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Diet and Feeding-Related Morphometrics of the Blackstripe Topminnow, *Fundulus notatus*, in Allotropic and Syntopic Populations with the Blackspotted Topminnow, *Fundulus olivaceus*

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The University of Southern Mississippi

DIET AND FEEDING-RELATED MORPHOMETRICS OF
THE BLACKSTRIPE TOPMINNOW, *FUNDULUS NOTATUS*,
IN ALLOTOPIC AND SYNTOPIC POPULATIONS WITH
THE BLACKSPOTTED TOPMINNOW, *FUNDULUS OLIVACEUS*

by

Charles Michael Champagne

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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August 2011

ABSTRACT

DIET AND FEEDING-RELATED MORPHOMETRICS OF THE BLACKSTRIPE TOPMINNOW, *FUNDULUS NOTATUS*, IN ALLOTOPIC AND SYNTOPIC POPULATIONS WITH THE BLACKSPOTTED TOPMINNOW, *FUNDULUS OLIVACEUS*

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The *Fundulus notatus* species complex consists of three described species: *F. notatus*, *F. olivaceus* and *F. euryzonus*. Both *F. notatus* and *F. olivaceus* have broad overlapping ranges with many populations being found within and outside of contact zones. Contact zones are generally found in mid-reaches with *F. olivaceus* dominating headwaters and *F. notatus* in larger rivers downstream. Both species share similar ecological niches so the mechanism allowing for stable coexistence in contact zones is unknown. The purpose of this study was to examine variability in diet and feeding morphology of *F. notatus* in syntopic and allotopic populations across three drainages. Both *Fundulus* species were sampled in Pascagoula River, Pearl River and Neches River contact zones in the summer of 2008. Fish were genotyped and feeding-related morphometrics were taken (standard length, body width, body depth, head length, head width, head depth, interorbital distance, preorbital length, orbit length, postorbital length, gape width, gape height, maxillary length, and

dentary length). Morphometric analyses were conducted to determine if there were ontogenetic shifts or sexual dimorphisms in allotopic and syntopic populations. Analyses were also conducted to determine if there were differences among species and syntopic-allotopic populations. Digestive tracts of the *F. notatus* were examined to determine prey items. There were significant differences in feeding-related morphometrics between age classes, sexes, and syntopic and allotopic populations for both *Fundulus*. There were also significant differences in diets of various groups of *F. notatus*.

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CHAPTER I

DIET AND FEEDING RELATED MORPHOMETRICS OF
THE BLACKSTRIPE TOPMINNOW, *FUNDULUS NOTATUS*,
IN ALLOTOPIC AND SYNTOPIC POPULATIONS WITH
THE BLACKSPOTTED TOPMINNOW, *FUNDULUS OLIVACEUS*

Introduction

A primary goal of evolution and ecology is understanding the mechanisms responsible for generating and maintaining species diversity. Competition has long been thought to be important in this regard (Begon et al. 2006) as competing individuals limit resource availability, which impacts growth, survivorship and ultimately fitness. The competitive exclusion principle, also known as Gause's principle, is simply stated as: *Complete competitors cannot coexist* (Hardin 1960). For example, if species A and B utilize the exact same ecological niche and if species A is more fit (i.e., higher fecundity, growth, and survival) then species B will be competitively excluded (Hardin 1960; Zaret & Rand 1971; Connell 1983). Exceptions to this pattern can occur when populations are supported by immigration or when habitats are sufficiently patchy to allow for some kind of competitive release. The former example is seen in the "paradox of the plankton" where seemingly homogeneous habitats (open ocean) support surprising diversity of zooplankton communities. The "answer" to the paradox is thought to be in habitat patchiness relevant to zooplankton populations.

Superficially, one might expect the competitive exclusion principle to limit biodiversity by limiting the number of species occupying any given habitat. However, competition between two species can have the opposite effect when competitive pressure results in shifts in habitat use or changes in morphology to fill empty niche space and relieve competitive pressure (Schoener 1974a; Connell 1980; Begon et al. 2006). Niche shifts occur when species change their realized niche, which allows access to resources not under heavy competition pressure. Over evolutionary time, morphological changes (character displacement) can occur when individuals with morphology that gives more efficient access to resources not under competitive pressure are selected for. Ultimately, both niche shifts and character displacement will allow the two species to coexist and increase local diversity. When two closely related species or congeners occur together, divergence in morphological or ecological traits (character displacement) can alleviate competitive pressure and ultimately increase diversity (Pritchard & Schluter 2001). While the strength of competitive interactions and selection affects character displacement (Slatkin 1980; Pritchard & Schluter 2001), caution should be used when invoking this as an explanation for contemporary patterns since evidence for historical competitive interactions is often elusive (Connell 1980, Schluter 2003).

Intraspecific competition occurs when individuals of the same species compete for the same limiting resource (density dependent effects, Begon et al. 2006). In general, one would expect intraspecific competition to be more intense than interspecific competition because individuals within a population will be

more similar. By extension, morphologically similar species and congeners are expected to experience more intense competition when densities are high and food resources are limited (Sardiña & Lopez Cazorla 2005). Ecologically and morphologically similar congeners that coexist are therefore good study systems to understand the nature of competitive interactions.

Niche shifts (either spatial or temporal) can alleviate competitive pressure. When two competing species occupy the same area at the same time, competition can be reduced by a separation in physical space (spatial partitioning) or by habitat and feeding segregation (niche shifts) (Hardin 1960; Nilsson 1965). Likewise, competition can be reduced by a separation in time (temporal partitioning) when the two species occupy the same location (Sardiña & Lopez Cazorla 2005) but exploit limiting resources at different times. Werner and Hall (1988) found that ontogenetic niche shifts can occur which result in increased food resources and growth rates. Juveniles, when syntopic with adults of the same species, are usually at a disadvantage; however, in certain situations juveniles can have a morphological or ecological advantage over adults in syntopic populations (Vincent et al. 2006). This is known as the compensation hypothesis. For example, one advantage in juvenile gape-limited predators, those which do not mechanically reduce their prey, is a larger mouth to body ratio (Vincent et al. 2006). Body size differences also contribute to reducing competition (Schoener 1974*b*).

In aquatic food webs food intake, energy partitioning, assimilation, and transfer are potential trophic interactions (Paine 1988). As fishes increase in age

and size, there is also an increase in mean prey size consumed to increase energy intake (Griffiths 1975; Cardinale 2000). Diet and morphology are important in determining ontogenetic shifts in fishes (Stoner & Livingston 1984).

Syntopic populations occur when two or more closely related species occupy the same habitat; therefore, they are capable of interbreeding (Rivas 1964). These areas are known as hybrid or contact zones and have long been viewed as natural laboratories of evolution. While some contact zones have been studied extensively as models for speciation or population genetic work (well studied systems include oak, crickets, butterflies and marine bivalves) the ecology of contact zones is not as well studied. Contact zones represent an ideal system to study the effects of competition on niche shifts and character displacement.

Study Species

The blackstripe topminnow, *Fundulus notatus* (Rafinesque), along with the blackspotted topminnow, *Fundulus olivaceus* (Storer), and the broadstripe topminnow, *Fundulus euryzonus* (Suttkus & Cashner), are members of the *Fundulus notatus* species complex. These species of topminnows have overlapping geographical ranges with morphological and ecological similarities (Thomerson & Wooldridge 1970; Blanchard 1996; Duvernell et al. 2007).

Fundulus notatus and *F. olivaceus* are found in the Mississippi River Valley and along the Gulf Coast; however, *F. notatus* has a more western and northern distribution (Fig. 1) (Thomerson & Wooldridge 1970; Boschung & Mayden 2004). Syntopic populations of *F. notatus* and *F. olivaceus* have been reported in

southern Illinois, southeastern Missouri, and western Kentucky (Brassch & Smith 1965; Duvernell et al. 2007). *Fundulus notatus* and *F. olivaceus* have terminal, upturned mouths with protrusible jaws, pointed snouts, and flattened dorsums, all contributing to their surface feeding habits (Etnier & Starnes 2001; Ross 2001). *Fundulus notatus* predominantly feed at the water surface on terrestrial invertebrates, which fall onto the water surface, and emergent aquatic insects (Thomerson & Wooldridge 1970; Etnier & Starnes 2001; Ross 2001). They also feed on littoral and benthic aquatic invertebrates and filamentous algae (Thomerson & Wooldridge 1970; Atmar & Stewart 1972; Etnier & Starnes 2001; Ross 2001). Thomerson and Wooldridge (1970) made casual observations in the field and aquarium and report that *F. notatus* and *F. olivaceus* have similar diets. *F. olivaceus* are typically found in the headwaters whereas *F. notatus* are typically found downstream. This research project focuses primarily on the blackstripe topminnow, *F. notatus* due to its broader geographic range.

The purpose of this study is to examine diet and feeding morphology of *F. notatus* populations across three drainages where the species is known to co-occur with *F. olivaceus* (i.e., contact zones). Within each drainage, I will attempt to compare diet and morphology of *F. notatus* at sites with (syntopic) and without (allotopic) *F. olivaceus*.

Morphometric Objectives

I hypothesize that feeding related structures are most likely to be under strong selective pressure due to competition for limiting food resources. Thus, the objectives for the feeding-related morphology portion of this study are to

determine if there are significantly different feeding-related morphologies (i) between *F. notatus* and *F. olivaceus*, (ii) between the drainages within each species, (iii) between *F. notatus* adults and juveniles (ontogenetic shift), and (iv) between syntopic and allotopic populations of *F. notatus* (character displacement).

Diet Objectives

To quantify a potential niche shift, I examined the diet (stomach contents) of *F. notatus* from the three contact zones. The objectives for the diet portion of this study are to determine if there are significant differences in diets i) between *F. notatus* in the three drainages, ii) between *F. notatus* adults and juveniles, and iii) between *F. notatus* in syntopic and *F. notatus* in allotopic populations.

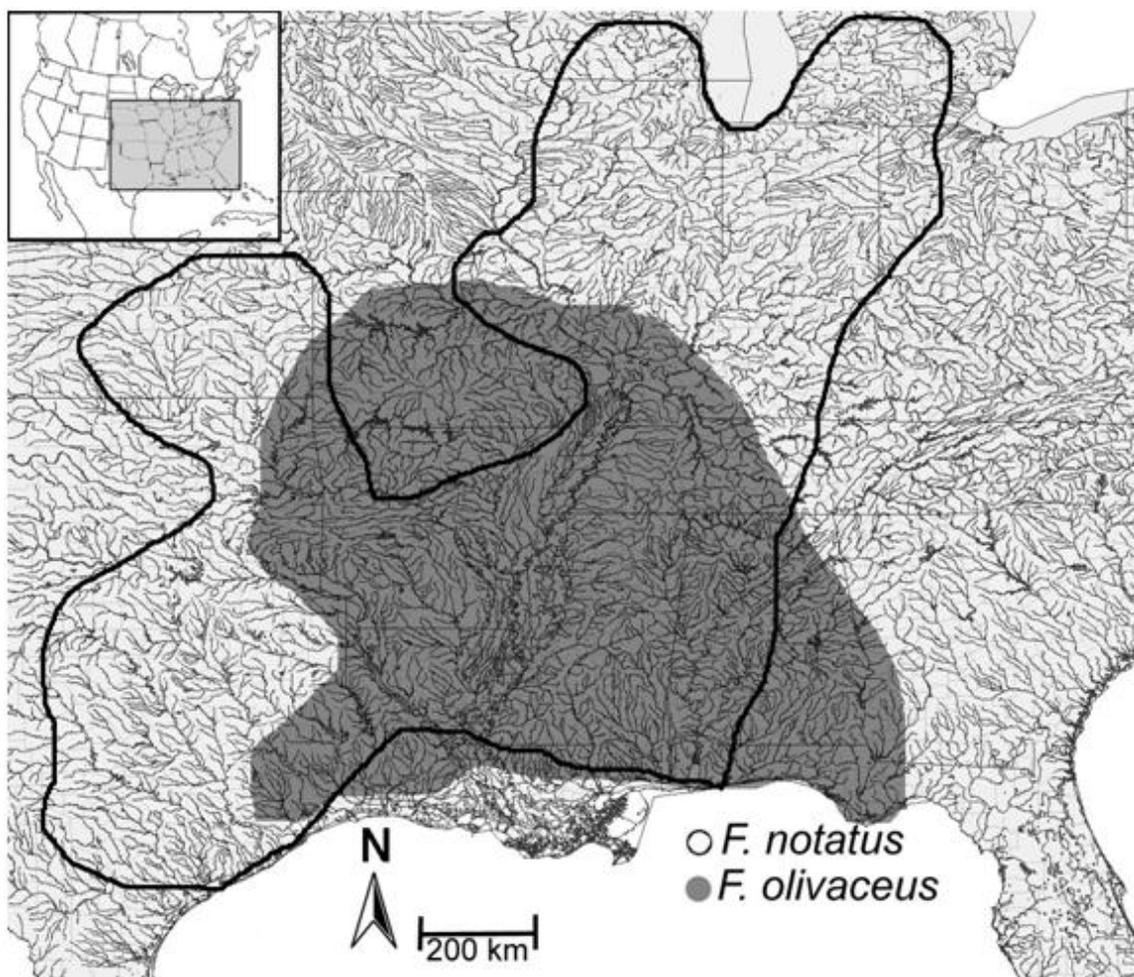


Fig. 1. Distribution of *F. notatus* and *F. olivaceus*.

Study Sites

In the summer of 2008, putative contact zones in three river drainages were sampled for *Fundulus notatus* and *F. olivaceus*. The contact zones are located in three drainages which flow in a southern direction and discharge into the Gulf of Mexico. Sampling sites were selected in an attempt to capture the center of the contact zone (syntopic sites) as well as allotopic *F. olivaceus* sites upstream (typically in tributaries) and allotopic *F. notatus* sites downstream. Many sites were accessed at bridge crossings where *Fundulus* were readily spotted from above. Other sites were accessed by boat sampling targeting

backwater areas of low flow and high structure where *Fundulus* are typically abundant.

Pascagoula River Drainage (PAS)

Located in southeastern Mississippi, USA, the Pascagoula River is about 130 km long and has a drainage area of about 23,000 km². It is the only large river in the continental United States that remains unimpounded (Dynesius & Nilsson 1994). The confluence of the Leaf River and Chickasawhay River form the northern portion of the Pascagoula River. The river generally flows in a southern direction where it discharges into the Mississippi Sound of the Gulf of Mexico. The main channel of the Pascagoula River (six sites) was sampled as well as the Black Creek tributary (four sites) (Fig. 2).

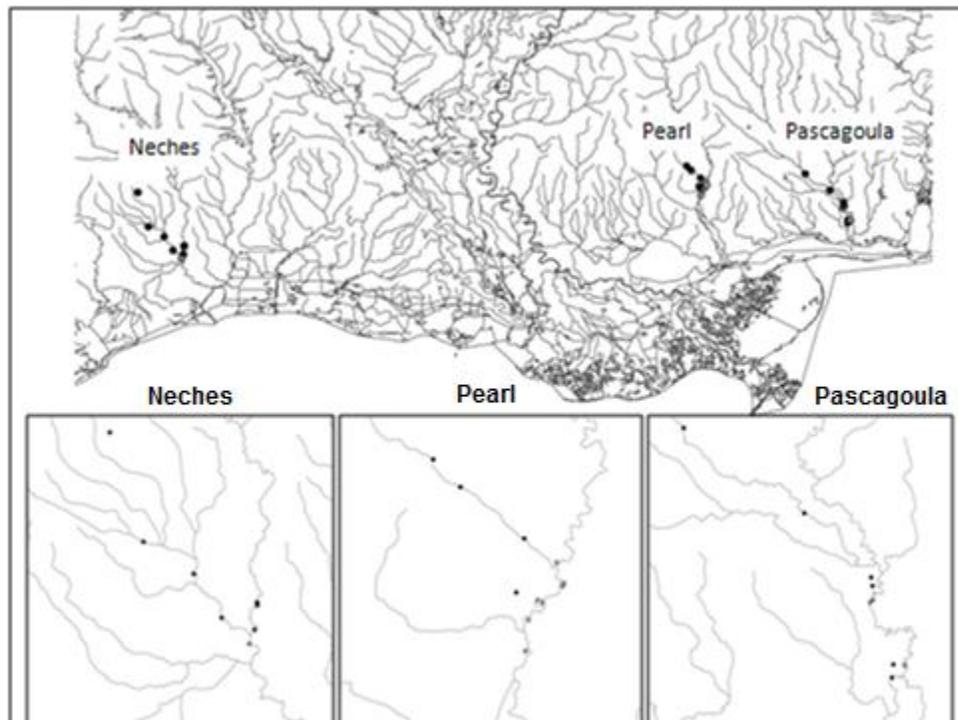


Fig. 2. Map of sites sampled in the three drainages.

Pearl River Drainage (PRL)

Located in Mississippi and Louisiana, USA, the Pearl River is about 790 km long and has a drainage area of about 22,688 km². The confluence of Nanaway and Tallahaga Creeks form the northern portion of the Pearl River. The river has undergone major anthropogenic modification. In 1962, the Ross Barnett Reservoir was constructed for water supply to the city of Jackson, MS. Since 1880, several dredging projects have resulted in major channelization of the Pearl River. In 1935, three locks were constructed to allow barge transportation up to the city of Bogalusa, LA. The river generally flows in a southern direction then forks forming East Pearl River and West Pearl River which both eventually discharge into the Gulf of Mexico. The main channel of the Pearl River (six sites) was sampled as well as the Pushpatapa Creek tributary (four sites) (Fig. 2).

Neches River Drainage (NEC)

Located in southeastern Texas, USA, the Neches River is about 669 km long and has a drainage area of about 25,928 km². There are two impoundments in the NEC, which create reservoirs, Lake Palestine and Lake B. A. Steinhagen. The river generally flows in a southern direction where it empties into Sabine Lake then into the Gulf of Mexico. The main channel of the Neches River (four sites) was sampled as well as Village Creek and Town Creek (four sites) tributaries (Fig. 2).

Materials and Methods

Collection of Fundulus

In the PAS nine sites were sampled (four syntopic; two allotypic *F. notatus*; three allotypic *F. olivaceus*). In the PRL thirteen sites were sampled (eight syntopic; zero allotypic *F. notatus*; five allotypic *F. olivaceus*). In the NEC eight sites were sampled (one syntopic; three allotypic *F. notatus*; four allotypic *F. olivaceus*).

At each site, I attempted to capture 30 individual *Fundulus* with the use of dip nets and seine (length 6.1m · depth 1.2m · mesh size 3.18 mm). After collection, a portion of caudal fin was removed and placed into a 1.5 mL microcentrifuge tube containing a preservation salt buffer solution (Seutin et al. 1991) along with an individual label identifying the drainage, date, and an individual fish identification number. A duplicate label and the individual fish were placed into a 50 ml BD Falcon centrifuge tube containing 10% formalin. The duplicate labeling allowed for the appropriate genotype data to be linked back to the individual fish for later morphological and diet analyses. Any putative hybrid individuals were removed from all analyses. Tissue samples were sent to Dr. David Duvernell at Southern Illinois University at Edwardsville, Edwardsville, IL, USA for genotyping (see Schaefer et al. 2011 for methods and genotype data). The fish were transported to The University of Southern Mississippi, Hattiesburg, MS, USA for morphometric and diet analyses described below.

Morphometric Data

All *Fundulus* were measured to the nearest 0.01 mm for 14 morphological measures (Table 1, Fig. 3) using digital calipers.

Table 1. Feeding-related metrics for each *Fundulus*.

Metric	Abbreviation
Standard length	SL
Body width	BW
Body depth	BD
Head length	HL
Head width	HW
Head depth	HD
Interorbital distance	IO
Snout length	SNOUT
Orbit length	ORB
Postorbit length	POST
Maxillary length	M
Dentary length	D
Gape width	GW
Gape height	GH

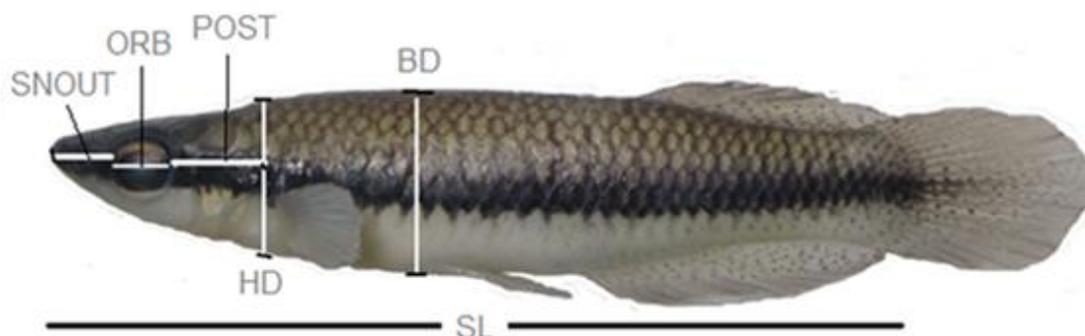


Fig. 3. *Fundulus notatus* with SL, BD, HD, SNOUT, ORB and POST indicated.

Fundulus mouths were measured for lengths of the maxillary (M), dentary (D), gape height (GH), and gape width (GW) with mouths fully opened, but not to the point of distortion. All feeding-related metrics were standardized and expressed as a percentage of SL. *Fundulus* become sexually mature around 35.00 mm SL (Schaefer et al. unpublished). Therefore, *Fundulus* < 35.00 mm SL

were categorized (age class) as juveniles while *Fundulus* > 35.00 mm SL were categorized as adults for the ontogenetic analyses in this study. Measurements were recorded and later analyzed using R statistical software (R Development Core Team 2009).

Morphometric Analyses

Principal Components Analysis (PCA) was used to summarize the 14 standardized morphological measures into two principal components (Peres-Neto et al. 2003). I used Non-Parametric Multivariate Analysis of Variance (NP-MANOVA) to partition variation among species, drainages, age-class and allotopic vs. syntopic sites. NP-MANOVA is analogous to MANOVA (Multivariate Analysis of Variance), except that permutation procedures are used to compare patterns of within and between group variance in a similarity matrix (Euclidean distance in PCA space in this case). Significance was assessed through 10,000 permutations at $\alpha = 0.05$ (Anderson 2001; Roberts & Taylor 2008).

Dietary Data

According to Prosser and Brown (1961) *Fundulus* do not have discrete stomachs. Thus, digestive tracts from the esophagus to the anus were removed through dissection and maintained in 10% formalin. Digestive tracts were dissected and contents were identified and enumerated with the use of a dissecting microscope (Wild-Heerzburg M5). Rose Bengal stain was used to facilitate identification of some prey items. Prey items were identified to the lowest taxonomic level possible using dichotomous identification keys (Merritt &

Cummings 1984; Thorp & Covich 2001). Once identified, the number of each type of prey in each digestive tract was recorded.

The fullness of each digestive tract was estimated visually (Table 2) and recorded. All digestive tract contents were then placed onto a Sedgwick-Rafter cell counting chamber (Hausser Scientific) to standardize the depth of the digestive tract contents. Total estimated prey volume (EPV) (mm^3) of digestive tract contents was then calculated by capturing a digital image of the counting chamber and measuring the area of digestive tract contents with digitization software (tpsDig version 2.16). This estimated prey volume (EPV) was then standardized by SL (mm).

Table 2. Digestive tract fullness scoring.

Visual Fullness	Empty-1/4	1/4-1/2	1/2-3/4	3/4-Full
Score	1	2	3	4

The single widest prey item in the digestive tract of randomly selected *F. notatus* was identified and its maximum width measured to the nearest 0.01mm using a digital microscope (Celestron digital microscope) and software (Digital Microscope Suite (DMS) Software). Maximum prey size (MPS) was defined as the ratio of the maximum width of the largest prey item to the gape width.

Diet Analyses

Complete stomach contents were visually summarized in two dimensional ordination space using Non-Metric Multidimensional Scaling (NMDS) with Bray-Curtis similarities using 500 iterations (Trexler et al. 2005). I used Indicator Species Analysis (ISA) (Dufrene & Legendre 1997; Roberts & Taylor 2008) to

identify prey species that were significant indicators for various groupings of *F. notatus*. ISA calculates the frequency of occurrence and relative abundance of each species in a group. In this context, a perfect indicator would be a prey item that was always found exclusively in one group in high relative abundance. Indicator values range from 1 (perfect indicator) to 0 (random occurrence among groups). The significance of indicator values is then assessed by permutation of the raw data. I used ISA to identify prey indicators for *F. notatus* among drainages, age classes (adults vs. juveniles) and in allotopic vs. syntopic populations. Both NMDS and ISA were performed in R statistical software (vegan package: R Development Core Team 2009). Finally, I used *t*-tests to compare mean EPV and MPS between age classes (adults vs. juveniles), sexes and allotopic vs. syntopic populations.

Results

Morphological Analyses

A total of 738 *Fundulus* were analyzed for feeding-related morphometric differences. A total of 311 *F. notatus* were analyzed from the three drainages [Pascagoula n = 129 (female n = 61; male n = 68); Pearl n = 115 (female n = 64; male n = 51); Neches n = 67 (female n = 43; male n = 24)]. A total of 427 *F. olivaceus* were analyzed from the three drainages [Pascagoula n = 109 (female n = 53; male n = 56); Pearl n = 183 (female n = 94; male n = 89); Neches n = 135 (female n = 72; male n = 63)]. Ten hybrids (Pascagoula n = 6; Neches n = 4) were collected and excluded from all analyses (Schaefer et al. 2011). In the Pearl River, there were no sites that were allotopic for *F. notatus*. Thus, all analyses

testing for allotopic-syntopic shifts focus only on the Pascagoula and Neches samples.

The first two principal components explained 63.52% (PC I 40.65%; PC II 22.87%) of the variance in feeding-related morphometrics. A number of measures loaded negatively on the first principal component including measures of head size (hd, hw), gape and mouth size (gh, gw, m and d) and body depth (bd) (Table 3). Body mass was positively correlated with the second principal component while head length, orbit and interorbit distance were all negatively correlated with the second principal component. Thus, fish with higher axis 1 scores generally had smaller head, gape, and mouth size with shallower bodies. Fish with higher axis 2 scores were larger but had shorter and narrower heads (Table 3).

Table 3. Principal components analysis loadings for the first four axes of all standardized morphological measures of all measured *Fundulus*. Loadings less than 0.1 are not listed and higher loadings over 0.3 are bolded.

METRIC	PC I	PC II	PC III	PC IV
mass	-0.302	0.603	-0.276	0.115
bd	-0.415	0.308	0.134	
bw	-0.288		0.308	
hl	-0.306	-0.425	-0.641	0.210
hd	-0.396			
hw	-0.371	-0.251	0.368	
io	-0.262	-0.328	0.254	
orb		-0.330		-0.110
post	-0.142	-0.219	-0.100	
snout	-0.102		-0.384	
gh	-0.272			
gw	-0.259			
m	-0.102		-0.121	-0.794
d	-0.123			-0.530
% Variance	40.65	22.87	10.09	5.78

There were significant differences between the two species and just over 17% of the morphological variation was explained by species differences (NP-MANOVA, $F_1 = 203.43$, $R^2 = 0.1724$, $p < 0.001$). *Fundulus notatus* were generally clustered to the right on PCA axis 1 indicating they had smaller heads, gape and mouth size and shallower bodies (Fig. 4).

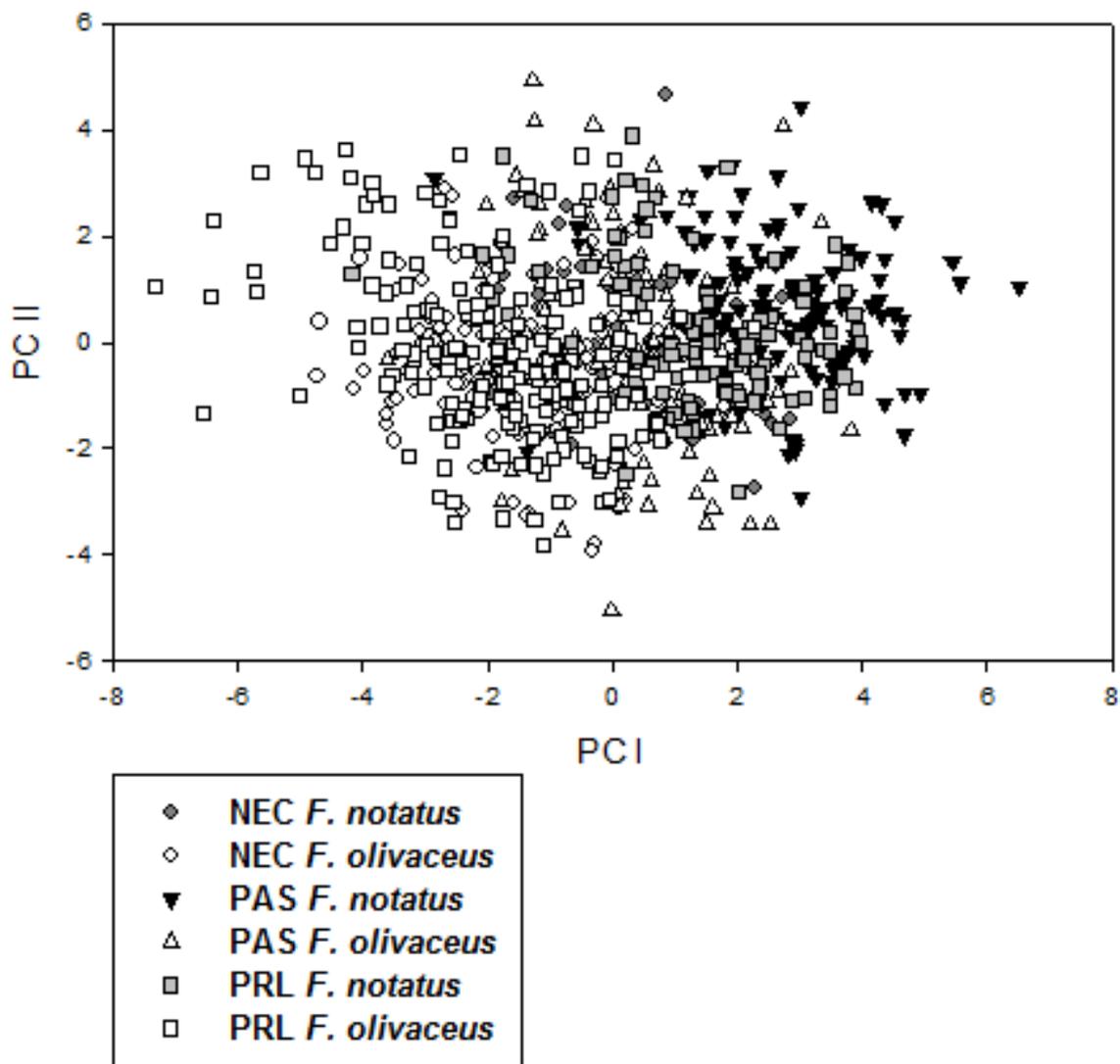


Fig. 4. PCA of all *Fundulus* plotted by species and drainage. Filled symbols are *F. notatus* while open symbols are *F. olivaceus*. Symbols represent different drainages.

There were also significant differences among the three drainages and these drainage differences accounted for just over 19% of the variation in the first two PCA axes scores ($F_2 = 113.40$, $R^2 = 0.1923$, $p < 0.001$). Individuals from both species in the PAS drainage (triangles in Fig. 4) were clustered to the right compared to individuals of both species in the PRL (squares in Fig. 4) or NEC

(circles in Fig. 4). This indicates that both species have smaller heads, gape and mouth size and shallower bodies in the PAS. Finally, because there was a significant interaction between species and drainages, NP-MANOVA were run on *F. notatus* alone to test for drainage and allotopic/syntopic differences.

There were significant differences between *F. notatus* from the three drainages and just over 26% of the variation was explained by drainage differences alone (NP-MANOVA, $F_2 = 93.08$, $R^2 = 0.2679$, $p < 0.001$, Table 4). The *F. notatus* from the PAS were generally clustered to the right on PCA axis 1 indicating they had smaller head, gape and mouth size and shallower bodies (Fig. 4) than those in the PRL (Fig. 4, filled squares) or NEC (Fig. 4, filled circles).

There were significant allometric/ontogenetic differences in *F. notatus* morphology (all morphological measures were standardized before conducting PCA). Changes in morphology with age class accounted for just over 26% of the variation in the first two PCA axes scores (NP-MANOVA, $F_1 = 181.07$, $R^2 = 0.2606$, $p < 0.001$). Juveniles have higher PCA axis 1 scores, while adults have higher PCA axis 2 scores. Adults (lower PCA axis 1 scores) were generally clustered to the left (Fig. 5 top) compared to juveniles on PCA axis 1. On the other hand, juveniles have lower PCA axis 2 scores (Fig. 5 bottom). This indicates that adults have relatively smaller head, gape and mouth size than juveniles. On the other hand, juveniles have smaller mass, body depth, head width, and head depth, but have larger head lengths than adults. In general as *Fundulus* get bigger, their heads and mouths get smaller in relation to SL.

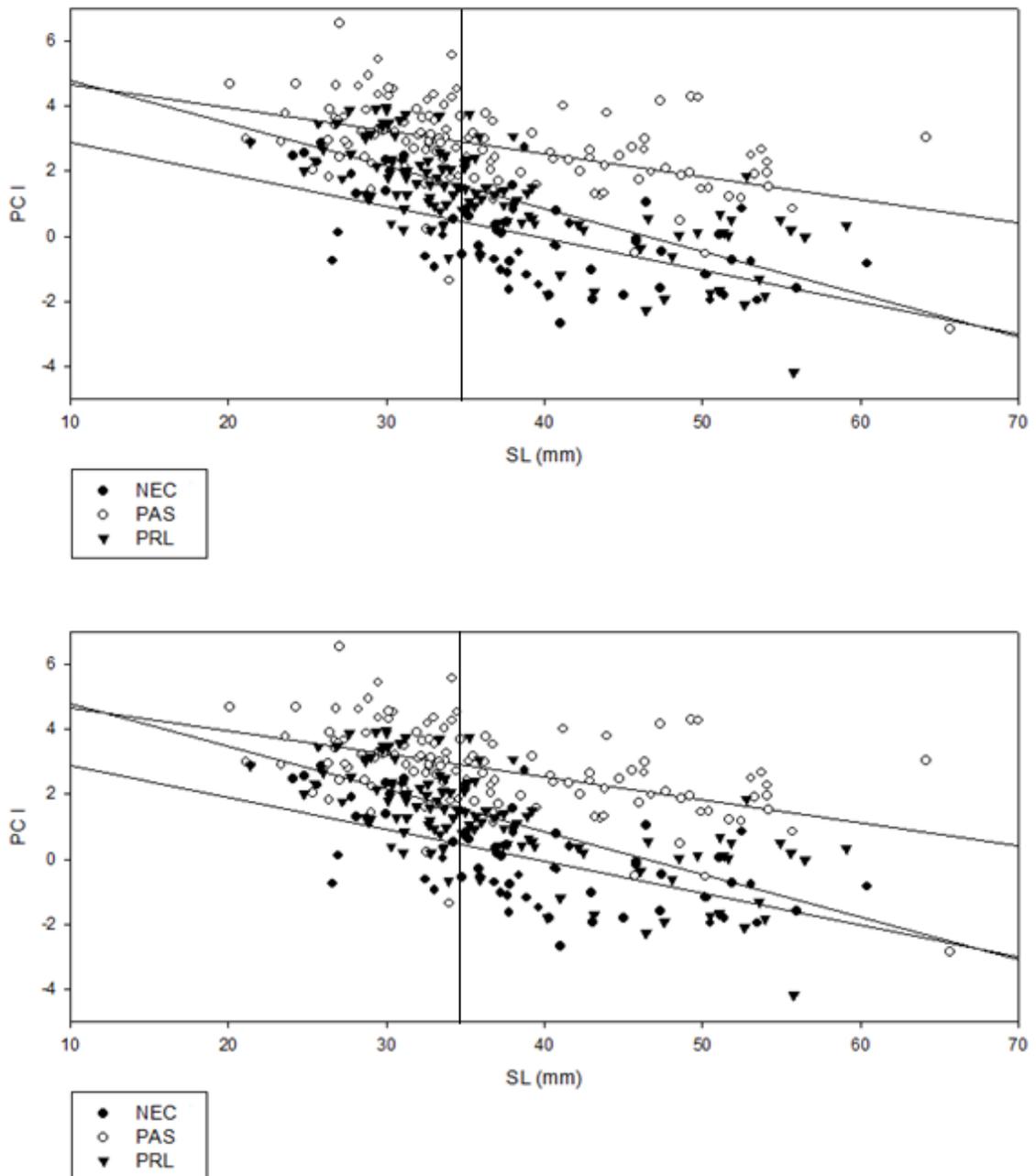


Fig. 5. PC I and PC II by SL of all *F. notatus*. Regression lines represent the best fit for each drainage. Vertical lines at 35 mm SL indicates size class separation (juveniles < 35.00 mm and adults > 35.00 mm)

There were significant differences between syntopic and allotopic populations of *F. notatus* in the NEC and PAS. While significant, these differences accounted for just over 1.6% of the variation in the first two PCA axes scores (NP-MANOVA, $F_1 = 11.195$, $R^2 = 0.0161$, $p = 0.001$). Syntopic populations were generally clustered to the right on PCA axis 1 compared to allotopic populations of *F. notatus* (Fig. 6; syntopic populations generally have larger interorbitals, postsnout, snout, gape height, gape width, maxillary and dentary). Overall, most of the morphological variability (56.7%) in *F. notatus* was accounted for by drainage and age class with an unaccounted for residual of 43.3% of the variation (Table 4).

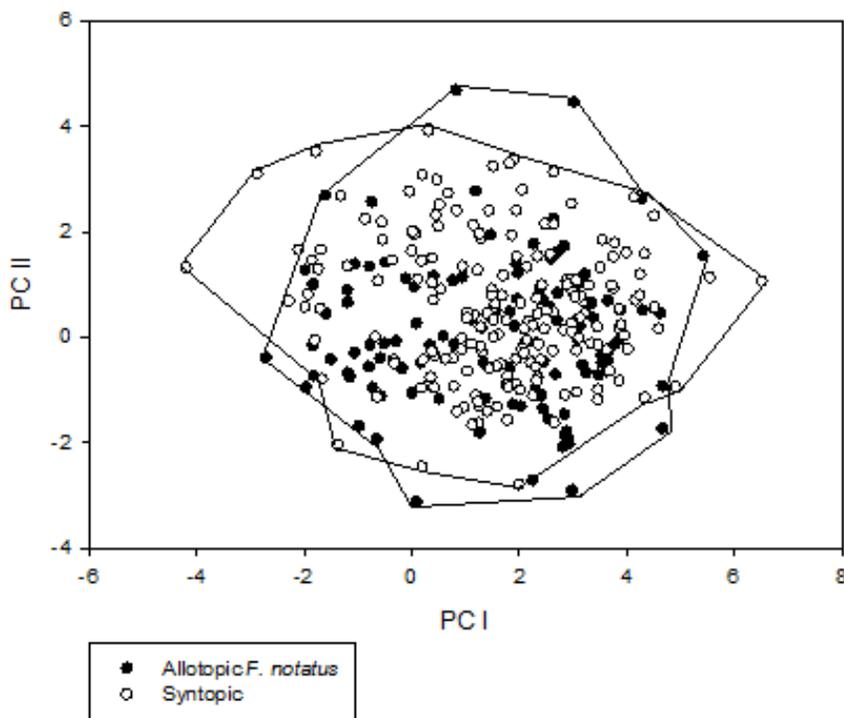


Fig. 6. PCA of all *F. notatus* by syntopic or allotopic populations.

Table 4. NP-MANOVA results for morphological data (PCA axes) for *F. notatus* from the two drainages with syntopic and allotopic sites.

ALL <i>F. notatus</i> from the PAS and NEC drainages				
	F ₂	R ²	d.f.	<i>p</i>
Drainage	142.70146	0.3105	1	0.001 ***
Syn-Allotopic	11.38481	0.0248	1	0.001 ***
Sex	8.95612	0.0195	1	0.001 ***
Age class	100.96181	0.2197	1	0.001 ***
Drainage*Syn-Allotopic	2.61065	0.0057	1	0.052 n.s.
Drainage*Sex	1.25080	0.0027	1	0.244 n.s.
Drainage*Age class	2.16532	0.0047	1	0.104 n.s.
Sex*Age class	1.10442	0.0024	1	0.346 n.s.
Sex*Syn-Allotopic	0.23934	0.0005	1	0.846 n.s.
Age class*Syn-Allotopic	1.59685	0.0035	1	0.176 n.s.
Drainage*Syn-Allo*Age class	1.73240	0.0037	1	0.142 n.s.
Drainage*Syn-Allo*Sex	1.16160	0.0025	1	0.334 n.s.
Drainage*Age class*Sex	0.62884	0.0014	1	0.583 n.s.
Syn-Allo*Age class*Sex	2.43259	0.0053	1	0.067 n.s.
Residuals		0.3938	181	

Diet Analyses

Identification of some prey items was difficult because some were partially digested and unidentifiable even with the use of Rose Bengal stain. Those prey items were enumerated and classified as unidentifiable. A total of 311 *F. notatus* digestive tracts were analyzed (PAS n = 129; PRL n = 114; NEC n = 68). Non-Metric Multidimensional Scaling (NMDS) was used to visualize diet data in ordination space where stomach contents from the three drainages generally clustered separately, although there is somewhat broad overlap (Fig. 7).

ISA identified different significant indicator prey items (Table 5) in each of the three drainages. Chironomidae, Scirtidae, and *Solenopsis invicta* (fire ants) were significant indicator species ($p = 0.002$, 0.001 and 0.001 , respectively) for the PAS. Notonectidae, Hemiptera, Mollusca and invertebrate eggs were

significant indicators ($p = 0.003, 0.036, 0.034$ and 0.001 respectively) for the PRL. The NEC had Cladoceran and unidentifiable prey items as significant indicators ($p = 0.003$ for both). There was a large number of invertebrate eggs ($n = 2242$) in the digestive tracts of *F. notatus*. One fish consumed 700 invertebrate eggs, which were probably consumed as one large cluster. Chironomidae ($n = 1133$) made up the largest percentage (19.30%) of prey items. Fire ants (*Solenopsis invicta*) ($n = 446$) were the terrestrial invertebrate that was consumed the most (7.60%). Aquatic insect larvae ($n = 348$) was another highly consumed prey item (5.93%).

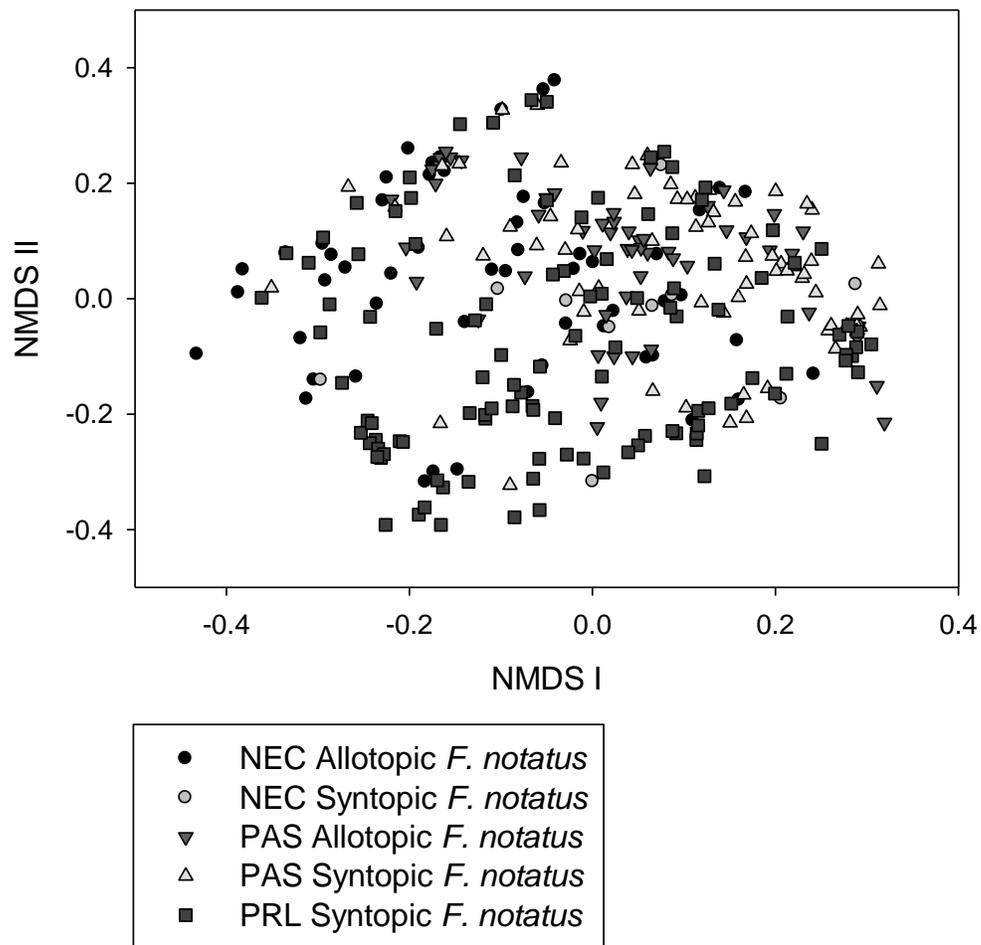


Fig. 7. NMDS of *F. notatus* diet data by drainage and syntopic and allotopic populations.

Table 5. Relative frequency (proportion of stomachs containing that item), relative abundance (percentage of items in each category), indicator value and the significance of that indicator value for all prey items found in *F. notatus* stomachs by drainage. Indicator values that are statistically significant are bolded.

Prey Item	Relative Frequency %			Relative Abundance %			Indicator Value			Sig.
	PAS	PRL	NEC	PAS	PRL	NEC	PAS	PRL	NEC	
Fire ant	70.08	36.84	52.94	46.04	22.16	32.9	0.3226	0.08	0.1707	0.003
Chironomidae	79.53	48.53	48.53	58.36	26.3	15.75	0.4642	0.1622	0.0757	0.001
Scirtidae	21.26	2.63	2.94	94.09	2.38	3.99	0.2	0.0006	0.0011	0.001
Gerridae	15.75	5.26	13.24	54.65	12.45	33.92	0.0861	0.0064	0.044	0.103
BlackAnt	3.1	1.75	1.47	43.05	36.53	20.42	0.0133	0.0064	0.003	0.694
Diptera	12.6	18.42	11.76	15.08	23.93	61.63	0.02	0.0437	0.072	0.681
Coleoptera	23.26	20.18	13.24	40.74	43.8	15.46	0.0948	0.0884	0.0205	0.456
Coccinellidae	0	0.88	1.47	0	37.36	62.64	0	0.0033	0.0092	0.704
Notonectidae	3.15	11.4	0	15.22	84.98	0	0.0048	0.0967	0	0.001
Hemiptera	0.78	6.14	2.94	3.5	83.21	13.29	0.0003	0.0511	0.0039	0.033
Homoptera	3.1	2.63	0	54.09	45.91	0	0.0168	0.0121	0	0.532
Trichoptera	14.73	11.4	2.94	40.12	40.86	19.03	0.0591	0.0466	0.0056	0.385
Orthoptera	1.55	2.63	1.47	27.43	46.56	26.02	0.0043	0.0123	0.0038	0.859
Ephemeroptera	1.57	1.75	2.94	27.74	32.07	53.76	0.0004	0.0049	0.0138	0.68
Thrip	0	4.39	2.94	0	64.15	35.85	0	0.0281	0.0105	0.194
Spider	4.65	10.53	5.88	20.39	53.83	25.78	0.0095	0.0567	0.0152	0.143
Cladoceran	0	0	5.88	0	0	100	0	0	0.0588	0.004
Megaloptera	0.78	0.88	1.47	9.91	33.66	56.43	0.0008	0.003	0.0083	0.945
Algae	2.33	14.04	13.24	6.55	39.56	53.89	0.0015	0.0555	0.0713	0.093
Vegetation	2.33	6.14	5.88	40.85	14.86	44.29	0.0095	0.0091	0.0261	0.683
FishScales	6.98	0.88	2.94	63.77	10.31	25.92	0.0445	0.0009	0.0076	0.07
Watermites	4.65	6.14	13.24	36.42	25.19	38.39	0.0169	0.0155	0.0508	0.334
Larva	20.16	41.23	39.71	33.67	35.72	30.61	0.0679	0.1473	0.1215	0.42
Pupa	13.18	2.63	10.29	43.73	39.45	16.82	0.0576	0.0104	0.0173	0.359
Invertebrate Eggs	0	42.11	2.94	0	56.73	43.27	0	0.2389	0.0127	0.001
Mollusca	0.78	4.39	0	0.24	99.76	0	0	0.0438	0	0.035
Unidentifiable	40.16	37.72	54.41	29.19	22.95	47.86	0.1247	0.0843	0.2535	0.003
Parts	8.66	11.4	8.82	29.8	44.1	26.09	0.0275	0.0489	0.0224	0.544

Table 6. Relative frequency (proportion of stomachs containing that item), relative abundance (percentage of items in each category), indicator value and the significance of that indicator value for all prey items found in *F. notatus* stomachs in allotopic or syntopic sites in the Neches and Pascagoula drainages. Indicator values that are in bold are significant indicator values.

Prey Item	Relative Frequency %		Relative Abundance %		Indicator Value		Sig.
	Allotopic	Syntopic	Allotopic	Syntopic	Allo	Syn	
Fire ant	65.79	47.18	58.83	41.17	0.387	0.1942	0.002
Scirtidae	6.14	12.82	9.14	90.86	0.006	0.1165	0.003
Gerridae	19.3	6.67	83.99	16.01	0.1621	0.0107	0.001
Coccinellidae	0	1.03	0	100	0	0.0103	0.008
Thrip	1.75	2.56	36.31	63.69	0.0064	0.0163	0.003
Invertebrate Eggs	0.88	25.13	0.07	99.92	0	0.2511	0.001
Unidentifiable	57.02	33.85	41.98	58.02	0.4016	0.1	0.001

There were no significant differences in MPS between sexes, age classes or syntopic-allotopic populations (Table 7).

Table 7. Sample size, mean and standard deviation maximum prey size (MPS) and results of t-tests comparing MPS among various groups of *F. notatus*.

	n	Mean	STD	<i>p</i>
Females	121	0.5113	0.1761	0.2773
Males	112	0.5446	0.1948	
Adults	104	0.5178	0.1923	0.4977
Juveniles	129	0.5350	0.1806	
Syntopic	171	0.5055	0.1834	0.8743
Allotopic	62	0.5876	0.1841	

There were significant differences in EPV between *F. notatus* sexes (Table 8). Females had a significantly greater ($p < 0.0204$) EPV than males (0.3281 and 0.3075 respectively). Adult *F. notatus* (0.3690 mm³/mm) had a significantly ($p < 0.0001$) greater EPV than juveniles (0.3690 and 0.2671

respectively). Finally, allotopic populations had significantly greater ($p < 0.0360$) EPV than syntopic populations (0.3282 and 0.3141, respectively) (Table 8).

Table 8. Sample size, mean and standard deviation of standardized estimated prey volume (EPV) and results of t-tests comparing EPV among various groups of *F. notatus*.

	n	Mean mm ³ /mm	STD	<i>p</i>
Females	151	0.3281	0.212	0.0204
Males	120	0.3075	0.1728	
Adults	138	0.3690	0.2189	<0.0001
Juveniles	133	0.2671	0.1522	
Syntopic	176	0.3141	0.1826	0.036
Allotopic	95	0.3282	0.2182	

Discussion

As expected, there were significant differences in the feeding-related morphology between *F. notatus* and *F. olivaceus*. There were also significant differences between the drainages for both species, which was unexpected. It was also clear that both species changed in similar ways among the drainages, primarily in having smaller head and mouth measures in the PAS. There were significant differences between size classes (ontogenetic shift) and between syntopic and allotopic populations of *F. notatus* (character displacement), which were also expected. There were significant differences in *F. notatus* diet between the three drainages, between the age classes and between syntopic and allotopic populations. While ontogenetic and syntopic/allotopic changes were generally expected and consistent with ecological theory, the dramatic differences among drainages in both diet and morphology were not expected and changed the dynamics of the study.

Although the two species have been reported to have morphological similarities (Thomerson & Wooldridge 1970; Blanchard 1996), Schaefer et al. (2011) studied general body shape and reported (i) differences between the species, (ii) substantial amounts of variability among drainages within species and (iii) fish from the Pascagoula (both species) were shaped differently from other drainages. These findings are consistent with the patterns I observed in feeding-related morphometric differences in the three drainages sampled as part of this study. While both species have similar body forms, *F. olivaceus* are typically found in faster flowing, clear headwaters, whereas *F. notatus* are typically found in turbid, slack backwater and downstream habitats (Braasch & Smith 1965; Etnier & Starnes 2001; Ross 2001). Schaefer et al. (2011) hypothesized that these general habitat differences contributed to body shape patterns (either through local adaptation or plastic responses to flow regimes). The variations in head morphologies found within this study could similarly be due to habitat adaptation, feeding adaptations or plasticity. Between habitats where the two species are usually found, allochthonous input of terrestrial invertebrates is expected to be much higher in headwater habitats (greater canopy cover and subsequent exposure to riparian vegetation) that typically favor *F. olivaceus*. Thus, species differences in feeding morphology might be attributed to these differences in available prey. If studied, I would predict *F. olivaceus* diets to consist of more terrestrial invertebrates.

There were highly significant differences in feeding-related morphometrics between the three drainages. In the PAS, both species had measures consistent

with smaller heads and mouths. These drainage level differences actually accounted for more morphological variation than species level differences, an unexpected pattern. Possible explanations for the variability among drainages could be due to local adaptation to the individual systems, ecological/morphological drift or plasticity. Phenotypic plasticity, which is variation, under environmental influence, in the phenotype associated with a single genotype, can be adaptive by allowing individuals to achieve a higher fitness. Plasticity can evolve and is adaptive when it allows individuals to adjust their phenotype to increase their fitness in a particular environment. Given that fish head and mouth morphology is known to be plastic and that the diets differed across drainages, plastic responses could well be responsible for the observed variability among drainages. However, in a separate study, Schaefer et al. (unpublished) reared PAS *F. olivaceus* and PRL *F. notatus* in common garden mesocosms in syntopic and allotopic treatments. When the same morphological traits were measured in those fish as adults, the same drainage-specific patterns were observed indicating these are likely not plastic responses.

Local adaptation to individual systems occurs when change in a trait increases individuals' ability to survive or reproduce compared to individuals without the trait. If a population is reproductively isolated, the frequency of that trait will increase. Ecological or morphological drift could result in random change of a trait (not under strong selection) if a population is isolated, resulting in variability among drainages over time. However, feeding related morphology was specifically chosen for this study because it is directly related to acquisition of

resources and likely under some selective pressure that would not allow random drift. Given the differences in *F. notatus* diet among drainages, local adaptation seems more likely than drift. Additional data on variability in *F. olivaceus* diet among drainages would provide additional support for this hypothesis.

There were significant feeding-related morphometric differences in *F. notatus* females and males in all three contact zones indicating that *F. notatus* do exhibit sexual dimorphism. However, the sexually dimorphic differences were not consistent between drainages. The PAS female *F. notatus* had significantly larger BW, HW, GH and GW than the males. In the PRL, females had larger BW, HW, IO and ORB whereas males had larger mouths and head morphometrics. In the NEC, *F. notatus* females had significantly larger BW, HW, IO and ORB than *F. notatus* males. There are a number of sexually dimorphic traits in these species including males having elongated dorsal and anal fins and a greater density of dorsolateral spots (Ross 2001; Boschung & Mayden 2004).

Ontogenetic differences were evident in all three drainages, but were again not consistent among drainages. In PAS and PRL, the juvenile *F. notatus* have larger HL, HW, IO, ORB, POST and SNOUT. While in the NEC, the juveniles had larger HW, IO, ORB and POST. Juveniles in the three drainages have larger ORB than adults indicating a possible visual advantage. Juveniles in the PAS and PRL have larger HL and SNOUT than adults which could be due to a swimming or hydrodynamic advantage. Killifishes characterized as benthic (*Orestias albus* and *O. luteus*) and feed primarily on molluscs have larger opercle

and longer heads than those characterized as littoral or pelagic, *O. jussiei* and *O. agassi* respectively (Maldonado et al. 2009).

The amount of feeding-related morphological variation could be sufficient enough to allow the continued coexistence of both species in syntopic populations. The PAS *F. notatus* in syntopic populations with *F. olivaceus* have larger BD, HD, GH, GW, M and D than PAS allotopic populations. *F. notatus* in syntopic populations with *F. olivaceus* (PAS and NEC) were found to have significantly larger BD, HD, GH, GW, M and D than *F. notatus* in allotopic populations. This could be a result of competitive interactions with *F. olivaceus* in the PAS. Again, syntopic and allotopic populations differences were only about 1.5% of the variation.

Although the morphological differences are not consistent across the three drainages, the different variations in each of the three drainages could be similar in other contact zones within those drainages. It should be noted that *F. olivaceus* are found in *F. notatus* type habitats where the contact zones were encountered and sampled. These significant differences in feeding-related morphology could contribute to differences in diet. Attempting to locate and document new contact zones in these drainages and comparing these multiple contact zones within a single drainage could be very informative in future ecological studies of these two species.

Diet

Diet of *F. notatus* has been reported to be predominantly terrestrial invertebrates, littoral and benthic aquatic invertebrates and filamentous algae

(Thomerson & Wooldridge 1970; Atmar & Stewart 1972; Etnier & Starnes 2001; Ross 2001). While the present study documents generally similar diets, there were diet differences among drainages as well as between syntopic populations and allotopic populations. The PAS *F. notatus* have the most significant indicator species. PAS allotopic populations have the greatest number of indicator species (5), Gerridae, Diptera, Trichoptera, pupa and unidentified prey items. *Solenopsis invicta*, which is an invasive species, is a significant indicator species in the PAS. *F. notatus* will swim upstream and downstream along the stream margin and capture *S. invicta* (fire ants) when they are drawn from the bank onto the water surface by ripples from wave action caused by wind (personal observation).

The variability in diet between drainages could be due to the spatial difference in drainages (e.g., local land use) as well as temporal difference (e.g., daily weather) in sampling of the three drainages. Attempting to quantify prey availability when these contact zones were sampled may have contributed to the diet portion of this study. However, all three drainages were sampled during a two week period. If the available prey items are the same across the three drainages, then these *F. notatus* may be actively selecting different prey items within those drainages. A prey selection experiment involving *F. notatus* and *F. olivaceus* in syntopic and allotopic mesocosms from each of the three drainages would be beneficial in addressing this question.

Understanding the diet of two ecologically and morphologically similar species is important in the persistence of the two species. When the two species occur in contact zones and food resources become limited, having knowledge of

their respective diets could potentially lead to conservation implications which can be beneficial to the continued existence of the two species.

Again, attempting to locate and document new contact zones in these drainages and comparing these multiple contact zones within a single drainage could be very informative in future ecological studies of these two species. For example, if this study had been conducted on the three contact zones within one drainage, instead of three contact zones in three drainages the conclusions might have been quite different. On the other hand, while the convergence on different morphology by both species in the PAS was problematic in this study, it is an interesting result that raises a number of new questions. It should also be noted that in this study there were a suite of environmental parameters measured at the three contact zones during collection of *Fundulus*, however these parameters were not included in the analyses of this study. Incorporating the environmental parameters in future studies may also reveal important information concerning these contact zones. Locating contact zones in other drainages especially on a latitudinal scale (i.e., northern contact zones) from the three drainages in this study may also reveal morphological and dietary differences. Identification and analyses of digestive tract contents from *F. olivaceus* should be examined and compared to that of *F. notatus* in allotopic and syntopic populations. This would help clarify whether the same prey items are being utilized by the two species and if there is direct competition in prey selection. If they are utilizing the same prey items, quantifying available prey during sampling of *Fundulus* could be important in determining the degree of competition for prey.

In syntopic populations, there may be some interspecific competition, but the two species are able to coexist, to some extent, due to allochthonous input of prey items. The amount of interspecific competition is similar to if not less than the amount of intraspecific competition within one of the species. Therefore, their coexistence is possible.

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APPENDIX

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE APPROVAL FORM



The University of
Southern Mississippi

Institutional Animal Care
and Use Committee

118 College Drive #5147
Hattiesburg, MS 39406-0001
Tel: 601.266.6820
Fax: 601.266.5509
www.usm.edu/spa/policies/animals

**INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE
NOTICE OF COMMITTEE ACTION**

The proposal 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 three year 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 (see attached) 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: **10100107**
PROJECT TITLE: **Collaborative Research: Phylogeography, Ecology and Reproductive Isolation in the *Fundulus notatus* Complex**
PROPOSED PROJECT DATES: **09/01/2007 to 09/31/2013**
PROJECT TYPE: **Renewal of a Previously Approved Project**
PRINCIPAL INVESTIGATOR(S): **Jake Schaefer, Ph.D.**
COLLEGE/DIVISION: **College of Science & Technology**
DEPARTMENT: **Biological Sciences**
FUNDING AGENCY/SPONSOR: **National Science Foundation**
IACUC COMMITTEE ACTION: **Full Committee Review Approval**
PROTOCOL EXPIRATION DATE: **09/30/2013**


Robert C. Bateman, Jr., Ph.D.
IACUC Chair

9/21/2010
Date