

# Diet and Movement of the Killifish, *Fundulus heteroclitus*, in a Maine Salt Marsh Assessed Using Gut Contents and Stable Isotope Analyses

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**ABSTRACT:** Killifish are ecologically important components of salt marsh ecosystems, but no studies have determined the importance of locally produced versus allochthonous food sources on a scale of less than multiple kilometers. The goal of our study was to examine diet and movement of the killifish, *Fundulus heteroclitus*, collected from a Maine salt marsh to assess the importance of locally produced versus allochthonous food sources on a scale of several hundred meters. We compared the gut contents and stable isotope signatures of *F. heteroclitus* from four regions along the central river of a Maine salt marsh to the distinct food sources and isotopic signatures of the region of the marsh in which they were caught. *F. heteroclitus* were relying on locally produced food sources even on the scale of several hundred meters. They fed daily in a small area less than 6 ha and maintained relatively strong site fidelities over the course of several months. Phytoplankton and salt marsh detritus both contributed to the high production of *F. heteroclitus*; terrestrial plant detritus was not an important component of their diet. The diet and feeding patterns of *F. heteroclitus* from this small Maine salt marsh were similar to the patterns found in much larger salt marshes, suggesting that locally produced organic matter is essential to the production of these ecologically important fish.

## Introduction

Killifish are some of the most abundant and ecologically important fish in estuarine ecosystems throughout Africa, Asia, the Americas, and parts of Europe (Valiela et al. 1977; Kneib 1986; Gutierrez-Estrada et al. 1998; Hrbek and Meyer 2003). Killifish, such as *Fundulus heteroclitus*, feed on flooded marsh surfaces during high tide and return to creeks with the ebb tide to digest their food. In the creeks, *F. heteroclitus* become prey for higher trophic-level consumers, such as striped bass (*Morone saxatilis*), bluefish (*Pomatomus saltatrix*), white perch (*Monroea americana*), and blue crabs (*Callinectes sapidus*), which enter the marsh to feed and then return to open water (Kneib and Stiven 1978; Allen et al. 1994; Kneib 2000; Fell et al. 2003). *F. heteroclitus* are important as trophic links between the productive marsh surface and the adjacent open estuary.

Despite years of research on the habitat use and feeding ecology of *F. heteroclitus*, little is known about which sources of primary production support this important fish species. There has been great debate over the importance of locally produced (salt marsh organic matter) versus allochthonous (terrestrial and marine organic matter) food

sources in supporting highly productive estuarine food webs. Upland plant organic matter carried by terrestrial runoff, marine phytoplankton, and  $C_4$  salt marsh plants cycled through a detrital pathway have all been proposed to explain the high production of estuarine consumers such as *F. heteroclitus* (Nixon et al. 1986; Day et al. 1989; Keller et al. 1990; Mallin and Paerl 1994; Deegan et al. 1995). Determining the diet and movement of *F. heteroclitus* within a marsh is crucial to understanding the importance of locally produced versus allochthonous food sources for *F. heteroclitus*.

There is distinct spatial heterogeneity in the distribution, abundance, and isotopic composition of the dominant food sources within most salt marshes (Allen et al. 1994; Peterson 1999; Riera et al. 1999). The three major sources of primary production are upland-terrestrial  $C_3$  plant organic matter,  $C_4$  vascular salt marsh plants, and phytoplankton (Peterson and Howarth 1987).  $C_3$  terrestrial plants dominate the watershed surrounding the salt marsh and have relatively depleted  $\delta^{13}C$  ( $-29\text{‰}$ ) and  $\delta^{15}N$  ( $0\text{‰}$ ) values.  $C_4$  salt marsh plants are prevalent in the lower and middle marsh region and have the most enriched  $\delta^{13}C$  ( $-13\text{‰}$ ) values with intermediate  $\delta^{15}N$  ( $6\text{‰}$ ) values. Marine phytoplankton enter the marsh from the adjacent estuary and have intermediate  $\delta^{13}C$  ( $-21\text{‰}$ ) values and highly enriched  $\delta^{15}N$  ( $9\text{‰}$ ) values (Peterson 1999).

The presence of regionally distinct food sources makes it possible to evaluate the contribution of

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local and allochthonous food sources to the diet of *F. heteroclitus* using gut contents (Allen et al. 1994) and stable isotope analyses (Rau et al. 1983; Fry and Sherr 1984; Deegan and Garritt 1997; Riera et al. 1999). Gut content analyses present an instantaneous picture of diet and movement for a single feeding episode. *F. heteroclitus* are opportunistic, omnivorous feeders that consume food in proportion to its occurrence in the environment (Kneib and Stiven 1978), so gut contents indicate where in the marsh these fish are feeding on a daily time frame. Stable isotope analyses of *F. heteroclitus* reveal patterns of feeding habits integrated over several months (DeNiro and Epstein 1978, 1981; Peterson et al. 1986; Fry and Sherr 1984). Used in tandem, gut contents and isotopic analyses elucidate the short term (days) and long term (months) patterns of *F. heteroclitus* diet and movement that are crucial to understanding which food sources support the high production of *F. heteroclitus*.

Numerous studies have examined the importance of locally produced versus allochthonous food sources in salt marsh food webs; these studies were conducted on the scale of kilometers to hundreds of kilometers (Findlay et al. 1996; Deegan and Garritt 1997; Muylaert and Raine 1999; Riera et al. 1999; Bouillon et al. 2000). Previous research has assumed that in small estuaries, which are strongly influenced by terrestrial runoff and marine processes, allochthonous food sources would play a much more important role in the estuarine food web than in large estuaries (Busby 1995; Muylaert and Raine 1999). The tremendous variability in sources of primary production on the scale of hundreds of meters within a salt marsh (Donnelly and Bertness 2001; Ewanchuk and Bertness 2003) means that the diet and movement of *F. heteroclitus* must be examined on a much finer scale than previously assessed. Most studies on the diet and movement of *F. heteroclitus* were conducted in the southeastern United States (Teal 1962; Lotrich 1975; Baker-Dittus 1978; Kneib 1986; Teo and Able 2003). Although a few recent studies in the lower northeastern U.S. have expanded upon such knowledge (Connecticut: Allen et al. 1994; Massachusetts: Deegan and Garritt 1997), no studies have investigated the importance of locally produced versus allochthonous food sources for *F. heteroclitus* in the northern extent of its range (i.e., north of Massachusetts).

The goal of our study was to examine diet and movement of *F. heteroclitus* collected from the Sprague River salt marsh in Phippsburg, Maine, to determine the dietary contribution of locally produced versus allochthonous food sources on the scale of several hundred meters. We hypothesized that *F. heteroclitus* were feeding on local food sources rather than traveling throughout the marsh to feed,

and that their gut contents and stable isotopic composition would reflect the food items distinct to the areas in which they were caught. To test this hypothesis, we collected *F. heteroclitus* and possible food sources from four regions of the Sprague River salt marsh during the fall of 2003. Gut content analyses of *F. heteroclitus* were used to determine daily patterns of diet and movement, and stable carbon and nitrogen isotope analyses of *F. heteroclitus* muscle tissue were used to examine longer term (months) feeding habits. This study extends our knowledge of *F. heteroclitus* feeding habits to include its northern range and examines diet and movement of *F. heteroclitus* on a finer scale than previously assessed.

## Materials and Methods

### SITE DESCRIPTION

The Sprague River salt marsh is a 30 ha back barrier marsh in the Bates-Morse Mountain Conservation Area, Phippsburg, Maine (43°43.8'N, 69°49.7'W; Fig. 1). The Sprague River is a north to south trending river that bisects the salt marsh. It experiences a seasonal tidal range of 0.6 to 1.7 m, and has a strong mean summer salinity gradient from the embankment at the causeway (9‰) to the mouth of the river (31‰). Vegetation of the Sprague River salt marsh is characteristic of a typical New England salt marsh. Smooth cordgrass, *Spartina alterniflora*, dominates along the main channel and drainage ditches in the low marsh, and fields of salt meadow cordgrass, *S. patens*, inland saltgrass, *Distichlis spicata*, and some saltmarsh rush, *Juncus gerardii*, occupy the high marsh. Virginia glasswort, *Salicornia virginica*, is also present but comprises a relatively small proportion of the total plant matter biomass. The higher high marsh is primarily *J. gerardii* and upland shrubs transitioning to a forest of red maples, *Acer rubrum*.

### SAMPLE COLLECTION AND PREPARATION

All samples were collected during daylight hours at low tide on September 23, 2003 (0.3 m above mean low water), and October 21, 2003 (0.4 m). Samples for analyses were randomly selected from a pooled group consisting of samples from both collection dates. Samples were pooled to increase replicates for gut content analyses with the assumption that there would be minimal differences in the diet and stable isotopic composition of *F. heteroclitus* within the short sampling period. *F. heteroclitus* were collected from three sites along the Sprague River: at the northern end just south of the causeway (embankment site), the middle reach (middle site), and the river mouth (mouth site) with approximately 600 m between adjacent sites (Fig. 1). *F.*

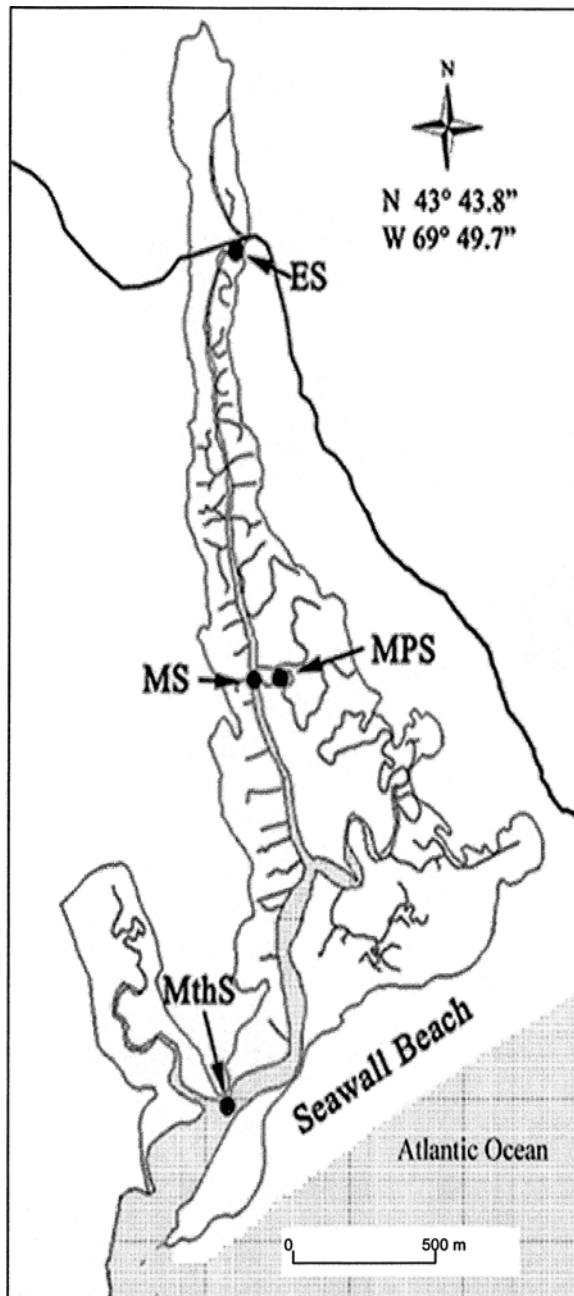


Fig. 1. The Sprague River salt marsh in the Bates-Morse Mountain Conservation Area, Phippsburg, Maine:  $43^{\circ}43.8'N$ ,  $69^{\circ}49.7'W$ . ES = embankment site, MS = middle site, MPS = middle pool site, and MthS = mouth site. Stippling represents water and the stippled line bisecting the marsh represents the Sprague River. Modified from Johnson et al. (In Press).

*heteroclitus* were also collected from the main marsh surface pool (middle pool site), which was approximately 20 m from the middle site (Fig. 1). At each site, one minnow trap, baited with chicken liver wrapped in cheesecloth to prevent consumption of the liver, was placed approximately 1 m deep and

1 m from the water's edge, parallel to shore to control for effects of varying access to the traps. The traps were left in the water for 1 h and collected in the order of their dispersal for optimum fish collection with minimal loss of small fish or digestion of food (Kneib and Stiven 1978). *F. heteroclitus* used for gut content analyses were immediately preserved in 12% formalin and transferred into 70% isopropyl alcohol after 24 h. Samples used for stable isotope analyses were packed on ice in the field and frozen in the lab at  $-20^{\circ}C$ .

Probable food sources of *F. heteroclitus* were collected from the Sprague River salt marsh on the days of *F. heteroclitus* collection. The following vascular plant species were sampled from the marsh surface between the middle and middle pool sites at the base of their stems: *S. alterniflora*, *S. patens*, *J. gerardii*, *D. spicata*, and *S. virginica*. *A. rubrum* was the dominant upland vegetation contributing to the terrestrially-derived organic matter in the Sprague River salt marsh. Recently fallen *A. rubrum* leaves were collected from the marsh surface at the embankment site. Macroalgae from the river bottom, such as knotted rack, *Ascophyllum nodosum*, and sea lettuce, *Ulva lactuca*, were collected from the middle and embankment sites, respectively. Daggerblade grass shrimp, *Palaemonetes pugio*, which are common prey items of *F. heteroclitus*, were collected from the river bottom at the middle site and adjacent ponds with dip nets.

One liter of river water was collected from each of the three river sites and passed through an ashed ( $500^{\circ}C$ ) GF/F filter ( $0.25 \mu m$ ) to extract particulate organic carbon (POC). Because POC represents a mixture of plant detritus, phytoplankton, and mineral material, atomic carbon to nitrogen (C : N) ratios were used to identify the dominant source of organic matter comprising the POC (terrestrial plant C : N  $>20$  and phytoplankton C : N  $<7$ ; Meyers 1994).

#### GUT CONTENT ANALYSIS

Gut content analyses were performed under a dissecting microscope on stomachs isolated from 25 *F. heteroclitus* from each site randomly selected from the pooled sample, resulting in a total of 100 fish analyzed. To evaluate the abundance of various food items in the diet, the relative volume of every food type in each gut was estimated visually and assigned a percentage of the total food volume. Following an F max test for homogeneity, non-homogeneous percent data were transformed with an arcsine square root transformation. A one-way analysis of variance (ANOVA) with a Bonferroni post hoc test was used to compare the abundance of gut components among sites. A two-tailed *t*-test was

TABLE 1. Characteristics and the percent volume (mean [ $\pm$  SD]) of the gut components in *Fundulus heteroclitus* caught at four sites along the central river of the Sprague River salt marsh, Phippsburg, Maine. Differences among regions were determined using a one-way ANOVA (\*\*\*) $p < 0.0001$ , (\*\*) $p < 0.01$ , (\*) $p < 0.05$ , (°) $p > 0.05$ . Means with the same letter are not significantly different from each other based on a Bonferroni post hoc test ( $p < 0.05$ ).  $n = 3$  fish for isotope analyses and 25 fish for all other analyses.

	Embankment	Middle	Middle Pool	Mouth	F ratio
<b>Characteristics</b>					
$\delta^{13}\text{C}$ (‰)	-19.3 (1.9) <sup>a</sup>	-16.2 (0.8) <sup>b</sup>	-21.4 (3.5) <sup>a</sup>	-20.7 (1.7) <sup>a</sup>	3.2*
$\delta^{15}\text{N}$ (‰)	9.6 (0.1)	9.0 (0.7)	9.8 (0.8)	8.8 (0.8)	1.7 <sup>°</sup>
Length (mm)	52.6 (9.9) <sup>a</sup>	45.9 (2.3) <sup>a</sup>	22.0 (5.4) <sup>b</sup>	47.6 (3.8) <sup>a</sup>	59.2***
Weight (g)	1.6 (0.7) <sup>a</sup>	1.4 (0.3) <sup>a</sup>	0.7 (0.1) <sup>b</sup>	1.4 (0.3) <sup>a</sup>	22.9***
<b>Gut components</b>					
Unidentified plant detritus	72 (18) <sup>ab</sup>	58 (26) <sup>a</sup>	78 (33) <sup>ab</sup>	78 (41) <sup>b</sup>	19.1***
<i>Acer rubrum</i>	8 (10) <sup>a</sup>	1 (3) <sup>b</sup>	0 <sup>b</sup>	0 <sup>b</sup>	12.7***
<i>Gammarus</i> sp.	6 (15)	6 (15)	14 (26)	14 (34)	2.2 <sup>°</sup>
<i>Ulva lactuca</i>	11 (14) <sup>a</sup>	5 (9) <sup>ab</sup>	2 (8) <sup>b</sup>	0 <sup>b</sup>	6.7**
Marine mite	3 (5)	6 (17)	1 (2)	8 (28)	3.4 <sup>°</sup>
Nematoda	0 <sup>a</sup>	13 (15) <sup>b</sup>	0 (2) <sup>a</sup>	0 <sup>a</sup>	17.4***
<i>Mytilus edulis</i>	0 <sup>a</sup>	16 (31) <sup>b</sup>	2 (6) <sup>a</sup>	0 <sup>a</sup>	6.3**
Foraminifera	0 <sup>a</sup>	1 (2) <sup>b</sup>	0 (0) <sup>a</sup>	0 <sup>a</sup>	7.4**
<i>Philoscia</i> sp.	0 <sup>a</sup>	0 <sup>a</sup>	3 (7) <sup>b</sup>	0 <sup>a</sup>	4.5*
<i>Littorina littorea</i>	0	0	0 (1)	0	2.1 <sup>°</sup>
Total plant	91 (16) <sup>a</sup>	64 (26) <sup>b</sup>	80 (34) <sup>a</sup>	78 (41) <sup>a</sup>	22.9***
Total animal	9 (16) <sup>a</sup>	36 (26) <sup>b</sup>	20 (34) <sup>a</sup>	22 (41) <sup>a</sup>	22.9***

used to assess differences in percent volume of total plant and animal matter among fish.

#### STABLE ISOTOPE ANALYSIS

Stable carbon and nitrogen isotope analyses were performed on three individuals for each species. *F. heteroclitus* of similar size to the individuals used in gut content analyses were selected for isotopic analyses. All samples were freeze dried prior to stable isotope analysis. Approximately 0.4–0.6 mg of tail muscle samples from *F. heteroclitus* and *P. pugio*, leaf samples from vascular plants, thallus samples from macroalgae, and POC from water column filters were analyzed for stable carbon and nitrogen isotope signatures and C : N ratios using a ThermoFinnigan Delta Plus Advantage stable isotope ratio mass spectrometer (IRMS) coupled to a Costech elemental analyzer via a Conflo III combustion interface in the Environmental Geochemistry Laboratory, Department of Geology, Bates College. Stable isotope ratios were expressed in  $\delta$  notation according to the following definition:

$$\delta X(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is  $^{13}\text{C} : ^{12}\text{C}$  or  $^{15}\text{N} : ^{14}\text{N}$ , and the standards were vienna pee dee belemnite (VPDB) and air for carbon and nitrogen, respectively. The accuracy and precision of the IRMS was determined by multiple analyses of a working standard (acetanilide:  $\text{C}_8\text{H}_9\text{NO}$ ) run every sixth sample. The reproducibility for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements was  $\pm 0.2\text{‰}$ . Following an F max test for homogeneity, differences in the isotopic signatures of *F. heteroclitus* among sample sites were

compared using a one-way ANOVA and Bonferroni post hoc test.

#### Results

Large quantities of fallen terrestrial *A. rubrum* leaves were observed on the marsh surface and river bottom at the embankment site. The middle site was the only area of the salt marsh with a visible population of *Mytilus edulis*, and the middle pool site was the only site where *Littorina littorea* were observed. *F. heteroclitus* from the middle pool site were significantly shorter than the *F. heteroclitus* from the three river sites (Table 1). *F. heteroclitus* from the river sites had a mean total length of  $48.7 \pm 6.8$  mm within a range of 41.1–70.0 mm, while *F. heteroclitus* from the middle pool site averaged  $22.0 \pm 5.5$  mm within a range of 14.1–36.5 mm. *F. heteroclitus* from the middle pool site,  $0.7 \pm 0.1$  g, were also significantly lighter than the *F. heteroclitus* from the three river sites,  $1.5 \pm 0.5$  g. Based on the *F. heteroclitus* size-age determinations of Kneib and Stiven (1978), the larger *F. heteroclitus* from the river sites were all adults in their second growing season; *F. heteroclitus* from the middle pool site were young of the year (i.e., within their first growing season).

#### GUT CONTENT ANALYSIS

All 100 *F. heteroclitus* stomachs examined contained food. *F. heteroclitus* from the middle pool site had the highest species richness in their diet (9 species; Table 1). The percent volume of plant and algal matter (78%) was significantly greater than animal matter (22%) in the stomachs of *F. heteroclitus* at the Sprague River salt marsh. Marine mites

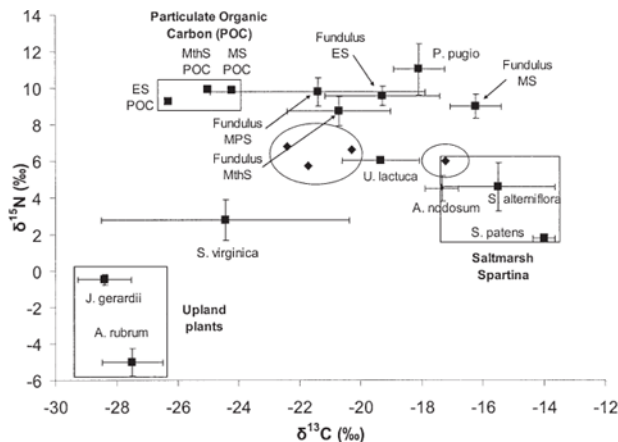


Fig. 2. Dual stable isotope plot of nitrogen versus carbon for *Fundulus heteroclitus*, their theoretical diet and possible food items from four regions in the Sprague River salt marsh, Phippsburg, Maine. The isotopic composition of the theoretical food sources of *F. heteroclitus* (circled diamond symbols) were determined using a 1‰ carbon and 3‰ nitrogen correction factor applied to the muscle tissue of *F. heteroclitus*, which accounted for isotopic fractionation during protein synthesis (Peterson and Howarth 1987). ES = embankment site, MS = middle site, MPS = middle pool site, and MthS = mouth site.  $n = 3$  individuals except for POC where  $n = 1$ .

(Halacaridae) and vascular plant detritus were found in *F. heteroclitus* at all four sites; but marine mites accounted for less than 8% of the total volume of gut components at any given site. *F. heteroclitus* from the embankment site had significantly greater quantities of *U. lactuca* and *A. rubrum* in their stomachs than *F. heteroclitus* from any other site (Table 1). *F. heteroclitus* from the middle site consumed a significantly greater volume of *M. edulis* than *F. heteroclitus* from any other site. *F. heteroclitus* from the middle pool site were the only fish to have *L. littorea* in their stomachs.

#### STABLE ISOTOPE ANALYSIS

The stable isotope composition of the dominant food sources available (upland-terrestrial  $C_3$  plant organic matter,  $C_4$  vascular salt marsh plants, and POC) defined three distinct fields in a  $\delta^{13}C$  versus  $\delta^{15}N$  plot (Fig. 2). Terrestrial  $C_3$  plants were depleted with respect to  $^{15}N$  relative to all other sources of food, and the  $C_4$  salt marsh plants were enriched with respect to  $^{13}C$  relative to all other sources of food. The POC from the mouth and embankment sites had C : N ratios of 7, while POC from the middle site had a C : N ratio of 22, indicating a higher abundance of phytoplankton at the embankment and mouth sites relative to the middle site. The macroalgae were isotopically similar to the  $C_4$  salt marsh plants.

*F. heteroclitus* from the middle site were significantly enriched in their  $\delta^{13}C$  signatures compared to

*F. heteroclitus* from the other sites. There was no significant difference in the  $\delta^{13}C$  values of *F. heteroclitus* from the embankment, mouth, or middle pool sites. The  $\delta^{15}N$  values of *F. heteroclitus* were not significantly different among the four sites (Table 1).

#### Discussion

The diets of *F. heteroclitus* from the Sprague River salt marsh were composed predominantly of primary producers (78%) according to gut content analyses (Table 1). These results support findings for *F. heteroclitus* from other salt marshes in the northeastern U.S. (Allen et al. 1994; Deegan and Garritt 1997), but differ from *F. heteroclitus* in the southeastern U.S., which consumed more annelids and crabs than plant matter (Baker-Dittus 1978; Kneib and Stiven 1978).

Much of the primary production in the stomachs of *F. heteroclitus* from the Sprague River salt marsh was unidentifiable plant detritus, but stable isotope analysis of *F. heteroclitus* elucidated information on the dominant types of primary producers being consumed in the salt marsh. The  $\delta^{13}C$  and  $\delta^{15}N$  composition of *F. heteroclitus* from the middle site indicates these fish were consuming primarily  $C_4$  salt marsh vegetation or macroalgae, whereas fish from the embankment and mouth sites were consuming a mixture of POC and  $C_4$  salt marsh plants (Fig. 2).

The isotopic signature of *F. heteroclitus* at the middle site is primarily a  $C_4$  salt marsh plant signal rather than a macroalgal signal, because the relative abundance of macroalgae to  $C_4$  salt marsh plants at the Sprague River salt marsh is very low. Previous studies have shown that vascular salt marsh plant matter can be made into a nutritious food source following bacterial degradation (Tenore 1983; Newell and Langdon 1986; Mann 1988). The employment of stable sulfur isotope analyses would help to determine the relative contribution of macroalgae and  $C_4$  salt marsh plants to the diet of *F. heteroclitus* (Peterson 1999).

Given that the POC at the mouth site was primarily phytoplankton and isotopically similar to POC values measured elsewhere in the Gulf of Maine (Incze et al. 1982), *F. heteroclitus* from the mouth site appear to be relying on a mix of marine phytoplankton and  $C_4$  salt marsh plant matter (Fig. 2). Marine phytoplankton has been shown to be readily consumed and assimilated into biomass by many estuarine food web constituents (Bayne et al. 1987; Crosby et al. 1989; Riera et al. 1999). This supports the findings of Riera et al. (1999) who concluded that marine phytoplankton was a major component of the diet for salt marsh inhabitants near the open estuary in Aiguillon Bay, France.

The POC at the embankment site was primarily phytoplankton and isotopically similar to POC values measured from the less saline upper regions of other estuaries (Fogel et al. 1992). The  $\delta^{13}\text{C}$  signature of *F. heteroclitus* from the embankment site indicates a mixed diet of locally produced phytoplankton and  $\text{C}_4$  salt marsh plant matter. Although terrestrial detritus, such as *A. rubrum* leaves, were frequent food items in the stomachs of *F. heteroclitus* from the embankment site (Table 1), these fish were too enriched in their  $\delta^{15}\text{N}$  signatures to be assimilating upper marsh-terrestrial  $\text{C}_3$  detritus into biomass (Fig. 2). This may be due to the high phenol levels of upland plants, which make them significantly less palatable compared to organic matter from the lower marsh (Bayne et al. 1987; Crosby et al. 1989; Goranson et al. 2004). These results support the conclusions from previous research that indicate terrestrial detritus is not a significant part of most estuarine food webs (Coffin et al. 1989; Fry et al. 1992; Peterson et al. 1994; Deegan and Garritt 1997). *F. heteroclitus* are generally surface sediment feeders (Baker-Dittus 1978), and the frequent occurrence of *A. rubrum* leaf particles in the stomachs of *F. heteroclitus* from the embankment site was probably a by-product of consuming other food sources, such as *Gammarus* sp. from the benthos.

It was difficult to compare the diet of juvenile *F. heteroclitus* from the middle pool site to the adult fish from the adjacent river due to the much higher and more variable lipid content in the juvenile muscle tissue (de Vlaming et al. 1978; Kneib and Stiven 1978; Prinslow et al. 1974; Gutjahr-Gobell 1998). The  $\delta^{13}\text{C}$  signatures of lipids are significantly more depleted than proteins due to enhanced fractionation associated with lipid synthesis (DeNiro and Epstein 1977). In our study, the muscle tissue of *F. heteroclitus* was not solvent extracted, and we did not compare the diet of juvenile *F. heteroclitus* to that of the adults using stable isotope analyses.

Once the diet of *F. heteroclitus* at the Sprague River salt marsh was determined, the movement of these fish among the study sites could be assessed using gut contents and stable isotope analyses. Our results confirmed our hypothesis that *F. heteroclitus* were feeding on locally produced food sources even on the scale of several hundred meters. On a daily time frame, *F. heteroclitus* in the Sprague River salt marsh fed on locally produced food in the areas in which they were caught. Food items unique to a single location within the marsh were significantly more abundant in the stomachs of *F. heteroclitus* from those sites than from other sites within the marsh (Table 1). *M. edulis*, for example, were found in the stomachs of *F. heteroclitus* from the middle site but not the mouth and embankment sites, despite these

sites being less than 600 m apart. Although our data did not examine the night time feeding habits of *F. heteroclitus*, these fish are visual predators that feed primarily during the day (Weisberg et al. 1981), and the gut contents of these fish during the day are an accurate reflection of their normal feeding habits. These fish appear to be feeding on locally produced food sources within a range of less than 6 ha ( $600 \times 100$  m) of accessible marsh surface area. These findings support previous work by Lotrich (1975) and Teo and Able (2003), which suggest *F. heteroclitus* exhibit strong site fidelities over a short period of days to weeks.

The juvenile *F. heteroclitus* at the middle pool site showed even stronger site fidelities than the adults, according to gut content analyses. The juveniles were feeding primarily within the pool from which they were collected. These fish consumed large quantities of very small *L. littorea* (only located at this site), but relatively few of the similarly sized *M. edulis* from the middle site 20 m away. Salt marsh pools serve as nurseries for juvenile *F. heteroclitus* (Bartholomew 2002) and these fish were probably not traveling far beyond the protection of their pool.

As seen on a daily time frame, *F. heteroclitus* appeared to maintain relatively strong site fidelities over the course of several months according to stable isotope analyses. If *F. heteroclitus* were feeding throughout the marsh on a monthly time frame, then *F. heteroclitus* should express a homogenized isotopic signature no matter where in the marsh they were collected. We found that individuals adopted local isotopic signatures distinct to the region in which they were caught. *F. heteroclitus* from the middle site had an isotopic signature reflecting their enriched  $^{13}\text{C}$  ( $\text{C}_4$ ) surroundings, while *F. heteroclitus* from the mouth and embankment sites had isotopic signatures influenced by both depleted phytoplankton and enriched  $\text{C}_4$  salt marsh plant matter (Fig. 2). This indicates that *F. heteroclitus* were relying on locally produced food rather than traveling throughout the marsh to feed over the course of several months, even on the scale of several hundred meters. This does not support the model that in small estuaries allochthonous food sources would be the dominant food source in the estuarine food web (Busby 1995; Muylaert and Raine 1999).

Although our conclusions about the diet and movement of *F. heteroclitus* were based upon data collected in September and October, these findings do reflect the normal activity of *F. heteroclitus* during their most active period (i.e., late spring, summer, and early fall; Baker-Dittus 1978; Fell et al. 2003). Unlike some marsh fish, such as *Menidia menidia*, which move offshore during the winter changing their isotopic signature seasonally, *F. heteroclitus*

remain within their native estuary year-round and retain the isotopic signature of that estuary (Griffin and Valiela 2001). There is some seasonal fluctuation in the  $\delta^{13}\text{C}$  signatures of salt marsh constituents, with the winter signal (January) becoming more depleted than the summer signal (July–August; Simenstad and Wissmar 1985). If the  $\delta^{13}\text{C}$  signatures of the *F. heteroclitus* from our study were depleted compared to their July–August signal, then our conclusions would underestimate the importance of locally produced salt marsh plant matter to the diet of *F. heteroclitus* at the Sprague River salt marsh.

The diet of *F. heteroclitus* from the Sprague River salt marsh consisted primarily of locally available primary production even on the scale of several hundred meters. *F. heteroclitus* fed daily in a small area of less than 6 ha and maintained relatively strong site fidelities over the course of several months. Phytoplankton and salt marsh plant detritus both contribute to the high production of *F. heteroclitus*, and terrestrially derived organic matter was not an important component of their diet. The diet and feeding patterns of *F. heteroclitus* from Sprague River salt marsh in Phippsburg, Maine, were similar to the patterns found in larger salt marshes (Deegan and Garritt 1997; Bouillon et al. 2000, 2004; Allen et al. 1994; Akpan and Isangedighi 2004), indicating that locally produced organic matter is essential to the production of important estuarine constituents such as *F. heteroclitus*.

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