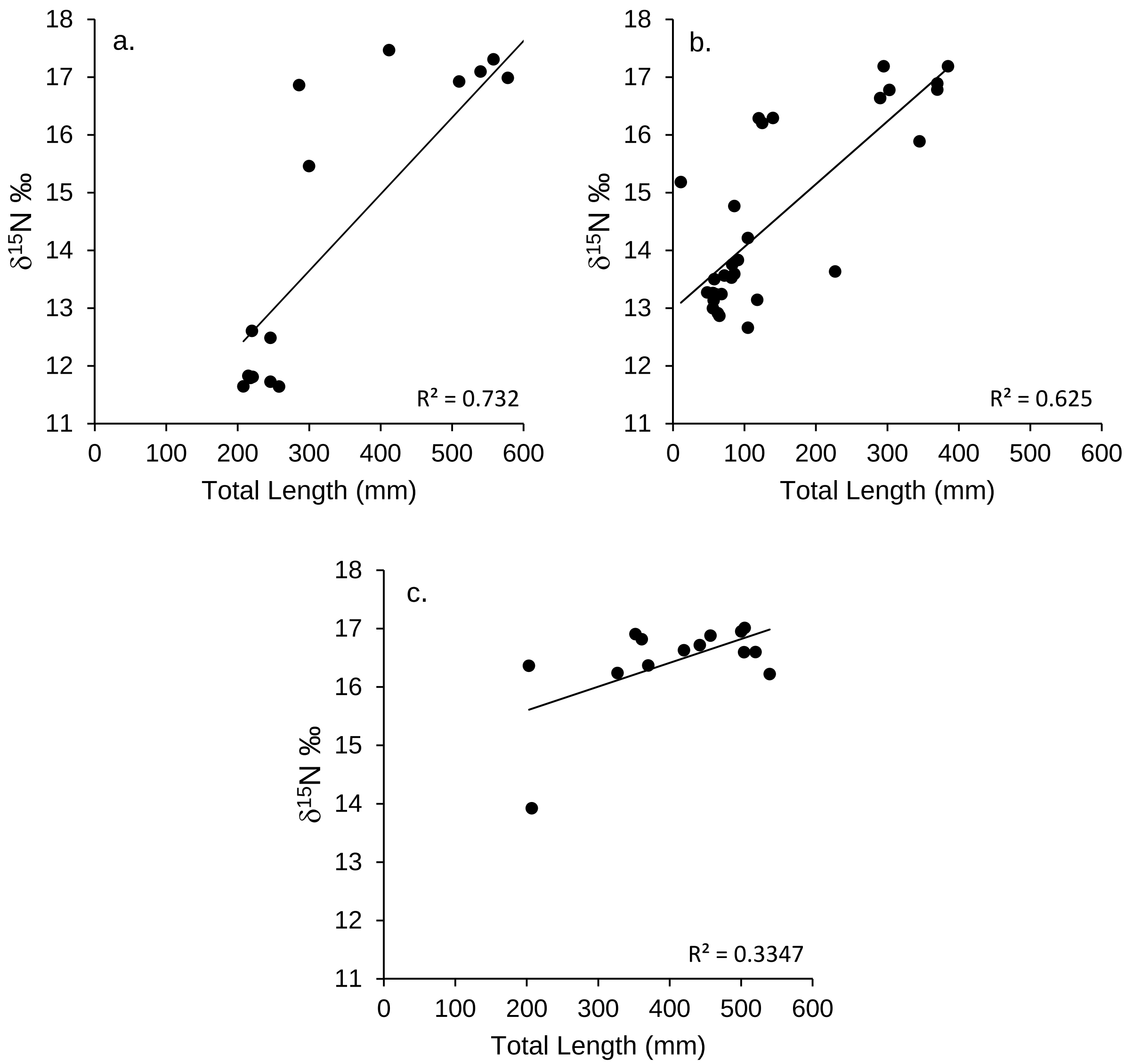


Figure 5.6. Nitrogen ($\delta^{15}N$) isotope signatures of walleye (a), largemouth bass (b), and hybrid striped bass as a function of total length (mm) for fish sampled in Three Mile Lake, Iowa during 2012.

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CHAPTER 6. GENERAL CONCLUSIONS

General Discussion

The original hybrid striped bass *Morone saxatilis* × *chrysops*, known as the palmetto bass, was first produced by Robert Stevens in 1965 by crossing a female striped bass Morone *saxatilis*, with a male white bass Morone *chrysops.* The reciprocal cross *M. chrysops* x *M. saxatilis* (sunshine bass) was developed later as an alternative (Kerby and Harrell 1990). Many natural resource agencies that have access to the brood striped bass females continue to use the original cross although supply can be somewhat unreliable due to environmental conditions, e.g., drought conditions and temperature swings that limit access (Mischke 2012). In contrast, the private sector is almost entirely comprised of the reciprocal cross as the private aquaculture industry either does not have access to wild female striped bass from public water ways or choose not to maintain the large female striped bass in captivity.

From our hatchery survey, it appears the culture differences between the two hybrid striped bass taxa need to be better addressed in actual field applications. Because stocking density, feeding rate, and fertilization rate all appear to have an effect on production, accepted culture methods still need to be established using peer-reviewed scientific research. It is unclear whether this disconnect is caused by limited resources within agencies, poor communication between researchers and managers, or perhaps operational constraints placed on the hatcheries, e.g., availability of fry and multiple species being cultured at a specific hatchery. The two major disconnects identified between practice and the peer-reviewed literature is stocking time and pond fertilization.

The use of pond fertilization has long been used to increase fish production within earthen ponds (Swingle and Smith 1938; Boyd 1981; Geiger 1983a; Murad and Boyd 1987; Boyd 1990). Although not statistically significant, we saw greater survival in fertilized than non-fertilized earthen ponds. While greater final fish biomass within fertilized ponds may have been related to the inherent difficulties of comparing production between ponds with unequal survival (Morris and Muncy 1988), we did not find a difference between fish length, weight, or growth, as would be expected (Brewer and Rees 1992). Our analysis indicates that the fertilization treatment increased both copepod and cladoceran taxa during the second week of culture; as typically observed (Geiger 1983a, b; Parmley and Geiger 1985; Johnson and Schlosser 1991; Ludwig1999).

As indicated by low NO_3-N:P ratios within our fertilized ponds, it was likely that they were dominated by filamentous blue-green cyanobacteria (Smith 1986; Culver et al. 1992). Since zooplankton populations are negatively affected by the presence of cyanobacteria (Gliwicz and Lampert 1990; DeMott et al. 1991), copepod and cladoceran populations within our fertilized ponds did not respond as effectively as would be expected with the addition of fertilizer.
High total phosphorus (TP) concentrations within our source water and initially high TP levels seen in our ponds may also have been an indication that nitrogen was the limiting nutrient (Rogge et al. 2003; Stone et al. 2012).

In addition to pond fertilization to boost natural prey populations, many culturists whom we surveyed indicated using a commercial fish feed during phase I culture of the palmetto hybrid. Similar to fingerling striped bass production (Fitzmayer et al. 1986) and hybrid striped bass production (Morris 1988), our results suggest that final fish biomass can be enhanced though feeding during phase I culture in plastic-lined ponds. However, the actual role of a commercial diet has been characterized as both an expensive organic fertilizer and as a selected food item for larval hybrid striped bass (palmetto) *Morone saxatilis* × *M. chrysops* culture operations. Using stable carbon and nitrogen isotopes, we were able to determine how much of the commercial fish feed contributed to fish growth.

Although we found that the hybrid striped bass do utilize prepared diets during phase I culture, fish production performance did not appear to greatly benefit with feed supplementation over pond fertilization alone. However, greater fish length and biomass may warrant the use of a commercial diet. The high percentage (≥ 80%) in which natural pond biota continued to account for in the fishes isotopic signatures indicate the importance of keeping a good zooplankton population throughout phase I, whether or not feed is offered. Enriched carbon and nitrogen signatures within the zooplankton and chironomid larvae also suggested that the natural pond biota was altered though the feed application.

The purpose for hybrid striped bass production by the Iowa Department of Natural Resources (IDNR) is to stock local waters for enhancing recreational fisheries and biologically control overabundant gizzard shad *Dorosoma cepedianum* populations. However, the debate over the utilization of nonnative fish introductions is currently a global issue that is highly polarized within the scientific community and the general public (Gozlan 2008; Vitule et al. 2009; Gozlan et al. 2010). The possibility of negative ecological impacts of new species introductions make it essential for risk assessment to predict future problems based on past failures and current circumstances. Unfortunately, much of what is known about the impacts of introduced fish taxa is from post hoc correlations made between introductions and subsequent changes in native populations (Taylor et al. 1984).

Within Three Mile Lake, Iowa, hybrid striped bass were introduced as a preemptive attempt at biologically suppressing a probable future yellow bass population. It was hypothesized that hybrid striped bass would selectively feed on the pelagic yellow bass much like what is typically observed in reservoirs with shad populations (Ott and Malvestuto 1981; Borkowski and Snyder 1982; Gilliland and Clady 1984; Jahn et al. 1987; Dettmers et al. 1998). What made Three Mile Lake an interesting case study, was that the successful establishment of hybrid striped bass had occurred in the absence of a shad prey-base. Using a dual approach of gut content analysis and stable isotope analysis, we were able to determine that the diets of hybrid striped bass included black crappie *Pomoxis nigromaculatus* (40-120 mm), bluegill *Lepomis macrochirus* (<40 mm), largemouth bass *Micropterus salmoides* (40-79 mm), and yellow bass *Morone mississippiensis* (40-79 mm) because of overlapping $\delta^{13}C$ values (Olson et al. 2007).

Although hybrid striped bass are typically thought of as open-water pelagic fish, their $\delta^{13}C$ values from Three Mile Lake appear to reflect a more littoral habitat preference. Because $\delta^{13}C$ values differ among primary producers (France 1995; Hecky and Hesslein 1995; Johnson et al. 2002), we assumed that carbon signatures of consumers reflected spatial separation of feeding habits within Three Mile Lake. Using the $\delta^{13}C$ values of largemouth bass and adult yellow bass as proxies for littoral and pelagic fish, it is apparent that hybrid striped bass δ values resembled those of the common littoral fish taxa (largemouth bass).

Using two methods for determining niche overlap from carbon and nitrogen bi-plot analysis (Layman et al. 2007; Hammerschlag-Peyer et al. 2011); we determined that the hybrid striped bass had resource overlap with largemouth bass and walleye. There is also some evidence that overlap with the largemouth bass niche was greater than walleye. Our analysis appears to indicate that the hybrid striped bass have overlapped with the largemouth bass's niche space instead of “filling” the perceived empty pelagic niche of the reservoir. The current success of this introduction is most likely due to the highly disturbed nature of reservoirs in general (Herbold and Moyle 1986) and coexistence with other top predators is likely (Jahn et al. 1987; Neal et al. 1999; Olson et al. 2007).

Management Implications

The approaches to developing culture guidelines for the two *Morone* hybrids have taken different approaches. Limited availability of female striped bass for private sector hatcheries has forced operations to focus on sunshine bass culture although phase I survival rates were historically half that of the palmetto bass. A review of the peer-review literature in conjunction with our survey indicates a continued improvement in pond survival rates of sunshine bass to levels that now approach palmetto bass. Recent research and extension publications on sunshine bass culture reflect the role of federal research programs in addressing private culture needs for furthering sunshine bass culture operations; noted improvements addressed the needs for economic viability within the private aquaculture industry. Continued research, along with an examination of both public and private production techniques will be necessary for future success in both taxa. Although much research has focused on pond fertilization regimes, the specificities required for geographic location and different fish taxa suggest that continued research may be necessary on a hatchery by hatchery basis. An attempt at producing a meta-analysis of pond fertilization techniques in both public and private culture facilities based on location will be an important next step in furthering the culture of both taxa.

Since we were able to obtain numerically but not significantly greater survival and biomass within fertilized ponds at the Mount Ayr Fish Hatchery, continued efforts should include producing pond-specific fertilization regimes onsite. Although phosphorus concentrations should be monitored and maintained to current agency minimum levels, the use of highly eutrophic source waters will diminished the need of additional phosphorus. Low NO_3:P ratios suggest that it may also be beneficial to use a nitrogen based inorganic fertilizer or an organic fertilizer with a higher nitrogen concentration, e.g., soybean meal. Future fertilization protocols should focus more on proper nutrient ratios.

With high feed costs, supplemental feeding should only be used if the fish are retained for additional culture beyond the initial 40+-d culture period. Although production performance was minimally affected with the addition of a commercial feed, the unpredictable nature of zooplankton populations and culture conditions make feeding a safer alternative to pond fertilization alone.

The combination of gut content analysis and stable isotope analysis was useful in determining that hybrid striped bass did not selective feed on the newly introduced yellow bass in Three Mile Lake. Somewhat surprising to local fishery managers though, yellow bass populations in Three Mile Lake continue to be minimal. For this reason, we cannot be conclusive that hybrid striped bass had no effect on yellow bass populations. If future fish assemblages appear to shift towards a greater dominance of yellow bass within Three Mile Lake, it would be beneficial to revisit our study and determine if the hybrid striped bass would switch to a more pelagic feeding behavior. It is likely that yellow bass predation was not dominant due to low overall abundance within the reservoir. Highly overlapping niche space, as indicated via stable isotope analysis, suggest that continued monitoring of other gamefish populations will be necessary to determine any negative ecological effect from the introduction of hybrid striped bass.

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