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Blue crab *Callinectes sapidus* dietary habits and predation on juvenile winter flounder *Pseudopleuronectes americanus* in southern New England tidal rivers

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ABSTRACT: Blue crabs *Callinectes sapidus* have expanded their geographic range northward in the NW Atlantic with possible trophodynamic effects on benthic communities. In this study, we examined the blue crab's diet in 2 southern New England tidal rivers (USA) and expounded on their predator–prey interaction with juvenile winter flounder *Pseudopleuronectes americanus*. Blue crabs (8–185 mm carapace width [CW]; n = 1835) were collected from the Seekonk River, Rhode Island, and Taunton River, Massachusetts, between May and August 2012 to 2016, and their feeding habits were assessed via stomach content, stable isotope, and molecular genetic analyses. Blue crabs were found to be generalist carnivores–omnivores with diets varying throughout ontogeny, yet shifts in prey composition had no effect on size-based nitrogen isotope signatures and trophic position (3.50 ± 0.35, mean ± SD). Carbon isotope values indicated that detritus–macroalgae were the dominant carbon source to the food web, with additional contributions from terrestrially derived organic matter and phytoplankton in oligohaline and polyhaline waters, respectively. The main prey of blue crabs ≤49 mm CW were amphipods, shrimp, and unidentified crustaceans, and larger conspecifics fed on bivalves, crabs, and fish. Winter flounder remains, e.g. sagittal otoliths, were identified in the diet of 2.5% of field-collected blue crabs, whereas PCR-based assays detected winter flounder DNA in 17.7% of crab stomachs. Blue crabs 23 to 160 mm CW preyed on winter flounder ranging from 26 to 66 mm total length, with occurrences of predation most closely associated with increases in crab size. Blue crab predation on winter flounder also varied spatially in the rivers, reflecting site-specific differences in flounder densities, abundances of other preferred prey, and dissolved oxygen concentrations that altered predator–prey dynamics. Lastly, the current predatory impact of blue crabs on juvenile winter flounder is nearly equivalent to other portunid crab species. Anticipated temperature-mediated increases in blue crab densities at northern latitudes, however, will intensify the predator-induced mortality of winter flounder and likely hinder their recovery in southern New England.

KEY WORDS: *Callinectes sapidus* · *Pseudopleuronectes americanus* · Blue crab · Winter flounder · Tidal river · Diet · Carbon isotope · Nitrogen isotope · PCR · Predation · Trophic position

1. INTRODUCTION

The blue crab *Callinectes sapidus* Rathbun is a portunid crustacean with a broad distribution extending from Cape Cod (Massachusetts, USA) to Argentina. Blue crabs are mainly concentrated in the Middle and South Atlantic Bight along the eastern USA (Delaware to Florida), and their bio-ecological characteristics have been extensively studied in this region (Kennedy & Cronin 2007). In contrast, there

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are few investigations focused on blue crabs at higher latitudes, with these areas historically sustaining smaller and, in some instances, ephemeral populations (Johnson 2015). The northern extent of the blue crab's geographic range is constrained by its physiological tolerance to cold water temperatures (Smith & Chang 2007, Bauer & Miller 2010). Blue crab growth, for example, is reduced at temperatures <10°C, and appreciable mortality occurs in juveniles and adult females at <3°C (Rome et al. 2005, Brylawski & Miller 2006), consequently limiting population sizes at northern latitudes (Bauer & Miller 2010). Recent studies, however, document a northward expansion in the distribution of blue crabs, such that crabs periodically occupy the Gulf of Maine (Johnson 2015) and larger, permanent populations are established in southern New England estuarine and nearshore coastal habitats (Taylor & Fehon 2021). The range extension and population increase of blue crabs at northern latitudes is presumably caused by warming water temperatures and the associated increase in crab over-wintering survival and growth (Hines et al. 2010, Hare et al. 2016, Glandon et al. 2019), which, in turn, may have repercussions to the trophic ecology and benthic community composition of the area.

Blue crabs are dominant opportunistic predators and scavengers throughout their geographic range, capable of regulating benthic prey populations (Hines et al. 1990, Seitz et al. 2005). The expansive dietary breadth of this species includes plant material, detritus, polychaetes, mollusks, and crustaceans (Hines 2007). Visual analyses of blue crab stomach contents also indicate that pelagic and epibenthic fish are an important food resource in select habitats (Hines 2007), accounting for 14 to 38%, by volume or weight, of the total diet (Ropes 1989, Hines et al. 1990, Fitz & Wiegert 1991). However, with few exceptions (e.g. Laughlin 1982), empirical studies that directly analyzed blue crab feeding habits rarely identified piscine prey to the genus or species taxonomic level.

Direct examination of a predator's stomach contents is the fundamental method for determining trophic interactions in natural systems. While providing valuable insights into diet composition and feeding ecology, stomach content analysis is inherently limited (Amundsen & Sánchez-Hernández 2019). First, direct analysis of stomach contents only reflects recent feeding activity of the predator, and gastric evacuation rates vary across prey taxa. To address this limitation, stable isotope analysis is routinely used to quantify the trophic position of an individual as a function of its time-integrated diet history (Michener & Schell 1994). Nitrogen isotopic signatures ($^{15}N/^{14}N$), specifically, are effective at quantifying the trophic position of an organism because enrichment of the heavier isotope (^{15}N) occurs incrementally across trophic levels at a constant rate (~3–4‰; Michener & Schell 1994). Carbon isotopic signatures ($^{13}C/^{12}C$), in contrast, are relatively consistent across trophic levels, i.e. <1‰ change between primary producer and consumer (Fry & Sherr 1984), but are valuable biomarkers for identifying different sources of primary production, including autochthonous (phytoplankton, benthic micro- and macroalgae) and allochthonous (terrigenous organic matter) sources (Peterson & Howarth 1987, France 1995).

Stomach content analysis is secondarily limited by the rapid deterioration of prey after initial ingestion (Amundsen & Sánchez-Hernández 2019); therefore, recovered tissues are unrecognizable at fine-scale taxonomic levels. The direct observation of crustacean stomach contents is especially problematic because chelae, mandible, and gastric mill actions macerate tissues, often precluding species-specific identifications of ingested prey (Taylor 2004, 2005a). Biochemical techniques offer a complementary approach to visual observations by identifying prey proteins or genetic material in a predator's stomach. Molecular genetic assays, for example, have successfully identified specific fish species in the stomachs of decapod crustaceans, including crangonid shrimps and portunid crabs (Saitoh et al. 2003, Albaina et al. 2010, Collier et al. 2014). These investigations further posit that predatory decapods are an important source of mortality of flatfish during the benthic juvenile stage, and, thus, ostensibly regulate year-class strength and recruitment success. With respect to blue crabs, PCR-based methods detected the DNA of winter flounder *Pseudopleuronectes americanus* in the diet of ~22% of crabs collected from the Shinnecock Bay (New York, USA), suggesting that blue crabs are an important predator of post-settlement winter flounder (Collier et al. 2014).

Winter flounder are a pleuronectid flatfish that traditionally supported robust fisheries along the NW Atlantic coast, ranging from Nova Scotia, Canada, southward to Maryland, USA (see Pereira et al. 1999 for winter flounder life history characteristics). Since the early 1980s, winter flounder populations have declined precipitously in the southern New England region and have yet to rebound (NEFSC 2017). Although overexploitation was paramount in the initial population decline, a multitude of factors continue to adversely affect winter flounder recruitment (NEFSC

2017) and, thus, keep adult populations at depressed levels. The precipitous decline in winter flounder abundance in northern temperate estuaries, for example, coincides with a significant warming trend in NW Atlantic coastal and inshore waters (Fulweiler et al. 2015). Elevated temperatures, in turn, may intensify the predator-induced mortality of post-settlement juvenile winter flounder by increasing the metabolism and consumption rate of local predators (Taylor & Collie 2003, Taylor & Peck 2004). Further, as noted above, subtle increases in temperature have caused a poleward shift in the distribution of more southerly located species, including blue crabs, resulting in a spatiotemporal overlap with juvenile winter flounder and newly formed predator–prey interactions (Taylor et al. 2019, Taylor & Fehon 2021). The decline of winter flounder abundance in southern New England habitats, coupled with changes in climatic conditions, has raised the question of whether these previously overexploited stocks can recover in the face of altered trophic dynamics.

The principal objective of this study was to examine the diet composition and foraging ecology of blue crabs collected from the Seekonk and Taunton Rivers (Rhode Island and Massachusetts, USA, respectively), 2 tidally influenced rivers that are contiguous with the Narragansett Bay Estuary and serve as nurseries for both blue crabs and post-settlement winter flounder (Taylor et al. 2016, Taylor & Fehon 2021). Importantly, blue crabs have recently experienced significant increases in abundance in this geographic area (Collier et al. 2014, Taylor & Fehon 2021), which may have implications to local benthic community structure and survival of juvenile winter flounder. This investigation, therefore, sought to (1) explore ontogenetic and spatiotemporal variations in the diet composition of blue crabs through conventional stomach content analysis and stable isotope measurements; (2) quantify the incidence of blue crab predation on winter flounder, as revealed by the aforementioned stomach analysis as well as novel molecular genetic techniques; and (3) examine the effect of abiotic and biotic factors on the crab–flounder predator–prey interaction.

2. MATERIALS AND METHODS

2.1. Field sampling

For a complete description of the study area and field sampling methodology, refer to Taylor & Fehon (2021). Briefly, blue crabs and juvenile winter flounder were collected annually from the Seekonk and Taunton Rivers from May through August 2012 to 2016 (Fig. 1). Fortnightly sampling occurred at 3 sites per river using a beach seine set (15 × 1.8 m; 0.64 cm mesh size and 0.48 cm bunt). One seine haul was performed at each site per sampling date during daylight (~08:00–16:00 h) and ±2 h of low tide. The area swept by each seine effort was recorded and varied due to tidal stage and beach profiles (mean ~850 m^2; range = 243–1774 m^2). Captured crabs were enumerated (no. of ind. 10 m^{-2}, Table 1 and reported in Taylor & Fehon 2021) and immediately preserved in 95% ethanol for subsequent stomach content and molecular genetic analyses (see Sections 2.2 and 2.4). From 2012 to 2015, random subsamples of crabs had their chelae immediately removed after capture, after which the chelipeds were placed on ice for trans-

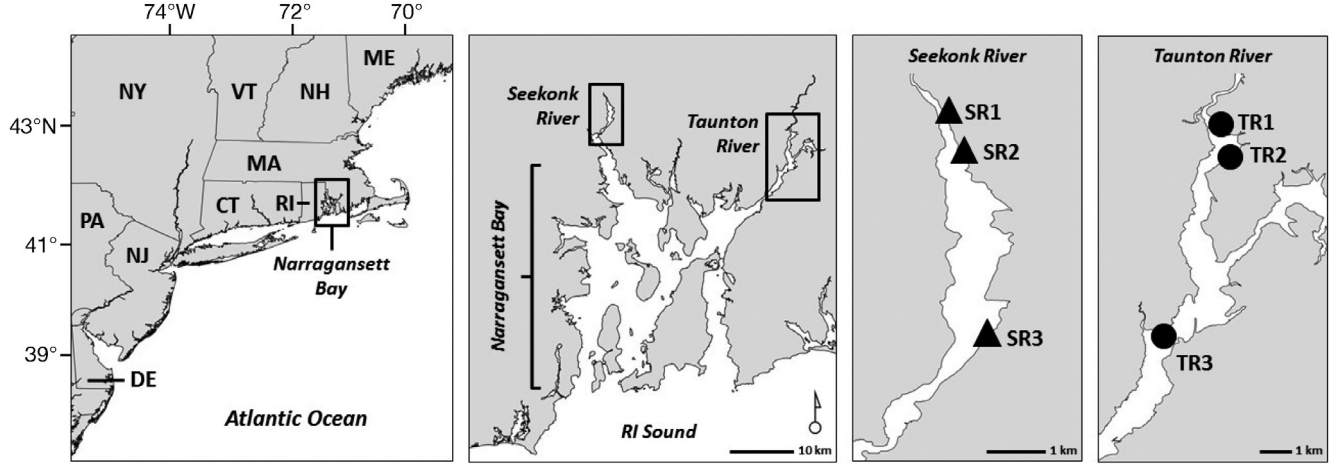

Fig. 1. Seekonk River (SR), Rhode Island, and Taunton River (TR), Massachusetts (USA), with points demarcating collection sites of blue crabs. Three sites were sampled fortnightly in each river (SR1–3 and TR1–3) from May to August 2012–2016, with the exception of SR1, which was not surveyed in 2014

Table 1. Summary of blue crab and winter flounder population density (no. of ind. 10 m^{-2}) and body size (carapace width and total length; mm) at individual sites in the Seekonk River (SR) and Taunton River (TR). Values are monthly means averaged across years, and ranges are reported in parentheses. Sites are defined in Fig. 1

	Seekonk River			Taunton River		
	SR1	SR2	SR3	TR1	TR2	TR3
Blue crab[a]						
Density	0.88 (0.06–1.92)	0.58 (0–3.56)	0.37 (0.04–2.01)	0.15 (0–0.67)	0.47 (0–1.40)	0.16 (0–0.50)
Carapace width	66.8 (30.1–107.0)	67.0 (23.6–103.9)	79.5 (24.5–154.0)	90.6 (34.3–140.0)	76.0 (28.2–116.3)	86.1 (27.8–172.0)
Winter flounder[a]						
Density	19.6 (0–95.7)	6.9 (0–68.1)	2.0 (0–8.4)	0.3 (0–0.8)	1.1 (0–5.0)	1.0 (0.04–4.2)
Total length	47.6 (26.9–71.3)	45.4 (26.4–65.3)	50.9 (32.8–65.9)	66.9 (43.8–81.7)	57.8 (29.0–74.7)	52.2 (40.0–61.0)

[a]Portions of dataset published in Taylor & Gervasi (2017) and Taylor & Fehon (2021)

portation (without ethanol preservation) and frozen at −20 °C in the laboratory for stable isotope analysis (see Section 2.3). Winter flounder collected from each sampling effort were enumerated (no. of ind. 10 m^{-2}) and measured for total length (TL; ±1 mm) (Table 1; Taylor & Gervasi 2017). In 2010 and 2013, random subsamples of captured winter flounder were immediately placed on ice and frozen for stable isotope analysis (Taylor & Gervasi 2017). Flounder not retained for laboratory analyses were returned to their place of capture.

Water temperature (°C), salinity (ppt), and dissolved oxygen (DO; mg l^{-1}) were measured with YSI meters at each site per date in the Seekonk and Taunton Rivers, and these results were reported in Taylor & Fehon (2021). Briefly, mean monthly water temperatures were generally consistent across sites but varied temporally, ranging from 17.9 to 29.0°C with maximal temperatures in July. Salinity gradually increased over time and differed markedly among sites, with the upper reaches of the rivers characterized as oligohaline waters (SR1 and TR1; mean salinity ≤5 ppt) and the mid-portion and lower portion defined as mesohaline (SR2–3 and TR2; salinity = 6–18 ppt) and polyhaline (TR3; salinity ≥19 ppt) (Fig. 1). DO ranged from 1.9 to 13.3 mg l^{-1} across sites, and concentrations were greater in the Seekonk River relative to the Taunton River. Mean monthly DO also differed temporally, decreasing from spring to late summer.

2.2. Visual analysis of blue crab stomach contents

In the laboratory, blue crabs previously preserved in 95% ethanol were measured to the nearest millimeter for carapace width (CW; range = 8–185 mm) and identified by sex and life stage based on abdomen external morphology (juvenile and adult for females only; Jivoff et al. 2007). The cardiac–pyloric stomachs of crabs were removed and visually inspected for proportional fullness (fullness values = 0.0, 0.2, 0.4, 0.6, 0.8, and 1.0; n = 1835). The effect of crab sex on the frequency of empty stomachs was analyzed with a chi-square test of independence, and mean differences in proportional stomach fullness with respect to river and month were evaluated with a 2-way ANOVA model. For the latter, fullness data were clustered by seine effort (see below), and the response variable was arcsine square-root transformed prior to statistical testing.

Prey extracted from blue crab stomachs were identified to the lowest possible taxon with the aid of stereoscopic microscopes. Each prey taxon's contribution to the diet of a crab was expressed by 2 indices: (1) frequency of occurrence (%F), which is the number of crab stomachs containing a prey item divided by the total number of stomachs with food contents (i.e. non-empty stomachs); and (2) volumetric percentage (%V), which is the visual determination of a prey item's volumetric contribution to the total volume of food recovered from a crab stomach. Each seine effort yielded a cluster of crabs; therefore, these individuals likely have greater dietary similarities to each other relative to crabs collected at different sites or dates (Bogstad et al. 1995). Accordingly, estimates of %F and %V were recalculated by using a cluster-sampling estimator (see Taylor & Gervasi 2017 for cluster-sampling calculations and variance estimates), and these data were used in subsequent analyses (i.e. permutational multivariate analyses and hierarchical cluster).

Differences in the diet composition of blue crabs by sex were examined using a 1-way permutational multivariate ANOVA (PERMANOVA) model, as provided in PRIMER 7.0 (PRIMER-E) (Anderson et al. 2008). A resemblance matrix of the log(x+1)-transformed diet data (%V) was created using the Bray-

Curtis similarity method, with each element in the matrix represented by sex (male and female), river (Seekonk and Taunton Rivers), month (May–August), and year (2012–2016). There was no significant difference in the diet of male and female crabs (see Section 3.1); therefore, subsequent statistical analyses did not include sex as an explanatory variable.

Ontogenetic (body size) variations in blue crab diet were examined using hierarchical cluster analysis of the %V data. Crabs were grouped into 20 mm CW size class intervals, and %V was reevaluated using the cluster-sampling estimator (Taylor & Gervasi 2017), i.e. 1 seine effort resulted in ≥2 clusters when multiple size classes were evident. Again, a Bray-Curtis resemblance matrix of log(x+1)-transformed data was created using PRIMER. Hierarchical cluster analysis was then performed on the resulting matrix by using the similarity profiling routine (SIMPROF), which identifies statistically distinct groupings among samples (Clarke et al. 2014). A dendrogram derived from the cluster analysis was produced to visually characterize the dietary similarities among crab size classes, and SIMPER was used to identify the prey taxa responsible for the dietary similarities or differences within or among groupings.

Variations in the size-dependent diet of blue crabs between rivers were examined using a 2-way PERMANOVA model. A Bray-Curtis resemblance matrix of log(x+1)-transformed data was created using the previously described methods, with the exception that %V was recalculated using the cluster-sampling estimator (Taylor & Gervasi 2017) by grouping crabs into distinct size categories based on cluster analysis results (see Section 3.1). Each element in the resemblance matrix, therefore, represented the mean %V for a given crab size (small, medium, and large) and river. Also, for each river, separate 2-way PERMANOVA models were used to examine fine-scale spatial (site) and temporal (month) variations in crab diet. If significant main effects were obtained in the PERMANOVA models ($p < 0.05$), SIMPER analyses were conducted to determine which prey taxa contributed to the observed differences in crab size-dependent diet across rivers, sites, and months. Moreover, principal coordinate (PCO) analysis was used to visualize the diet composition data and facilitate the interpretation of the PERMANOVA results. This method provides a direct projection of data points in space according to their actual dissimilarities, and PCO axes quantify the amount of variation within the resemblance matrix that is attributed to each PCO axis (expressed as percentage of total variation) (Anderson et al. 2008). By using Pearson correlations, vectors of the dominant prey taxa (%$V > 4$%) were superimposed onto the PCO biplots, which correspond to the monotonic relationships between a prey's dietary importance and the PCO axes (Anderson et al. 2008).

2.3. Stable isotope analysis of blue crabs and winter flounder

In the laboratory, propodus and carpus muscle tissue of partially thawed blue crabs was excised from chelae (44–185 mm CW; n = 257), and the gastrointestinal tracts of winter flounder were removed (20–73 mm TL; n = 170). All crab muscle and flounder whole-body tissue samples were freeze dried for at least 48 h (Labconco FreeZone 4.5 Liter Benchtop Freeze Dry System), homogenized with clean stainless steel spatulas, and stored at room temperature in borosilicate vials. Subsamples of crab and flounder tissue samples (~1 mg dry weight) were analyzed for $^{15}N/^{14}N$ and $^{13}C/^{12}C$ isotopes at the Boston University Stable Isotope Laboratory (Boston, Massachusetts, USA) using automated continuous-flow isotope ratio mass spectrometry (CF-IRMS). Crab and flounder tissues were not pre-treated with lipid extraction due to the relatively low lipid contents (C:N ratio = 3.2 ± 0.2, mean ± SD; Patterson & Carmichael 2016). Isotopic ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ were expressed in delta notation (δ) as the relative per mil (‰) difference between the samples and international standards (atmospheric nitrogen [$^{15}N_{air}$] and Vienna Peedee Belemnite carbonate [$^{13}C_{V\text{-}PDB}$]):

$$\delta Z = (R_{sample}/R_{standard} - 1) \times 1000(‰) \quad (1)$$

where $Z = {}^{15}N$ or ^{13}C, and $R = {}^{15}N/{}^{14}N$ or $^{13}C/^{12}C$. The sample isotope ratio was compared to a secondary gas standard, whose isotope ratio was calibrated to international standards ($R_{standard}$). For $^{15}N_{air}$, the gas was calibrated against atmospheric N_2 and ammonium sulfate standards (IAEA standards N-1, N-2, and N-3). For $^{13}C_{V\text{-}PDB,}$ the gas was calibrated against NBS 20 (Solenhofen limestone). All international standards were obtained from the National Bureau of Standards in Gaithersburg, Maryland, USA. The precision of the CF-IRMS method, as determined by the analysis of internal reference material (peptone and glycine), was 0.7‰ for nitrogen and 0.2‰ for carbon.

Nitrogen isotope signatures were used to calculate the trophic position of individual blue crabs (TP_{BC_i}) at each riverine site (i; SR1–3 or TR1–3) according to the following equation:

$$TP_{BC_i} = TP_{WF_i} + \frac{(\delta^{15}N_{BC_i} - \delta^{15}N_{WF_i})}{3.2} \quad (2)$$

where $TP_{WF_i} = (\delta^{15}N_{WF_i}/14.07) \times 3.1$ (TP_{WF_i} is the trophic position of winter flounder at site *i*), $\delta^{15}N_{BC_i}$ and $\delta^{15}N_{WF_i}$ are the respective nitrogen isotope signatures of a blue crab and winter flounder at site *i*, 3.2 is the constant nitrogen isotope enrichment (‰) per trophic level (Post 2002, Sweeting et al. 2007), 14.07 is the mean nitrogen isotope value of winter flounder across all riverine sites (see Section 3.2), and 3.1 is the previously measured mean trophic position of juvenile winter flounder from the Narragansett Bay estuarine complex (24–85 mm TL, n = 313; Payne & Taylor 2010, Szczebak & Taylor 2011).

Winter flounder was selected as the baseline species from which trophic positions were calculated in this study because (1) as mentioned, the trophic status of similarly sized winter flounder was recently quantified in the Narragansett Bay, which includes the contiguous tidal rivers; (2) winter flounder were readily available from the 6 river sites, i.e. SR1–3 or TR1–3, and post-settlement juveniles exhibit high site fidelity (Saucerman & Deegan 1991), thus enabling site-specific analyses of isotopic signatures; and (3) juvenile flounder are an important prey resource for blue crabs (see Sections 3.1 and 3.3).

Carbon isotope signatures of each blue crab and winter flounder at site *i* were adjusted to account for trophic fractionation, such that:

$$\delta^{13}C_{corrected(i)} = \delta^{13}C_{raw(i)} - 0.39\,(TP_i - 1) \quad (3)$$

where $\delta^{13}C_{corrected(i)}$ is the carbon isotopic signature adjusted for trophic fractionation, $\delta^{13}C_{raw(i)}$ is the unadjusted (original) isotope value, TP_i is the trophic position of an individual crab or flounder (Eq. 2), and 0.39 is the rate of carbon isotope enrichment (‰) per trophic level (Post 2002).

Differences in blue crab isotope signatures ($\delta^{15}N$ and $\delta^{13}C_{corrected}$) and trophic position as a function of sex (male and female), body size (small, medium, and large; see Section 3.1), and river (Seekonk and Taunton Rivers) were analyzed with 3-way ANOVA models. Also, for each river, 2-way ANOVA models were used to examine variations in crab isotope values and trophic position across sites (SR1–3 and TR1–3) and months (May–August). The post hoc examinations of the response variables across 3 levels of crab size, 6 sites, and 4 mo were contrasted Ryan-Einot-Gabriel-Welsch (Ryan's *Q*) multiple comparison tests. Further, response variables were log(*x*) transformed prior to statistical testing, noting that absolute values were used for $\delta^{13}C_{corrected}$ data.

2.4. Molecular genetic analysis of blue crab predation on winter flounder

Molecular genetic techniques were implemented in this study to complement the visual dietary analyses and further elucidate the predator–prey interaction between blue crabs and juvenile winter flounder. Specifically, a PCR-based method using intraspecific oligonucleotide primers (WF208; Collier et al. 2014) targeted winter flounder mitochondrial control region DNA in the stomach contents of potential blue crab predators. Before the analysis of field-collected crabs, several initial procedures were performed to ensure the efficacy of the molecular approach, including (1) testing the sensitivity and specificity of the assay, and (2) determining detection limits. First, the winter flounder specific primer set used in this study (WF208) is discussed in Taylor et al. (2019) and includes a detailed description of the PCR methods and analysis of the assay's sensitivity (self-reactivity) and specificity (cross-reactivity). Second, in the current study, the effect of digestion time on detecting winter flounder DNA in blue crab stomachs was determined in laboratory feeding experiments. Crabs used in feeding trials were collected from the Seekonk River in 2014 and 2015, as described above (see Section 2.1), transferred to the laboratory, and maintained individually in 19 l flow-through tanks (19–26°C and 27–28 ppt). Crabs measuring 69–167 mm CW were starved for ~3 d to ensure that all previous stomach contents were evacuated prior to the initiation of feeding experiments (McGaw & Reiber 2000). Previously frozen whole-body winter flounder were partially thawed, weighed, and offered to starved crabs (wet weight of offered flounder: mean = 1.7 g; range = 0.6–4.6 g). After a crab completed a feeding event, remaining flounder tissue, if any, was removed from the tank and reweighed (weight of consumed flounder: mean = 1.4 g; range = 0.1–3.9 g). Crabs were sacrificed at 8 time points after initial feeding (0–16 h post feeding; n = 2–10 per time point; total n = 49) by immersion in 95% ethanol and then frozen at −20°C until further processing. The stomachs of partially thawed crabs were removed using dissection instruments previously scoured by sand abrasion; rinsed in a series of deionized water, 10% bleach, and 95% ethanol baths; and flame sterilized with an alcohol lamp. Extracted stomachs were transferred to 1.5 ml microcentrifuge tubes with 0.5× TE buffer (5 mM Tris-HCl, 0.5 mM EDTA, pH 7.5) and frozen at −20°C until further analysis (Collier et al. 2014). The DNA extraction, PCR protocol,

and gel electrophoresis methods described in Taylor et al. (2019) were applied to a 100 µl aliquot of each prepared sample (i.e. stomach content–buffer mixture) to evaluate the amplification of flounder DNA in crab stomachs at various time points after initial feeding. For this experiment, a small-scale PCR reaction was modified from procedures described in Taylor et al. (2019) by combining 6.25 µl of 2× MyTaq^{TM} Red DNA polymerase (Bioline), 0.313 µl of a 10 mg ml^{-1} BSA (Ambion), 0.5 µl of each WF208 primer, and 5 µl of crab stomach DNA.

Multivariate logistic regression analysis, employing a stepwise selection process, was used to examine the effect of several variables on the detection of winter flounder DNA in laboratory-fed blue crabs. The explanatory variables incorporated into the regression model were crab CW (mm), time after initial ingestion of flounder (h), and amount of flounder tissue consumed (g wet weight). Chi-square values were calculated to test the significance of each explanatory variable because data were treated as frequency responses (presence or absence of flounder in crab stomachs) rather than continuous responses, and for a given explanatory variable, the significance level for entry and retention into the regression model was $p < 0.05$. The natural logarithm of the ratio of response frequencies (logits) was used to estimate parameters of the regression model (Taylor et al. 2019).

PCR-based assays were performed on field-collected blue crabs after defining the ability of WF208 primers to amplify winter flounder DNA in known samples. Random subsamples of male and juvenile female crabs (11–169 mm CW; n = 232) were collected from the Seekonk and Taunton Rivers (2014–2016; see Section 2.1), and their respective stomachs were extracted, prepared, and assayed as previously described (Taylor et al. 2019, this study). The positive identification of winter flounder in crab stomachs was determined by the WF208 primer set amplifying a product at the expected size of the targeted gene sequence, which was produced and visualized using the small-scale PCR-based and gel electrophoresis methods (Taylor et al. 2019, this study). Chi-square tests were used to analyze differences in the presence–absence of flounder in crab stomachs as a function of river, site, and month. The post hoc separation of mean response frequencies across 3 levels of site (per river) and 4 mo was performed using pairwise comparisons with Bonferroni corrections of p-values (i.e. critical p-values = 0.017 for site and 0.0083 for month).

2.5. Factors affecting blue crab predation on winter flounder

The visual identification of winter flounder in the diet of blue crabs was mainly determined by the presence of recognizable, i.e. morphologically unique, sagittal otoliths in crab stomach contents (see Section 3.1). Winter flounder otoliths recovered from crab stomachs, in turn, were used to examine predator–prey size relationships. Specifically, the maximum linear lengths of recovered winter flounder otoliths (OL) were measured with the aid of stereoscopic microscopes equipped with stage micrometers (±0.05 µm). Otolith lengths were then incorporated into a non-linear (exponential) least-squares regression model to predict the original TL of the consumed winter flounder (modified from Taylor et al. 2019):

$$\text{Log}(TL) = 0.3477 \times OL + 1.1958 \quad (4)$$

A non-linear (log-log) least-squares regression model was used to examine the relationship between the CW of blue crabs visually confirmed to have fed on winter flounder and the predicted TLs of flounder recovered from crab stomachs.

Multivariate logistic regression analyses were used to examine the effect of several biotic and abiotic explanatory variables on 2 response variables (i.e. occurrence of winter flounder in blue crab stomachs as revealed by visual and PCR analysis). The explanatory variables incorporated into each regression model were specific to a field sampling effort (i.e. seine haul by date and riverine site) and included blue crab CW (mm; measured for each crab during visual or PCR analysis), mean winter flounder TL (mm), winter flounder population density (no. of ind. 10 m^{-2}), date of crab capture (day of year), water temperature (°C), salinity (ppt), and DO (mg l^{-1}). Logits were again used to calculate parameters of each linear model and estimate the proportion of field-collected crab stomachs containing winter flounder (Taylor et al. 2019).

3. RESULTS

3.1. Visual analysis of blue crab stomach contents

The stomachs of 1243 blue crabs from the Seekonk River and 592 crabs from the Taunton River were examined for food contents, of which ~42% contained ingested material (Table 2). The frequency of empty stomachs differed significantly between male crabs (60% empty; total no. of male stomachs = 1204)

Table 2. Contribution of prey taxa to the diet of blue crabs, expressed as frequency of occurrence (%*F*) and volumetric percent (%*V*). Mean values (±1 SD) were calculated using a cluster-sampling estimator (Taylor & Gervasi 2017). Blue crabs were collected from the Seekonk River and Taunton River

Prey taxon	Seekonk River		Taunton River	
	%*F*	%*V*	%*F*	%*V*
Crustaceans				
Amphipods	18.8 ± 7.9	13.2 ± 7.2	13.7 ± 7.0	10.5 ± 7.6
Isopods	0.2 ± 0.4	0.2 ± 0.4	0	0
Crabs	19.4 ± 6.0	11.7 ± 4.6	25.9 ± 8.7	16.8 ± 6.2
Shrimps	15.4 ± 7.4	11.8 ± 6.2	5.7 ± 6.0	3.8 ± 3.7
Unidentified crustaceans	18.9 ± 7.4	14.4 ± 6.6	25.4 ± 11.6	22.6 ± 11.3
Insects	0.5 ± 0.7	0.1 ± 0.2	0.3 ± 0.6	<0.1
Polychaetes	10.4 ± 5.4	6.5 ± 4.7	6.7 ± 5.4	3.9 ± 3.4
Mollusks				
Bivalves	43.0 ± 7.5	27.5 ± 6.4	38.0 ± 10.9	24.1 ± 8.5
Urosalpinx cinerea (Atlantic oyster drill)	0.1 ± 0.2	<0.1	2.0 ± 2.9	0.3 ± 3.6
Fish				
Pseudopleuronectes americanus (winter flounder)	2.9 ± 2.3	2.2 ± 2.1	0.1 ± 0.6	<0.1
Unidentified fish	8.3 ± 3.6	3.5 ± 2.4	4.7 ± 4.1	2.7 ± 3.6
Seaweeds and sediments	2.3 ± 1.8	0.5 ± 0.4	1.2 ± 1.4	0.4 ± 0.5
Unidentified prey (including detritus)	9.5 ± 4.8	8.5 ± 4.7	15.2 ± 9.6	14.7 ± 9.6
Total number of stomachs examined (n_t)	1243		592	
Percent of empty stomachs (%)	62.6		49.3	
Percent stomach fullness, including empty (%)	12.3 ± 18.9		18.1 ± 22.8	
Total number of clusters (seine hauls with crabs; n_c)	77		76	
Unique prey per stomach (mean ±1 SD)	1.6 ± 0.8		1.5 ± 0.7	

and female crabs (55% empty; total no. of female stomachs = 631) (chi-square: $\chi^2_{1,1834} = 3.9$, $p < 0.05$), but the proportional fullness of their stomachs were similar (mean proportional fullness: male = 0.14 ± 0.19; female = 0.14 ± 0.21). Further, the fullness of crab stomachs varied by river but not month, nor was there a significant river–month interaction effect (2-way ANOVA; river: $F_{1,151} = 4.0$, $p < 0.05$; month: $F_{3,151} = 1.8$, $p = 0.153$; river × year: $F_{3,151} = 2.3$, $p = 0.081$) (Table 2).

Twelve unique prey were identified in blue crab stomachs, excluding unidentifiable prey and detritus, and the mean number of prey taxa per stomach was 1.5 ± 0.7 (max. no. of taxa in 1 stomach = 5). The dominant identifiable prey in crab stomachs were bivalves, crustaceans (e.g. crabs, shrimps, and amphipods), polychaetes, and fish. With respect to volumetric contributions (%*V*), these prey accounted for 88.5% of the crab's diet and had a mean frequency of occurrence (%*F*) of 18.8 ± 11.2% (range = 9–41%). Unidentified prey also comprised a relatively large portion of the material consumed by crabs (overall %*V* ~ 11%), and hereafter this prey category was excluded from statistical analyses.

There was no significant difference in diet composition between male and female blue crabs (Table 3). However, hierarchical cluster analyses revealed 3 distinct dietary groups of crabs based on their body sizes (Fig. 2A). After accounting for the size-dependent effects on crab diet, the cor-

Table 3. Summary statistics for 1- and 2-way permutational multivariate ANOVA models used to examine differences in blue crab diet as a function of sex (male and female), river (Seekonk and Taunton Rivers), body size (small, medium, and large; Fig. 2), sites (SR1–3 and TR1–3; Fig. 1), and month (May–August)

Factor	pseudo-*F* (df)	p
Rivers combined (n = 133)		
Sex	2.06 (1)	0.110
Rivers combined (n = 81)		
Size	9.30 (2)	<0.001
River	6.17 (1)	<0.003
Size × River	0.68 (2)	0.701
Seekonk River (n = 49)		
Site	1.39 (2)	0.239
Month	1.55 (3)	0.113
Site × Month	0.61 (6)	0.880
Taunton River (n = 43)		
Site	2.66 (2)	<0.05
Month	2.74 (3)	<0.05
Site × Month	1.36 (6)	0.192

rected cluster sample size (n_c) was 233 for the Seekonk and Taunton Rivers, i.e. 1 seine effort resulted in >1 cluster when multiple size categories were present. Small crabs (≤49 mm CW; n = 194) had a dietary similarity of 91.5% (SIMPROF; π = 0.0, p = 1.0) and mainly consumed amphipods (%*V* = 29%), shrimp (%*V* = 24%), and unidentified crustaceans (%*V* = 21%), with lesser dietary contributions from bivalves, crabs, and polychaetes (%*V* = 5–10%) (Fig. 2B). Crabs in the medium size category (50–89 mm CW; n = 286) had a dietary similarity of 93.0%, and large crabs (≥90 mm CW; n = 285) had a similarity of 90.8% (SIMPROF; π = 0.0–0.64, p = 1.0–0.58). Consumption of polychaetes remained relatively constant at the medium and large crab body sizes (%*V* = 4–7%) (Fig. 2B). In contrast, increases in crab size resulted in precipitous declines in the dietary importance of shrimp and amphipods (%*V* = 4–13%), whereas bivalves and crabs became increasingly more pervasive in blue crab stomach contents (%*V* = 23–40%) (Fig. 2B). The largest crabs also readily consumed fish (%*V* = 9%) (Fig. 2B), with winter flounder representing the only fish taxon identified to the species level (Table 2). Winter flounder occurred in 19 different crab stomachs (%*F* = 2.5%), and 84% of these occurrences were determined by the presence of flounder sagittal otoliths in the stomach contents. Specifically, the unique morphology of juvenile winter flounder otoliths allowed their positive identification among other fish remains. The mean number of winter flounder sagittal otoliths recovered from crab stomachs was 1.5, with a range of 1 to 3 otoliths per stomach, indicating that in some instances, >1 flounder was consumed by an individual crab.

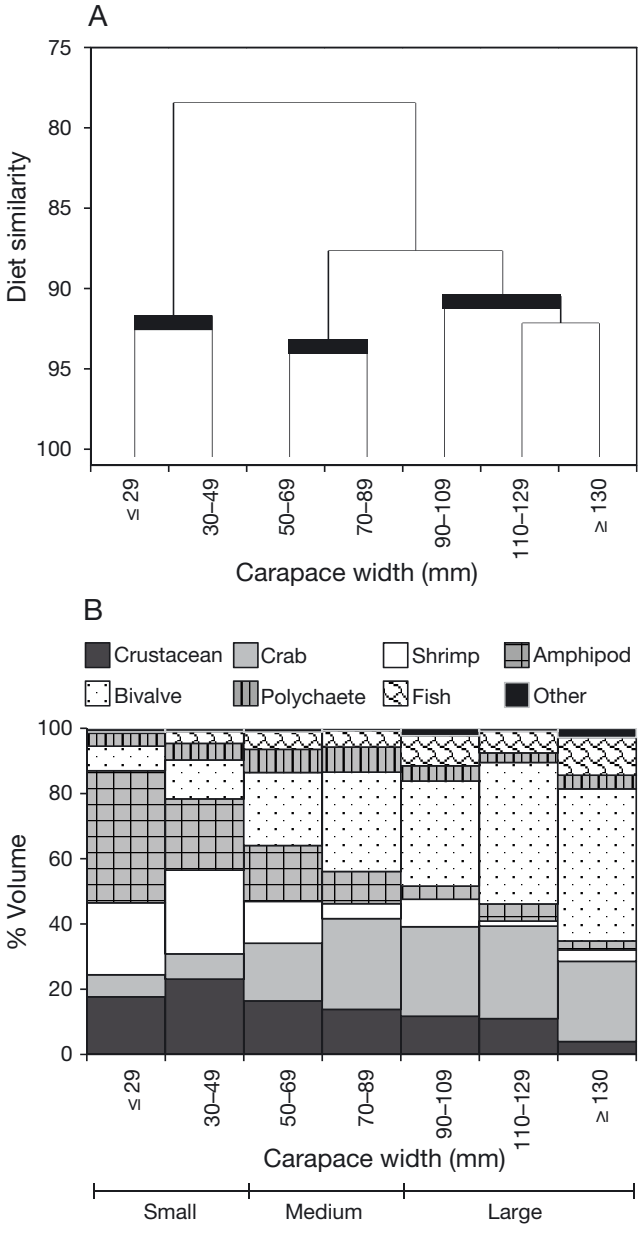

Fig. 2. (A) Dendrogram derived from hierarchical cluster analysis that represents the dietary similarities among blue crabs at 20 mm carapace width (CW) increments. Thick horizontal bars represent distinct dietary groups, as determined from cluster analyses and similarity profiling. (B) Prey contribution to the diet of blue crabs as a function of CW, expressed as percent volumetric contributions. Thin horizontal line with brackets represents distinct (size-based) dietary groups, as determined from hierarchical cluster analyses and similarity profiling

Blue crab diet statistically varied by size and river (Table 3). PCO analysis revealed that body size most closely corresponded to the first PCO axis and accounted for 36.4% of the explainable variation in crab diet, and river was associated with the second PCO axis and described 29.1% of the diet variation (Fig. 3). Vectors of dominant prey taxa superimposed on PCO biplots affirmed ontogenetic dietary shifts, with smaller blue crabs feeding predominantly on amphipods and shrimp and subsequently transitioning to crabs, bivalves, and fish with increasing size (Fig. 3). Differences in crab diet between rivers were mainly attributed to the increased importance of bivalves, shrimp, and fish in the Seekonk River (combined taxa %*V* = 45%) relative to the Taunton River (%*V* = 31%) (Table 2; Figs. 3 & 4). The latter includes crabs feeding on winter flounder, of which 95% were from the Seekonk River (n = 18). Moreover, in the Seekonk River, blue crabs consumed lower proportions of crab prey and unidentified crustaceans (%*V* = 20%) in comparison to conspecifics from the Taunton River (%*V* = 32%) (Table 2; Figs. 3 & 4).

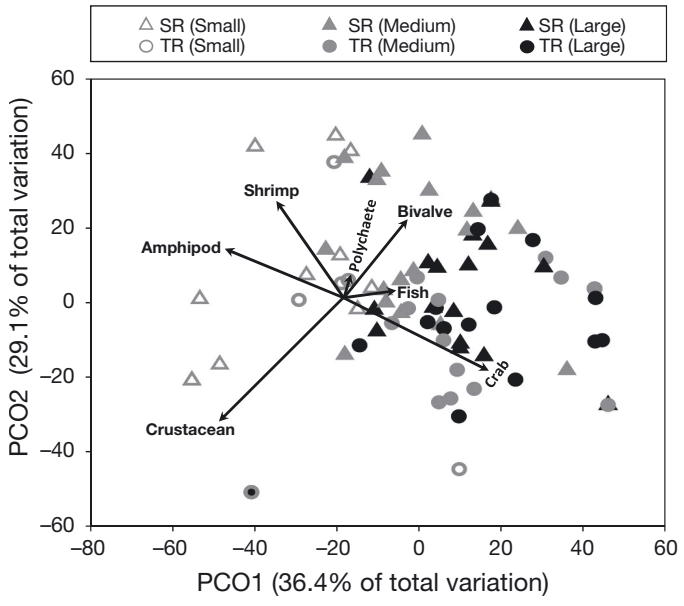

Fig. 3. Principal coordinate (PCO) plot that represents the dietary dissimilarities of blue crabs from the Seekonk River (SR) and Taunton River (SR). Each data element reflects the crab diet by year, month, and body size: small (≤49 mm carapace width [CW]), medium (50–89 mm CW), and large (≥90 mm CW). Solid lines superimposed on the PCO plot represent vectors of dominant prey taxa, which correspond to the monotonic relationships between a prey's dietary importance and the ordination axes. The first (PCO1) and second (PCO2) ordination axes correspond to body size and river, respectively, and quantify the percent of total variation in crab diet

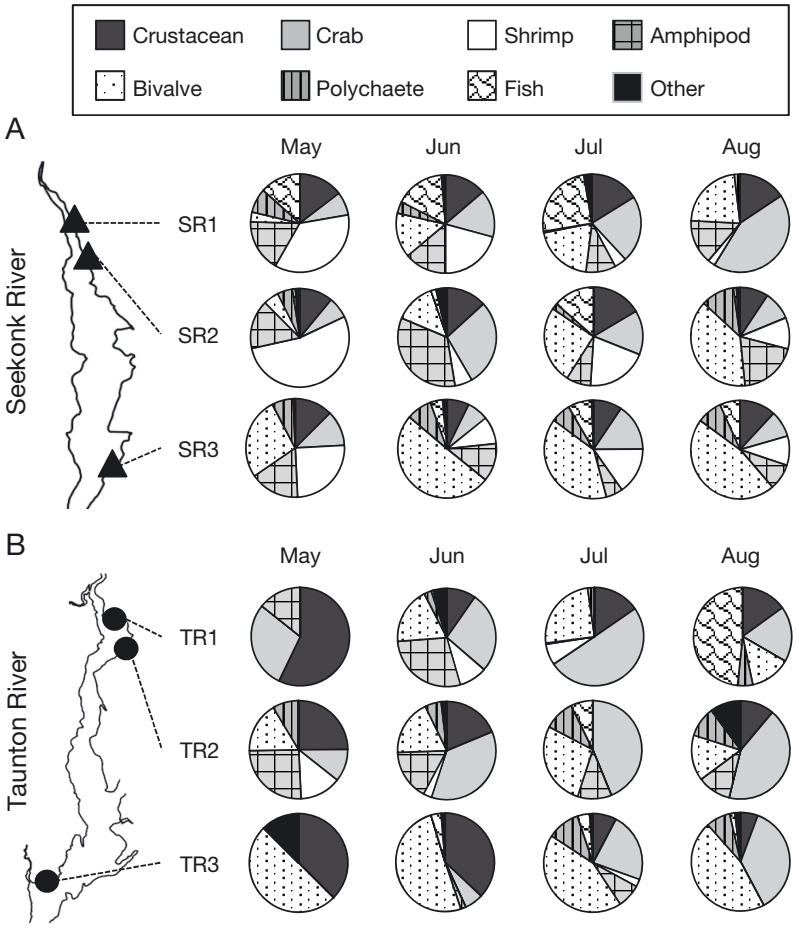

Fig. 4. Prey contribution to the diet of blue crabs in the (A) Seekonk River (SR) and (B) Taunton River (TR) as a function of site (SR1–3 and TR1–3) and month (May–August). Dietary contributions of prey are expressed as percent volumetric contributions

The food habits of blue crabs from the Taunton River differed across sites and months (Table 3; Fig. 4B). Fine-scale spatial differences in crab diet were attributed to the greater contribution of bivalves in the lower reaches of the river (%*V* for TR3 = 48% and TR1–2 = 18%), whereas identifiable crustaceans (amphipods, shrimp, and crabs) were more prevalent in the upper and mid-river sites (%*V* for TR1–2 = 55% and TR3 = 24%). Monthly variations in crab diet were due to the importance of amphipods, shrimp, and unidentified crustaceans at the onset of the Taunton River survey (%*V* for May = 63% and August = 11%). These prey were subsequently replaced by bivalves, crabs, and fish in later months (%*V* for May = 30% and August = 77%). Blue crabs from the Seekonk River demonstrated similar spatiotemporal variations in diet relative to crabs from the Taunton River (Fig. 4A), although the feeding patterns were less pronounced (Table 3). Amphipods, shrimp, and unidentified crustaceans comprised 68% by volume of the diet of Seekonk River crabs in May, which declined to 34% in August. Similarly, the dietary importance of bivalves and crabs increased monthly in the Seekonk River (%*V* for May = 24% and August = 59%). The contribution of fish to crab diet varied inconsistently across months in the Seekonk River (maximal in July; %*V* = 14%), and predation on winter flounder was most prevalent in the upper river (SR1; 74% of all crab–flounder interactions; n = 14).

3.2. Stable isotope analysis of blue crabs and winter flounder

Stable carbon ($\delta^{13}C$) isotope signatures were used to differentiate among sources of carbon to the tidal river food web, and stable nitrogen ($\delta^{15}N$) values approximated the trophic position (TP) of blue crabs (Table 4). These results were compared to $\delta^{13}C$ and trophic values measured for juvenile winter flounder and alternative crab prey, the latter assessed during recent studies in the Narragansett Bay and adjoining tidal rivers (Payne & Taylor 2010, Szczebak & Taylor 2011) (Fig. 5). Alternative prey of blue crabs included fish (mummichog *Fundulus heteroclitus* and striped killifish *F. majalis*), crabs (Atlantic mud crab *Panopeus herbstii*), shrimp (sand shrimp *Crangon septemspinosa* and grass shrimp *Palaemonetes* spp.), amphipods (*Gammarus* spp.), polychaetes (*Nereis* spp.), clams (softshell clam *Mya arenaria*), and mussels (ribbed mussel *Geukensia demissa*). Stable $\delta^{13}C$ and $\delta^{15}N$ signatures for the alternative prey were derived from whole-body tissues, noting that shells were removed for bivalves. Interspecific comparisons between blue crabs and prey, including winter flounder, should be interpreted with caution because of discrepancies in analyzed tissue types, i.e. muscle or whole body. However, it is noteworthy that in the portunid crab *Carcinus meanas*, the extent of $\delta^{13}C$ enrichment observed in muscle tissues, relative to whole-body samples, did not preclude the identification of primary production sources, and $\delta^{15}N$ values did not statistically differ between muscle and whole-body tissues (Curtis et al. 2017).

The mean $\delta^{13}C$ of blue crabs corrected for trophic fractionation ($\delta^{13}C_{corrected}$; Eq. 3) equaled −18.9 ± 1.8‰, whereas winter flounder had a mean $\delta^{13}C$ of −21.1 ± 1.9‰. Among alternative prey, mean $\delta^{13}C$ ranged from −16.5 to −21.4‰ (Fig. 5). The mean $\delta^{15}N$ of blue crabs was 15.4 ± 1.1‰, corresponding to a trophic position of 3.50 ± 0.35 (Table 4). In comparison, winter flounder had a mean $\delta^{15}N$ of 13.8 ± 1.1‰ and trophic position of 3.05 ± 0.25 (Table 4). Each alternative prey taxon maintained a lower trophic position than blue crabs and winter flounder (Fig. 5). Killifish, decapod crustaceans, and polychaetes occupied trophic levels between 2.6 and 2.9, whereas the trophic positions of amphipods and bivalves were <2.3 (Fig. 5). Finally, the collective trophic position of prey eaten by blue crabs within defined size categories (Fig. 2A) was calculated *a posteriori* using the following equation:

$$TP_{\bar{P}rey} = \sum_{k=1}^{6} V_k \times TP_{Prey_{(k)}} \quad (5)$$

Table 4. Summary of body size (mm), stable carbon ($\delta^{13}C_{raw}$) and nitrogen ($\delta^{15}N$) isotope signatures, and trophic position of blue crabs and winter flounder at individual sites in the Seekonk River (SR) and Taunton River (TR). Mean values, ranges (in parentheses), and sample sizes (n) are presented. Body sizes reported as crab carapace width and flounder total length are for individuals used in isotope and trophic analyses. Sites are identified in Fig. 1

	Seekonk River			Taunton River		
	SR1	SR2	SR3	TR1	TR2	TR3
Blue crab	(n = 28)	(n = 45)	(n = 64)	(n = 26)	(n = 36)	(n = 58)
Carapace width	107.6 (59–146)	94.8 (44–153)	111.3 (47–168)	113.3 (54–170)	100.3 (55–173)	96.9 (48–185)
$\delta^{13}C$	−18.19 (−21.06 to −16.21)	−17.65 (−19.98 to −15.38)	−17.74 (−20.77 to −14.16)	−19.80 (−24.36 to −15.61)	−19.40 (−25.15 to −14.79)	−16.64 (−21.37 to −12.97)
$\delta^{15}N$	14.82 (12.27–17.08)	14.91 (12.55–17.51)	15.80 (13.41–18.21)	15.84 (13.80–18.06)	16.25 (14.34–18.12)	14.74 (13.54–18.58)
Trophic position	3.43 (2.63–4.13)	3.49 (2.75–4.30)	3.64 (2.89–4.40)	3.61 (2.97–4.31)	3.69 (3.09–4.27)	3.22 (2.85–4.42)
Winter flounder	(n = 43)	(n = 26)	(n = 58)	(n = 2)	(n = 4)	(n = 37)
Total length	44.3 (28–66)	38.1 (25–64)	45.3 (30–70)	26.5 (20–33)	51.0 (37–73)	51.0 (30–68)
$\delta^{13}C$	−22.19 (−24.58 to −18.66)	−20.62 (−21.98 to −17.71)	−20.16 (−22.69 to −16.57)	−23.16 (−24.05 to −22.26)	−21.06 (−22.91 to −19.83)	−17.63 (−21.60 to −15.05)
$\delta^{15}N$	13.09 (11.57–14.65)	12.72 (11.21–13.95)	14.06 (11.98–15.59)	14.50 (14.17–14.84)	15.06 (14.37–15.81)	15.00 (13.60–15.66)
Trophic position	2.88 (2.55–3.23)	2.80 (2.47–3.07)	3.10 (2.64–3.43)	3.20 (3.12–3.27)	3.32 (3.17–3.48)	3.30 (3.00–3.45)

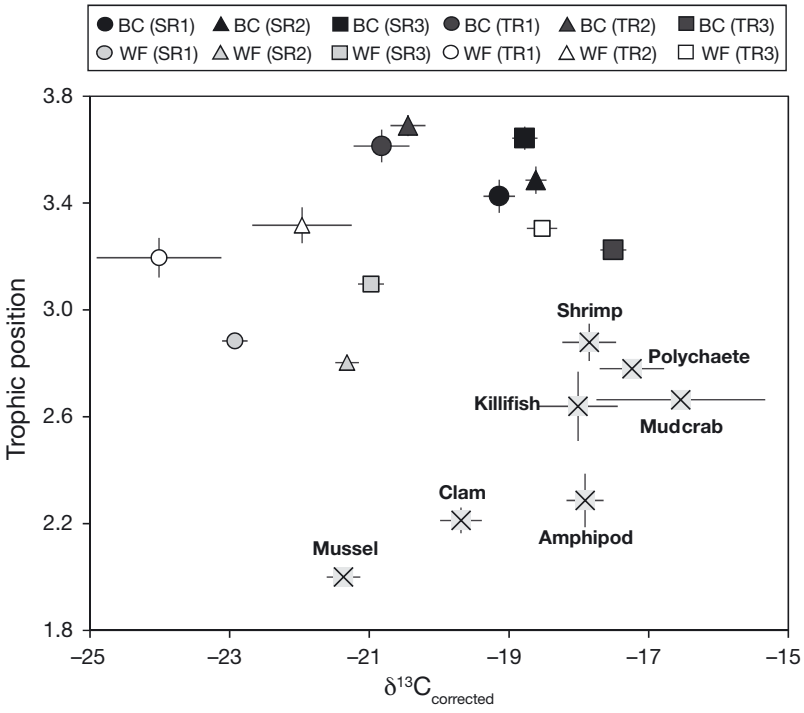

Fig. 5. Mean trophic position of blue crab (BC), winter flounder (WF), and alternative crab prey as a function of stable carbon isotope ($\delta^{13}C$) signatures. Trophic position and corrected $\delta^{13}C$ values were calculated using Eqs. (2) and (3), respectively. Data points for blue crab and winter flounder represent means for each site in the Seekonk River and Taunton River (SR1–3 and TR1–3, respectively; Fig. 1). Error bars represent ±1 SE, and alternative prey are identified in Section 3.2

where $TP_{\bar{P}rey}$ is the trophic position of cumulative prey consumed by small (≤49 mm CW), medium (50–89 mm CW), or large (≥90 mm CW) crabs; V_k is the proportional volumetric contribution of prey taxon k to the diet of differently sized crabs, of which there were 6 prey taxa (fish [winter flounder and *Fundulus* spp.], crabs, shrimp, amphipods, polychaetes, and bivalves [clams and mussels]; Table 2); and $TP_{Prey_{(k)}}$ is the trophic position of prey taxon k (Fig. 5). Accordingly, the $TP_{\bar{P}rey}$ of prey eaten by small blue crabs was 2.53, whereas medium and large crabs ate prey with trophic positions of 2.47 and 2.43, respectively.

Blue crab $\delta^{15}N$ and trophic values were consistent across sex and body-size categories (Table 5), but small crabs had significantly enriched $\delta^{13}C$ signatures relative to medium and large individuals ($\delta^{13}C$ for small crabs = −16.6 ± 1.3‰ [mean ± SD] and medium–large crabs = −19.0 ± 1.7‰). Blue crab $\delta^{15}N$ and $\delta^{13}C$ did not differ between rivers, but crabs in the Seekonk River had a higher trophic position than conspecifics from the Taunton River (Tables 4 & 5; Fig. 5). A more focused analysis of each river revealed that crab $\delta^{15}N$ and trophic positions varied significantly by site but not month (Table 5). In the Seekonk River, mean crab $\delta^{15}N$ and trophic values were maximal in the lower river (SR3); conversely, in the Taunton River, upper and mid-river crabs had higher $\delta^{15}N$ and trophic status (TR1 and TR2)

Table 5. Summary statistics for 3- and 2-way ANOVA models used to examine differences in blue crab stable carbon ($\delta^{13}C_{corrected}$) and nitrogen ($\delta^{15}N$) isotope signatures and trophic position as a function of sex (male and female), body size (small, medium, and large; Fig. 2), river (Seekonk River [SR] and Taunton River [TR]), site (SR1–3 and TR1–3; Fig. 2), and month (May–August)

	$\delta^{13}C$		$\delta^{15}N$		Trophic position	
Factor	*F* (df)	p	*F* (df)	p	*F* (df)	p
Rivers combined (n = 257)						
Sex	1.76 (1)	0.186	0.51 (1)	0.475	0.24 (1)	0.627
Size	10.4 (2)	<0.0001	0.41 (2)	0.667	0.34 (2)	0.711
River	0.89 (1)	0.348	0.46 (1)	0.499	3.87 (1)	<0.05
All interactions	0.01–1.9	0.15–0.99	0.36–1.29	0.28–0.70	0.19–1.15	0.32–0.82
Seekonk River (n = 137)						
Site	2.02 (2)	0.137	13.1 (2)	<0.0001	6.09 (2)	<0.01
Month	0.82 (3)	0.483	0.96 (3)	0.416	1.13 (3)	0.376
Site × Month	0.57 (6)	0.751	0.99 (6)	0.436	1.05 (6)	0.398
Taunton River (n = 120)						
Site	27.8 (2)	<0.0001	18.0 (2)	<0.0001	18.8 (2)	<0.0001
Month	1.29 (3)	0.282	0.42 (3)	0.742	0.31 (3)	0.821
Site × Month	0.44 (6)	0.854	1.27 (6)	0.275	1.39 (6)	0.224

(Table 4; Fig. 5). Crab $\delta^{13}C$ values were significantly depleted in the upper and middle reaches of the Taunton River ($\delta^{13}C$ for TR1–2 = –20.6 ± 1.72‰ and TR3 = –17.5 ± 1.5‰), whereas $\delta^{13}C$ did not vary spatially in the Seekonk River (Tables 4 & 5; Fig. 5). Further, $\delta^{13}C$ signatures of crabs from both rivers were consistent across months (Table 5).

3.3. Molecular genetic analysis of blue crab predation on winter flounder

Taylor et al. (2019) affirmed that the intraspecific primer set (WF208; Collier et al. 2014) and molecular assays implemented in this study have high sensitivity and specificity. However, the ability to detect flounder DNA in crab stomachs declined significantly after initial ingestion (Table 6). The absolute detection limit of flounder in crab stomachs was projected to occur at 12 h post feeding (% detection = 0%), but reliable detection was limited to the first 10 h after initial ingestion of flounder tissue (% detection > 83%) (Fig. 6). Further, detection levels were not affected by crab CW or the amount of flounder tissue consumed.

The analysis of field-collected blue crabs revealed that 41 of 232 stomachs contained winter flounder DNA (17.7% positive detection). The incidence of predation was significantly higher in the Seekonk River (24.6%; 28 of 114 stomachs) compared to the Taunton River (11.0%; 13 of 118 stomachs) (chi-square: $\chi^2_{1,231} = 20.1$, $p < 0.01$). Within each river, the occurrence of flounder in crab stomachs was independent of site (chi-square: $\chi^2_{1,57–91} = 0.002–4.35$, $p = 0.037–0.961$) and month (chi-square: $\chi^2_{1,76–154} = 0.003–5.78$, $p = 0.016–0.953$). Finally, a subsample of blue crabs that were visually confirmed to have fed on winter flounder (n = 8) and unidentified fish (n = 15) was also examined using PCR analysis. Of these stomach content samples, 37.5% (n = 3) and 26.7% (n = 4) tested positive for winter flounder DNA.

Table 6. Summary statistics and mean parameter estimates (SE) for logistic regression analyses of the proportion of blue crab stomachs containing winter flounder, as determined from laboratory feeding experiments (detection limit) and the visual and PCR analysis of field-collected crabs. Regression variables include time after crab initial ingestion of flounder (h), crab carapace width (mm), flounder total length (mm), flounder density (no. of ind. 10 m^{-2}), salinity (ppt), and dissolved oxygen concentrations (mg l^{-1})

Method/variable	Parameter estimate (SE)	Chi-square	p
Laboratory-fed crabs: detection limit analysis			
Intercept	5.341 (1.740)	9.43	<0.005
Time after ingestion	–0.492 (0.168)	8.63	<0.005
Field-collected crabs: visual analysis			
Intercept	1.482 (1.763)	0.706	0.401
Carapace width	0.0214 (0.007)	8.28	<0.005
Flounder length	–0.0573 (0.028)	4.18	<0.05
Flounder density	0.0123 (0.0059)	4.31	<0.05
Salinity	–0.151 (0.054)	7.68	<0.01
Dissolved oxygen	–0.496 (0.121)	16.7	<0.0001
Field-collected crabs: PCR analysis			
Intercept	–1.249 (0.447)	7.79	<0.01
Carapace width	0.011 (0.005)	4.28	<0.05
Salinity	–0.116 (0.030)	15.0	<0.0001

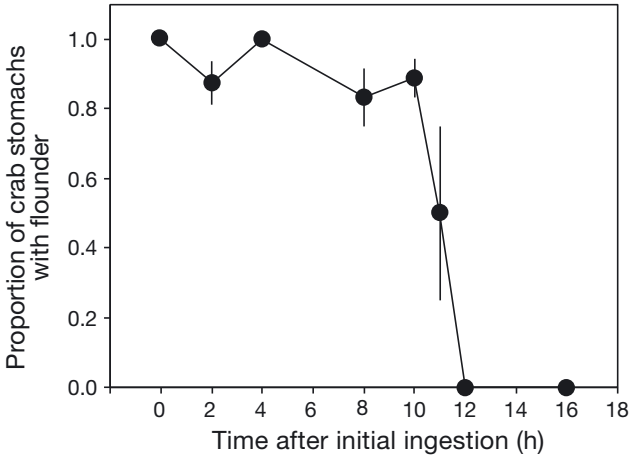

Fig. 6. Mean proportion of blue crab stomachs testing positive for winter flounder DNA as a function of time after initial ingestion (h) (n = 2–10 per time point; total n = 49). Error bars denote ± 0.5 SE

3.4. Factors affecting blue crab predation on winter flounder

The majority of winter flounder visually identified in blue crab stomachs were recognized by the presence of sagittal otoliths in the predator stomach contents. These otoliths, in turn, were used to estimate the original body size of each flounder recovered from crab stomachs (Eq. 4). Accordingly, the mean TL of consumed flounder equaled 47.0 ± 10.3 mm (range = 26–66 mm). The predatory blue crabs had a mean body size of 93.7 ± 37.9 mm CW (range = 27–154 mm) based on visual dietary analysis and 82.0 ± 35.3 mm CW (range = 23–160 mm) from the molecular assays. There was a significant positive relationship between the CW of crabs visually con-

firmed to feed on winter flounder and the predicted TL of flounder recovered from predator stomachs (regression: $F_{1,17}$ = 20.2, R^2 = 0.559, p < 0.0001) (Fig. 7).

The prevalence of winter flounder in the diet of blue crabs, as determined by visual analysis of stomach contents, was strongly associated with predator and prey body sizes (Table 6), such that increases in crab CW and decreases in mean flounder TL coincided with higher probabilities of predation (Fig. 8A,B). The occurrence of winter flounder in blue crab stomachs was also positively correlated to flounder population density (Fig. 4D) and inversely related to salinity and DO concentrations (Table 6; Fig. 8C), the former reflecting the higher %*F* values at the oligohaline sites in the Seekonk River (Table 2). Through molecular-based assays, positive detection of winter flounder DNA in blue crab stomachs was directly related to predator size (Fig. 8A) and negatively correlated to salinity (Table 6; Fig. 8C).

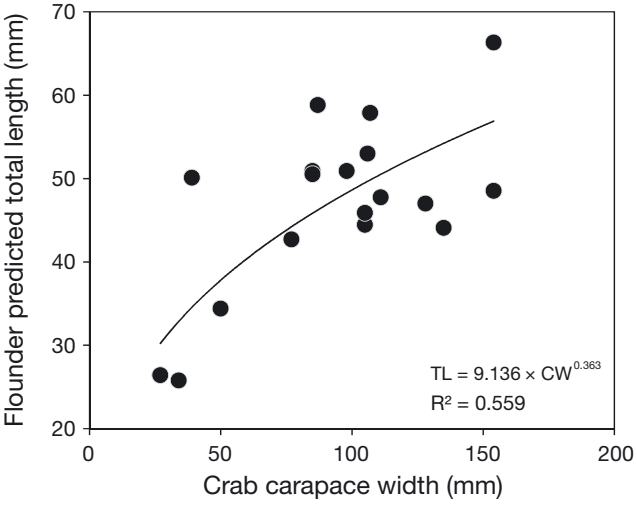

Fig. 7. Relationship between the carapace width (CW) of blue crabs and the predicted total length (TL) of consumed winter flounder (Eq. 4). Solid line represents the non-linear (log-log) least-squares regression model fit to the full data set (n = 18), and the equation and R^2-value are presented

4. DISCUSSION

4.1. Blue crab dietary habits: visual analysis

Blue crabs from the Seekonk and Taunton Rivers exhibited a generalist foraging strategy, as evidenced by the breadth of prey taxa recovered from extracted stomachs. Crabs collected from these rivers fed mainly on bivalves (e.g. *Mya arenaria*, *Modiolus modiolus*, and *Geukensia demissa*), amphipods (e.g. Gammaridae), crabs (e.g. Xanthidae), shrimp (e.g. *Crangon septemspinosa*, Palaemonidae, and Mysidacea), polychaetes (e.g. Nereididae), fish (e.g. winter flounder), and vegetal–detrital material. These results are consistent with previous studies that examined the feeding habits of blue crabs from southern New England, the Middle and South Atlantic Bight, and the Gulf of Mexico (Hines 2007; Table 7). Spatial variations in crab diet are also evident, especially when viewed at greater taxonomic resolution, and likely reflect distinct prey assemblages across latitudes and among diverse habitat types, e.g. soft bottom, vegetated, biogenic reefs, and salt marshes (Table 7).

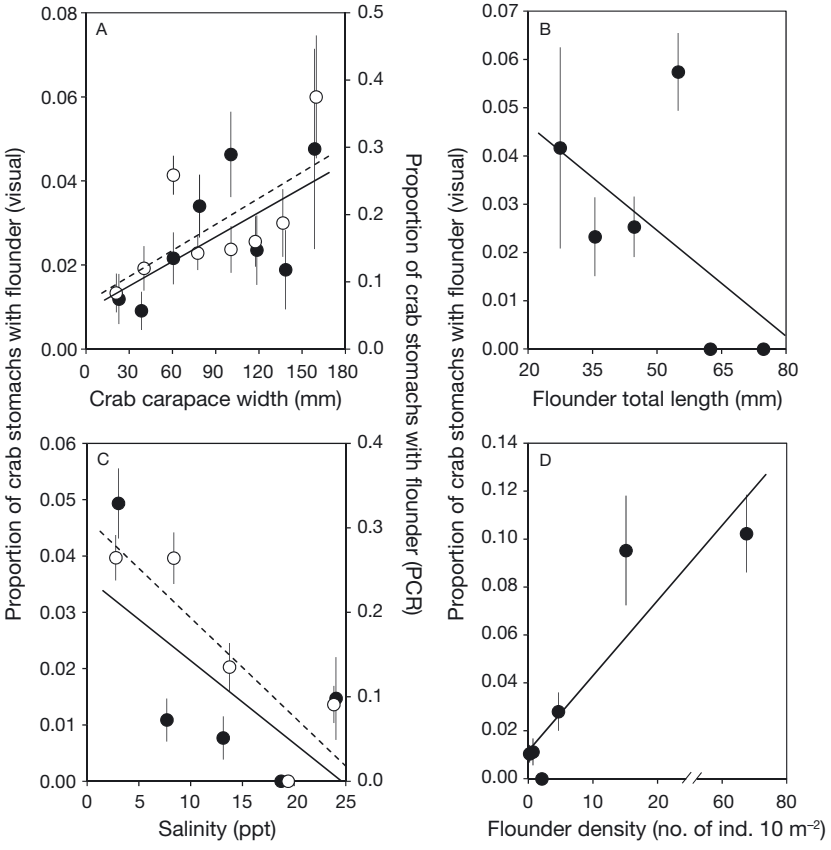

Fig. 8. Proportion of blue crab stomach contents containing winter flounder. Occurrences of predation were analyzed as a function of (A) crab carapace width, (B) mean flounder total length, (C) salinity, and (D) flounder population density. Data points are presented for visual (solid circles) and PCR (open circles) analysis of crab stomach contents. Logistic regression models were fit to the visual (solid lines) and PCR (dashed lines) full data sets, but data were binned across the *x*-axis for graphical representation. Error bars denote ±0.5 SE

Table 7. Literature review of blue crab dietary habits in the NW Atlantic and Gulf of Mexico (USA). The following information is provided for each source document: study location, crab carapace width (CW; mm), and prey taxa identified in crab stomachs and their corresponding contribution to diet (%), expressed as frequency of occurrence (%*F*), volumetric percent (%*V*), or weight percent (%*W*). With the exception of cannibalized *Callinectes sapidus*, only prey taxa with dietary contributions ≥4% are reported

Location	CW	Prey	Contribution	Index	Literature source
Pettaquamscutt River (Rhode Island)	20–160	Bivalves (e.g. *Gemma gemma* and *Mytilus edulis*)	49	%*F*	Ropes (1989)
		Crabs	40		
		Small crustaceans	33		
		Fish	18		
		Annelids (e.g. *Nereis* sp.)	17		
		Gastropods (e.g. *Hydrobia* sp.)	12		
		Algae–plant matter	19		
Hudson-Raritan Estuary (New York and New Jersey)	40–185	Crabs (e.g. Xanthidae, *Pagurus* sp., and *Ovalipes ocellatus*)	39	%*V*	Stehlik et al. (2004)
		Bivalves (e.g. *Mulinia lateralis* and *M. edulis*)	32		
		Gastropods (e.g. *Nassarius* spp.)	11		
		C. sapidus	<0.1		
		Organic matter	9		
Navesink River-Sandy Hook Bay (New Jersey)	10–180	Bivalves (e.g. *Mya arenaria* and *M. edulis*)	31	%*V*	Meise & Stehlik (2003)
		Non-portunid crabs (e.g. Xanthidae and *Pagurus* spp.)	20		
		Mollusks, unidentified	12		
		C. sapidus	10		
		Shrimps (*Crangon septemspinosa* and *Palaemonetes* spp.)	6		
		Gastropods (e.g. *Nassarius* spp.)	6		
		Amphipods	5		
Rhode River (Maryland)	125	Clams	33	%*V*	Hines et al. (1990)[a]
		Amphipods (*Leptocheirus plumulosus* and *Corophium lacustre*)	28		
		Digested animal tissue	15		
		Fish	14		
		Polychaete (*Nereis succinea*)	4		
		C. sapidus	3		
Rappahannock and York Rivers (Virginia)	4–40	Clams	23	%*V*	Seitz et al. (2011)[b]
		Polychaetes	14		
		Amphipods	8		
		Small crustaceans (shrimps, copepods, and ostracods)	8		
		Crabs	6		
		Gastropods	5		
		Plant–detrital matter	17		
Rappahannock and York Rivers (Virginia)	>60	Bivalves (*M. arenaria*, *Macoma* sp., *M. lateralis*, *Anadara* sp., *Crassostrea virginica*, and mussels)	55	%*V*	Mansour (1992), Lipcius et al. (2007)
		C. sapidus	30		
		Polychaetes (*Nereis* sp., *Glycera*, and *Pectinaria*)	5		
		Crustaceans (e.g. shrimps, crabs, and amphipods)	5		
		Other (including fish)	5		
Duplin River and South End Creek (Georgia)	50–163	Crabs (e.g. *Uca* sp., Grapsidae, and Xanthidae)	43	%*V*	Fitz & Wiegert (1991)
		Fish	38		
		Shrimps and amphipods (e.g. Penaeidae, Palaemonidae, and Peracaridea)	12		
St. Johns River (Florida)	5–200	Clams (Sphaeriidae, *Rangia cuneata*, and *M. lateralis*)	19	%*V*	Tagatz (1968)
		Fish	19		
		Mussels (e.g. *Geukensia demissus* and *Musculus niger*)	18		
		Amphipods (e.g. *Gammarus fasciatus* and *Corophium* sp.)	6		
		Crabs (e.g. *C. sapidus* and Xanthidae)	4		
		Organic–algal–plant matter	24		

(Table continued on next page)

Table 7 (continued)

Location	CW	Prey	Contribution	Index	Literature source
Apalachicola Estuary (Florida)	<31 to >60	Bivalves (e.g. *R. cuneata*, *Brachidontes* sp., and *Crassostrea* sp.)	36 12	%*W*	Laughlin (1982)[c]
		Fish (*Anchoa mitchilli*, *Micropogonias undulates*, *Microgobius*, *Etropus*, and *Trinectes*)	11		
		Xanthid crabs (e.g. *Rhithropanopeus harrisii*)			
		C. sapidus	9		
		Shrimps (*Penaeus* sp. and *Palaemonetes* sp.)	5		
		Gastropods (*Neritina reclivata*, *Odostomia* sp., and *Bittium* sp.)	5		
		Organic–plant–detrital matter	11		
Lake Pontchartrain (Louisiana)	30–197	Bivalves (*R. cuneata* and *Mytilopsis leucophaeata*)	47	%*V*	Darnell (1958)[d]
		Crabs and crustaceans, unidentified	16		
		C. sapidus	6		
		Gastropods	4		
		Organic–detrital matter	19		

[a]Crab CW = mean value; volumetric percentages averaged across muddy and sandy sediments for June survey
[b]Volumetric percentages estimated graphically and represent maximum values observed at any one of 4 coves surveyed
[c]Volumetric percentages pooled across 3 crab size classes: <31, 31–60, and >60 mm CW
[d]Volumetric percentages averaged across 3 crab size classes: 30–74, 125–147, and 148–197 mm CW

Riverine blue crabs exhibited pronounced ontogenetic dietary shifts. Small crabs (≤49 mm CW) mainly consumed amphipods, shrimp, and unidentified crustaceans, transitioning to bivalves, crabs, and fish with increased blue crab body sizes. Comparable size-dependent patterns in crab diet were reported in Chesapeake Bay tributaries (Hines et al. 1990, Lipcius et al. 2007); Apalachicola Bay and St. Johns River, Florida (Laughlin 1982, Tagatz 1968); Tamiahua Lagoon, Mexico (Rosas et al. 1994); and Laguna Joyuda, Puerto Rico (Stoner & Buchanan 1990). The preponderance of studies indicate that young blue crabs (<40 mm CW) have a broad dietary breadth, including small epibenthic organisms and shallow-dwelling infauna (e.g. amphipods, mysids, polychaetes, and vegetal–detrital matter). Larger crabs (>60 mm CW), in contrast, have a more restricted diet often limited to deeply buried infauna (e.g. clams) and mobile prey (e.g. fish and decapod crustaceans) (Lipcius et al. 2007). The ontogenetic dietary shift undergone by late juvenile and adult crabs is practicable due, in part, to enlarged chelae and improved ability to detect, excavate or capture, and process bivalves and evasive fauna (Blundon & Kennedy 1982, Mascaro et al. 2003, Aronhime & Brown 2009).

The main discrepancy in the diet data presented herein compared to prior investigations is the absence of conspecifics in blue crab stomachs, i.e. cannibalism (Table 7). Cannibalized prey are a significant dietary component of blue crabs >40 to 60 mm CW in several estuarine systems (Dittel et al. 1995, Hines & Ruiz 1995, Moksnes et al. 1997, Table 7). In this study, with respect to predation on decapod crustaceans, blue crabs >70 mm CW chiefly targeted xanthid crabs. It is conceivable that some unidentified crabs or crustaceans were the remains of *Callinectes sapidus*; however, substantial maceration of prey often precluded species-specific identifications. Alternatively, cannibalism may not have occurred in this study because (1) potentially cannibalistic blue crabs foraged on more abundant and profitable prey species, i.e. bivalves instead of smaller crab conspecifics (Lipcius et al. 2005, Seitz et al. 2005); and (2) abundances of riverine blue crabs were below a threshold level at which density-dependent cannibalism occurs (0.1–1.0 crab m^{-2}) (Lipcius et al. 2005, Posey et al. 2005, Seitz et al. 2011).

The feeding habits of blue crabs in this study varied spatially and temporally. This observation is partly assigned to differences in the intraspecific size structure between the Seekonk and Taunton Rivers (Taylor & Fehon 2021), the latter causing ontogenetic dietary shifts. Further, owing to their opportunistic foraging strategy, the diet composition of blue crabs is also governed by locational and seasonal patterns in prey abundance and availability (Laughlin 1982, Hines et al. 1990, Meise & Stehlik 2003). Spatiotemporal variations in prey assemblages were not assessed in this study, but previous studies in the upper and mid-Narragansett Bay Estuary documented significant changes in the abundance of benthic epi-

fauna and infauna among sampling locations and months (Rudnick et al. 1985, Calabretta & Oviatt 2008). Many of these benthic fauna, in turn, constitute important prey for blue crabs, including bivalves (*Mytilus edulis*, *Mulinia lateralis*, and *Gemma gemma*; Seed 1982, Ropes 1989, Hines et al. 1990, this study), littorinid gastropods (Laughlin 1982, Seed 1982), gammarid amphipods (Laughlin 1982, Martin et al. 1989, this study), and polychaetes (Glyceridae and Nereidae; Ropes 1989, Hines et al. 1990, this study).

4.2. Blue crab trophic ecology: stable isotope analysis

Stable carbon ($\delta^{13}C$) isotope signatures were used to differentiate among sources of primary production to the tidal river ecosystem, including the identification of autochthonous and allochthonous carbon sources (Peterson & Howarth 1987, France 1995). The $\delta^{13}C$ compositions of estuarine primary producers are often isotopically distinct across taxa (Hoeinghaus & Davis 2007), and specific to the Seekonk and Taunton Rivers, basal carbon sources are derived from detritus–benthic macroalgae ($\delta^{13}C < -18$‰), phytoplankton ($\delta^{13}C \sim -18$ to -22‰), and terrigenous material ($\delta^{13}C > -22$‰) (Peterson & Fry 1987, Payne & Taylor 2010). The relative contribution of these carbon sources to the food web also varies in response to spatially explicit abiotic conditions (Bucci et al. 2007, Pruell & Taplin 2015). In this study, mean $\delta^{13}C$ values across biota ranged widely from −16.5 to −24.0‰, indeed suggesting multiple sources of carbon to the rivers. Moreover, the respective $\delta^{13}C$ signatures of blue crabs and winter flounder varied spatially along a salinity gradient. Detritus–benthic macroalgae is posited as the main carbon source to the riverine food web, as reported elsewhere (Dittel et al. 2000, 2006, Bucci et al. 2007, Hoeinghaus & Davis 2007), and this production source is ubiquitous throughout the study area (D. Taylor pers. obs.). In contrast, the oligo- and mesohaline regions of the rivers (SR1 and TR1–2) were secondarily supported by allochthonous carbon sources (i.e. terrestrially derived organic material), whereas high-salinity waters in closer proximity to the main estuary (TR3) received substantial carbon inputs from phytoplankton (Dittel et al. 2000). Comparable direct relationships between salinity and $\delta^{13}C$ were observed for juvenile winter flounder in the Narragansett Bay, including a contiguous tidal river, and blue crabs from North Carolina estuaries (USA), attributable to biota consuming $\delta^{13}C$-depleted food resources in upper bay and river habitats (Bucci et al. 2007, Pruell & Taplin 2015). Finally, in this study, blue crabs exhibited a pronounced size-based shift in their $\delta^{13}C$ isotopic composition. The more enriched mean $\delta^{13}C$ signature of crabs ≤49 mm CW (mean $\delta^{13}C = -15.6$‰) implies that early juveniles principally derived carbon from benthic trophic assemblages. This supposition is supported by the stomach content data, i.e. crustaceans and polychaetes accounted for >86%, by volume, to the diet of small crabs, and these prey had a mean $\delta^{13}C$ signature of −17.4‰. Conversely, the more depleted $\delta^{13}C$ signature of late juvenile and adult crabs (>50 mm CW; $\delta^{13}C \sim -18.2$‰) suggests an increased reliance on phytoplankton-based carbon, which is effectively explained by the dietary contribution of suspension-feeding bivalves in larger crabs (% V = 27–40%, $\delta^{13}C = -20.5$‰).

Stable nitrogen ($\delta^{15}N$) signatures were used to quantify the trophic position of blue crabs as a function of their time-integrated feeding history (Michener & Schell 1994), where crab chelae muscle tissue has a $\delta^{15}N$ isotope half-life of ~22 d (Llewellyn & La Peyre 2011). Blue crabs occupied a mean trophic position of 3.5 (range = 2.6–4.4) in the Seekonk and Taunton Rivers, indicating that crabs were mainly secondary–tertiary consumers in the rivers, with some evidence of persistent omnivory (~5% of crabs had TP < 3.0). Similar trophic positions were cited for juvenile and adult blue crabs from native western Atlantic and Gulf of Mexico coastal habitats (Baird & Ulanowicz 1989, Akin & Winemiller 2008, Rodríguez-Graña et al. 2008) and non-indigenous environments (Carrozzo et al. 2014, Mancinelli et al. 2016), with trophic levels ranging from ~2.6 to 4.3. The broad range of trophic values calculated in this study (~2 full levels), irrespective of crab size, underscores the trophic flexibility and opportunistic foraging strategy of this species (Hines 2007, Mancinelli et al. 2017).

Visual analysis of blue crab stomach contents affirmed ontogenetic variations in diet, yet shifts in prey composition across crab sizes did not result in corresponding changes in their trophic status. Crabs delineated into small, medium, and large size classes had remarkably consistent trophic positions (TP = 3.53–3.60). The comparable trophic levels of these crabs are adequately explained by the near equivalent TPs of their respective prey (i.e. $TP_{\bar{P}rey}$; Eq. 5), which ranged between ~2.4 and 2.5. Numerous studies report a strong positive relationship between trophic position (or $\delta^{15}N$) and body size for many aquatic taxa (Keppeler et al. 2020 and references therein), but this

relationship is absent for *Callinectes* sp. in several geographic areas, including the Delaware Bay, Delaware (for crabs >30 mm CW; Dittel et al. 2006); Apalachicola Bay, Florida (Wilson et al. 2009); Guadalupe Estuary, Texas (Hoeinghaus & Davis 2007); and non-native coastal systems of the Ionian and Adriatic Seas (Mancinelli et al. 2017). In this study, the lower than anticipated trophic position of late juvenile and adult blue crabs, relative to smaller conspecifics, is attributed to their increased dietary reliance on bivalves, which, in turn, occupy a low trophic level in the tidal rivers and adjoining estuary (TP ~ 2.1). This is further supported by the depleted $\delta^{13}C$ signatures in bivalves, which implies this taxon consumes phytoplankton (e.g. pelagic diatoms; Shumway et al. 1985), as noted above. Lastly, the $\delta^{15}N$ signature and trophic status of blue crabs exhibited fine-scale spatial differences among riverine sites. Crabs occupying the lower reaches of the Taunton River, in particular, demonstrated a reduced trophic position (TP at TR3 = 3.2) because of the increased dietary contribution of bivalves at this site (%V = 48%). Alternatively, the added effect of site-specific abiotic conditions (e.g. nutrient loading and nitrogen biogeochemistry) may affect crab $\delta^{15}N$ signatures at varying riverine locations (Pruell et al. 2006, Bucci et al. 2007), perhaps most markedly in the middle and upper reaches of the Seekonk River (Doering et al. 1990).

4.3. Blue crab predation on winter flounder: visual and genetic analyses

Predation during the early juvenile stage is critical in regulating year-class strength and recruitment success of flatfish (Bailey 1994), but assessing the extent of predator-induced mortality during this life stage is often constrained by methodology. Foremost, direct visual examination of a predator's diet is problematic when consumed prey are unrecognizable after tissue maceration and digestion (Amundsen & Sánchez-Hernández 2019). In this study, stomach content analysis was coupled with molecular genetic techniques to ascertain the frequency of blue crab predation on winter flounder in natural populations. Winter flounder were visually identified in the diet of 2.5% of crabs collected from the Seekonk and Taunton Rivers, with the majority of flounder identified by the recovery of their otoliths from predator stomachs. In comparison, the PCR-based assay used in this study detected winter flounder DNA in 17.7% of field-collected crabs. These results are consistent with incidences of blue crab predation on winter flounder in the Shinnecock Bay, where 21.8% of crabs >54 mm CW had target flounder DNA in their gut contents (12 of 55 samples; Collier et al. 2014). The discrepancy in flounder detection rates between direct and genetic methodologies is mostly attributed to piscine prey being visually unidentifiable to the species level owing to tissue deterioration (Amundsen & Sánchez-Hernández 2019) and partial predation events that did not result in crabs ingesting recognizable flounder remains, e.g. otoliths (Seikai et al. 1993). Moreover, in this study, PCR analysis revealed that ~27% of prey initially categorized as unidentified fish by visual inspection were indeed winter flounder.

PCR-based assays may overestimate the prevalence of winter flounder in blue crab stomachs if the procedure erroneously amplified non-target DNA (methodological false positivity; Taylor 2004). The intraspecific primer set used in this study (WF208), however, exclusively amplified winter flounder DNA without cross-reacting with the genetic material of blue crabs or non-target fish and invertebrate prey (i.e. primer set has high sensitivity and specificity; Taylor et al. 2019). Further, sequencing of winter flounder PCR products verified that the WF208 primers amplified the expected mitochondrial non-coding control region of the flounder genome (Taylor et al. 2019), which negates methodological false-positive results. It is noteworthy that the occurrence of winter flounder in blue crab stomachs does not necessarily constitute a direct predator–prey interaction (circumstantial false positivity; Taylor 2004). For example, blue crabs may scavenge on dead or moribund flounder (Hines 2007), and kleptoparasitism commonly occurs in portunid crabs (Quinn & Boudreau 2016). Circumstantial predation may also result from crabs opportunistically feeding on flounder within sampling nets or via secondary detection, wherein crabs consume prey whose own gut contents contain flounder (Taylor 2004, 2005a, Collier et al. 2014). These circumstantial events, however, are unlikely because net predation was not directly observed in this study (D. Taylor pers. obs.), and secondarily consumed winter flounder are probably undetectable using molecular techniques because multiple predatory interactions exacerbate the degradation of target DNA and extend the cumulative digestion period beyond the PCR assay's detection limits (Collier et al. 2014, this study).

The molecular techniques implemented in this study may alternatively underestimate blue crab predation on winter flounder by failing to amplify target genetic material within a predator's alimentary sys-

tem (methodological false negativity; Taylor 2004). The WF208 primers were moderately efficacious in detecting winter flounder DNA in the stomachs of confirmed crab predators (~38% amplification), which is due, in part, to the primers' ineffectiveness in amplifying biological variants of the target DNA region (Collier et al. 2014). Further, the inability to generate PCR products for some ingested flounder may result from extensive digestion and degradation of the target DNA (Collier et al. 2014, Taylor et al. 2019). Successfully amplifying consumed flounder DNA depends on preserving the stomach contents of suspected predators shortly after foraging events. *Callinectes* spp. demonstrate a broad array of diel feeding patterns, including a propensity for diurnal (Fitz & Wiegert 1991), nocturnal–crepuscular (Paul 1981, Ryer 1987), or indiscriminate (Bell et al. 2003a) foraging activity. Laboratory experiments presented in this study verified that PCR assays reliably detected flounder DNA 10 h after its initial ingestion by crab predators (at 19–26°C), which is consistent with Collier et al.'s (2014) original study (7 h detection limit at 23°C) and reported blue crab gastric evacuation rates (8–10 h at 20°C; McGaw & Curtis 2013). Field collections of blue crabs from the tidal rivers occurred from morning to mid-day (08:00–16:00 h). Accordingly, PCR analysis adequately surveyed the crabs' diet over a complete diel cycle, with the exception of dusk and early night foraging events. Given the possibility for false negativity (e.g. DNA variants and degradation, testing beyond detection limits), the results presented herein are conservative because some unidentified piscine prey (>27%) are likely winter flounder. Notwithstanding these limitations, the PCR-based assays used in this study improved the detection of biological interactions between blue crabs and winter flounder in natural populations.

The predator–prey interactions between blue crabs and winter flounder observed in this study were most affected by their respective body sizes. Visual and genetic dietary analyses affirmed that occurrences of flounder in crab stomachs were positively related to increases in predator size and decreases in prey length. Moreover, the CWs of predatory crabs were 23 to 160 mm (mean ~86 mm CW; n = 60), and they consumed flounder ranging from 26 to 66 mm TL (mean ~47 mm TL; n = 18). These results closely correspond to previous laboratory experiments that examined size-dependent predation by green crabs *Carcinus meanas* (family Portunidae) on post-settlement winter flounder and plaice *Pleuronectes platessa* (family Pleuronectidae) (Van der Veer & Bergman 1987, Fairchild & Howell 2000). In these investigations, green crab predation on juvenile flatfish was limited to individuals >20 and 26 mm CW, respectively, beyond which predation rates progressively increased at larger crab sizes (Van der Veer & Bergman 1987, Fairchild & Howell 2000). Juvenile plaice also achieved a size refuge from green crab predation at 51–50 mm TL, irrespective of predator size (maximum crab size = 50 mm CW; Van der Veer & Bergman 1987). Lastly, immunological dietary analysis of green crabs collected from the Niantic River (Connecticut, USA; crab size range = 14–74 mm CW) revealed that individuals <29 mm CW did not feed on winter flounder, and maximal predation rates occurred at a mean flounder size of 35 mm TL (Taylor 2005a). The size relationships governing portunid crab predation on flatfish is attributed to larger crabs having increased foraging efficiency on mobile and elusive fish prey (Mascaro et al. 2003) and, conversely, large-bodied flounder benefitting from improved responsiveness and escape capabilities after a predatory attack (Taylor 2003).

The prevalence of winter flounder in the diet of blue crabs was density dependent and influenced by spatially explicit abiotic conditions in the tidal rivers. First, visual and genetic analyses documented 60 unique predator–prey interactions between crabs and flounder, 77% of which occurred in the Seekonk River. Visual examination of crab stomach contents also revealed that the frequency of predatory events was directly related to flounder density. The mean monthly density of flounder was 12× greater in the Seekonk River (9.5 flounder 10 m^{-2}) compared to the Taunton River (0.8 flounder 10 m^{-2}), which likely increased the number of interspecific encounters and subsequent predation events (Taylor 2003). Second, the extent of crab predation on flounder was negatively associated with salinity, such that 82% of confirmed interspecific interactions occurred at <10 ppt. This apparent salinity effect coincides with the elevated densities of flounder in oligohaline waters (Taylor et al. 2016), including the upper reaches of the Seekonk River, where predation rates were maximal (mean monthly density at SR1 ~20 flounder 10 m^{-2}). Conversely, blue crab foraging on winter flounder may be lower in polyhaline habitats (SR3 and TR3) because these areas support greater abundances of bivalves, an alternative and preferred dietary item of blue crabs (Seed 1982, Ropes 1989, Hines et al. 1990, this study). Finally, occurrences of winter flounder in blue crab stomachs, as determined by visual inspection, were inversely related to DO concentrations. Approximately 2% of crabs collected

under normoxic conditions fed on winter flounder (>4 mg DO l^{-1}; 12 of 690 stomachs), whereas %*F* increased to 14% in moderately hypoxic waters (2–4 mg DO l^{-1}; 7 of 50 stomachs). Previous studies report that deficiencies in DO adversely affect numerous estuarine biota, with the severity of effects differing among taxa (Diaz et al. 2004). Juvenile winter flounder, in particular, are highly sensitive to oxygen stress (Howell & Simpson 1994, Stierhoff et al. 2006, Meng et al. 2008), and exposure to even sub-lethal, moderate hypoxia can have detrimental effects on their antipredator behaviors, including locomotory performance and responsiveness to predatory attempts (Domenici et al. 2007, Brady & Targett 2010). It is noteworthy that blue crab predation on winter flounder was not observed during severe hypoxia (<2 mg DO l^{-1}; 0 of 25 stomachs), which is consistent with an earlier biotelemetry study that reported a decrease in blue crab feeding activity at DO concentrations <2 mg l^{-1} and complete foraging cessation at ~1 mg 1^{-1} (Bell et al. 2003b).

4.4. Potential effect of blue crab predation on winter flounder natural mortality

Prior studies ascribed decapod crustaceans as important sources of predator-induced mortality for juvenile winter flounder in southern New England nurseries and, thus, possible determinants of flounder year-class strength and recruitment success (Taylor 2005a,b). Dietary immunoassays of green crabs surveyed in the Niantic River, for example, detected winter flounder proteins in ~7% of the crab stomachs (Taylor 2005a). Deterministic model simulations further estimated that green crabs contributed ~10% to the annual cumulative mortality of the juvenile winter flounder year class. The predatory impact of blue crabs described hereafter is inferred from deterministic model simulations executed for green crabs (see Taylor 2005a and Taylor & Fehon 2021 for model description), noting 3 key points: (1) prey size constraints are comparable between the portunid crab species, i.e. winter flounder obtain a size refuge from blue crab and green crab predation at 66 mm TL and 50 to 70 mm TL, respectively (Fairchild & Howell 2000, this study); (2) the 2 crab species have similar spatiotemporal density patterns in southern New England estuaries (Taylor 2005a, Taylor & Fehon 2021), although blue crabs are ~2.6× less abundant than green crabs (mean peak seasonal abundance estimated from normal density functions = 0.64 blue crab 10 m^{-2} and 1.7 green crabs 10 m^{-2}; Taylor 2005a, Taylor & Fehon 2021); and (3) the frequency of winter flounder in blue crab stomachs is 2.5× greater than occurrences in the green crab diet (%*F* = 17.7 for blue crabs and 7.1% for green crabs; Taylor 2005a, this study). A cursory estimate of the blue crab's contribution to winter flounder mortality (*M*) was calculated as the product of the total mortality attributed to green crab predation (*M* = 10.2%) and the proportional interspecific differences in peak crab abundances (0.64 blue crab 10 m^{-2}/1.7 green crabs 10 m^{-2} = 0.38) and frequencies of flounder in crab stomachs (17.7%/7.1% = 2.49). Accordingly, blue crabs have a projected predatory effect on post-settlement winter flounder comparable to green crabs, currently consuming 9.6% ($M = 10.2 \times 0.38 \times 2.49 = 9.6$) of the year class annually. Because of the relatively high prevalence of winter flounder in the blue crab diet, however, subtle increases in crab density will have pronounced negative effects on juvenile flounder survival. Anticipated temperature-mediated increases in blue crab densities at northern latitudes may, therefore, hinder the recovery of winter flounder in this geographic area (Hines et al. 2010, Hare et al. 2016, Glandon et al. 2019). It follows that subsequent research should systematically monitor the biotic interactions between blue crabs and winter flounder in diverse habitat types within southern New England estuarine complexes.

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