Observations on the Life History of the Bayou Killifish, Fundulus pulvereus (Everman 1899) in Mississippi Tidal Marshes

Leah Corrine Gaines
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OBSERVATIONS ON THE LIFE HISTORY OF THE BAYOU KILLIFISH,
FUNDULUS PULVEREUS (EVERMAN 1899) IN
MISSISSIPPI TIDAL MARSHES

by

Leah Corrine Gaines

A Thesis
Submitted to the Graduate School
of the University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

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May 2015
ABSTRACT

OBSERVATIONS ON THE LIFE HISTORY OF THE BAYOU KILLIFISH, 
*FUNDULUS PULVEREUS* (EVERMAN 1899) IN 
MISSISSIPPI TIDAL MARSHES 

by Leah Corrine Gaines

May 2015

This study provides insight into the life history of the relatively unknown intertidal marsh fish *Fundulus pulvereus* (Everman), the bayou killifish. Monthly sampling for *F. pulvereus* took place in three different Mississippi tidal marshes over a period of 17 months (n=445). Observations on the diet, parasite fauna, and fecundity of the bayou killifish were documented on all specimens collected. Percent Occurrence and Percent Number coupled along with multivariate analyses indicated seasonal, site, and ontogenetic shifts in the diet of *F. pulvereus*. Dominant prey items consumed by *F. pulvereus* were copepods, mites, chironomids, and terrestrial and aquatic insects. The bayou killifish is an opportunistic omnivore that utilizes marsh floodplains to forage for prey, and is a link in the trophic interactions taking place within estuaries. Parasitological observations resulted in the documenting of fourteen species; all but two of which were not previously reported from *F. pulvereus*. The presence of trophically-transmitted parasites indirectly indicates consumption of prey items not observed during the examination of stomach contents within bayou killifish (e.g., *Cyrenoida floridana*); as well as, the role of this marsh fish as trophic intermediate host for piscivorous predators such as alligators, herons, and raccoons. Analysis using
the Gonadosomatic Index indicated a spawning season that began in February and extended through August. The information provided here should give a greater understanding of the function of fundulids within the northern Gulf of Mexico tidal marsh systems and serves as a baseline for future studies.
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SB         Site 1 Simmons Bayou
WB         Site 2 Weeks Bayou
MP         Site 3 Moss Point
SL         Standard Length in mm
TL         Total Length in mm
MG         Mouth Gape in mm
MG*        Log_{10} transformed Mouth Gape in mm
%N         Percent number
%O         Percent occurrence
Fo         Frequency of Occurrence
ANOSIM     Analysis of Similarities
ANOVA      Analysis of Variance
BF         Batch Fecundity
BF*        Log_{10} transformed Batch Fecundity
RF         Relative Fecundity
RF*        Log_{10} transformed Relative Fecundity
GSI*       Arcsine square root transformed Gonado Somatic Index
GFBW       Gonad Free Body Weight in
CHAPTER I
INTRODUCTION

Many species of the genus *Fundulus* have been well studied, resulting in hundreds of publications detailing their life history, ecology, genetics, and embryology. This coverage is biased towards the larger fundulids, specifically the *Fundulus heteroclitus – Fundulus grandis* species-complex (Able and Hata 1984), with over 300 papers on *F. heteroclitus* alone (Able and Castagna 1975; Foster 1967). Much less information exists on the smaller fundulids such as *Fundulus pulvereus*. The present study provides new information to address some of these knowledge gaps. Observations will be made on the trophic ecology, life history, and parasite fauna of the poorly studied bayou killifish, *F. pulvereus*. The primary objectives of this research are to (1) determine if ontogenetic and seasonal shifts in diet are present, (2) document the endo- and ectoparasites occurring in this host to better understand the role of the Bayou killifish as a trophic indicator between *F. pulvereus* and its parasite fauna (Valtonen et al. 2010), (3) determine batch and relative fecundity (4) and delineate a preliminary spawning season using the Gonadosomatic Index (GSI).

**Hypotheses:**

1. The diet of *F. pulvereus* shifts seasonally with available food sources within its diet-breadth.

2. The diet of *F. pulvereus* shifts ontogenetically with an increase in size of fish.
3. Representative parasites are present in *F. pulvereus* and serve as ecological (trophic) tags.

4. The batch- and relative fecundities of *F. pulvereus* follow the pattern seen in similar fundulids.

5. The spawning season of this semilunar batch spawner begins in the early spring and continues through late summer.

The information gained from this study should provide a greater understanding of the function of the bayou killifish within the northern Gulf of Mexico tidal marsh system.

Literature Review

*Previous studies on the diets of F. pulvereus and other tidal marsh species of Fundulus*

The diets of many North American fundulids occurring in estuarine habitats have been well documented (Akin and Winemiller 2008; Allen et al. 1994; Byrne 1978; Harrington and Harrington 1961; 1972; Kneib 1978; Lopez 2009; Lopez et al. 2010; Odom and Heald 1972; Rozas and LaSalle 1990; Sullivan and Moncreiff 1990; Weisberg et al. 1981; Weisberg 1986), but there is little previous information on the diet of the bayou killifish. In an overview paper on salt marsh food webs, Sullivan and Moncreiff (1990) reported that δ^{15}N stable isotopes showed that *F. pulvereus* was one of the few consumers in the Mississippi salt marsh to utilize *Spartina* sp. as a carbon source. With a δ^{15}N signature of less than 9% *F. pulvereus* is placed into the category of deposit and suspension feeders as established by Peterson and Howarth (1987). Akin and
Winemiller (2006), found *F. pulverous* to be an omnivore/primary carnivore with a trophic level of 3.00, using the formula by Adams et al. (1983). The bayou killifish was grouped with *Lucania parva* (rainwater killifish) and *Gobiosoma bosc* (naked goby). These species showed high dietary overlap feeding on similar prey items such as amphipods and chironomid larvae (Akin and Winemiller 2006). Akin and Winemiller (2006) showed an increase in trophic level from deposit and suspension feeder to a more carnivorous diet. The only observations on the diet of the bayou killifish are anecdotal references to unidentified aquatic insects and an isopod in the stomachs of two specimens of *F. pulvereus* collected in a Texas saltmarsh (Simpson and Gunter 1956).

Detailed information is available on the diets of the larger killifishes, *F. grandis* and *F. heteroclitus*, which utilize wider foraging habitats such as the upper marsh and the intertidal creeks of the lower marsh (Butner and Brattstrom 1960; Kneib 1986; Rozas and LaSalle 1990; Valiela et al. 1977). Both *F. grandis* and *F. heteroclitus* are opportunistic omnivores, and consume both terrestrial and aquatic insects and larval fish. Amphipods and polychaetes also make up a portion of their diets (Baer 1974; Baker-Dittus 1978; Harrington and Harrington 1961; Kneib 1986; Odum 1970; Rozas and LaSalle 1990; Springer and Woodburn 1960). However, *F. grandis* and *F. heteroclitus* rely heavily upon the upper marsh food supply during high tide to sustain them. As an example of its opportunistic feeding strategy and niche placement, *F. grandis* includes fiddler crabs from the intertidal marsh, in its diet (Odum 1970; Rozas and LaSalle 1990; Springer and Woodburn 1960). Rozas and LaSalle (1990) also reported that
when *F. grandis* gained access to the marsh flood plain they were able to consume a greater volume of prey items.

Active feeding by *F. heteroclitus* occurred during high tides even if tidal height was insufficient enough to inundate the marsh flood plain (Weisberg et al. 1981). Weisberg et al. (1981) also reported similar findings as those of previous authors (Baer 1974; Fritz 1974; Kneib and Stiven 1978; Schmelz 1964); when the marsh flood plain was inundated the diet of *F. heteroclitus* was representative of the flood plain faunal composition. Feeding chronology for *F. heteroclitus* revealed that tidal patterns had a greater effect than temporal patterns on prey consumption, but a diurnal feeding pattern was observed (Weisberg et al. 1981). Baer (1974); Clymer (1978); and Weisberg et al. (1981) reported that nocturnal feeding was greatly reduced since *F. heteroclitus* was most likely a visual feeder. Other fundulids (*Fundulus majalis*, *Fundulus notatus*, and *Fundulus similis*) have been observed to increase feeding during the daylight hours (Atmar and Stewart 1972; Baer 1974; Bennett 1973). The varied diet of fundulids seems to be dependent on access to the high marsh flood plains. It suggests that they are opportunistic, taking advantage of the high tides and intertidal pools as important foraging grounds (Harrington and Harrington 1961).

Seasonal differences in the diet of these 3 fundulids (*F. heteroclitus*, *F. majalis*, and *Fundulus diaphanous*) showed that polychaetes were most utilized in the spring and fall (Baker-Dittus 1978). *F. majalis* continued to consume polychaetes throughout the summer, but *F. heteroclitus* consumed more plant material. Baer (1974) found that *F. heteroclitus* selects for a few seasonally
available prey items (fish eggs and brachyuran larvae) and supplements with other prey items year round (polychaetes, amphipods, detritus, and fishes). Baer (1974) also showed that, due to its body morphology, \textit{F. majalis} fed more efficiently on benthos than did \textit{F. heteroclitus}, the diet of which was more generalized. \textit{F. heteroclitus} has a blunter snout than \textit{F. majalis} and therefore consumes more detrital material along with benthic prey.

Prey size has been shown to shift ontogenetically among size classes within several species of \textit{Fundulus}. A study by Baker-Dittus (1978) revealed that prey size among different size classes of \textit{F. heteroclitus} and two other sympatric fundulids (\textit{F. majalis} and \textit{F. diaphanous}) remained relatively constant with only a minor increase in prey size in larger fish. The size of terrestrial insects (dipterans, hemipterans, and hymenopterans), amphipods, and gastropods increased progressively with the size of \textit{Fundulus jenkinsi}. While crab zoea and springtails were consumed in all size classes there was a shift to larger prey items in adult \textit{F. jenkinsi}.

Documentation also exists on the diets of \textit{Fundulus luciae}, \textit{Fundulus confluentus}, and \textit{F. jenkinsi}; which, like \textit{F. pulvereus}, forage primarily in tidal marsh habitats such as saw grass and salt marshes (Boschung and Mayden 2004; Byrne 1978; Harrington and Harrington 1972; Kneib 1978; Lopez 2009). Both \textit{F. luciae} and \textit{F. confluentus} utilize the upper marsh to forage on terrestrial insects (Akin and Winemiller 2008; Harrington and Harrington 1961; Kneib 1978). Harrington and Harrington (1961, 1972) found the stomachs of \textit{F. confluentus} primarily contained terrestrial insects and mosquito larvae, and small
crustaceans. Odum (1970) reported unpublished findings of Billy Drummond (1965-66), who found that chironomid larvae and bivalves were also prominent prey items. Both Harrington and Harrington (1961, 1972) and Odum (1970) reported that *F. confluentus* exhibited piscivory of larval fish. Odum (1970) had a small sample size, but results concurred with those of Drummond. Akin and Winemiller (2006) reported *F. confluentus* at a slightly lower trophic level (2.70) than *F. pulverous* (3.00), which implies that *F. confluentus* consumes more plant material than *F. pulverous*. Both diets were reflective of summer sampling months only. Neither species was caught during winter sampling events (Akin and Winemiller 2006).

Seasonal changes in diet have been observed in several species of fundulids. Byrne (1978) and Kneib (1978) reported that the diet of *F. luciae* varied seasonally in relation to prey availability and temperature in the isolated pools of the upper marsh. Insects and amphipods were the primary prey items of *F. luciae* in the winter and prey importance shifted in spring to phytoplankton. In summer and fall sand rich detritus was most prevalent (Kneib 1978). Furthermore, Kneib (1978) found that *F. luciae* foraged on detritus, terrestrial insects, diatoms, and aquatic arthropods (e.g., copepods and ostracods) year round except in colder months. This led Kneib (1978) to believe that temperature changes were responsible for the seasonal diet shift. A seasonal shift in diet was observed by Lopez (2009), who found that there was a seasonal difference in the diet of *F. jenkinsi*, where diet breadth was greatest in the spring with the narrowest being summer. In this case Lopez (2009) suggested a temporal patter
as the reason for seasonality of prey items. Winter on the Mississippi Gulf Coast has nocturnal high tides while the rest of the year high tides usually occur during the day. These tidal fluctuations allow for differential access to the tidal flood plains during certain times of the year, and therefore access to different prey items (Lopez 2009).

There has been much controversy over which prey indices depict the truest diet parameters of a species. Frequency of Occurrence is a quick and effective method for categorizing stomach contents and prey preferences (Chapman et al. 1989; Hyslop 1980). Percent Occurrence (%O) lends itself to a diet study on *F. pulvereus* due to its tendency to masticate food, and because a portion of its diet contains detritus, algae, and plant matter (Crisp et al. 1978). Hynes (1950) and Hellawell and Able (1971) both showed that gravimetric and numerical analyses have a significant correlation and that both give an accurate picture of common prey items. Liao et al. (2001) suggested that %O is a good measure of predator prey preference and how readily available those taxa are in the environment. Liao et al. (2001) also noted that component indices are most useful when dominant prey taxa are of a similar size, and may be a more accurate picture than the percent index of relative importance (IRI). Liao et al. (2001) found that, like IRI, %O was unbiased towards prey size. However %O tends to give higher importance values than other indices (Liao et al. 2001). Ostracods, amphipods, and insects were consumed frequently along with detritus, sand, and unidentified organic matter. Even though %N and %O have been criticized as being too narrow (Hallawell and Abel 1971), many researchers
believe that combining multiple indices will give an accurate overall picture of diet (Hellawell and Able 1971; Hynes 1950; Liao et al. 2001).

*Known Parasites of Fundulus pulvereus*

Using trophically transmitted parasites as bio-indicators of predator prey interactions is a common practice (Valtonen et al. 2010) and has been shown to provide valuable insight into the diet of a host (Williams et al. 1992). Because certain parasites have complex life cycles with specific hosts, they increase perception when building trophic linkages within a habitat. The presence of a parasite within a host organism reflects the prevalence of the other host species in that parasites life cycle. Predator prey relationships are usually required for the transmission of parasites from one host to another (Lafferty et al. 2008; Marcogliese and Cone 1997; Marcogliese 2003; Minchella and Scott 1991) and host trophic levels usually increase with parasite life stages. Studies that have incorporated parasite fauna information have been shown to be helpful in developing an overall view of the host species trophic interactions (Huxham et al. 1995; Valtonen et al. 2010). In some instances the composition of the parasite fauna alone may give a more accurate picture of trophic position than stomach contents; by combining knowledge on diet and parasite fauna there is an increased resolution in trophic linkages per fish species (Valtonen et al. 2010). The occurrence of a parasite within a specific habitat can confirm the presence and trophic position of the hosts involved in its life cycle. Presence in the host can also show long-term prey preference since a parasite may infect a host for periods of time much longer than the average digestion rate. Ontogenetic shifts
in diet may also be reflected by a species parasite fauna which will shift along with the host. This makes parasites biological indicators of individual species and the ecosystem as a whole (Marcogliese and Cone 1997; Marcogliese 2003).

Members of the genus *Fundulus* can and do play host to multiple species of parasites as both an intermediate host, or as the only host (Harris and Vogelbein 2006). Valtonen et al. (2010) found that omnivorous fish, like fundulids, displayed the highest diversity in diet and parasite fauna, and there are numerous species of parasites that occur in multiple species of *Fundulus*. Harris and Vogelbein (2006) have summarized the taxonomy, distribution, and literature dealing with the parasites infecting or infesting *F. pulvereus* and other species of *Fundulus*.

The known parasites of *F. pulvereus* are restricted to four species, the coccidian *Calyptospora funduli*, digenean metacercaria of *Sticodora cursitans* (Harris and Vogelbein 2006), the monorchid *Lasiotocus minuta* (Smedly 2000), and the copepod *Ergasilus funduli* (Harris and Vogelbein 2006).

*F. pulvereus* was found to be a natural definitive host for *C. funduli* (formerly *Eimeria funduli*) (Solangi and Overstreet 1980). The grass shrimp *Palamonetes pugio* is the intermediate host for *C. funduli*, and the definitive host can be one of several Atheriniform fishes, including fundulids. The sporozoites occur in the gut basal cells of *P. pugio*, and, once ingested by a fish host, they migrate to the liver via the bloodstream. Other sites of infection can be the pancreas in *F. pulvereus* and other common fundulids, or possibly the skin and intestines in *F. grandis* (Solangi and Overstreet 1980).
The encysted metacercaria of the digenean, *S. cursitans*, naturally occurs in the tissues of fundulids (e.g. *F. grandis*, *F. similis*) along the West Coast of Florida. There have been no reports of this parasite using *F. pulvereus* as a natural or second intermediate host. Under laboratory conditions, however, it can become infected with the metacercaria of *S. cursitans* after exposure to cercariae being shed by the first intermediate host, the gastropod *Cerithidea scalariformis* (Kinsella and Heard 1974). Natural infections were seen in the closely related *F. confluentus*, and two other fundulids (*F. similis* and *F. grandis*). The preceding intermediate host, *C. scalariformis*, is a snail and the definitive hosts range from rice rats (*Oryzomys palustris*), opossums (*Didelphis virginiana*), and raccoons (*Procyon lotor*) (Kinsella and Heard 1974). The bayou killifish, along with other fundulids (e.g. Gulf killifish, *F. grandis*), is the final or definitive vertebrate host for the monorchid digenean, *Lasiotocus minuta* (Smedly 2000). Killifish become infected when they ingest the small marsh clam, *Cyrenoida floridana*, which harbors both the asexual developmental stages and serves as the trophic second intermediate host for this small digenean parasite.

The copepod *E. funduli* was reported in 1863 by Krøyer in an unidentified species of *Fundulus*. Krøyer (1863) believed it to be *F. limbatus*; however, Wilson (1911) and Roberts (1970) considered the species to be *F. confluentus*. The sample site was located in New Orleans, Louisiana, and at the time *F. confluentus* was believed to be a synonym for *F. pulvereus*. Since the known range of *F. confluentus* is confined to the Florida Coast and only extends to Mobile Bay (Ghedotti and Davis 2013; Relyea 1965), this leads to the uncertain
conclusion that *E. funduli* could utilize *F. pulvereus* as a host. Roberts (1970) indicated that there is some contention on the actual species of *Ergasilus* involved. Certain specimens were labeled as *E. funduli* by Krøyer (1863) but for a time *E. manicatus* and *E. funduli* were considered synonyms (Johnson and Rogers 1973; Roberts 1970; Wilson 1911). Kabata (1986) reexamined the type specimens and agreed with the original taxonomic classification and determined that *E. funduli* and *E. manicatus* remain separate species. The copepod infects the gills of *Fundulus* species such as *F. heteroclitus*, possibly *F. confluentus*, *F. ocellaris*, *F. grandis*, and *F. similis* (Harris and Vogelbein 2006; Johnson and Rogers 1973; Roberts 1970; Santiago et al. 2007; Wilson 1911).

**Current taxonomic status and distribution of Fundulus pulvereus /confluentus complex**

In the past, scientists have debated whether or not *F. pulvereus* and *F. confluentus* were truly separate species. Their similar coloration and ability to hybridize with each other led to the conclusion that these two species might be conspecifics (Foster 1967; Hardy 1980 a, b; Relyea 1965). However the morphometrics and genetic analysis have shown that *F. pulvereus* and *F. confluentus* are closely related, but separate, sister species (Ghedotti and Davis 2013; Whitehead 2010; Wiley 1986). The range for *F. pulvereus sensu stricto* is from Corpus Christi to the approximately 100 km Mississippi-Alabama coastal region where overlap and hybridization with the closely related *F. confluentus sensu stricto* occurs. The range of *F. confluentus* extends eastward and
southeast along the Florida Gulf and then northward up the Florida East Coast to the lower York River in Virginia (Ghedotti and Davis 2013; Relyea 1965).

Reproduction and Spawning of Fundulus tidal marsh species

Most members of Fundulidae, including *F. pulvereus*, are iteroparous batch spawners (Byrne 1978; Greeley and MacGregor 1983; Greeley 1984; Greeley et al. 1986; Greeley et al. 1988; Harrington and Harrington 1972; Lang 2010; Lang et al. 2012; Lopez 2009). Fundulids also employ a multi-clutch spawning strategy and possess a single ovary (Taylor and Burr 1997). Cyprinodonts are capable of spawning daily over the course of several weeks during a reproductive season (Foster 1967; Greeley et al. 1988). The reproductive efforts of *F. pulvereus* are no exception. Like *F. grandis*, *F. heteroclitus*, and other fundulids *F. pulvereus* lay their eggs over a period of 13 to 15 days during spring tides. The bayou killifish utilizes the higher inundation levels of spring tides to lay its eggs in the mid to high marsh (Byrne 1978; Greeley and MacGregor 1983; Greeley 1984; Greeley et al. 1986; 1988; Harrington and Haeger 1958; Lang 2010; Lang et al. 2012; Taylor et al. 1979; Taylor 1991). Greeley (1984) suggested that both *F. pulvereus* and its relative *Adinia xenica* are semilunar batch spawners. Greeley (1984) also suggests that the yoking of tidal cycles and spawning may be a familial trait among the Cyprinodonts. The reproductive season of most fundulids begins in early spring, around March, and lasts until August or September (Byrne 1978; Greeley and MacGregor 1983; Greeley 1984; Greeley et al. 1986; Hardy 1978; Harrington 1959; Kneib 1978; Kneib and Stiven 1978; Lopez et al. 2010; Lang 2010).
Evidence suggests that midsummer reproductive rates slow slightly before a final increase in late summer (Harrington 1959; Kneib and Stiven 1978; Lang et al. 2012). The spawning season of *F. pulvereus*, a semilunar batch spawner, may closely resemble that of most fundulids including its counterparts *F. jenkinsi*, *F. heteroclitus*, *F. luciae*, and *F. similis* (Byrne 1978; Hardy 1978; Kneib 1978; Lang et al. 2012). Lang, (2010) found that for several days before and after spring tides *F. jenkinsi* continued to spawn but during neap tides no spawning occurred. Spawning for *F. grandis* and *F. similis* occurred on late flooding tides especially when coinciding with spring tides (Greeley and MacGregor 1983; Greeley et al. 1986) whereas *F. jenkinsi* also spawned during early descending tides (Lang 2010). With a few exceptions all three species spawned in correlation with the declination of the moon (Greeley and MacGregor 1983; Greeley et al. 1986; Lang 2010). While much research has been done on the spawning capabilities of other fundulids the reproductive season and fecundity of *F. pulvereus* has not been properly documented.

**Known biological information for Fundulus pulvereus**

Though quite abundant in many northern Gulf tidal marsh habitats, *F. pulvereus* has been poorly studied and very little or nothing has been published on the various aspects of its ecology, life history, and reproductive biology (Akin and Winemiller 2008; Boschung and Mayden 2004; Greeley 1984; Griffith 1974; Newman 1909; Relyea 1965; Simpson and Gunter 1956; Sullivan and Moncreiff 1990). The bayou killifish is an intertidal marsh resident that is restricted to the northern Gulf of Mexico tidal marsh systems and represents one of the smaller,
slimmer estuarine species of Fundulidae. The females are usually larger (Ruebsamen 1972) and are counter shaded olive to a steel grey dorsally, fading to a creamy gold on the ventral surface. Both sides of the trunk are dotted with black spots. Pale vertical stripes may be present (Simpson and Gunter 1956). Relyea (1965) recorded the maximum total length for *F. pulvereus* as 65 mm. Males of *F. pulvereus* have similar body coloration to the females but have 12-17 bluish black vertical stripes along either side and most lack spots. Male anal fin coloration has a yellowish hue as in most males of the family Fundulidae (Boschung and Mayden 2004; Newman 1909; Simpson and Gunter 1956). Like *F. jenkinsi* and *F. confluentus*, *F. pulvereus* is a euryhaline species found most often in low salinities. However, they can tolerate hypersalinites exceeding 70 and can adapt to freshwater environments indefinitely (Griffith 1974).

In general members of the cyprinodontiform family Fundulidae play a vital role in the trophic relay taking place within tidal marsh estuarine systems. During high tide, killifish utilize the inundated marsh plains to forage for food. As the tide subsides, they retreat to intertidal creeks and fringing estuarine waters. During this retreat, energy from the high marsh is transferred to low marsh areas through excretion of waste, or as these small fish become prey to larger resident and transient tidal marsh predators (Butner and Brattstrom 1960; Kneib 1997; Lefeuvre et al. 1999; Odum and Heald 1975; Rountree and Able 2007; Rozas and LaSalle 1990; Talbot and Able 1984; Valiela et al. 1977; Weisberg et al. 1981). Fundulids are an important prey source for higher trophic level fishes both commercial and recreational, as well as piscivorous birds (e.g., herons,
ducks, and ibises) and mammals (e.g., raccoons, minks, and rice rats) (Kneib and Stiven 1978; Valiela et al. 1977). Completing the loop, many species of fundulids also facilitate the input of allochthonous organic matter into the marsh system through feeding on fallen plant matter and resident insect fauna (Valiela et al. 1977).
CHAPTER II

METHODS

Sampling Methods

Sampling was conducted in three different Mississippi tidal marshes: Simmons Bayou (SB) [30° 22'23.17"N 88° 45'11.16"W], Weeks Bayou (WB) [30° 24'7.07"N 88° 48'51.75"W], and Moss Point (MP) on the Pascagoula River [30° 26'35.67"N 88° 33'0.36"W] (Figure 1). Both SB and WB are mesohaline marshes located in Ocean Springs, MS, and are composed mostly of *Spartina alterniflora* edge. Patchy islands of *Juncus roemerianus* can be found in small areas of higher elevation within the marsh and adjacent to the shoreline. Both are fringed on three sides by high marsh flora (wax myrtle and pine). Through a sub tidal channel, WB has direct access to the Mississippi Sound, while SB is located slightly farther inland within the Mississippi marsh system. Access to the sound from SB requires passage through Davis Bayou. MP is a restored saw grass marsh on the Pascagoula River located farther to the North than WB or SB. It was restored after Hurricane Katrina in 2005 and has reverted quickly to its pre-Katrina state (Figure 1). Here the water is mostly fresh with a salinity of less than one and the flora consists primarily of saw grasses surrounding intertidal pools and channels. This marsh is also fringed on three sides by local high marsh fauna (cypress trees and pine) and opens into a larger bayou with access to the Pascagoula River and its in-fluxing salt wedge.
Sampling was conducted monthly for an entire year during high spring tides. Spring tides were defined as the six highest tidal ranges close to the full and new moons. Tidal predictions were taken from NOAA’s tide table predictions for each specified area, Hollingsworth Point, Davis Bayou, and Pascagoula respectively. Target species for collection was: *Fundulus pulvereus*. Approximately 10 to 20 fish were collected from each site per month. Fish were sampled using both Breder traps and baited and unbaited standard minnow traps. Bait, when present, was enclosed in a permeable cloth bag. Standard minnow traps are vinyl coated and have 2.54 cm openings with 6.54 mm mesh. Breder traps are Plexiglass boxes (30X15X15cm) with one open end that has two pieces of Plexiglass (30X15cm) that fan out to guide fish into the trap (Fulling et al. 1999). Traps were set near marsh perimeters and checked every hour until
a representative sample was obtained or until the tide receded to a level where fish were unable to enter traps. Rivulets and marsh pools were utilized in placing traps since fundulids utilize these when moving onto and off of the marsh flood plain (Rozas et al. 1988). Water quality data: salinity and temperature (°C) were recorded.

Upon collection fish were immediately taken via a cervical cut using dissection scissors and placed on ice. This eliminated the possibility of further digestion or regurgitation of stomach contents (Windell 1968) and reduced stress. Samples were transferred to 10% formalin within one to four hours. In the lab wet weight (WW) to the nearest 0.001 mg, Standard Length (SL, mm), Total Length (TL, mm), and Mouth Gape (MG, mm) to the nearest 0.01 mm were recorded for each fish. The entire digestive tract and gonads were removed (Windell 1968).

Two 24-hour sampling days were chosen, once in the early spring when high tides still occur at night and once in the late summer when high tides occur during the day. Sampling took place in SB. Traps were set and checked every four hours, and up to 12 fish were collected each time period.

Diet

Once the digestive tract was removed, stomach fullness (i.e. food presence or absence) was visually assessed and recorded. A diagram was marked to show where food was located within the digestive tract. Because fundulids do not have discrete stomachs (Babkin and Bowie 1928; Barrington 1957), the length of the digestive tract from the esophagus to the first bend (the
fore-gut) was examined. All prey taxa were counted and keyed out to the lowest possible taxonomic level (Heard 1982, McCafferty 1983, Overstreet 1978). Dietary data was partitioned into three seasons: spring (March-May), summer (June-September), and winter (October-February) according to sampling dates. While Windell (1968) suggests discerning dominant prey taxa using both volumetric and numerical analysis, the idea of using two indices was also suggested by Liao et al. (2001), who states that using a combination of %IRI with another index is more powerful than using just a numerical index by itself. Percent IRI would be difficult with cyprinodonts since they macerate their food (Hallawell and Able 1971); and volumetric analyses are very time consuming and highly impractical for small prey items with little volume (Hallawell and Abel 1971). Volumetric analyses would also be problematic since macerated food items are difficult to separate. Volumetric analysis (including squash method) does not account for parasites within the stomachs; these are not prey items but represent a very relevant set of information. The squash method also uses subjective measurements for smaller stomachs/fish, even though the error is found to be small (Hallawell and Abel 1971), and makes comparisons between results difficult since user error is greatly increased. Quicker less accurate processing of larger samples has been shown to yield higher accuracy in results and a truer picture of diet than highly analyzed small samples (Hallawell and Abel 1971; Hynes 1950). This study aimed to show an overall view of the diet of *F. pulvereus* by increasing accuracy, keeping processing time to a minimum, and employing a variety of analyses. Two prey indices and a multivariate analysis
were used to discern dominant prey taxa: percent occurrence (%O), percent number (%N), and permutation analysis (ANOSIM).

%N was calculated as a percentage of the total number of prey taxa in all prey categories (Crisp et al. 1978; Ikusemiju & Olaniyan 1977). %O was obtained using frequency of occurrence (Fo), and expressed as the average number of occurrences per prey item (Frost 1946; 1954; Hunt and Carbine 1951). %O and %N were analyzed by season, size class, and sampling site to ascertain if any seasonal or ontogenetic shifts in food availability or preference occurred (Harrington and Harrington 1972). Prey taxa with > 5 %O or %N were considered common. A two – way crossed Analysis of Similarity (ANOSIM) was run with replicates using Primer to assess dominant prey taxa and those that contribute to the major differences in diet between seasons (n = 3), sites (n = 3), year (n=2), day vs. night, and sex (n=2). This analysis did not include detritus, plant material, or unidentified items since this data was only collected in presence or absence form.

Parasites
Parasites found in the body cavity and foregut of all fish examined during the course of the study were recorded and keyed out to the lowest possible taxonomic level. In addition, ten adult *F. pulvereus* were collected in the spring using the aforementioned sampling method. Upon return they were examined for infection. Observations were made on the external anatomy, the heart, the gills, the digestive tract, and the body cavity. All endoparasites and ectoparasites found were removed, counted, and identified to the lowest practical taxonomic
level. Standard length, sex, and wet weight were recorded for all fish examined. Parasite data are expressed in terms of prevalence (% infected = # of fish infected/ # of fish total), and mean intensity (# of a particular parasite species/ # of fish infected) (Bush et al. 1997). Confidence intervals of 95% are also reported.

Reproduction

Wet weight to the nearest 0.001 g, gonad weight to the nearest 0.001 g, standard length (SL, mm), and total length (TL, mm) were recorded for all *F. pulvereus* specimens. A Shapiro – Wilk test for normality was performed on all data sets: SL, TL, wet weight, gonad weight, and gonad free body weight (GFBW) to see if they were normally distributed. If data was not normally distributed a suitable transformation was performed on each data set. SL, TL, wet weight, gonad weight, and gonad free body weight (GFBW) were log$_{10}$ transformed and denoted with an asterisk * (i.e. an analysis using SL* used data that has been log$_{10}$ transformed). Ovaries were examined visually and reproductive status was determined using modified Taylor and Burr 1997 descriptions on the oocyte phases of *Fundulus dispar* (northern starhead topminnow). Ovary stage was assigned using a modified version of Brown-Peterson terminology (Brown-Peterson et al. 2011) (Table 1).
Table 1


<table>
<thead>
<tr>
<th>OVARY STAGE</th>
<th>BROWN PETERSON ET AL. (2011)</th>
<th>OVARY DESCRIPTION (Lopez 2009)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature</td>
<td>Immature</td>
<td>White, small immature oocytes</td>
</tr>
<tr>
<td>Developing</td>
<td>Early/Late Developing</td>
<td>White/yellow, few mature oocytes</td>
</tr>
<tr>
<td>Fully Developed</td>
<td>Capable/ Actively Spawning</td>
<td>Yellow, large oocytes, mostly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mature</td>
</tr>
</tbody>
</table>

A variation on volumetric analysis was performed on three representative fish to determine size frequency of eggs. As stated previously all fish were preserved in 10% formalin. Research has found that preservation in formalin can cause changes to the weight and size of ovaries (Joseph 1963, Ramon and Bartoo 1997). To reduce this effect preservation methods remained the same throughout the study and fish remained preserved for several months before dissection so that weight could stabilize (Ramon and Bartoo 1997). Eggs from each ovary were teased apart and placed in 50ml of water and stirred using a magnetic stirrer and stirring plate. The planar motion allowed for a heterogeneous mixture (Jons and Miranda 1997). One ml of solution was removed by means of a micropipette. All eggs within the one ml sample were counted and their diameter measured (mm). This process was repeated two more times for a total of three aliquots. A size frequency distribution was plotted to show the mean egg size for *F. pulvereus*.

Batch fecundity (BF) is defined as the total number of eggs released during a single spawning event (Mura et al. 2003). To determine batch fecundity,
the ovaries were teased apart and the mature (largest) oocytes were counted in all fully developed females. Batch fecundity was found to be not normally distributed thus a $\log_{10}$ transformation was performed (denoted BF*). A linear regression analysis was run on fish with fully developed ovaries to determine if a correlation was present between SL* as the dependent variable and the number of oocytes (BF*) as the independent variable (Kneib and Stiven 1978).

Relative fecundity was defined as the number of eggs per gram of fish (De Vlaming 1983). Relative fecundity was found to be not normally distributed so a $\log_{10}$ transformation was run (denoted RF*). Both batch and relative fecundity are reported in the form of mean ± standard error. The mean is the raw data mean. Relative fecundity is a pertinent measure of reproduction since it allows for the cross species comparison of spawning capability (De Vlaming 1983).

GSI has been shown to be a rudimentary estimate of spawning season (Jons and Miranda 1997, Lang 2010). Jons and Miranda 1997 suggested that ovarian weight is both affected by the number and size of the oocytes. They also found that ovarian weight, when standardized for fish weight, (i.e. GSI) roughly corresponds to spawning seasons when examined for longer periods of time. GSI was estimated, to determine spawning preparedness, utilizing the equation from Greeley et al. (1986), a modified version of Pickford’s 1953 equation.

$$GSI = \left( \frac{\text{Gonad Weight}}{\text{Gonad Free Body Weight}} \right) \times 100$$

Prior to analysis, Levene’s statistic was used to test for homogeneity of variance and the Kolmogorov-Smirnov test was used to test for normality. If data were found to be not normally distributed a revitalization was done and an arcsine
square root transformation was calculated (denoted GSI*) (Lang 2010, Clardy 2012). GSI* values were compared to GFBW* using a linear regression to ascertain if a relationship exists between GSI* and GFBW*. A Kruskal-Wallis H test was conducted on both male and female and GSI separately to see if there was a significant difference in GSI per month.
CHAPTER III

RESULTS

A total of 445 fish were captured 230 females and 215 males; 133 from WB, 241 from SB, and 71 from MP. Fish were captured on high spring tides monthly from March 2012 through August 2013 with the exception of November 2012, and July 2013 when weather prevented sampling or no fish were captured during attempts. Males ranged from 18-51 (mm) SL with a mean of 34.6 (mm) ± 0.5 and 23-63 (mm) TL with a mean of 43.0 (mm) ± 0.6. Weight ranged from 0.1-3.4 (g) with a mean of 1.1 (g) ± 0.0. Mouth gape ranged from 1.8-5.9 (mm) with a mean of 3.8 (mm) ± 0.1. Females ranged from 17-60 (mm) SL with a mean of 35.1 (mm) ± 0.6 and 22-76 (mm) TL with a mean of 43.5 (mm) ± 0.7. Weight ranged from 0.1 -7.5 (g) with a mean of 1.3 (g) ± 0.1, and mouth gape ranged from 0.3-6.0 (mm) with a mean of 3.8 (mm) ± 0.1. Seasonal patterns of temperature and salinity remained relatively consistent at each site (Table 2) with WB having the greatest fluctuations in Temperature (Figure 2).

Table 2

Average temperature (°C) and salinity from three different Mississippi tidal marshes: Simmons Bayou (SB), Weeks Bayou (WB), and Moss Point (MP)

<table>
<thead>
<tr>
<th>Site</th>
<th>Temperature (°C)</th>
<th>Salinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB</td>
<td>22</td>
<td>4.9</td>
</tr>
<tr>
<td>WB</td>
<td>20.1</td>
<td>2.1</td>
</tr>
<tr>
<td>MP</td>
<td>18.3</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 2. Average temperature by month at Simmons Bayou, Weeks Bayou, and Moss Point, three different Mississippi tidal marsh systems, over the course of this study.

The salinity was also consistently the highest at this site. This was most likely due to closer proximity of WB to the Mississippi Sound. Salinity at MP was consistently zero since it was located further inland on the Pascagoula River (Figure 3).
Figure 3. Average salinity at Simmons Bayou, Weeks Bayou, and Moss Point, three different Mississippi tidal marsh systems, over the course of this study.

Diet

There were 46 different prey taxa, not including fragments, detritus, plant matter, or unidentifiable organic matter (Table 3). MP had the lowest prey richness with only 16 different prey taxa. SB and WB had greater species richness than MP with 35 and 31 different prey taxa, respectively. Observations of all 445 fish studied revealed only 14.8% of fish had empty stomachs (n=445). A species area curve was plotted to show that the diet was well represented by the sample size (Figure 4). Only 7.6% of sampling took place at low tide (n= 34) but 29.4% of fish sampled at low tide had empty stomachs. Only 13.6% of fish sampled at high tide had empty stomachs (n=411). Overall more fish were sampled at high tide, regardless of time of day or night, and 86.4% of those fish had prey taxa in their stomach.
Figure 4. Number of different prey items found within the stomachs of *Fundulus pulvereus* includes miscellaneous prey items such as detritus, plant matter, and arthropod fragments.

Table 3

List of Prey Items found in the Stomachs of Fundulus pulvereus (n = 445)

- Detritus
- Organic matter
- Inorganic matter
- Kingdom Chromalvaeolata
  - Phylum Heterokontophyta
    - Class Bacillariophyceae - Diatoms
- Kingdom Plantae
  - Chlorophyta
    - Green algae
  - Seeds from unidentified vascular plant
  - Plant matter
Table 3 (continued).

- Phytoplankton
- Phylum Annelida
  Subphylum Polychaeta
    Family Nereidae
    Polychaete setae
  Subphylum Clitellata
    Class Oligochaeta
      Terrestrial oligochaete
      Aquatic oligochaete
- Phylum Mollusca
  Class Gastropoda
    Terrestrial snail
    Order Neotaenioglossa
      Family Hydrobiidae
    Order Neritopsina
      Family Neritidae
        *Neritina usnea* (Roding 1798), olive nerite
- Phylum Arthropoda
  Arthropod fragments
  Subphylum Chelicerata
    Class Arachnida
      Order Araneae - Spiders (2 spp.)
      Subclass Acari - Mites
  Subphylum Crustacea
    Unidentified crustacean fragments
    Class Ostracoda
    Class Maxillipoda
      Subclass Copepoda
    Class Malacostraca
      Order Tanaidacea
### Table 3 (continued).

<table>
<thead>
<tr>
<th>Order Amphipoda</th>
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</thead>
<tbody>
<tr>
<td>Unidentified amphipod</td>
</tr>
<tr>
<td><strong>Suborder Gammaridae</strong></td>
</tr>
<tr>
<td><em>Gammarus mucronatus</em> Say 1818</td>
</tr>
<tr>
<td><em>Melita</em> sp.</td>
</tr>
<tr>
<td><em>Orchestia</em> sp.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Order Isopoda</th>
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</thead>
<tbody>
<tr>
<td>Terrestrial isopod</td>
</tr>
<tr>
<td>Aquatic isopod</td>
</tr>
<tr>
<td><strong>Suborder Asellota</strong></td>
</tr>
<tr>
<td><em>Caecidotea</em> sp.</td>
</tr>
<tr>
<td><strong>Suborder Oniscidea</strong></td>
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</table>

<table>
<thead>
<tr>
<th>Order Decapoda</th>
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</thead>
<tbody>
<tr>
<td>Crab megalope</td>
</tr>
<tr>
<td><strong>Family Ocypodida</strong></td>
</tr>
<tr>
<td><em>Uca</em> sp.</td>
</tr>
<tr>
<td><strong>Family Portunidae</strong></td>
</tr>
<tr>
<td><em>Callinectes</em> sp.</td>
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<table>
<thead>
<tr>
<th>Subphylum Hexapoda</th>
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<tbody>
<tr>
<td><strong>Class Collembola</strong></td>
</tr>
<tr>
<td>Springtail - juveniles</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Class Insecta</th>
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<tbody>
<tr>
<td>Unidentified Insects – eggs, larvae, pupae, and adult</td>
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<tr>
<td><strong>Order Odonata</strong> – odonate larvae</td>
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<tr>
<td><strong>Order Coleoptera</strong></td>
</tr>
<tr>
<td><strong>Order Diptera</strong></td>
</tr>
<tr>
<td>Family Ceratopogonidae – larvae, pupae</td>
</tr>
<tr>
<td>Family Chironomidae – larvae, pupae, and midges</td>
</tr>
<tr>
<td>Family Tabanidae – larvae</td>
</tr>
</tbody>
</table>
Table 3 (continued).

<table>
<thead>
<tr>
<th>Taxa</th>
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<tbody>
<tr>
<td>Dipteran – larvae (2 spp.), adult</td>
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<tr>
<td>Unidentified fly - adult</td>
</tr>
<tr>
<td>Order Hymenoptera</td>
</tr>
<tr>
<td>Family Mymaridae - Fairyfly adults</td>
</tr>
<tr>
<td>Family Formicidae - ants</td>
</tr>
<tr>
<td>Order Hemiptera</td>
</tr>
<tr>
<td>- Phylum Chordata</td>
</tr>
<tr>
<td>Larval fish</td>
</tr>
<tr>
<td>Fish eggs (possibly Fundulus)</td>
</tr>
<tr>
<td>DIET KNOWN FROM PARASITES</td>
</tr>
<tr>
<td>- Phylum Mollusca</td>
</tr>
<tr>
<td>Class Bivalvia</td>
</tr>
<tr>
<td>Order Veneroida</td>
</tr>
<tr>
<td>Family Cyrenoidida</td>
</tr>
<tr>
<td>Cyrenoida floridana, Florida marsh clam</td>
</tr>
<tr>
<td>- Phylum Arthropoda</td>
</tr>
<tr>
<td>Class Decapoda</td>
</tr>
<tr>
<td>Order Caridea</td>
</tr>
<tr>
<td>Family Palaemonida</td>
</tr>
<tr>
<td>Palaemonetes pugio, grass shrimp</td>
</tr>
</tbody>
</table>

Percent Occurrence and Percent Number of Prey Taxa in Diet of F. pulvereus.

Frequently consumed prey taxa found in the stomachs of F. pulvereus were copepods, chironomid larvae and midges, mites (subclass Acari), detritus and organic matter, terrestrial insects, ceratopogonid larvae, and ostracods (Figure 5). The two highest %O in F. pulvereus stomachs were copepods (32.36%) and chironomid larvae (21.1%). The highest %N was also copepods
with (14.8%) but when mites were consumed they were consumed in larger numbers than chironomid larvae with a %N of 12.9% (mites) and 11.2% (chironomid larvae) (Figure 6).

Figure 5. Percent occurrence (%O) of prey taxa in *Fundulus pulvereus* from all sites, seasons, and size classes grouped together for an overall examination of diet.
Certain trends were found when %O and %N were analyzed by site, season, and size class. Copepods, chironomid larvae, and mites were consumed in the highest abundance in almost every category, including seasons. Fish from the MP site had consumed more copepods (42.3%O) and chironomid larvae (40.9%O) than those from SB or WB bayou. Differences in sites, seasonality of available prey taxa, and ontogenetic shifts, were also observed. WB was the most dissimilar. Hydrobiid snails, spiders, Orchestia sp. amphipods, crab megalope (decapodid stage), Uca fragments, and a wide variety of terrestrial insects (tabanid larvae, and unidentified insect adults) were only consumed in high numbers in WB (Figure 7). Springtail juveniles (subclass Collembola), Leptochelia rapax, and Gammarus mucronatus amphipods were only consumed in high numbers in SB. One F. pulvereus from SB was found to
have consumed insect eggs in high enough quantities to result in 11.7 %N of the diet in SB (Figure 8).

Figure 7. Differences in prey consumption and availability at each study site, Moss Point (MP), Simmons Bayou (SB) and Weeks Bayou (WB), in *F. pulverus* using percent occurrence (%O).
Seasonal differences showed that spring and summer were the most dissimilar (Figure 9). Insect larvae (tabanids and other dipterans) had the highest %O and %N in the spring along with *G. mucronatus*. Insect adults had the highest %N in the spring as well (Figure 10). In summer consumption of *L. rapax* increased along with springtail juveniles, ants, crab megalope, and *Uca* sp. In winter consumption of copepods and chironomid larvae increased dramatically with %O of chironomids more than doubling from around 17.0% in spring and summer to 45.2% in the winter. Copepod %O increased seasonally with spring being the lowest (28.3%) and winter being the highest (38.7%) (Figure 9).
Figure 9. Seasonality of prey taxa consumed by *F. pulvereus* using percent occurrence (%O).
Size class data revealed several ontogenetic trends in prey consumption. Copepods and chironomid larvae were consumed in high numbers by all size classes (Figure 12) with the smallest size class (<20mm) having the highest %O of both of these prey items (87.5% for copepods and 50.0% for chironomids) (Figure 11). Detritus also had a high %O in all size classes with %O almost doubling in fish longer than 50 mm. Ostracods, ceratopogonid larvae, and mites were consumed by all but the smallest size class (<20mm) and fish >50mm had the highest %O of these prey taxa. All but the largest *F. pulvereus* (>50mm) had a high %O of *G. mucronatus* in their diet. Larger fish (>50mm) were the only consumers of larval fishes.

*Figure 10.* Seasonality of prey taxa consumed by *F. pulvereus* using percent number (%N).
Figure 11. Ontogenetic differences in prey item consumption in *F. pulvereus* using percent occurrence (%O).
Figure 12. Ontogenetic differences in prey item consumption in *F. pulvereus* using percent number (%N).

A Two-way crossed ANOSIM showed that prey assemblages in *F. pulvereus* varied among the three study sites (ANOSIM Global R = 0.153, p = 0.001), with prey assemblages from SB being different from both WB (r = 0.121, p = 0.001) and MP (r = 0.232, p = 0.012). Likewise, assemblages varied between WB and MP (r = 0.330, p = 0.018). Prey assemblages also varied between genders (ANOSIM Global R = 0.165, p = 0.001) as well as among size classes (ANOSIM Global R = 0.057, p = 0.037). However, p values in pairwise comparisons among size classes were not significant; and one comparison (fish >50mm vs. fish 10-19mm) failed to run because the sample size was too small. Differences in prey assemblages among seasons, however, were significant.
(ANOSIM Global R = 0.113, p = 0.014). Spring assemblages were significantly different from winter assemblages (r = 0.576, p = 0.001) but not from summer (r= 0.057, p= 0.146). Summer was also not significantly different from winter (r= 0.913, p= 0.063). There was no significant variation in prey assemblages between years (ANOSIM Global R= 0.085, p=0.067) or between collection time of day (day versus night) (ANOSIM Global R= 0.007, p=0.439).

Parasite Prevalence and Intensity

The ten specimens of *F. pulvereus* collected from Simmons Bayou specifically for parasitological examination yielded eight different species of metazoan parasites (Table 4). *Phagicola diminuta* and *Echinochasmus* sp. were found in the gills of almost all fish studied (prevalence =80%) (Figure 13) with a mean intensity of 216.25 ± 85.75 *P. diminuta* and 16.25 ± 8.39 *Echinochasmus* sp. (Figure 14). The monorchid *Lasiotocus minuta* was found throughout the digestive tract of seven of the 10 fish studied (prevalence 70%) (Figure 13) with a mean intensity of 19.14 ± 6.25 parasites per host (Figure 14).
Figure 13. Prevalence of parasite species in *Fundulus pulvereus* from a tidal marsh in Simmons Bayou Ocean Springs, Mississippi (n=10). Prevalence expressed as the percentage of hosts infected by species.
Figure 14. Mean intensity of parasite species in *Fundulus pulvereus* from a tidal marsh in Simmons Bayou Ocean Springs, Mississippi (n=10). Mean intensity expressed as the as the average number of a parasite species per infected hosts (Bush et al. 1997).

Another trematode, *Homalometron* sp. A, was found in 40% of fish digestive tracts, mostly in the lower intestine, with a mean intensity of 1.50 ± 0.27 (Figure 14) parasites per infected host. The metacercaria of *Proctocaecum coronarium*, an alligator fluke, was found in the fin rays of 60% (Figure 13) of fish with a mean intensity of 7.66 ± 1.96 parasites per infected host (Figure 14).

Three parasites: *Neoechinorhynchus* sp., *Ergasilus funduli*, and a monogenean, *Gyrodactylus* sp. were found in 20% of fish studied (Figure 13).
Figure 15. Parasite prevalence found in and attached to the stomach of *Fundulus pulvereus* in three Mississippi tidal marshes (n=445). Prevalence expresses as % of hosts infected by species.
The parasite assemblage in all 445 fish in this study was typically comprised of digenean trematodes, with at least nine different species of parasites infected in and attached to the stomachs of *Fundulus pulvereus* (Table 4). Forty percent of all specimens examined contained parasites in and attached to the stomach (n=445). The most common parasite found was the monorchid digenean, *L. minuta* (prevalence = 27.42% (Figure 15); mean intensity = 6.27 ± 0.33) (Figure 16). Cestodes, nematodes, and two species of the digenean *Homalometron spp.* were also common. One species was identified as *Homalometron palmeri*. The other species, *Homalometron sp.* A remains unidentified and may be undescribed.
Table 4

Parasites occurring on or in Fundulus pulvereus from Mississippi tidal marshes

<table>
<thead>
<tr>
<th>PARASITE GROUP &amp; TAXON</th>
<th>LIFE CYCLE STAGE</th>
<th>SITE OF INFECTION</th>
<th>REFERENCE</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>COCIDAIA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Calyptospora funduli</em> Duszynski, Solangi, and Overstreet, 1979</td>
<td>Adult</td>
<td>Liver, viscera</td>
<td>Solangi and Overstreet 1980</td>
</tr>
<tr>
<td><strong>DIGENEA (Digenetic trematodes)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family Heterophyidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Phagicola diminuta</em> Stunkard and Haviland, 1924</td>
<td>Metacercaria</td>
<td>Gill filaments</td>
<td>Present study</td>
</tr>
<tr>
<td>Family Cryptogonimidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Proctoecaecum coronarium (Cobbold, 1861)</em></td>
<td>Metacercaria</td>
<td>Fins, under scales</td>
<td>Present study</td>
</tr>
<tr>
<td>Family Apocreadidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Homalometron palmeri</em> (Manter, 1931)</td>
<td>Adult</td>
<td>Intestine</td>
<td>Present study</td>
</tr>
<tr>
<td><em>Homalometron sp.</em></td>
<td>Adult</td>
<td>Intestine</td>
<td>Present study</td>
</tr>
<tr>
<td>Family Echinostomatidae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Echinochasmus sp.</em></td>
<td>Metacercaria</td>
<td>Gill filaments</td>
<td>Present study</td>
</tr>
<tr>
<td>Family Monorchidae</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4 (continued).

<table>
<thead>
<tr>
<th>CESTODA (Tape worms)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Family Gryporhynchidae</strong></td>
</tr>
<tr>
<td><em>Glossocercus</em> sp.</td>
</tr>
<tr>
<td><strong>MONOGERNTIC TREMATODE</strong></td>
</tr>
<tr>
<td><em>Gyrodactylus</em> sp.*</td>
</tr>
<tr>
<td><strong>ACANTHOCEPHALA (Thorny headed worms)</strong></td>
</tr>
<tr>
<td><em>Neoechinorhynchus</em> sp.*</td>
</tr>
<tr>
<td>Unidentified</td>
</tr>
<tr>
<td><strong>NEMATODA (Round worms)</strong></td>
</tr>
<tr>
<td><strong>Family Dioctophymatidae</strong></td>
</tr>
<tr>
<td><em>Eustrongylides</em> sp.*</td>
</tr>
<tr>
<td><strong>Family Raphidascarididae</strong></td>
</tr>
<tr>
<td><em>Hysterothylacium reliquens</em> (Norris and Overstreet, 1975)</td>
</tr>
<tr>
<td><strong>CRUSTACEA</strong></td>
</tr>
<tr>
<td>Copepoda</td>
</tr>
<tr>
<td><strong>Family Ergasilidae</strong></td>
</tr>
<tr>
<td><em>Ergasilus funduli</em> (Krøyer, 1863)</td>
</tr>
</tbody>
</table>
Reproduction

There were 156 fully developed females, 23 developing/redeveloping (Lang 2010) females, 46 immature females, two unknowns collected over the course of this study. Ovaries of female *F. pulvereus* collected ranged in weight from 0.0 – 0.6 (g) (n = 221) with a mean of 0.07 ± 0.0 (g). Male testes ranged from 0.0 - 0.036 (g) (n = 191) with a mean of 0.007 ± 0.0 (g). The number of eggs per female ranged from 15-157 with a mean of 60.7 eggs per fish.

The mean egg diameter was calculated for three fully developed representative females. Mean oocyte diameter (n=3) was 2.3 mm ± 1.7 (untransformed mean), with a minimum of 0.2 mm and maximum of 7.1 mm.

Mean batch fecundity was 58.8 ± 2.7 (n=156) with a standard deviation of 33.1 (Figure 17) for all fully developed females. The range was 150.0 oocytes with a minimum of 7.0 and a maximum of 157.0. The mean for RF was slightly smaller at 52.6 ± 2.8 oocyte/g (n=156) with a standard deviation of 35.5 (Figure 18). The range was 278.8 oocytes/g with a minimum of 3.4 oocytes/g and a maximum of 282.2 oocytes/g. BF was highest in the spring and summer beginning in April and dropping off around August. Fish caught in July had the highest BF with an average of 106.7 ± 15.6 ripe oocytes per FD female. Fish caught in September had the lowest BF with an average of 25.8 ± 5.7 (Table 5).
Figure 17. Average batch fecundity (untransformed) per month for fully developed female *F. pulvereus*.

Figure 18. Average relative fecundity (untransformed) by month for fully developed female *Fundulus pulvereus*.
Table 5

*Batch fecundity descriptives for fully developed female Fundulus pulvereus by month*

<table>
<thead>
<tr>
<th>Month</th>
<th>N</th>
<th>Min</th>
<th>Max</th>
<th>Mean</th>
<th>Std. error</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>5</td>
<td>21.0</td>
<td>64.0</td>
<td>42.6</td>
<td>7.1</td>
</tr>
<tr>
<td>February</td>
<td>1</td>
<td>41.0</td>
<td>41.0</td>
<td>41.0</td>
<td>0.0</td>
</tr>
<tr>
<td>March</td>
<td>17</td>
<td>11.0</td>
<td>93.0</td>
<td>38.7</td>
<td>4.7</td>
</tr>
<tr>
<td>April</td>
<td>50</td>
<td>16.0</td>
<td>154.0</td>
<td>58.9</td>
<td>4.7</td>
</tr>
<tr>
<td>May</td>
<td>30</td>
<td>23.0</td>
<td>134.0</td>
<td>74.1</td>
<td>6.1</td>
</tr>
<tr>
<td>June</td>
<td>18</td>
<td>31.0</td>
<td>113.0</td>
<td>74.4</td>
<td>6.0</td>
</tr>
<tr>
<td>July</td>
<td>3</td>
<td>81.0</td>
<td>136.0</td>
<td>106.7</td>
<td>16.0</td>
</tr>
<tr>
<td>August</td>
<td>20</td>
<td>11.0</td>
<td>157.0</td>
<td>54.5</td>
<td>7.4</td>
</tr>
<tr>
<td>September</td>
<td>10</td>
<td>7.0</td>
<td>65.0</td>
<td>25.8</td>
<td>5.7</td>
</tr>
</tbody>
</table>

Both BF and RF were not normally distributed and were log_{10} transformed for all further analysis (Denoted BF* and RF* in turn). BF* for all fully-developed females was correlated with Log_{10} transformed SL (R^2=0.311, p<0.001) (Figure 19) and GFBW* or ovary weight (R^2=0.311, p=<0.001) (Figure 20). RF* for all fully developed females was also correlated with Log_{10} transformed SL (R^2=0.283, p <0.001) and GFBW* (R^2=0.304, p <0.001).
Figure 19. Linear Regression analysis of log_{10} transformed batch fecundity and log_{10} transformed standard length (mm) from fully developed female *F. pulvereus.*
Figure 20. Linear Regression analysis of log_{10} transformed batch fecundity and log_{10} transformed gonad free body weight (g) from fully developed female *F. pulvereus*.

Average GSI for female and male *F. pulvereus*, using untransformed data was 6.2 ± 0.5 (n= 220) and 0.9 ± 0.05 (n= 191) respectively. GSI values, for both males and females, were not normally distributed, therefore an arcsine square root transformation was performed (denoted as GSI*) and used for all further calculations unless otherwise stated.
A linear regression analysis indicated that female *F. pulvereus* GSI* values were not correlated with GFBW* ($R^2 = 0.0$, $p = 0.9$) ($n=221$), (Figure 21). Male *F. pulvereus* GSI* values were correlated with GFBW* ($R^2 = 0.325$, $p < 0.0001$) (Figure 22). The general trend seen is an inverse relationship between GSI* and GFBW*. As GSI* increases the GFBW* decreases; and with an $R^2$ value of 0.325, 32.5% of the variance in GSI* can be explained by GFBW*.
Figure 22. Linear regression analysis of male *F. pulvereus* shows that GFBW* and GSI* are correlated.
Figure 23. Average GSI per month for both male and female *F. pulvereus*.

A Kruskal-Wallis H test was conducted on GSI* for each sex showing that there was a significant difference in both female and male GSI* per month, Female: $\chi^2 (9) = 129.807, p < 0.0001$; Male: $\chi^2 (9) = 94.381, P < 0.0001$. Mean female GSI* began rising in February, peaked in March and steadily began decreasing until September when it drops down below two for the winter (Figure 23). Following the pattern of *F. jenkinsi* and other fundulids (Harrington 1959; Kneib and Stiven 1978; Lang 2010) there is evidence oocyte production slows down in midsummer but this decrease is too slight to be biologically significant. No fully developed females were seen in December. Male GSI* values followed a similar pattern to Female GSI values. GSI began to increase in February and peaked in March.
CHAPTER IV
DISCUSSION

Diet

The marsh surface functions as a vital foraging habitat for estuarine fundulids and is rich in resources (Byrne 1978; Harrington and Harrington 1961; 1972; Kneib 1978; Meador and Kelso 1990a, b; Odom and Heald 1972; Ruebsamen 1972; Weisberg et al. 1981; Weisberg 1986; Vanderkooy et al. 2000). Lopez (2009) suggested that the wide variety of prey items seen in fundulids could be attributed to prey availability and differential access to the tidal flood plains of the marsh. During this study my data indicated that, *F. pulvereus*, like its congeners *F. jenkinsi, F. confluentus, F. grandis*, and *F. luciae* utilizes the increased inundation of the upper marsh and adjacent supratidal zones during spring tides to feed on terrestrial and aquatic insect instars in the upper marsh (Atmar and Stewart 1972; Forman 1968; Harrington and Harrington 1961; Kneib 1978; Lopez 2009; Rozas and LaSalle 1990; Ruebsamen 1972).

The varied diet of *F. pulvereus* was composed of terrestrial and aquatic insects, benthic, and epibenthic organisms, detritus and plant material. The most common prey taxa found in the stomachs of *F. pulvereus* were copepods, chironomids (larvae and adults), mites, detritus, organic matter, terrestrial insects, ceratopogonids (larvae and adults), and ostracods. Copepods, chironomid larvae, and mites were the most commonly occurring organisms in the diets of all ontogeny, during the entire study at all three sites. Mites, when present, were consumed in large numbers suggesting that, when readily
available, they are an energetically efficient food source indicating that *F. pulvereus* is an opportunistic feeder. Both Odum (1970) and Harrington and Harrington (1961, 1972) found that *F. confluentus*, a sister species of *F. pulvereus*, is an opportunistic mesopredator with an omnivorous diet that can easily adapt to alternative feeding patterns. While the diet reflected in the *F. pulvereus* from this study is indicative of an upper water column feeder, but also utilizes benthic prey organisms (e.g. chironomid larvae, hydrobiid snails, tanaidaceans).

The large variety of prey taxa occurring within the stomachs of *F. pulvereus* varied by site. Fish from the Moss Point (MP) sampling location had the least diverse diet while diets of *F. pulvereus* caught at Simmons Bayou (SB) and Weeks Bayou (WB) were at least twice as diverse. Proportionally at WB, the consumption of hydrobiid snails and spiders by *F. pulvereus* led to the greatest variety of dominant prey items. WB was also the only site where crab megalope and *Uca* fragments were found in the stomachs of the bayou killifish. The closer proximity to the Mississippi Sound or the higher salinity waters at WB may have given *F. pulvereus* greater access to crab megalope and *Uca* sp. as prey. Furthermore the marsh system at WB had a great deal of tree cover and during high tides the water reached well past the marsh flats and into the high marsh zone; which resulted in higher abundances of terrestrial insects consumed by *F. pulvereus* here than at MP or SB. The greater proportional presence of *S. alterniflora* may have also functioned as the source of terrestrial insects in the diet of *F. pulvereus* at WB, similar to that of LaSalle and Bishop (1987) who
found that the lower marsh, containing *S. alterniflora*, had greater densities of insects than did the mid marsh *Juncus roemerianus* zone. The large number of terrestrial insects found in the diet of *F. pulvereus* may have been biased since spring tides, when sampling took place, allowed fish better access to the high marsh. During the period of my study springtail juveniles, *L. rapax*, and *Gammarus mucronatus* were consumed in higher abundances at SB indicating that these organisms may have been more abundant at SB than at WB or MP. The variation in prey preference among the three sites, is reflective of the opportunistic and adaptive diet of *F. pulvereus*.

The diet of *F. pulvereus* displayed a seasonal effect, similar to that of other fundulids (Kneib 1978; LaSalle and Bishop 1987; Lopez 2009; Ruebsamen 1972). Insect larvae and adults were consumed by *F. pulvereus* at higher frequencies and greater abundance in spring. During the summer months crab megalope are more readily available in the environment (Hovel and Morgan 1997), which accounts for the higher consumption of decapod larvae by *F. pulvereus* during this season. Ruebsamen (1972) found crustaceans were consumed all year by *F. pulvereus* but there was a seasonal shift from chironomid larvae in winter to polychaetes in spring. Chironomids were consumed at higher abundances in the winter. Since the consumption of copepods and chironomid larvae by *F. pulvereus* more than doubled in winter, these items may have been readily available in the marsh system and more easily consumed. The presence of polychaetes in the diet of *F. pulvereus* were noted in a similar manner to that of Forman (1968) who studied the diets of six
Cyprinodonts, by setae (especially the aciculi) and fragments. In my study, however, polychaetes were not found to be an integral part of the diet. Since Baker-Dittus (1978) found that all three fundulids he studied consumed polychaetes in large numbers, polychaetes and oligochaetes were possibly underrepresented in the diet of *F. pulvereus*. Quick digestion rates of soft bodied animals in comparison to the exoskeletons of crustaceans and insects could have been the cause of under representation.

Cosmopolitan consumption of copepods and chironomid larvae were documented in all size classes of *F. pulvereus* at all three study sites. Even though copepods were found in the highest number of stomachs, across almost all size classes, only 32.4% of fish were found to have consumed them. Hyslop (1980) suggests that a high %N and low %O indicates that only a portion of the population are using that food source; whereas if both are high then the prey consumption is cosmopolitan. Contrary to Hyslop’s findings, my data indicate that, even though copepod consumption had a high %N and low %O these small crustaceans were consumed across all size classes, study sites, and seasons so the greater majority of the populations were utilizing copepods as a food source. Ruebsamen (1972) proposed that the diets of opportunistic fish foragers may give an indication of the availability of certain prey items within various marsh systems. During my study the low %O of copepods suggests, while readily available year round, they are not present in large enough numbers to have a higher frequency in the diet of *F. pulvereus*. Copepods may not be as energetically efficient and only taken when present in large numbers and readily
available for consumption. Detritus and algae have been reported in the digestive tracts of fundulids and have been considered as incidentally consumed during the capture of prey (Atmar and Stewart 1972; Baer 1974; Kneib and Stiven 1978; Odum 1970). In this study detritus occurred in all size classes of *F. pulvereus* especially larger fish. In such high occurrences accidental uptake does not seem as likely, but again this material may represent the tube material of prey. Baker-Dittus (1978) did not find detritus in the diets of *F. majalis*, *F. heteroclitus*, or *F. diaphanous* but, similar to my study, did find that plant matter and algae were consumed, along with all other available prey. Ruebsamen (1972) noted that non-detritivores consumed detritus during colder months, and suggests that this may be a way to supplement their diet, utilizing the detritus or the microorganisms that colonize it. In this study, however, I found that detritus was consumed at higher rates during the spring and summer, and became relatively unimportant during the winter months. Ostracods, ceratopogonid larvae, and mites were utilized by all but the smallest size classes with increasing importance in larger size classes. Piscivory was rare. Only the largest of *F. pulvereus* studied were found to be larvivorous cyprinodonts. Piscivory has been seen in other fundulids as well (Harrington and Harrington 1961; 1972); including large *F. confluentus* (Odum 1970) and *F. grandis* (Forman 1968; Harrington and Harrington 1961; Odum and Heald 1972; Rozas and LaSalle 1990; and Ruebasamen 1972).

There was not a significant difference found in the prey assemblages from year to year in my study. However, prey assemblages have been found to vary
within the marsh from year to year. Past research on Mississippi marshes (R. Heard and L. Gaines personal observations) has revealed a greater percentage of *L. rapax* in the diets of *F. pulvereus* (Table 6). During the year 2008, the marshes were inundated by tides and rain often resulting in considerable algal growth. This led to an increase in *L. rapax, Gammarus mucronatus, and Corophium louisianum* and their availability as an important food source for opportunistic *F. pulvereus* (R. Heard and L. Gaines personal observations).

Table 6

*Percent Occurrence and Percent Number of Prey Taxa in the stomachs of F. pulvereus (n = 41) from February 2008 (unpublished data)*

<table>
<thead>
<tr>
<th></th>
<th><em>Leptochelia</em> Rapax</th>
<th>Chironomid larvae</th>
<th>Harapacticoid copepods</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>%O</strong></td>
<td>61.0</td>
<td>36.6</td>
<td>26.8</td>
</tr>
<tr>
<td><strong>%N</strong></td>
<td>28.0</td>
<td>61.0</td>
<td>11.0</td>
</tr>
</tbody>
</table>

ANOSIM results concurred with %O and %N that there were differences in the prey assemblages among study sites, size classes, and seasons. While prey assemblages were shown to vary among all sites, SB was found to be the most dissimilar. With the greatest variety of prey taxa found within fish caught in SB, this is not surprising. Gender, which was not analyzed by %O or %N also showed a difference, this could be due to the slightly larger size of females in the fish studied. Females were larger and slightly heavier than males on average, which corresponds to the findings of both Simpson and Gunter (1956) and
Ruebsamen (1972) who also found that female *F. pulvereus* were slightly larger than males. This could indicate that females have a greater energy requirement and were therefore consuming more energy efficient prey items. Timmerman et al. (2000) found that mouth diameter limits prey size in largemouth bass. Mouth diameter could have contributed to the difference in prey assemblages between the male and female *F. pulvereus* in my study, since female mouth gape was found to be slightly larger than male mouth gape. Prey assemblages did vary among size classes but there was not a significant enough comparison between them to distinguish a pattern using ANOSIM. Baer (1974) also found an increase in prey size in larger *F. heteroclitus*. A larger sample size, would help determine the source of the ontogenetic differences in diet found using ANOSIM. In concurrence with %O and %N data, spring was shown to have the most variety in prey assemblages. Summer, however, was not significantly different from spring or winter, leading us to believe that summer had high occurrences of the more common prey taxa and only a few different prey taxa. Winter was dissimilar from spring, more than likely due to the lack of variety in prey assemblages since only the most common prey taxa were consumed in winter.

Parasites

Of the fourteen different species of parasites discovered infecting *Fundulus pulvereus* from Mississippi tidal marsh habitats during my research, only two, the digenean *Lasiotocus minuta* and the copepod *Ergasilus funduli*, have been previously reported from this fish host (Table 4) (Harris and Vogelbein 2006, Smedley 2000). New parasite host records established during this study
include; five digeneans (two larval forms and three adult stages), one monogenean, one cestode larva (metacestode stage), and two nematodes (3rd stage larvae) (Table 4). Two of the five parasites, the coccidian *Calyptospora funduli* and helminth *Sticodora cursitans* (Kinsella and Heard 1974) previously reported from the bayou killifish (Harris Voglebein 2006), were not observed or recorded during my study.

The presence of the microscopic stages of *C. funduli* were not documented since my study was limited to metazoan parasites, and consequently no liver squashes were done to corroborate the presence of this coccidian in the fish examined. *Calyptospora funduli*, however, was originally reported by Solangi and Overstreet (1980) as infecting *F. pulvereus* from marsh habitats in the vicinity of my general study area. The dagger-blade grass shrimp, *Palamonetes pugio*, is the trophic intermediate host for *C. funduli* and methods of trophic transfer are through very young grass shrimp (Solangi and Overstreet 1980). Even though *P. pugio* was not found in any of the stomach contents examined during my study, the presence of this coccidian would indicate that at certain points in its ontogeny *F. pulvereus* utilizes grass shrimp in its diet.

Of the six digenetic trematodes found parasitizing *F. pulvereus* during my study, three (*Phagicola diminuta, Proctocaecum coronarium*, and *Echinochasmus* sp.) were larval stages (encysted metacercaria) and three (*Homalometron palmeri, Homalometron* sp. A, *L. minuta*) were adults occurring the intestinal tract of the bayou killifish.
The monorchid trematode, *L. minuta*, infects *C. floridana*, a small marsh clam, and was the most common parasite found in the stomach and digestive tract of *F. pulvereus* (Smedley 2000). However, the presence of the clam host (*C. floridana*) was not recognized in the stomachs of *F. pulvereus* examined. This may have been due to digestion rates or, since *F. pulvereus* masticates its food, it could have mistakenly been listed as unidentified organic matter. Notwithstanding, the presence of *L. minuta* is a bio indicator for the presence of *C. floridana* at all three tidal marsh study sites and is consumed by the bayou killifish.

At my study sites estuarine snails belonging to the family Hydrobiidae serve, or appear to serve, as the first intermediate hosts for asexual stages of the remaining five digenean species infecting *F. pulvereus* (R. Heard personal observations, S. Curran et al. 2013 a, b, Overstreet 1978). Three of these digeneans, *Echinochasmus* sp., *Phagicola diminuta*, and *Proctocaecum coronarium* utilize the bayou killifish as a trophic intermediate host. The two apocreadid species *Homalometron palmeri* and *Homalometron* sp.*A use hydrobiid snails as their first intermediate and their trophic second intermediate host. The bayou killifish and gulf killifish (*F. grandis*) serve as their final vertebrate host. Circumstantially this supports the occurrence of the life cycles of these five digeneans by the common occurrence of several species of hydrobiid snails in Mississippi brackish marshes (Heard 1982) and specifically their presence at my study sites during sampling. Of the two species of *Homalometron* spp. found in the digestive tract of the bayou killifish, one was positively identified
as *Homalometron palmeri*, which has historically been misidentified as *H. palladium* (Curran et al. 2013a). There is some discrepancy as to whether or not *Homalometron* sp. A is a described species or represents a new cryptic species. Since it is often difficult and impractical to distinguish some species of *Homalometron* morphologically (Curran et al. 2013), analysis of ribosomal DNA is recommended (Curran et al. 2013 a, b) for future studies to determine the taxonomic status of *Homalometron* sp. A infecting *F. pulvereus* in the Mississippi marsh systems. As mentioned previously hydrobiid snails appear to be involved as both first and trophic intermediate hosts for species of *Homalometron* utilizing estuarine fishes as definitive hosts (Curran et al. 2013 a, b). It should be noted that hydrobiid snails occurred in the digestive tracks of *F. pulvereus* examined during my study.

*Phagicola diminuta* utilize hydrobiid snails as the first intermediate host and the free swimming larvae encyst in fish intermediate hosts (Overstreet 1978). The definitive or final host for *P. diminuta* and *Echinochasmus* sp. are fish-eating birds and mammals. In contrast the American alligator, *Alligator mississippiensis*, is the definitive vertebrate host for the cryptogonimid, *Proctocaecum coronarium* (Brooks and Overstreet 1977). Besides *F. pulvereus* several other cyprinodontiform fishes (e.g., *F. grandis, Cyprinodon variegatus*) may serve as the trophic second intermediate host for this alligator parasite. Like other digeneans this parasite can be used a bio-indicator. For example, after its complete inundation of Horn Island, MS by high salinity storm surge from Hurricane Katrina, R. Heard (personal observations.) used the presence of *P.*
coronarium metacercariae in the tail fin of *C. variegatus* as a bio-indicator for the recovery of brackish water ponds on this Mississippi barrier island. The presence of these metacercariae, which are visible to the naked eye, in the tail fin of *C. variegatus* indicated presence of both alligators and hydrobiid snails, which may not have been noticeably evident, in the pond system being surveyed. Because of the limited home range of *F. pulvereus* the same conclusions can be drawn for the presence of the first and definitive hosts of this parasite at my Simmons Bayou site (Brooks and Overstreet 1977).

The common occurrence of several species of hydrobiid snails, killifishes, and piscivorous birds and mammals in Mississippi brackish marshes, circumstantially supports the occurrence and completion of the life cycles of these five digeneans at my study sites (Heard 1982, R. Heard and R. Overstreet personal observations).

A metacestode larva (Gryporhynchidae) tentatively placed in the genus *Glosso cercus*, was found in the viscera of *F. pulvereus*. The gryporhynchids are thought to use crustaceans such as copepods as first intermediate hosts (R. Heard personal observations). The bayou killifish serves as the second intermediate host with the definitive host being a piscivorous bird such as a heron, clapper rail, or ibis.

At least two species of parasitic nematodes, *Eustrongylides sp.*, and *Hysterothylacium reliquens* were found associated with the viscera and body cavity of *F. pulvereus*. The members of genus *Eustrongylides* are reported to use oligochaetes as first intermediate hosts and small fishes such as *F.*
pulvereus as trophic second intermediate hosts. The adult parasite occurs in the esophagus of piscivorous birds (Overstreet 1978). During my study oligochaetes were found in the stomach contents of F. pulvereus. The Ascaridoidean nematode, H. reliquens uses a copepod as a first intermediate host and a fish (e.g., F. pulvereus) as a second intermediate host in its life cycle (Overstreet 1978); copepods were common components in the diet of the bayou killifish examined in the present study. A piscivorous fish serves as the final vertebrate host of H. reliquens (Overstreet 1978).

The adult of an acanthocephalan, Neoechinorhynchus sp., was found in the intestine of F. pulvereus. Members of the genus Neoechinorhynchus utilize ostracods as trophic intermediate hosts (Uglem, 1972). The adults of this usually freshwater acanthocephalan are a common parasite of largemouth bass, Micropterus salmoides (Richardson and Abdo 2011), which are known to occur in Simmons Bayou during periods of low salinity (R. Heard, personal observations). Ostracods were found in the stomach contents of the bayou killifish during my study.

The two ectoparasites, the monogenean, Gyrodactylus sp. and the ergasilid copepod, Ergasilus funduli, have no utility as trophic tags since their life cycles do not involve multiple hosts. The monogenean Gyrodactylus sp. was found on the body service of F. pulvereus. Since the life cycle of this monogenean is direct and monogeneans are usually host specific, F. pulvereus is probably the only host for this species and can be used as a bio indicator of the presence of this fish in tidal marsh habitats (Overstreet 1978). DNA
sequencing, and staining are needed to determine the specific identification of this species. The copepod parasite, *Ergasilus funduli* was found attached to the gill filaments of *F. pulvereus*. This genus of copepod has been previously recorded in fundulids with some contention over whether or not *F. pulvereus* or *F. confluentus* was reported host (Krøyer 1863; Roberts 1970; Wilson 1911).

Like *F. heteroclitus*, subpopulations of *F. pulvereus* have a restricted home range (Marcogliese and Scholz 1999, Harris and Vogelbein 2006). It can therefore be inferred from the presence of certain parasites that the entire host range for the parasite can be found within that marsh system. As an intermediate host to certain parasites, trophic linkages may be made to animals that are known to feed on *F. pulvereus* (Table 7) (George-Nascimento 1987; Campbell 1983; Zander et al. 1993; Groenewold et al. 1996; Marcogliese and Cone 1997). Further studies on the parasites occurring in *F. pulvereus* should include DNA sequencing for several parasite larval stages in attempting to identify them to genus or species.
Table 7

List of Trophic Linkages for Parasites found in Fundulus pulvereus

<table>
<thead>
<tr>
<th>Parasite</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; intermediate host</th>
<th>Trophic host</th>
<th>Definitive host</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phagicola diminuta</td>
<td>hydrobiid</td>
<td>Cyprinodontiform fish</td>
<td>heron, raccoon, Ibis</td>
</tr>
<tr>
<td>Echinochasmus sp.</td>
<td>hydrobiid</td>
<td>Cyprinodontiform fish</td>
<td>fish eating mammal or bird</td>
</tr>
<tr>
<td>Proctocaecum coronarium</td>
<td>hydrobiid</td>
<td>Cyprinodontiform fish</td>
<td>alligator</td>
</tr>
<tr>
<td>Lasiotocus. minuta</td>
<td>Cyrenoida floridana</td>
<td>Cyrenoida floridana</td>
<td>Fundulus spp.</td>
</tr>
<tr>
<td>Homalometron spp.</td>
<td>hydrobiid</td>
<td>hydrobiid</td>
<td>Fundulus spp.</td>
</tr>
<tr>
<td>Sticodora cursitans</td>
<td>Cerithidea scalariformis Fundulus spp.</td>
<td></td>
<td>raccoon</td>
</tr>
<tr>
<td>Glossocercus sp.</td>
<td>small crustacean</td>
<td>Fundulus spp.</td>
<td>heron, rail</td>
</tr>
<tr>
<td>Eustrongylides sp.</td>
<td>oligochaete</td>
<td>small fish</td>
<td>heron</td>
</tr>
<tr>
<td>Hysterothylacium reliquens</td>
<td>copepod</td>
<td>fish</td>
<td>piscivorous fish</td>
</tr>
<tr>
<td>Neoechinorhynchus sp.</td>
<td>podocopid ostracod</td>
<td>fish</td>
<td>or direct final fish host</td>
</tr>
</tbody>
</table>

Reproduction

Fundulids utilize high spring tides to lay their eggs in the upper reaches of the intertidal marsh (Byrne 1978; Greeley 1984; Greeley et al. 1986; 1988; Greeley and MacGregor 1983; Harrington and Haeger 1958; Lang 2010; Lang et al. 2012; Taylor 1991; Taylor et al. 1979). The eggs of fundulids are large compared to their body size. With a mean oocyte diameter of 2.3 mm, the eggs of *F. pulvereus* are no exception. Byrn (1978) observed a mean egg size for *F. luciae* as slightly smaller, at 1.96mm but the sample size was much smaller (n= 40 oocytes).
Batch fecundity and relative fecundity give relevant information on a fish’s reproductive rates. Fecundity comparisons across species are made using relative fecundity since it removes the size variable between fish species.

Average BF for *F. pulvereus* was low (58 oocytes) as was relative fecundity (52.6 oocytes/g). In comparison Clardy (2012) reported a RF of 213 ± 35.7 eggs/g for *Menticirrhus americanus*, a larger estuarine fish with much smaller eggs (mature oocytes were >350µm). The Mediterranean sardine also has a large relative fecundity of 360 eggs/g at its spawning peak (Ganias et al. 2004). In my study BF for *F. pulvereus* was highest from April through August with fish caught in September having the lowest BF. Both BF* and RF* were correlated with SL* and GFBW* in *F. pulvereus*. Jons and Miranda (1997), found similar results, but included the caveat that within fish of the same size class BF varied greatly. As a semi lunar spawner *F. grandis* has a similar reproductive pattern to *F. pulvereus* (Greeley 1984; Greeley et al. 1988). At the beginning of the spawning season *F. grandis* spawns almost daily (Greeley et al. 1988). Greeley et al. (1988) believed that this was to allow more offspring to develop in the favorable conditions of summer.

There have been several conflicting reports over the spawning season of *F. pulvereus*. Ruebsaman (1972) found spawning capable female *F. pulvereus* as early as January but reported that the spawning season ended in May. Simpson and Gunter (1956) reported a much later spawning season (from fall – winter) in Texas than any other reports. While Forman (1968) found that *F. pulvereus* did not spawn in winter. GSI* data from this study corresponded to
BF* and RF*, and suggested that *F. pulvereus* has a spawning season of February through August, with March and April being the most significant. The start to the spawning season of *F. pulvereus* is marginally earlier than most fundulids, whose spawning season begins in March (Kneib and Stiven 1978); and could be due to geographic variations in temperature since spawning seasons have been shown to lengthen in warmer waters (Burger 1939; Kneib and Stiven 1978). Similar to our study, Harrington (1959b) found that *F. confluentus* had a spawning season which also began in February but lasted through October in the warmer waters of Florida. Lopez et al. (2010) found 35% of *F. jenkinsi* were spawning capable in winter, via oocyte diameter data, Lang (2010) found none, by the use of histology. Lang (2010) ascertained that *F. jenkinsi* had a spawning season of April through August using GSI*. However, histology showed that March was the true beginning of the spawning season. Due to this variation in findings some discrepancy is advised when using oocyte diameter and GSI as an indication of spawning capable fish.

**Conclusion**

Previously, information on the life history of *F. pulvereus* was lacking. This study has sought to gain a better understanding of the function of *F. pulvereus* within the tidal marsh system. Two prey indices and multivariate analyses indicated that diet varied by season, site, and ontogenetically by size class. This suggests that *F. pulvereus* is most likely an opportunistic omnivore who utilizes the marsh floodplains to forage for prey items such as terrestrial and aquatic insects. These foraging habits link *F. pulvereus* to the trophic relay
taking place within estuaries. Furthermore, this would suggest that the
invertebrate community in marshes vary spatially and temporally. Additional
determination of diet breadth was achieved through the finding of fourteen
species of parasites infecting *F. pulvereus* which utilize intermediate hosts
typically not observed as prey items in the diet. Reproductive efforts of
cyprinodonts seem to be familial. Like other fundulids, *F. pulvereus* is a
semilunar batch spawner (Greeley, 1984). Fecundity and GSI analyses indicated
a spring and summer spawning season, also similar to that of other fundulids.
Spawning began slightly earlier than most fundulids, in February, and extended
through August. Sparse knowledge existed on the fecundity of *F. pulvereus* thus
this information will allow for inter-species comparisons and can serve as a
baseline for future studies.

Further efforts should focus on temporal and tidal feeding cycles of *F.
pulvereus*, and genetic sequencing of some parasites, such as *Homalometron*
sp. A, should be performed to identify parasites to species level. Also,
histological analysis of oocytes would determine the beginning of the spawning
season for *F. pulvereus*. Since it has been shown that individual *F. jenkinsi* are
capable of spawning multiple times within a tidal cycle (Lang 2010) it may also
establish if *F. pulvereus* has a similar spawning pattern. As an intertidal marsh
resident, *F. pulvereus* plays a role in the energy flow of the Mississippi tidal
marsh system. The findings of this study will allow for a more in-depth analysis
and a more holistic view of the fundulids intertidal existence.
APPENDIX A

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

The proposal amendment noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: 14010031
PROJECT TITLE: Feeding ecology, reproduction, and trophic position of Fundulus pellevaris
PROPOSED PROJECT DATES: 01/2014 - 09/2016
PROJECT TYPE: New
PRINCIPAL INVESTIGATOR(S): Robin Overstreet
DEPARTMENT: Coastal Sciences
FUNDING AGENCY/SPONSOR: N/A
IACUC COMMITTEE ACTION: Full Committee Approval
PROTOCOL EXPIRATION DATE: September 30, 2016

Frank Moore, PhD
IACUC Chair

January 9, 2014
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