



FEATURE ARTICLE

Piscivory in age-0 summer flounder *Paralichthys dentatus* with a focus on predator-induced mortality of post-settlement winter flounder *Pseudopleuronectes americanus*

David L. Taylor*, Kelly J. Cribari, Abigail Scro

Roger Williams University, Department of Marine Biology, One Old Ferry Road, Bristol, RI 02809, USA

ABSTRACT: We examined the piscivorous diet of age-0 summer flounder *Paralichthys dentatus* in southern New England tidal rivers, with a focus on their predatory impact on post-settlement winter flounder *Pseudopleuronectes americanus*. The population density, size-structure, and growth of age-0 summer flounder and winter flounder were evaluated in the Seekonk and Taunton Rivers (Rhode Island and Massachusetts, USA, respectively) between May and August/September 2009 through 2015. For a subsample of summer flounder collected during this time (20–181 mm total length, TL; $n = 743$), diet was assessed using direct visual analysis and PCR-based assays that detect winter flounder mitochondrial DNA within predator stomach contents. Summer flounder were generalist piscivores consuming 8 distinct fish prey taxa from both epibenthic and pelagic guilds. The most frequently observed fishes in the diet of summer flounder were age-0 winter flounder and herring (Clupeidae) with frequencies of occurrence, % F , of 2.6 and 2.0%, respectively, and overall % F of fish equal to 13.6%. Fish were absent in the stomachs of summer flounder <44 mm TL, beyond which piscivory increased significantly with increasing predator size. Summer flounder 50–153 mm TL preyed on winter flounder ranging from 19–54 mm TL, resulting in predator-to-prey size ratios of 2.2–3.6 (mean \pm SD = 2.8 ± 0.3). Incidences of summer flounder predation on winter flounder were positively related to body size ratios, and this relationship was attributed to the enlarged mouth gape and improved prey capture abilities of larger predators. Summer flounder predation on fishes, including winter flounder, also demonstrated significant spatiotemporal variability, reflecting riverine and seasonal differences in flounder population size structure and dynamics in prey composition and availability. Deterministic model simulations estimated that age-0 summer flounder account



Age-0 summer flounder *Paralichthys dentatus* (right) are predators of winter flounder *Pseudopleuronectes americanus* (left) in tidal rivers that adjoin the Narragansett Bay Estuary (Rhode Island/Massachusetts, USA).

Photos: J. Reynolds and D. Taylor

for 0.7% of the daily mortality of post-settlement winter flounder (range = 0.0–2.9%), and consumed 3.0% of the total winter flounder year-class annually (range = 0.0–12.8%). Therefore, relative to other predatory fishes and decapod crustaceans, age-0 summer flounder likely have a nominal effect on winter flounder populations in tidal river nurseries. Summer flounder predation may be substantial, however, when multiple age-classes are considered and elevated age-0 summer flounder densities elicit a strong effect on winter flounder survival, albeit at local scales.

KEY WORDS: *Paralichthys dentatus* · *Pseudopleuronectes americanus* · Summer flounder · Winter flounder · Predation · Diet · Piscivory · PCR · Size ratio · Mouth gape

*Corresponding author: dtaylor@rwu.edu

1. INTRODUCTION

Predation is a key source of mortality for early life history stages of marine fish (Bailey & Houde 1989, Chambers & Trippel 1997); therefore, fisheries research has focused on identifying factors that affect trophic interactions between piscivores and fish prey. Numerous investigations have demonstrated that predator-induced mortality of early-stage fish is strongly influenced by the relative body sizes of interacting individuals, i.e. predator-to-prey size ratios (Paradis et al. 1996, Claessen et al. 2002, and references therein). Successful foraging attempts by piscivorous predators, for example, are positively correlated with their body size due to concomitant increases in pursuit swimming ability and visual acuity (Ellis & Gibson 1997, Wootton 1998). Similarly, the responsiveness of fish prey to predatory attacks is size-dependent, such that reaction distance and swimming performance is enhanced with increasing size (Wootton 1998). Large-bodied fish prey may also be inaccessible to certain predators because of morphological constraints, e.g. prey size exceeds the mouth gape of the predator (Manderson et al. 2000, Nilsson & Brönmark 2000, Scharf et al. 2000). Accordingly, trophic interactions are dynamic throughout fish ontogeny and presumably respond to spatiotemporal variations in processes that affect predator-to-prey size ratios, e.g. interspecific developmental and growth rates (Taylor 2003, 2005a).

The progressive development of early-stage fish may result in age- and size-specific habitat requirements, consequently exposing individuals to novel predator types (Werner et al. 1983, Werner 1986). Flatfish, specifically, undergo a pronounced morpho-behavioral metamorphosis characterized by pelagic larvae transitioning into benthic juveniles (Able & Fahay 2010). This ontogenetic habitat shift changes predator species composition; for example, larval flatfish are vulnerable to pelagic coelenterates and zooplanktivorous fishes (Bailey & Batty 1984, Purcell 1985, Van der Veer 1986), whereas juvenile flatfish are consumed by decapod crustaceans, demersal fishes, and avian piscivores (Leopold et al. 1998, Manderson et al. 1999, 2000, Taylor 2005a,b, Collier et al. 2014). The transition in habitat usage following metamorphosis, and altered trophic interactions, may be critical in determining year-class strength and recruitment success of flatfish (Bailey 1994, Gibson 1994).

The winter flounder *Pseudopleuronectes americanus* is a pleuronectid flatfish occurring in north-west and mid-Atlantic waters from Nova Scotia, Canada, southward to Maryland, USA (Pereira et al.

1999). Coastal populations of winter flounder spawn demersal eggs inside estuaries during the winter and early spring (Pearcy 1962). After hatching ~14 to 21 d post-spawning, larval winter flounder are pelagic for ~60 d (Chambers & Leggett 1987), after which they transition to benthic juveniles during the late spring and early summer (Pearcy 1962). A variety of decapod crustaceans and demersal fishes feed on recently settled winter flounder (Manderson et al. 1999, 2000, Taylor 2005a,b, Collier et al. 2014), which is attributed to their small size at settlement (~8 mm total length; Able & Fahay 2010) and weak swimming and burying capabilities (Manderson et al. 1999, Phelan et al. 2001). The result is intense predator-induced mortality, and thus possible year-class determination during the early juvenile stage (Taylor 2005a,b).

The summer flounder *Paralichthys dentatus* is a paralichthid flatfish whose geographic distribution and habitat requirements vary considerably across life history stages. Adult summer flounder inhabit estuarine and inner continental shelf waters in the Southern New England-Middle Atlantic Bight from Massachusetts to North Carolina, USA (Packer et al. 1999). Summer flounder spawn offshore on the continental shelf during the fall and early winter, producing pelagic eggs that hatch ~3 d post-spawning (Packer et al. 1999). Planktonic summer flounder larvae recruit to inshore nurseries from October to May, after which they metamorphose into benthic juveniles. The geographic range of juvenile summer flounder, historically delineated as inshore nurseries between New Jersey and North Carolina, USA (Able & Kaiser 1994), has recently extended further poleward into southern New England estuaries and coastal habitats (Taylor et al. 2016). The range extension of juvenile summer flounder is caused by elevated water temperatures in the northwest Atlantic (Smith et al. 2010, Taylor et al. 2016, Morley et al. 2018) and their associated effects on the latitudinal distribution of the adult summer flounder spawning stock (Nye et al. 2009) and over-wintering survival of early-stage flounder spawned the previous fall (Malloy & Targett 1991).

The occurrence of juvenile summer flounder in southern New England habitats may have important consequences to resident, post-settlement winter flounder. Most notably, the spatiotemporal overlap of flounder species could promote predator-prey interactions. Taylor & Gervasi (2017) examined the feeding habits of juvenile (age-0) summer flounder from the Seekonk and Taunton Rivers (Rhode Island and Massachusetts, respectively), 2 tidal rivers that adjoin the Narragansett Bay Estuary and serve as function-

ally important nurseries (Taylor et al. 2016). Direct visual analysis of summer flounder stomach contents revealed ontogenetic dietary shifts toward piscine prey. Of the identifiable fish remains, winter flounder had the greatest contribution to summer flounder diet, thus verifying the existence of this predator–prey interaction in southern New England nurseries. This paper is an extension of Taylor & Gervasi (2017) and provides a more comprehensive analysis of piscivory in age-0 summer flounder, with a focus on their predatory impact on post-settlement winter flounder. For the latter, the objectives were 3-fold: (1) quantify the incidence of summer flounder predation on winter flounder, as revealed by conventional stomach content analysis and novel molecular genetic techniques; (2) ascertain the effect of spatiotemporally explicit abiotic and biotic factors on the predator–prey interaction; and (3) calculate the daily instantaneous and cumulative mortality rates of winter flounder, as caused by summer flounder predation, using a size-dependent deterministic model.

2. MATERIALS AND METHODS

2.1. Field sampling

A complete description of the field sampling methodology is provided by Taylor et al. (2016) and Taylor & Gervasi (2017). Briefly, age-0 summer flounder and winter flounder were collected from the Seekonk and Taunton Rivers from May through August/September each year from 2009 through 2015 (Fig. 1). Fort-

nightly sampling occurred at 3 to 4 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 at each site varied due to tidal stage and beach profiles (average and range of area sampled per site: 857 m² and 185–1848 m²). For each sampling effort, captured summer flounder and winter flounder were enumerated (no. ind. m⁻²) and measured for ‘fresh’ total length (TL_{fi} ±1 mm). A random subsample of flounder was immediately preserved in 70 % ethanol or placed on ice and frozen at –20°C in the laboratory, thereby preserving individuals for subsequent diet analysis and morphological measurements. Flounder not retained for laboratory analyses were returned to their place of capture.

Water temperature (°C), salinity (ppt), and dissolved oxygen (mg l⁻¹) were measured at each site per date by using a handheld YSI Model 85 meter, and these results were previously reported in Taylor & Gervasi (2017). Briefly, mean monthly water temperature and dissolved oxygen were comparable across river sites but varied temporally, ranging from 17.6–28.4°C (maximal in July) and 4.4–12.3 mg l⁻¹ (decreased monthly), respectively. Salinity gradually increased over time and differed markedly among river sites, with the upper reaches of the rivers characterized as oligohaline waters (mean salinity ≤5 ppt; sites SR1 and TR1) and the mid- and lower portions defined as mesohaline (salinity 6–18 ppt; sites SR2–SR4 and TR2–TR3) and polyhaline (salinity ≥19 ppt; TR4) (Fig. 1).

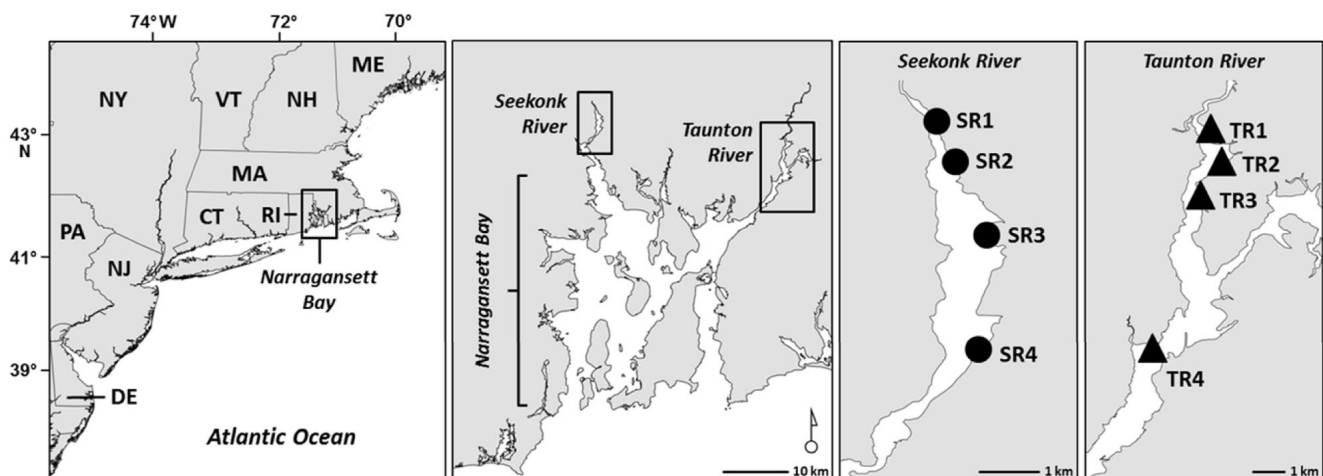


Fig. 1. Seekonk River (SR), Rhode Island, and Taunton River (TR), Massachusetts, (USA) with points denoting collection sites of age-0 summer flounder *Paralichthys dentatus* and winter flounder *Pseudopleuronectes americanus*. Four sites were sampled fortnightly in each river (SR1–SR4; TR1–TR4) from May to August/September 2009–2015, with the exception of SR3 and TR3, which were surveyed 2009–2011

2.2. Flounder size structure, population density, and growth rates

For each year (2009 through 2015) and river (Seekonk and Taunton), summer flounder and winter flounder length–frequency distributions were created by compiling monthly catch data (May through August/September) and organizing body sizes into intervals of 5 mm TL_f. As described by Taylor et al. (2016), intraspecific cohorts were identified by visually inspecting the length–frequency distributions, and then verified using the modal progression routine of FiSAT II (Gayanilo et al. 2002).

Summer flounder and winter flounder population densities analyzed separately by river and year were determined at daily increments by fitting a normal density function to catch data over time:

$$\rho_t = \left[\frac{1}{\sigma\sqrt{2\pi}} e^{-0.5[(t-\mu)/\sigma]^2} \right] \cdot \varepsilon \quad (1)$$

where ρ_t is the density of summer flounder or winter flounder per seine haul (no. ind. m⁻²) at time t (day of year, DOY), μ is the arithmetic mean of the distribution that indicates the DOY of maximum density, σ is the standard deviation of the distribution, and ε is a correction parameter that converts the probability mass function to units of density. The dates of maximum flounder density (μ) were determined by back-calculating intraspecific growth models (Eq. 2; see below) to the DOY on which summer flounder mean body sizes were 13 mm TL_f and winter flounder were 8 mm TL_f (i.e. size at settlement; Able & Fahay 2010). The remaining parameters of the normal density function (σ and ε) were determined by non-linear least-squares analysis. Finally, pairwise Kolmogorov-Smirnov (K-S) tests were performed on summer flounder and winter flounder catch data, pooled across years, to compare intraspecific density distributions from the Seekonk and Taunton Rivers against the null hypothesis that samples were from populations with the same parametric distribution (Sokal & Rohlf 1981). Here and elsewhere, statistical analyses were performed using SAS ver. 9.1, and significance was set *a priori* at $\alpha = 0.05$.

Summer flounder and winter flounder growth rates analyzed separately by river and year were determined by first fitting logarithmic regression models to body size data measured during field sampling:

$$TL_{f,t} = \alpha + \beta \times \ln(t) \quad (2)$$

where $TL_{f,t}$ is the mean ‘fresh’ TL of summer flounder or winter flounder per seine haul (mm) at time t , and α and β are intercept and slope parameters estimated

by least-squares analysis. Results from the regression models were used to estimate flounder growth rates during a standardized time period (21 May to 10 September; DOY = 141–253, which represents the range of dates examined in this study):

$$G = \frac{TL_{f,Final} - TL_{f,Initial}}{t_{Final} - t_{Initial}} \quad (3)$$

where G is the growth rate of summer flounder or winter flounder (mm TL_f d⁻¹), $TL_{f,Final}$ and $TL_{f,Initial}$ is the ‘fresh’ TL of flounder (mm) obtained on Day 253 (t_{Final}) and Day 141 ($t_{Initial}$), respectively. Lastly, analysis of covariance (ANCOVA) models were used to examine differences in summer flounder and winter flounder growth rates (and predator-to-prey size ratios; see Section 2.6) between the Seekonk and Taunton Rivers, with DOY (ln-transformed) as the covariate and river as the discrete explanatory variable. For these analyses, Eq. (2) was applied to intraspecific $TL_{f,t}$ data pooled across years.

2.3. Visual analysis of summer flounder diet

Summer flounder previously preserved in 70% ethanol were measured for ‘preserved’ TL (TL_p ; ± 1 mm) in the laboratory. Prey were then extracted from summer flounder stomachs, transferred to 3.7 ml borosilicate vials with 70% ethanol, and later visually identified to the lowest practical taxon using stereoscopic microscopes, as reported by Taylor & Gervasi (2017). Each fish taxon’s contribution to the diet of summer flounder was expressed as frequency of occurrence (% F), which equaled the number of summer flounder stomachs containing a specific fish taxon divided by the total number of examined stomachs. When possible, fish prey recovered from summer flounder stomachs were measured for TL_p using Vernier calipers (± 0.01 mm).

2.4. Molecular genetic analysis of summer flounder diet

The majority of fish prey recovered from summer flounder stomachs were categorized as ‘unidentified fish’ (Table 1; see Section 3.2). Accordingly, molecular genetic techniques were used to determine if the unidentified fish were winter flounder by testing for the presence of intraspecific genomic DNA. For each unidentified fish recovered from summer flounder stomachs ($n = 60$), ~5 mg wet weight of tissue were removed and transferred to 1.5 ml microcentrifuge

Table 1. Frequency of occurrence (%F) of fish prey in the stomachs of summer flounder *Paralichthys denatus* from 4 sites in the Seekonk River (SR1–SR4) and Taunton River (TR1–TR4) (Rhode Island and Massachusetts, USA, respectively; see Fig. 1). The numbers of stomachs analyzed from each river site (n) are reported. Winter flounder *Pseudopleuronectes americanus* occurrences in summer flounder stomachs were determined from visual inspection and PCR analysis confirmed via DNA sequencing. Mean preserved total lengths (ranges in parentheses, mm) are also presented for intact fish prey that were recovered from summer flounder stomachs

Fish taxon	Seekonk River				Taunton River				Prey total length
	SR1	SR2	SR3	SR4	TR1	TR2	TR3	TR4	
Unidentified fish	11.8	3.9	2.5	5.6	20.0	4.6	9.6	0.0	
Winter flounder	7.8	0.8	0.0	1.4	0.0	0.0	0.0	0.0	29.3 (17.5–51.2)
Herring (Clupeidae)	0.5	0.0	0.0	0.0	8.3	5.5	5.8	0.0	25.0 (20.0–29.2)
Northern pipefish <i>Syngnathus fuscus</i>	0.0	0.8	0.0	0.0	0.0	0.9	3.8	0.0	24.4 (17.5–30.7)
Gobies (Gobiidae)	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	38.0
White sucker <i>Catostomus commersoni</i>	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	22.0 (18.8–24.0)
Atlantic silverside <i>Menidia menidia</i>	0.0	0.0	0.0	0.7	0.0	0.9	0.0	0.0	27.7
American eel <i>Anguilla rostrata</i>	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	55.4
Largemouth bass <i>Micropterus salmoides</i>	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	34.0
Overall %F of fish	21.1	5.5	2.5	7.7	26.7	11.9	19.2	0.0	
n	204	127	40	143	60	109	52	8	

tubes. Tissue extraction and subsequent genetic analyses were also performed for (1) field-collected age-0 winter flounder, i.e. non-consumed winter flounder; (2) winter flounder recovered from summer flounder stomachs, i.e. consumed winter flounder (n = 17); and (3) field-collected age-0 summer flounder and other potential prey, i.e. predator and prey species that were not the target of molecular assays, hereafter referred to as 'non-target' predator and prey. Non-target prey of summer flounder included fish (windowpane flounder *Scophthalmus aquosus*, hogchoker *Trinectes maculatus*, mummichog *Fundulus heteroclitus*, striped killifish *F. majalis*, threespine stickleback *Gasterosteus aculeatus*, fourspine stickleback *Apeltes quadracus*, goby *Gobiosoma* sp., northern pipefish *Syngnathus fuscus*, and American eel *Anguilla rostrata*), crustaceans (sand shrimp *Crangon septemspinosa*, Atlantic mud crab *Panopeus herbstii*, blue crab *Callinectes sapidus*, and long-clawed hermit crab *Pagurus longicarpus*), polychaetes (*Nereis* spp.), and mollusks (eastern oyster *Crassostrea virginica*, softshell clam *Mya arenaria*, and eastern mud snail *Nassarius obsoleta*). Genomic DNA was extracted from each 5 mg sample using a Qiagen DNeasy Blood and Tissue Kit following a modified spin-column protocol for animal tissues. To increase DNA yield, specifically, the elution step was repeated twice with a single 100 µl volume of Buffer AE. The nucleic acid concentrations and quality of DNA of all tissue types were determined by spectrophotometry (NanoDrop 2000c Spectrophotometer, Thermo Scientific).

A species-specific polymerase chain reaction (PCR)-based method was used to amplify a 208 bp sequence

of winter flounder DNA from prepared samples. The oligonucleotide primers implemented in this study (WF208) were first identified by Collier et al. (2014) and were synthesized by Integrated DNA Technologies (Coralville, Iowa, USA). The WF208 primer set targets the mitochondrial non-coding control region (D-loop) of the winter flounder genome (GenBank accession number U12068), with 4 sections of the D-loop used to create the forward and reverse primers (Collier et al. 2014). The PCR-based and gel electrophoresis methods described herein follow the protocol of Collier et al. (2014) with minor modifications. A 12.5 µl small-scale PCR reaction was initiated by combining 10.5 µl of 2× MyTaq Red DNA polymerase (Bioline USA), 0.5 µl of each 10 µM WF208 primer, and 1 µl of template DNA. Note that when DNA concentrations of prepared samples were <15 ng µl⁻¹, 2 µl of template DNA were added to the reaction (total volume = 13.5 µl). The PCR reaction was performed in a Bio-Rad C1000 and DNA Engine Peltier thermal cycler under the following conditions: 2 min of initial denaturation at 95°C; 35 cycles of 30 s at 95°C, 30 s at 54°C, and 90 s at 72 °C; 10 min of final extension at 72°C; and hold at 4°C. The reaction products, i.e. amplicons, were visualized using gel electrophoresis in 1% agarose gels with 100 ml of a 1× Tris-Acetate-EDTA buffer and stained with 5 µl of GelRed (Biotium) per 100 ml of gel solution. PCR products were viewed using UV trans-illumination through a Kodak 1D 3.6 imager (Kodak Scientific Imaging Systems).

Finally, large-scale PCR reactions for the purposes of DNA sequencing were performed at a total vol-

ume of 47 μ l using previously described concentrations. Seven subsamples across 3 tissue types were chosen for this reaction because of their successful amplification of the targeted gene sequences during the small-scale PCR, including: non-consumed winter flounder ($n = 2$); consumed winter flounder, as determined by visual analysis ($n = 3$); and previously unidentified fish suspected to be winter flounder, as determined by PCR methods ($n = 2$). Gel electrophoresis was used to isolate the desired products, and subsequent extraction and purification of the resulting DNA followed the Wizard SV Gel and PCR Clean-Up System protocol (Promega). Single-strand Sanger sequencing was carried out at the Rhode Island Genomics & Sequencing Center (University of Rhode Island, Kingston, Rhode Island), and was performed using 2 μ l of 2.5 μ M of the WF208 forward primer and 2.5 ng of DNA per 100 bases, with a final volume of 12 μ l. Sequences were then identified to the species level by comparing against GenBank using BLAST (GenBank accession number U12068.1).

2.5. Flounder morphological relationships

Early-stage flounder kept in 70 % ethanol or other preservatives are prone to shrinkage and decreases in body length (Tucker & Chester 1984, Hjörleifsson & Klein-MacPhee 1992), thus making direct comparisons to TL_f problematic. In this study, to account for decreases in flounder length owing to ethanol preservation, linear least-squares regression models were used to examine the relationship between TL_f and TL_p . The TL_f – TL_p relationships were derived from the analysis of 87 summer flounder and 100 winter flounder collected from the Seekonk and Taunton Rivers. Flounder were measured for TL_f immediately after capture (mean \pm SD, TL_f : summer flounder = 87.0 ± 30.6 mm [range = 32–150 mm]; winter flounder = 61.4 ± 16.0 mm [range = 23–91 mm]), preserved in 70 % ethanol for ≥ 2 wk, and individually re-measured for TL_p (TL_p : summer flounder = 82.5 ± 28.9 mm [range = 30–143 mm]; winter flounder = 58.5 ± 15.3 mm [range = 22–89 mm]) in the laboratory. The pairwise length data were then regressed to produce intraspecific TL_f – TL_p linear models (Eqs. 4 and 5; see Section 3.4 and Table 2).

An important objective of this study was to examine summer flounder predation on winter flounder as a function of predator–prey size relationships. However, the winter flounder recovered from summer flounder stomachs were typically masticated (e.g. damaged caudal fins), thereby precluding direct

measurements of their original TL s. Accordingly, the sagittal otoliths of winter flounder recovered from summer flounder stomachs were extracted and prepared using routine procedures (Secor et al. 1991), and subsequently measured for otolith maximum linear length (OL) with the aid of stereoscopic microscopes equipped with stage micrometers (± 0.05 μ m). OLs were then incorporated into a non-linear (exponential) least-squares regression model to predict the original TL_p of consumed winter flounder (Eq. 6; see Section 3.4 and Table 2). The predictive TL_p –OL relationship was derived from the analysis of 51 winter flounder collected from the Seekonk and Taunton Rivers between May and August 2011 through 2013. Field-collected winter flounder were immediately preserved in 70 % ethanol, measured for TL_p and OL in the laboratory (mean \pm SD: $TL_p = 57.4 \pm 18.2$ mm [range = 25–89 mm]; OL = 1.6 ± 0.4 μ m [range = 0.7–2.2 μ m]), and pairwise length data were regressed to produce the TL_p –OL exponential model. A linear least-squares regression model was then used to examine the relationship between the TL_f of summer flounder visually confirmed to feed on winter flounder (TL_f converted from TL_p using Eq. 4) and the predicted TL_f of winter flounder recovered from summer flounder stomachs (Eqs. 5 and 6) (see Section 3.4 and Table 2).

Summer flounder mouth gape and winter flounder body depth were examined to evaluate the putative morphological constraints on the predator–prey interaction. In the laboratory, previously frozen flounder were thawed and measured for non-ethanol preserved TL using Vernier calipers (± 0.01 mm), hereafter also denoted as TL_f (mean \pm SD TL_f : summer flounder = 52.4 ± 22.7 mm [range = 20–137 mm]; winter flounder = 44.1 ± 12.2 mm [range = 17–79 mm]). Using the same calipers, summer flounder mouth gape (MG; $n = 247$) was measured as the maximum linear distance between the upper pre-maxillary and lower dentary jaw bones with the mouth stretched open (mean \pm SD MG = 8.3 ± 4.0 mm [range = 2.7–21.2 mm]). Winter flounder body depth (BD; $n = 278$) was measured as the maximum dorso-ventral linear distance with the dorsal and anal fins pressed against the body wall (mean \pm SD BD = 14.7 ± 4.4 mm [range = 5.4–27.1 mm]). Summer flounder MG and winter flounder BD were regressed with the flounders' respective TL_f to produce MG– TL_f and BD– TL_f linear models (Eqs. 7 and 8; see Section 3.4 and Table 2). Linear least-squares regression analysis was then used to relate summer flounder MG to the BD of winter flounder recovered from summer flounder stomachs (Eqs. 4–8; see Section 3.4 and Table 2).

2.6. Factors affecting summer flounder predation on fish and winter flounder

Multivariate logistic regression analyses, employing a stepwise selection process, were used to test for a significant relationship of 2 response variables (i.e. occurrence of 'fish' or 'winter flounder' in summer flounder stomachs) with several abiotic and biotic explanatory variables. The explanatory variables incorporated into the 'fish' regression model were specific to a field sampling effort (i.e. seine haul by date and river site) and included date of summer flounder capture (DOY), water temperature (°C), salinity (ppt), dissolved oxygen (mg l⁻¹), summer flounder population density (no. ind. m⁻²), and summer flounder TL_f (mm; TL_p measured directly for summer flounder used in stomach content analysis and converted to TL_f using Eq. 4). The 'winter flounder' regression model included the aforementioned explanatory variables, as well as winter flounder population density (no. ind. m⁻²), mean winter flounder TL_f (mm), and the predator-to-prey size ratio (individual summer flounder TL_f / mean winter flounder TL_f). Chi-squared values were calculated to test the significance of each explanatory variable because data were treated as frequency responses (presence or absence of fish or winter flounder in summer flounder stomachs, the latter determined by visual and molecular analysis) rather than continuous responses, and, for a given explanatory variable, the significance level for entry and retention into the regression model was $p < 0.05$. Finally, the natural logarithm of the ratio of response frequencies (Logits) was used to estimate parameters of each linear model. The proportion of summer flounder stomachs containing fish or winter flounder (P_t) at time t was equal to:

$$\text{Logit}(P_t) = \log\left(\frac{P_t}{1 - P_t}\right) = \alpha + \beta_1 X + \beta_2 Y + \beta_3 Z \dots \quad (9)$$

where α is the intercept parameter, β_{1-3} are vectors of the slope parameters, and X , Y , and Z are hypothetical explanatory variables that satisfied the entry and retention requirements for the regression analyses.

2.7. Modeling winter flounder mortality owing to summer flounder predation

The average and maximum daily instantaneous mortality rate (Z_{avg} and Z_{max}) and cumulative mortality rate (M) of age-0 winter flounder, as caused by summer flounder predation, were calculated using a deterministic model (Taylor 2005a,b). The model was

developed to track the daily growth and survival of a single cohort of post-settlement winter flounder in the Seekonk and Taunton Rivers for each year (2009 through 2015). The start period of the model was determined by using Eq. (2) to back-calculate winter flounder growth to the DOY when the mean body size equaled 8 mm TL_f. The total density of each winter flounder cohort was initially 8 ind. m⁻², and their temporal settlement pattern was modeled as a normal distribution (Eq. 1), with μ = DOY when mean winter flounder size was 8 mm TL_f and σ = 10 d. The peak of the normal distribution represents the maximum winter flounder density at time t (ρ_{max}) and was 0.319 ind. m⁻². The total abundance and temporal distribution of winter flounder modeled for this exercise were based on historical data from several north-west Atlantic estuaries during the spring and summer (Sogard et al. 2001, Curran & Able 2002, Yencho et al. 2015, Taylor et al. 2016).

For a given river and year, the deterministic predation mortality model was used to calculate the number of surviving winter flounder at daily time steps:

$$N_t = N_{t-1} - (\rho_{\text{SF},t} \times P_t) \quad (10)$$

where N_t is the number of winter flounder m⁻² surviving to time t , N_{t-1} is the number of winter flounder m⁻² at the previous daily time step, $\rho_{\text{SF},t}$ is the density of summer flounder (no. ind. m⁻²) of a mean TL_f at time t , and P_t is the proportion of summer flounder stomachs containing winter flounder, as estimated from the logistic regression model (Eq. 9). Only those explanatory variables significant at $p < 0.05$ were incorporated in the logistic model, and these variables were the predator-to-prey size ratio (R_t) and salinity (S_t ppt) at time t (see Section 3.5). The population density of summer flounder incorporated into Eq. (10) was re-calculated daily based on Eq. (1), whereas winter flounder density was estimated daily according to additions via benthic settlement and losses attributed to summer flounder predation. The size structures of summer flounder and winter flounder at daily increments were estimated with Eq. (2), and the results were used to calculate R_t (i.e. mean summer flounder TL_f / mean winter flounder TL_f) for inclusion in Eq. (9). For initial model simulations, S_t was held constant at 5 ppt and was representative of conditions in the upper reaches of the tidal rivers (Taylor & Gervasi 2017; Fig. 1). Further, predatory summer flounder were modeled to consume 1.24 winter flounder per feeding episode (i.e. mean no. winter flounder per predator stomach; see Section 3.4) and feed twice in a 24 h period (Malloy & Targett 1991, Yamamoto & Tominaga 2007).

Table 2. Summary statistics for univariate linear and exponential regression models used to examine morphological relationships in summer flounder (SF) and winter flounder (WF). Model variables include: SF and WF preserved and fresh total length (TL_p and TL_f , respectively; mm); WF sagittal otolith maximum length (OL, μm); SF mouth gape (MG; mm); and WF body depth (BD, mm). All models are significant at $p < 0.0001$. Equation numbers as sequentially listed in the text are also presented

Regression model	Equation	n	F	R ²
SF: $TL_f = 1.0549 \times TL_p - 0.1174$	(4)	87	26006	0.997
WF: $TL_f = 1.0436 \times TL_p + 0.3527$	(5)	100	25136	0.996
WF: $\text{Log}(TL_p) = 0.3502 \times \text{OL} + 1.1704$	(6)	51	578	0.922
SF: $MG = 0.1735 \times TL_f - 0.8124$	(7)	247	24948	0.990
WF: $BD = 0.356 \times TL_f - 1.0327$	(8)	278	16325	0.983

The daily instantaneous mortality rate of winter flounder, analyzed separately by river and year, was averaged from the projected day of settlement to Day 253 (Z_{avg}), and the maximum daily instantaneous mortality (Z_{max}) represented the largest value obtained during this time period. Annual cumulative mortality rates of winter flounder (M) in each river were calculated as the total number of individuals eaten by summer flounder m^{-2} divided by the initial cohort size (8 winter flounder m^{-2}). Finally, to account for natural variations in environmental conditions and flounder population dynamics, additional model simulations were performed to estimate winter flounder M over a broad range of salinity (0–30 ppt), predator-to-prey size ratios (1–4), summer flounder maximum density (i.e. peak of normal density function in Eq. 1; $0.01\text{--}0.8 \text{ ind. m}^{-2}$), and winter flounder initial cohort size (4–14 ind. m^{-2}). For these final simulations, flounder density and length models incorporated into Eq. (10) were generated by pooling data across rivers and years.

3. RESULTS

3.1. Flounder size structure, population density, and growth rates

Summer flounder and winter flounder early season length–frequency distributions verified the occurrence of 1 cohort per species, composed exclusively of age-0, post-settlement juveniles. In May, for example, flounder length frequencies were consistently unimodal, irrespective of year (2009 through 2015) and river (Seekonk and Taunton) (Fig. 2). The mean total length (TL_f averaged across years) of summer flounder in May was 44.5 mm (range = 23–82 mm) and 42.8 mm (range = 32–55 mm) in the Seekonk and Taunton Rivers, respectively. Winter flounder were

similarly sized in May, with a mean TL_f of 37.8 mm (range = 20–77 mm) in the Seekonk River and 41.9 mm (range = 28–57 mm) in the Taunton River.

Further analysis of length–frequency distributions indicated that flounder cohorts persisted in June and July, with distributions generally shifting to larger body sizes and broadening in range across months (Fig. 2). Despite these interspecific similarities, patterns in flounder size structure differed in several fundamental respects. First, summer flounder experienced more pronounced positive shifts in their length–

frequency distributions over time relative to winter flounder. Between May and July, for example, the mean TL_f of summer flounder increased by 49.3 mm (averaged across years and rivers), whereas winter flounder TL_f only increased by 19.4 mm during the same time period. Second, summer flounder occurred over a wider range of TL_f in June and July in comparison to winter flounder (mean difference between maximum and minimum TL_f = 103.3 and 65.0 mm for summer flounder and winter flounder, respectively), and these trends were consistent in the Seekonk and Taunton Rivers. Third, irrespective of species, flounder inhabiting the Taunton River in June and July were ~8–15% larger with respect to TL_f relative to conspecifics from the Seekonk River (Fig. 2).

Winter flounder unimodal length–frequency distributions persisted in August/September, yet the body size of these individuals increased modestly (mean increase in TL_f between July and August/September = 5.0 and 3.3 mm in the Seekonk and Taunton Rivers, respectively; Fig. 2). Conversely, summer flounder mean TL_f increased appreciably during this time (mean increase = 17.3 and 28.6 mm in the respective rivers), but the modality of the length–frequency distributions varied by river and year. In August/September, summer flounder length–frequency distributions were unimodal in the Taunton River, irrespective of year (2009 and 2015), as well as in the Seekonk River in 2011. However, bimodal distributions were characteristic of summer flounder in the Seekonk River in 2009 and 2013, with peak distributions occurring at approximately 75 and 150 mm TL_f (Fig. 2).

The magnitude and timing of maximal flounder population density differed by species, river, and year (Table 3). Summer flounder density was greatest in 2009 in the Seekonk and Taunton Rivers (1.05 and 0.69 ind. m^{-2} , respectively), and across years, densities were significantly higher in the Seekonk River relative to the Taunton River (mean \pm SD = 0.32 ± 0.38 and

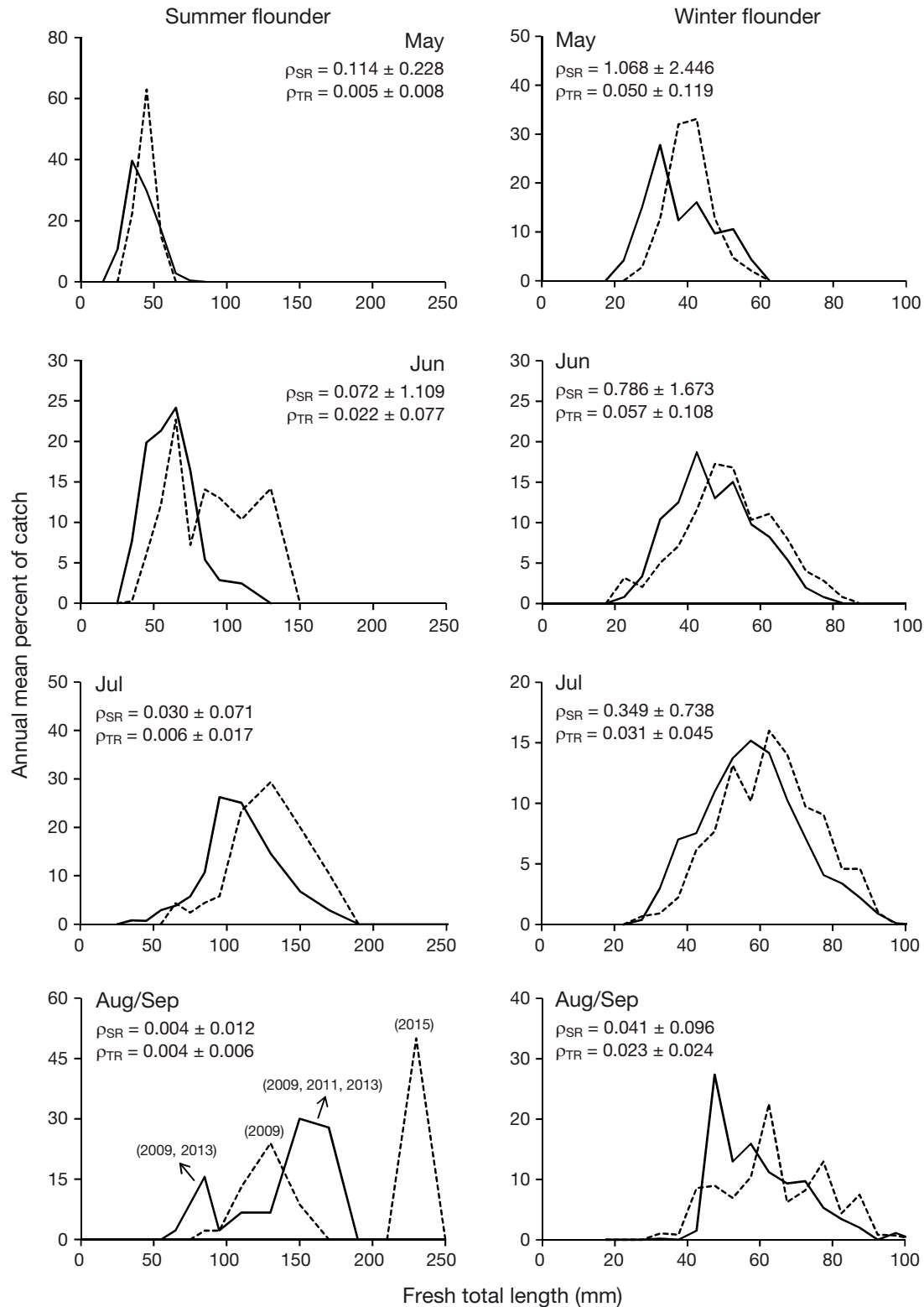


Fig. 2. Monthly length–frequency distributions of summer flounder and winter flounder collected from the Seekonk River (solid line) and Taunton River (dashed line). Lengths represent ‘fresh’ total length, i.e. measured immediately after capture, and frequencies were calculated as percent of total catch averaged across years (2009–2015). The mean monthly density (\pm SD) of summer flounder and winter flounder in the Seekonk River (ρ_{SR}) and Taunton River (ρ_{TR}) are provided. Additionally, collection years are reported in parentheses for summer flounder multi-modal length–frequency distributions in August/September

Table 3. Model simulations that estimate the total number of winter flounder consumed (WF; no. flounder eaten m^{-2}) by summer flounder (SF), and the resulting average and maximum daily instantaneous mortality rate (Z_{avg} and Z_{max} , respectively) and cumulative mortality rate (M ; %). Simulations were performed separately for the Seekonk and Taunton Rivers for the years 2009 through 2015 and accounted for annual variations in maximum flounder density (no. ind. m^{-2}), date of maximum flounder density (day of year [DOY], reported in parentheses), and flounder growth rates (mm fresh total length [TL_t] d^{-1}), with the latter affecting predator-to-prey size ratios (Ratio; SF mm TL_t / WF mm TL_t). Means \pm SD are reported across years

River/Year	Maximum density ^a (DOY ^b)		Growth ^c		Ratio ^c	No. of WF eaten	Z _{avg} ^d	Z _{max}	M
	SF	WF	SF	WF					
Seekonk River									
2009	1.05 (121)	0.71 (37)	0.66	0.17	1.15	1.03	6.86 × 10 ⁻⁴	2.09 × 10 ⁻³	12.83
2010	0.018 (131)	0.11 (99)	1.24	0.30	2.18	0.11	8.64 × 10 ⁻⁵	2.03 × 10 ⁻⁴	1.34
2011	0.14 (131)	0.70 (83)	1.26	0.28	1.93	0.22	1.65 × 10 ⁻⁴	5.68 × 10 ⁻⁴	2.74
2012	0.047 (124)	0.37 (58)	1.26	0.23	1.91	0.54	3.14 × 10 ⁻⁴	6.62 × 10 ⁻⁴	6.79
2013	0.45 (126)	18.7 (94)	1.18	0.36	1.87	0.37	2.81 × 10 ⁻⁴	1.44 × 10 ⁻⁴	4.64
2014	0.53 (142)	0.45 (64)	1.58	0.18	2.18	0.14	9.65 × 10 ⁻⁵	9.44 × 10 ⁻⁴	1.79
2015	0.029 (138)	0.73 (106)	1.30	0.41	1.68	0.053	4.20 × 10 ⁻⁵	1.33 × 10 ⁻⁴	0.66
Mean ± SD	0.32 ± 0.38 (130.4 ± 7.5)	3.11 ± 6.88 (77.3 ± 25.3)	1.21 ± 0.27	0.28 ± 0.09	1.84 ± 0.35	0.35 ± 0.34	2.39 × 10 ⁻⁴ ± 2.22 × 10 ⁻⁴	8.64 × 10 ⁻⁴ ± 7.02 × 10 ⁻⁴	4.40 ± 4.28
Taunton River									
2009	0.69 (123)	0.030 (89)	0.92	0.36	1.46	0.69	6.20 × 10 ⁻⁴	2.25 × 10 ⁻³	8.64
2010	0.00	0.008 (121)	–	0.47	–	0	0	0	0
2011	0.011 (139)	0.064 (150)	2.24	0.47	2.33	0.004	1.66 × 10 ⁻⁶	2.11 × 10 ⁻⁵	0.05
2012	0.016 (134)	0.11 (84)	2.06	0.40	2.08	0.022	1.56 × 10 ⁻⁵	6.19 × 10 ⁻⁵	0.27
2013	0.012 (122)	0.49 (65)	1.22	0.24	2.02	0.025	1.66 × 10 ⁻⁵	5.41 × 10 ⁻⁵	0.31
2014	0.011 (142)	0.046 (81)	1.86	0.29	2.07	0.049	3.55 × 10 ⁻⁵	9.73 × 10 ⁻⁵	0.62
2015	0.002 (142)	0.091 (77)	2.45	0.31	2.41	0.051	3.04 × 10 ⁻⁵	7.60 × 10 ⁻⁵	0.63
Mean ± SD	0.11 ± 0.26 (133.7 ± 9.1)	0.12 ± 0.17 (88.8 ± 18.6)	1.79 ± 0.60	0.36 ± 0.09	2.06 ± 0.33	0.12 ± 0.25	1.03 × 10 ⁻⁴ ± 2.29 × 10 ⁻⁴	3.66 × 10 ⁻⁴ ± 8.33 × 10 ⁻⁴	1.50 ± 3.16

^aMaximum flounder densities were estimated from the peak of normal density functions fitted to annual catch data over time (*t*) (Eq. 1);^bDates of maximum flounder densities were determined by back-calculating intraspecific growth models to the day of year on which summer flounder were 13 mm TL_f and winter flounder were 8 mm TL_f (i.e. size at settlement) (Eq. 2); ^cFlounder growth rates (Eq. 3) and predator-to-prey size ratios were averaged from May 21 to September 10 (Days 141 to 253); ^dWinter flounder average daily instantaneous mortality rates were calculated from the day of year mean body size was 8 mm TL_f (Eq. 2) to Day 253

0.11 ± 0.26 flounder m^{-2} , respectively; K-S = 2.659, $p < 0.0001$; Fig. 3A). Further, maximal summer flounder densities occurred in early and mid-May, and these temporal patterns were consistent across rivers and years (DOY of maximum density in Seekonk and Taunton Rivers = 130.4 ± 7.5 and 133.7 ± 9.1 , respectively).

Winter flounder population densities were maximal in 2013 in the Seekonk and Taunton Rivers (18.7 and 0.49 ind. m^{-2} , respectively) and, similar to summer flounder, abundances were significantly higher in the former river (3.11 ± 6.88 and 0.12 ± 0.17 ind. m^{-2} , respectively; K-S = 3.315, $p < 0.0001$; Table 3, Fig. 3B). The timing of peak winter flounder densities occurred earlier in the season relative to summer flounder, i.e. mid- to late March, and settlement patterns were substantially more variable across rivers and years (DOY of maximum density in Seekonk and Taunton Rivers = 77.3 ± 25.3 and 88.8 ± 18.6 , respectively).

The logarithmic regression models adequately fit the flounder body size data measured during field

sampling (May through August/September; Fig. 3C,D), as indicated by statistically significant model fits (mean $p < 0.005$; range <0.05 to <0.0001) and relatively high R^2 values (mean $R^2 = 0.722$; range = 0.279–0.989), irrespective of species, river, or year. The mean annual growth rate of summer flounder in the Seekonk and Taunton Rivers was 1.21 ± 0.27 and 1.79 ± 0.60 mm d^{-1} , respectively (range = 0.66–2.54 mm d^{-1}), whereas winter flounder growth was comparatively slower in the respective rivers (0.28 ± 0.09 and 0.36 ± 0.09 mm d^{-1} ; range = 0.17–0.47 mm d^{-1} ; Table 3). Intraspecific flounder growth rates did not differ between the Seekonk and Taunton Rivers, i.e. day–river interaction effects were not significant (ANCOVA; day \times river; summer flounder: $F_{1,132} = 0.06$, $p = 0.800$; winter flounder: $F_{1,202} = 1.30$, $p = 0.256$). However, the mean TL ($TL_{f,t}$) of both flounder species at time t (DOY) was significantly larger for individuals collected from the Taunton River (ANCOVA; river; summer flounder: $F_{1,132} = 7.47$, $p < 0.01$; winter flounder: $F_{1,202} = 15.9$, $p <$

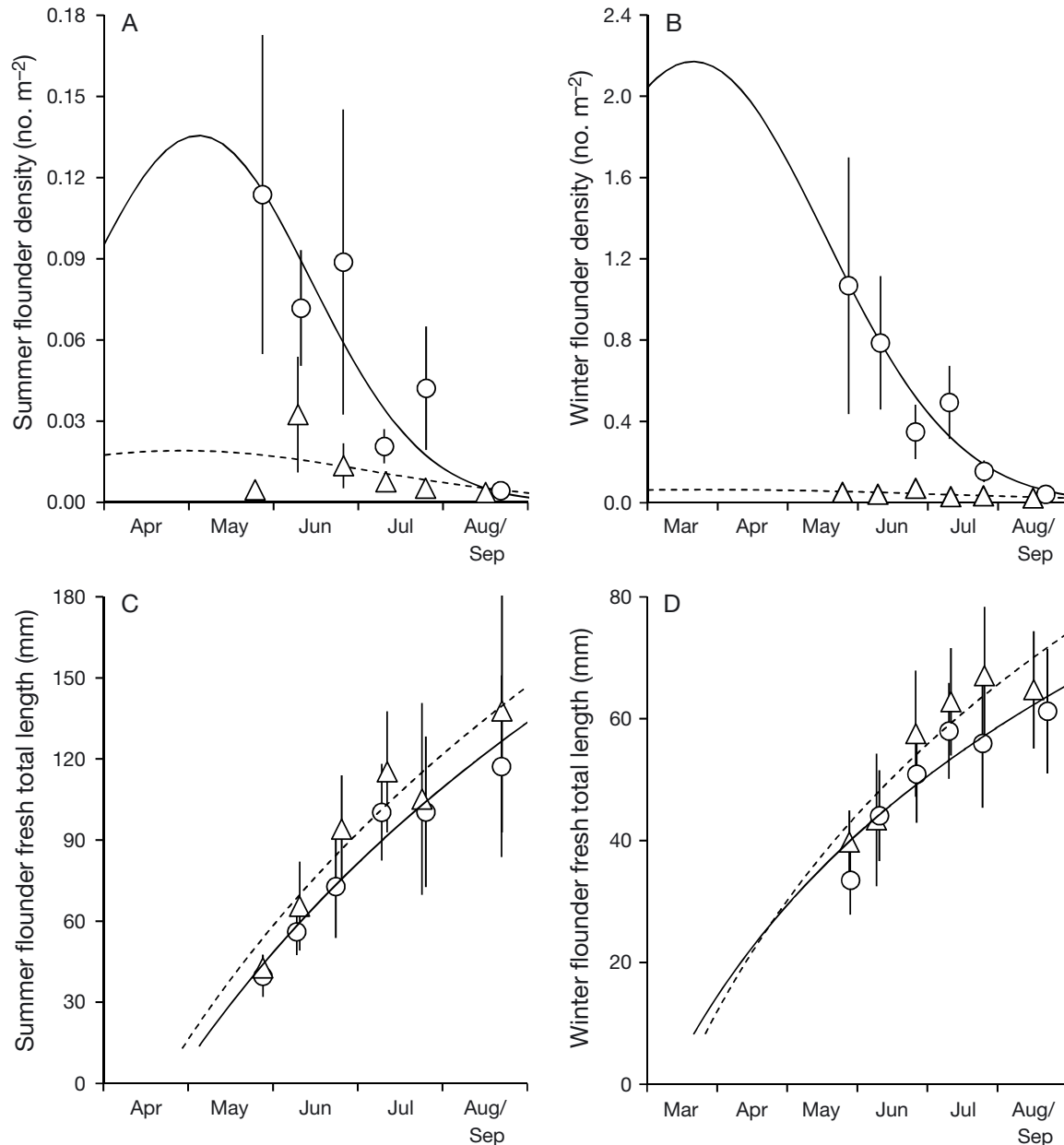


Fig. 3. (A,B) Monthly densities and (C,D) 'fresh' total lengths of summer flounder and winter flounder, respectively, collected from the Seekonk River (circles) and Taunton River (triangles). Data points represent means calculated across river sites (see Fig. 1) and years (2009–2015), and error bars denote \pm SE. Normal density functions (Eq. 1) and logarithmic regression models (Eq. 2) were fit to full data sets for the Seekonk River (solid lines) and Taunton River (dashed lines). The dates of maximum flounder densities were determined by back-calculating intraspecific growth models to the day of year on which summer flounder were 13 mm and winter flounder were 8 mm (i.e. size at settlement; Able & Fahay 2010)

0.0001; Fig. 3C,D). Moreover, for each river, intraspecific flounder growth rates were reevaluated from May through mid-July, as opposed to August/September being the terminal date. This truncated time interval eliminates or minimizes size-dependent processes that potentially confound the original growth results (e.g. emigration of late season, larger-bodied flounder; Rountree & Able 1992, Szedlmayer et al. 1992). This

reanalysis yielded very similar growth patterns. First, summer flounder grew significantly faster than winter flounder in both rivers (ANCOVA; day \times species; Seekonk: $F_{1,139} = 26.45$, $p < 0.0001$; Taunton: $F_{1,90} = 10.44$, $p < 0.005$). Second, mean $TL_{t,t}$ values at time t were significantly greater for Taunton River flounder (ANCOVA; river; summer flounder: $F_{1,97} = 10.69$, $p < 0.005$; winter flounder: $F_{1,131} = 5.15$, $p < 0.05$).

Intraspecific logarithmic growth models were used to calculate predator-to-prey size ratios (mean summer flounder TL_f / mean winter flounder TL_f) as a function of DOY. Accordingly, the mean flounder predator-to-prey size ratio, calculated from late May to early September, was 1.94 ± 0.35 (range = 1.15–2.41; Table 3). The respective size discrepancy between flounder species significantly increased over time (ANCOVA; day: $F_{1,109} = 34.1$, $p < 0.0001$), and was attributed to the faster growth rates of summer flounder relative to winter flounder (Table 3). Finally, seasonally calculated predator-to-prey size ratios were lower in the Seekonk River relative to the Taunton River (mean \pm SD = 1.84 ± 0.35 and 2.06 ± 0.33 , respectively), but these differences were not significant (ANCOVA; river: $F_{1,109} = 1.85$, $p = 0.177$).

3.2. Visual analysis of summer flounder diet

A total of 743 summer flounder from the Seekonk River (SR) and Taunton River (TR) were randomly selected for stomach content analysis (SR: $n = 514$, 20–171 mm TL_f ; TR: $n = 229$, 32–181 TL_f ; Table 1). Visual inspection revealed that 101 of these stomachs contained fish prey (% $F = 13.6\%$), and 167 individual fish were recovered from the stomach contents (1.65 individual fish stomach $^{-1}$). In total, 8 unique fish prey taxa were identified in summer flounder stomachs (excluding 'unidentified' fish), and 94.9% of these stomachs only contained a single fish taxon (1.05 taxa stomach $^{-1}$).

Summer flounder predation on fish exhibited considerable spatio-temporal variation. For example, summer flounder from the Seekonk River had a broader dietary breadth than conspecifics from the Taunton River (7 and 4 fish prey taxa consumed, respectively; Table 1). The overall occurrence of fish in the summer flounder diet, however, was greater in the Taunton River (% F : TR = 17.0%; SR = 12.1%, respectively), and predation on fish was markedly higher in the upper reaches of both rivers in comparison to other field sites (% F : TR1 = 26.7%; SR1 = 21.1%; other sites = 8.8%; Fig. 1). The incidence of fish in summer

flounder stomachs was relatively low during May in the Seekonk and Taunton Rivers (% $F = 2.3\%$), after which % F increased from June through mid-July (% $F = 18.4\%$; Fig. 4). Summer flounder predation on fish declined thereafter, remaining relatively constant in the Seekonk River from late July to September (% $F \sim 5.5\%$), whereas % F decreased from 23.3 to 0.0% in the Taunton River during the same time period.

The majority of fish encountered in the stomachs of summer flounder from the Seekonk and Taunton Rivers were unidentifiable to a specific taxon (unidentified fish % F : SR = 7.4%; TR = 9.6%; Table 1). Of the visually identifiable fish taxa, the dominant prey of summer flounder from the Taunton River were herring (% $F = 6.1\%$), while gobies, pipefish, and silversides were of lesser importance (% $F = 0.4$ – 1.3%). In the Seekonk River, winter flounder were the most common fish type in the diet of summer flounder (% $F = 3.3\%$), followed by suckers, pipefish, silversides, herring, eels, and largemouth bass (% $F = 0.2$ – 0.6%). Summer flounder consumed fish prey with a mean TL_p of 27.4 ± 7.8 mm (range = 17.5–55.4 mm; Table 1), and the mean body size of piscivorous summer flounder was 82.0 ± 26.7 mm TL_p (range = 42–172 mm).

The summer flounder–winter flounder predator–prey interaction varied spatially and temporally

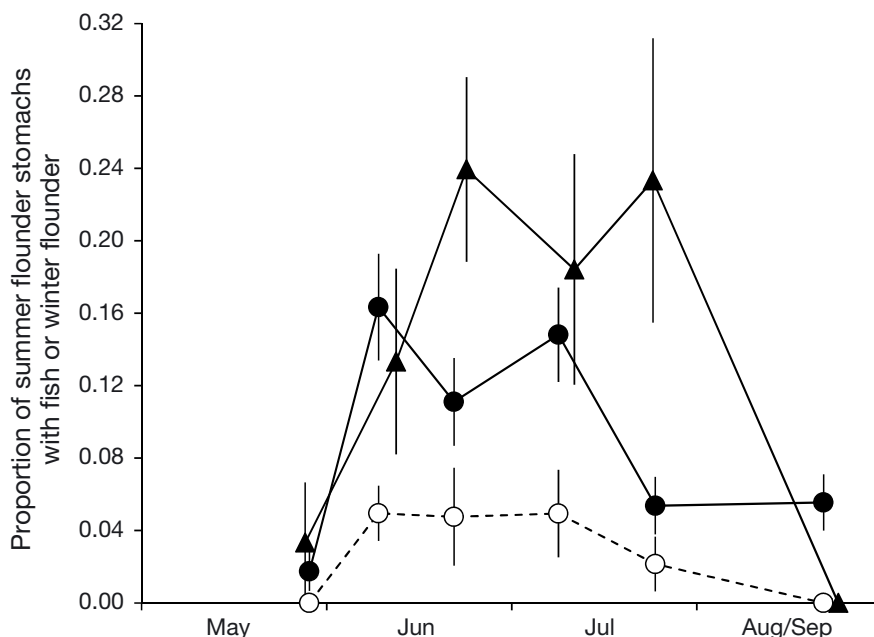


Fig. 4. Proportion of summer flounder stomach contents containing fish prey (solid symbols) or winter flounder (open symbols) from the Seekonk River (circles; $n = 514$) and Taunton River (triangles; $n = 229$). Data were grouped by months, and means were calculated across river sites (see Fig. 1) and years (2009–2015). Error bars denote \pm SE

(Table 1, Fig. 4), such that predation rates on winter flounder were maximal in the upper portion of the Seekonk River (%F: SR1 = 7.8%; other sites = 0.0–1.4%) and during June (%F: June = 4.8%; other months = 0.0–3.4%). Summer flounder predation on winter flounder was not directly observed in the Taunton River.

3.3. Molecular genetic analysis of summer flounder diet

DNA concentrations (mean \pm SD) varied by tissue type and were highest in field-collected summer flounder and non-target prey (83.5 ± 216.1 ng μl^{-1} ; $n = 16$), followed by non-consumed winter flounder (70.0 ± 84.7 ng μl^{-1} ; $n = 7$), consumed unidentified fish (63.1 ± 75.0 ng μl^{-1} ; $n = 60$), and consumed winter flounder (22.1 ± 38.2 ng μl^{-1} ; $n = 17$). DNA quality values (A260/A280) were comparable across tissue types (1.94 ± 0.27 ; range = 0.88–3.20). The species-specific primer set used in this study (WF208; Collier et al. 2014) consistently amplified DNA from field-collected (non-consumed) winter flounder, and conversely, no amplification occurred for field-collected summer flounder or non-target fish and invertebrate prey. With respect to prey recovered from summer flounder stomachs, PCR products were observed in 58.8% of the consumed winter flounder (10 of 17 samples), and 3.3% of the unidentified fish prey resulted in amplification of target DNA (2 of 60 samples). Sequencing of select PCR products confirmed that the WF208 primer set amplified the expected region of the winter flounder DNA for all tissue types (7 of 7 samples; GenBank accession number U12068.1).

3.4. Flounder morphological relationships

The linear and exponential regression models used to examine morphological relationships in summer flounder and winter flounder (Eqs. 4–8) were highly significant (mean $R^2 = 0.98$, range = 0.92–1.00; $p < 0.0001$; Table 2). For the winter flounder TL_p –OL regression (Eq. 6), ANCOVA models were used to examine the effect of river (Seekonk and Taunton) and year (2011 through 2013) on the relationship. As neither discrete explanatory variable significantly affected the TL_p –OL model (ANCOVA; river: $F_{1,50} = 0.25$, $p = 0.619$; year: $F_{2,50} = 0.25$, $p = 0.778$), data were pooled for further analysis (Fig. 5A). A total of 21 winter flounder were recovered from 17 summer

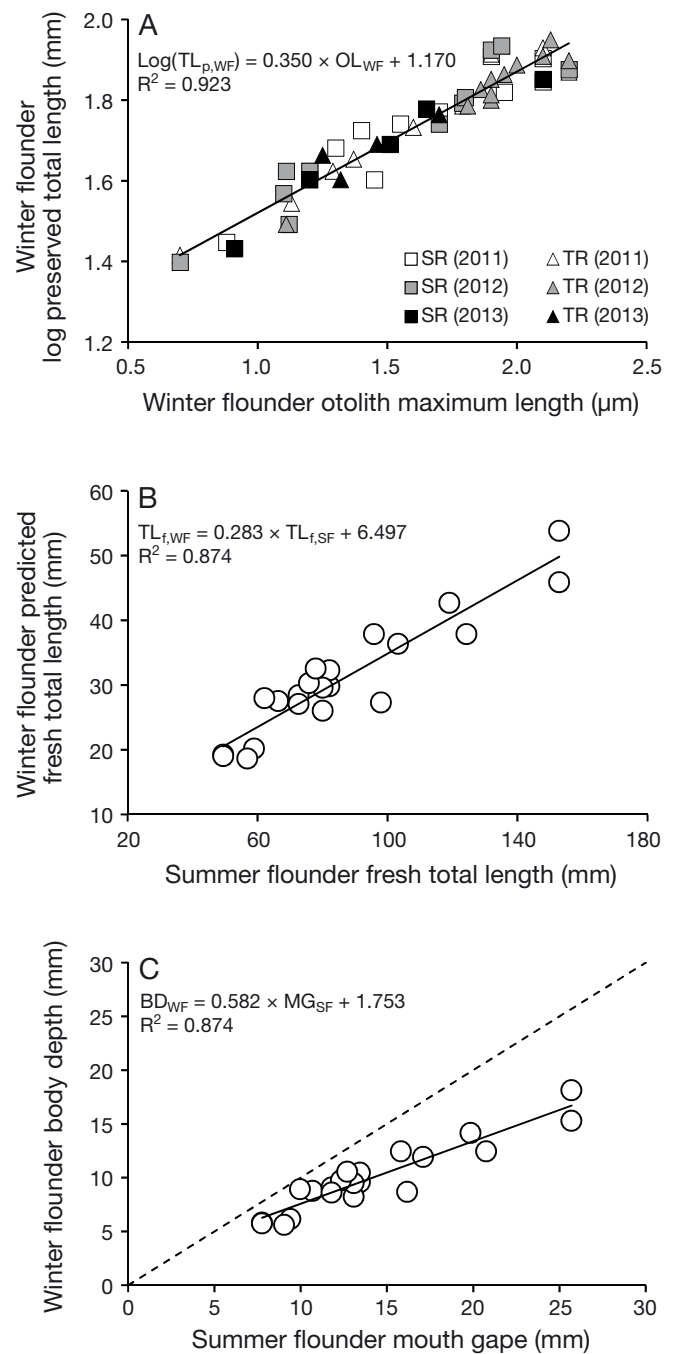


Fig. 5. (A) Relationship between the maximum linear length of sagittal otoliths (OL) and preserved total length (log-transformed; TL_p) of winter flounder (WF) collected from the Seekonk River (SR) and Taunton River (TR) from 2011 through 2013 ($n = 51$). (B) Relationship between the fresh total length (TL_f) of summer flounder (SF) and predicted TL_f of consumed winter flounder ($n = 21$). (C) Relationship between the predicted mouth gape of summer flounder (MG) and body depth of consumed winter flounder (BD) ($n = 21$). Solid lines represent linear least-squares regression models fit to the full data sets with the equations and R^2 -values provided, and the dashed line represents the flounder MG-to-BD 1:1 ratio

flounder stomachs (1.24 winter flounder stomach $^{-1}$), and the mean number of predatory events per year was 4.0 ± 3.2 (range = 1–9, as observed in 2013 and 2011, respectively). The mean TL_f of consumed winter flounder equaled 31.0 ± 9.1 mm (range = 18.7–53.8 mm), as determined from Eqs. (5) and (6). The predatory summer flounder had a mean body size of 86.3 ± 29.9 mm TL_f (range = 49.5–152.8 mm; converted from TL_p using Eq. 4), resulting in a mean predator-to-prey size ratio of 2.77 ± 0.34 (range = 2.22–3.59). There was a significant positive relationship between the TL_f of summer flounder visually confirmed to feed on winter flounder and the predicted TL_f of winter flounder recovered from summer flounder stomachs (linear regression: $F = 132.2$, $R^2 = 0.874$, $p < 0.0001$; Fig. 5B).

Summer flounder that preyed on winter flounder had an estimated mean MG of 14.2 ± 4.4 mm (range = 7.8–25.7 mm; Eq. 7), and the mean BD of the consumed winter flounder was 10.0 ± 2.2 mm (range = 5.6–18.1 mm; Eq. 8). Increases in summer flounder MG as a function of their TL_f resulted in the consumption of larger-bodied winter flounder, yet winter flounder BD never exceeded the MG of predatory summer flounder (Fig. 5C).

3.5. Factors affecting summer flounder predation on fish and winter flounder

The prevalence of fish in the diet of summer flounder was directly related to predator body size

(Fig. 6A). There was an absence of fish in the stomachs of summer flounder <44 mm TL_f , whereas piscine prey were progressively more common in larger summer flounder (maximum $TL_f = 181$ mm; $\beta = 0.041$; Table 4). The probability of fish occurring in summer flounder stomachs was also inversely related to DOY (t ; $\beta = -0.049$) and predator density (ρ_{SF} ; $\beta = -1.532$) (Table 4). These results may be attributed indirectly to body size effects, given that larger summer flounder occur later in the season when densities are also reduced (Figs. 2 & 3A,C). Finally, summer flounder predation on fish increased at lower salinities (S ; $\beta = -0.113$; Fig. 6B), as indicated by the high % F values observed in the upper reaches of the Seekonk and Taunton Rivers (i.e. oligohaline water; Table 1).

Summer flounder predation on winter flounder was significantly related to the predator-to-prey size ratios (Fig. 6C), such that increases in summer flounder TL_f , relative to winter flounder lengths, coincided with higher probabilities of predation (R ; $\beta = 1.695$; Table 4). The occurrence of winter flounder in summer flounder stomachs was also inversely related to salinity ($\beta = -0.161$; Table 4, Fig. 6D), again reflecting the higher % F values at the oligohaline sites in the Seekonk River (Table 1).

3.6. Modeling winter flounder mortality owing to summer flounder predation

Model simulations (2009 through 2015) estimated that summer flounder consume, on average, a total of 0.35 winter flounder m^{-2} in the Seekonk River (range = 0.05–1.03 winter flounder m^{-2} ; Table 3). This corresponds to a mean annual cumulative mortality (M) of 4.4 % (range = 0.7–12.8 %) and average daily instantaneous mortality (Z_{avg}) of 2.39×10^{-4} (range = 4.20×10^{-5} to 6.86×10^{-4}). The predatory impact of summer flounder was lower in the Taunton River, with 0.12 winter flounder consumed m^{-2} (mean) during the observation period (range = 0.0–0.69 winter flounder m^{-2} ; Table 3). This predation rate equates to a mean M of 1.5 % (range = 0.0–8.6 %) and Z_{avg} of 1.03×10^{-4} (range = 0.0 to 6.20×10^{-4}). The total daily instantaneous mortality rate of post-settlement winter flounder was previously reported between 0.0123 and 0.0400 (average $Z = 0.0235$; Taylor 2005a and references therein). Thus, on average, in the Seekonk and

Table 4. Summary statistics and mean parameter estimates for logistic regression analysis of the proportion (P) of summer flounder (SF) stomachs containing fish or winter flounder, as a function of summer flounder fresh total length (TL_f ; mm), day of year (DOY) (t), salinity (S), summer flounder density (ρ ; no. flounder m^{-2}), and predator-to-prey size ratio (R ; summer flounder mm TL_f / winter flounder mm TL_f). Proportions are the natural logarithm of the ratio of response frequencies (logits); logit (P) = $\log [P/1 - P]$

Prey type / Variable	Symbol	Parameter estimate (SE)	Chi-squared	p
Fish				
SF total length	TL_f	0.041 (0.006)	49.6	<0.0001
DOY	t	-0.049 (0.009)	33.1	<0.0001
Salinity	S	-0.113 (0.034)	10.9	<0.001
SF density	ρ	-1.532 (0.687)	5.0	<0.05
Intercept	α	4.444 (1.219)	13.3	<0.0005
Winter flounder				
Size ratio	R	1.695 (0.448)	14.3	<0.0005
Salinity	S	-0.161 (0.075)	4.7	<0.05
Intercept	α	-5.245 (0.814)	41.5	<0.0001

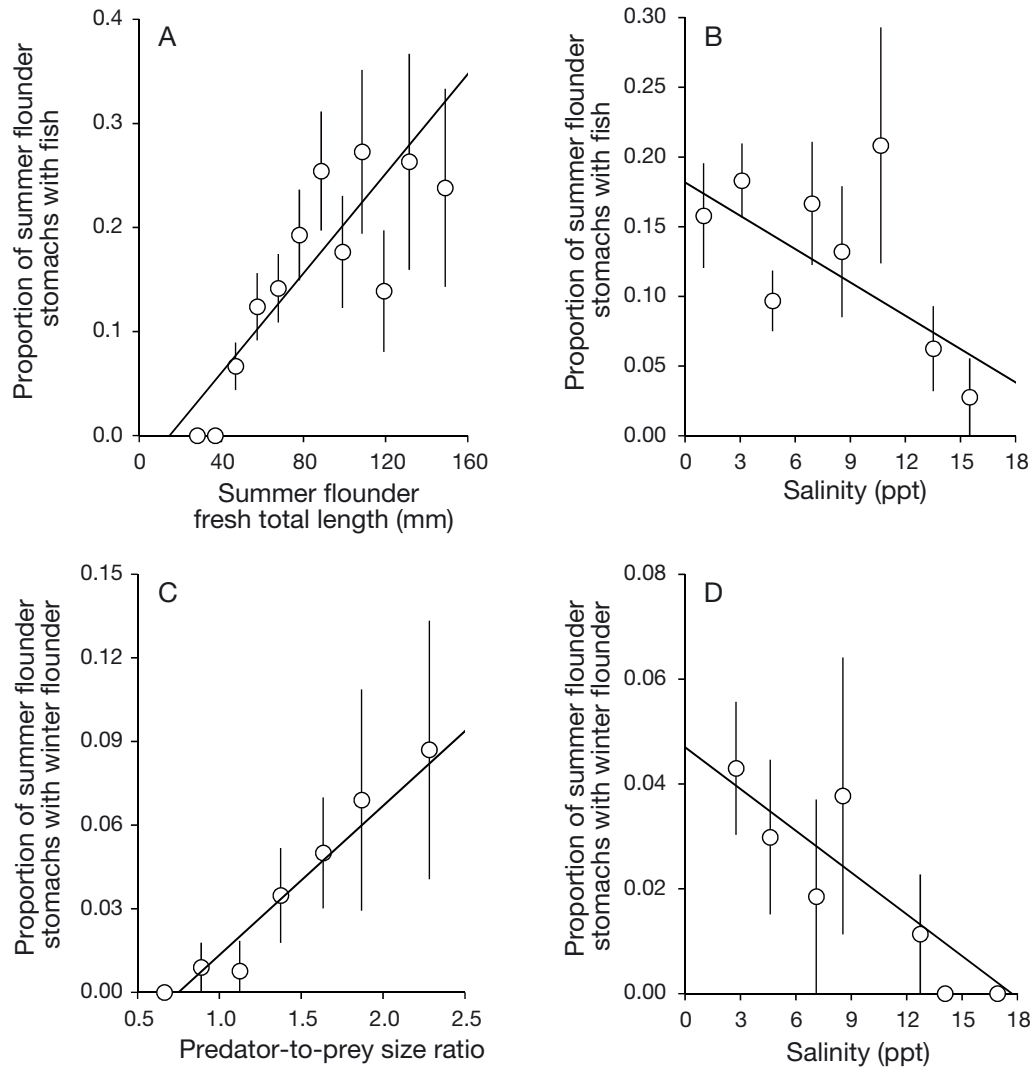


Fig. 6. Proportion of summer flounder stomach contents containing (A,B) fish or (C,D) winter flounder. Occurrences of predation were analyzed as a function of (A) summer flounder fresh total length (TL_f), (B,D) salinity, and (C) predator-to-prey size ratios (individual summer flounder TL_f / mean winter flounder TL_f). Solid lines represent logistic regression models fit to the full data sets ($n = 743$), but data were binned across the x-axis for graphical representation. Error bars denote \pm SE. Summary statistics for the logistic regressions are provided in Table 4

Taunton Rivers, respectively, 1.0% (range = 0.2–2.9%) and 0.4% (range = 0.0–2.6%) of the winter flounder's total daily mortality may be attributed directly to summer flounder predation.

Winter flounder cumulative mortality owing to summer flounder predation was evaluated over a broad range of biological and salinity conditions (Fig. 7). The relative size structure of both flounder species substantially affected predation rates. For example, incremental increases in predator-to-prey size ratios caused an acceleration in winter flounder mortality (Fig. 7A). At a salinity of 5 ppt (i.e. upper river conditions), changes in size ratios from 1.0–2.5 and 2.5–4.0 caused winter flounder M to increase

by 9.2 and 51.6%, respectively. Salinity also influenced estimates of winter flounder mortality by ostensibly approximating other causative factors that varied along an upriver–downriver gradient. The effect of salinity was marginal in polyhaline waters (19–32 ppt), and more pronounced in mesohaline (6–18 ppt) and oligohaline waters (<5 ppt). For example, at a fixed predator-to-prey size ratio of 2.77 (i.e. mean size ratio observed in this study), a decrease in salinity from 32 to 19 ppt led to a 1.6% increase in cumulative mortality of winter flounder, whereas declines from 18 to 6 ppt and 5 to 0 ppt increased M by 11.0 and 13.3%, respectively (Fig. 7A).

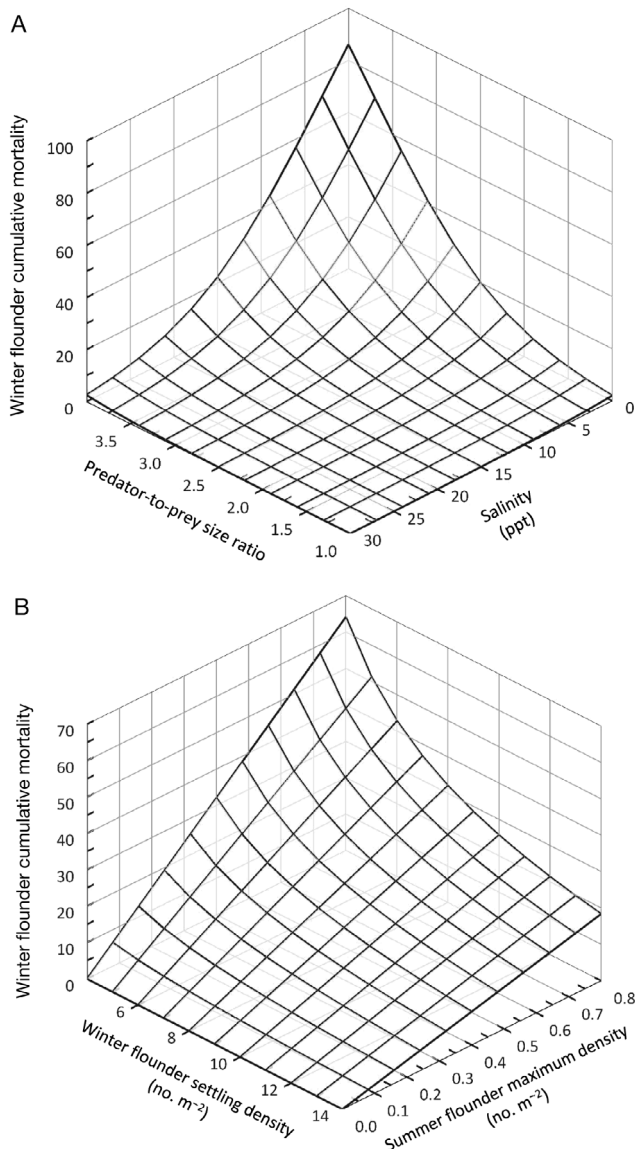


Fig. 7. Winter flounder cumulative mortality (M) owing to summer flounder predation (M = total number of winter flounder eaten by summer flounder m^{-2} divided by the initial cohort size). Winter flounder mortality was estimated using a deterministic model and calculated over a range of (A) predator-to-prey size ratios (individual summer flounder TL_f /mean winter flounder TL_i) and salinity and (B) winter flounder total cohort size and summer flounder maximum density

Interspecific flounder population dynamics also greatly affected winter flounder predator-induced mortality (Fig. 7B). For example, winter flounder settling at low densities (total cohort = 4 flounder m^{-2} ; $\rho_{max} = 0.16$ flounder m^{-2} ; $\sigma = 10$; $M = 25.9\%$) experienced a 18.5% increase in M relative to conspecifics at higher densities (total cohort = 14 flounder m^{-2} ; $\rho_{max} = 0.56$ flounder m^{-2} ; $\sigma = 10$; $M = 7.4\%$), assuming

a maximum summer flounder density of $0.323 m^{-2}$ (i.e. annual mean in the Seekonk River; Table 3). Moreover, a change in maximum summer flounder density (ρ_{max}) from 0.01 to $0.80 ind. m^{-2}$ caused a concomitant increase in winter flounder M from 0.4 to 32.1% (initial winter flounder cohort size = $8 ind. m^{-2}$).

4. DISCUSSION

4.1. Summer flounder predation on fishes

This study provides a detailed description of the piscivorous diet of juvenile (age-0) summer flounder to better understand their trophodynamic role in southern New England tidal rivers. Summer flounder in these rivers exhibited a generalist foraging strategy, consuming 8 distinct fish taxa. The most frequently observed fishes in the diet of summer flounder were winter flounder and herring, with fewer occurrences of pipefish, suckers, silversides, gobies, eels, and bass. The fishes consumed by summer flounder were categorized as age-0 juveniles based on their small body sizes (Jacobs & O'Donnell 2009, Able & Fahay 2010), with the exception of gobies that mature at ~ 20 mm TL (e.g. naked goby *Gobiosoma bosc*; Able & Fahay 2010). These results indicate that age-0 summer flounder consume early-stage fishes from both epibenthic and pelagic prey guilds, as reported for older conspecifics (Manderson et al. 2000, Staudinger & Juanes 2010a). To our knowledge, this study represents the first documentation of piscivory by age-0 summer flounder in southern New England waters (Taylor & Gervasi 2017).

The piscivorous behavior of age-0 summer flounder described herein is consistent with reports from the Middle and South Atlantic Bight (Packer et al. 1999), although geographic differences in diet composition are evident and attributed to broad spatial variations in fish prey assemblages (Taylor & Gervasi 2017). For example, in the Great Bay–Little Egg Harbor Estuary (New Jersey), the dominant piscine prey of summer flounder 167–305 mm TL ($n = 137$) were Atlantic silverside and mummichog ($\%F = 32$ and 16% , respectively), whereas striped killifish, sheepshead minnow *Cyprinodon variegatus*, and naked goby were encountered less frequently ($\%F \sim 1\%$) (Rountree & Able 1992). In the York River (Virginia), a major tributary of the Chesapeake Bay, fish were the main dietary item of summer flounder 98–192 mm TL ($n = 28$; TL converted from standard length, SL; Able & Fahay 1998), but no further taxonomic resolution of fish prey was provided beyond

the infraclass Teleostei (Smith et al. 1984). For summer flounder <225 mm TL collected from the Chesapeake Bay mainstem ($n = 128$), engraulids (bay anchovy *Anchoa mitchilli*) and sciaenids (weakfish *Cynoscion regalis*, Atlantic croaker *Micropogonias undulatus*, and spot *Leiostomus xanthurus*) accounted for ~12 % of the total diet (Latour et al. 2008). Similarly, in the Pamlico Sound (North Carolina), fishes occurred frequently in the stomachs of summer flounder 100–200 mm TL (% $F = 33$ %; $n = 313$), with the most substantial contributions from engraulids and sciaenids (Powell & Schwartz 1979).

In our study, the presence of fish in the diet of summer flounder directly corresponded to predator body size. Summer flounder reportedly undergo an ontogenetic transition to fish prey as predator size increases (Festa 1979, Link et al. 2002, Latour et al. 2008, Buchheister & Latour 2015), and this was attributed to the enlarged mouth gape and improved prey capture abilities of larger flounder (Buchheister & Latour 2011). Moreover, summer flounder predation on fishes in this study demonstrated significant spatiotemporal variability. These observations are likely due to site-specific differences in summer flounder body size, somatic growth, and the resultant ontogenetic transition to piscivory (Taylor et al. 2016). The diet composition of summer flounder may simultaneously reflect riverine and seasonal variations in prey composition, which affect the feeding habits of this species in other geographic locations (Powell & Schwartz 1979, Rountree & Able 1992, Manderson et al. 2000, Link et al. 2002, Latour et al. 2008). With the exception of age-0 winter flounder, the *in situ* abundance of fishes that constitute important prey of summer flounder were not quantified in this study. Previous investigations in southern New England and mid-Atlantic estuaries, however, have documented habitat-specific and seasonal variations in juvenile fish abundance, most notably herring, Atlantic silverside, northern pipefish, and American eel (Able & Fahay 1998, 2010). The diet composition of summer flounder ultimately reflects spatiotemporal dynamics in prey availability, as well as morphological constraints imposed by relative predator–prey body sizes (Manderson et al. 2000, Staudinger & Juanes 2010a).

4.2. Utility of molecular genetics to identify species-specific prey

The visual identification of prey species extracted from predator stomachs is often difficult and there-

fore hampers our understanding of trophic relationships in natural systems. For example, in this study, the majority of fishes encountered in the stomachs of summer flounder were unrecognizable to a detailed taxon, and ‘unidentified fish’ routinely accounted for the largest component of the summer flounder diet (% weight or volume dietary contribution ~12–21 %; Festa 1979, Link et al. 2002, Sagarese et al. 2011). The molecular genetic technique used in this study offers a supplementary approach to analyzing predator stomach contents by testing for the presence of intraspecific genomic DNA (Collier et al. 2014). The efficacy of this approach is contingent on minimizing errors in analysis associated with false-positive and false-negative results. The incorrect assertion that target prey are present in a predator’s stomach (false-positivity) is caused by amplification of non-target DNA. In this regard, the WF208 primer set used in this study was extremely effective at generating PCR products for field-collected (non-consumed) winter flounder (100 % amplification) without cross-reacting with the genomic material of the predator or alternative prey (i.e. primers have high sensitivity and specificity; Taylor 2004). Further, sequencing of PCR products confirmed that the WF208 primer set amplified the expected mitochondrial non-coding control region (D-loop) of the winter flounder genome, hence alleviating potential concerns of false-positive results.

The WF208 primers were moderately successful at amplifying the DNA of winter flounder extracted from summer flounder stomachs (~59 % amplification). The inability to generate PCR products for several of the consumed winter flounder (false-negativity) was mainly attributed to prolonged digestion and the degradation of target DNA regions (Collier et al. 2014). Although the effect of digestion time on detecting winter flounder DNA in summer flounder stomachs was not quantified in this study (i.e. detection limits; Taylor 2004), previous investigations that used PCR-based methods to evaluate piscivorous fish diets reported detection limits of 12 to 16 h (Rosel & Kocher 2002, Carreon-Martinez et al. 2011). Moreover, using the WF208 primer set, Collier et al. (2014) and Scro et al. (2014) amplified juvenile winter flounder DNA 7 and 10 h after initial ingestion by blue crabs, respectively. False-negative results may have secondarily occurred because of the ineffectiveness of WF208 primers to amplify biological variants of the target winter flounder DNA region, as confirmed by Collier et al. (2014). In our study, 60 summer flounder stomachs contained unidentified fish, as determined by direct visual inspection, and subsequent genetic analyses (PCR and DNA sequencing)

verified that 3.3% of these stomach samples contained winter flounder. Given the potential for false-negativity (e.g. testing beyond detection limits and biological variants), these results are conservative because additional unidentified fish prey may be winter flounder. Notwithstanding these limitations, the genetic techniques employed in this study improved the quantitation of the summer flounder–winter flounder predator–prey interactions.

4.3. Summer flounder predation on winter flounder

By coupling stomach content analysis with molecular genetic techniques, this study provides the first evidence of age-0 summer flounder predation on winter flounder in natural populations (Taylor & Gervasi 2017). Winter flounder occurred in ~3% of summer flounder stomachs, and predatory events were relatively common in the upper reaches of the Seekonk River ($%F = \sim 8\%$). Several investigations purport that age-1+ summer flounder are important predators of age-0 winter flounder in more southern estuaries, with occurrences of predation comparable to this study. For example, in the Little Egg Harbor Estuary, winter flounder were present in the diet of 8% of summer flounder 260–650 mm TL ($n = 13$) (Festa 1979). Similarly, in the Great South Bay (New York), 5% of the stomachs of summer flounder 260–649 mm TL contained winter flounder ($n = 141$) (Sagarese et al. 2011). Other studies have indicated that juvenile winter flounder are a more substantial component of age-1+ summer flounder diet. Most notably, age-0 winter flounder were the dominant piscine prey of summer flounder 252–648 mm TL ($%F = 16\%$; $n = 95$) in the Navesink River (New Jersey) (Manderson et al. 2000). Further, in the more expansive Navesink River/Sandy Hook Bay estuarine system, the annual $%F$ of winter flounder in age-1+ summer flounder stomachs ranged between 11 and 41% ($n = 207$) (Manderson et al. 2006).

Predator–prey relations between age-0 summer flounder and winter flounder in our study were affected by their relative body sizes. Summer flounder specifically consumed winter flounder that were ~28–45% of the predator's TL_f (mean \pm SD = $36.6 \pm 4.3\%$), and incidences of predation increased significantly with increasing predator-to-prey size ratios. These results closely correspond to previous laboratory experiments, whereby summer flounder 96–450 mm TL consistently ate age-0 winter flounder that were 30–40% of their length (maximum = 48%)

(Curran & Able 1998). Similarly, in the Navesink River, a predator-to-prey size ratio of 2.7 was observed between summer flounder (378 mm TL) and winter flounder (140 mm TL; 37% of predator length) (Manderson et al. 2000). However, in the aforementioned study, interspecific length ratios were typically lower in field-collected flounder, i.e. winter flounder (24–67 mm TL) were 6–19% of summer flounder length (252–648 mm TL; $n = 60$) (Manderson et al. 2000).

Morphological constraints, e.g. mouth gape limitations, are critical factors affecting the predator–prey relationship between age-0 summer flounder and winter flounder. In our study, summer flounder mouth gape increased proportionally with total length and resulted in the consumption of larger winter flounder. Moreover, the body dimensions of ingested winter flounder did not exceed the mouth gape of predatory summer flounder, which is consistent with previous research on the foraging ecology of age-1+ summer flounder (Manderson et al. 2000, Staudinger & Juanes 2010a). The smallest winter flounder collected in this study was 20 mm TL_f with a maximum body depth of 6.1 mm. The smallest summer flounder capable of consuming a winter flounder of this size (i.e. unconstrained by mouth gape) is 41 mm TL_f . This projected minimum-size threshold is below the smallest summer flounder identified as a predator of winter flounder (~50 mm TL_f), suggesting that other size-dependent factors restrict the predator–prey interaction, including size-specific limitations on a predator's pursuit and handling of mobile flatfish prey (Ellis & Gibson 1997, Manderson et al. 2000).

Temporal variations in the predator–prey interaction between flounder species were governed by their respective size structure. In this study, predation was maximal during June through mid-July, and conversely, absent in May and August/September. Summer flounder <50 mm TL_f comprised 77% of the population in May, and their small body size precludes winter flounder as viable prey. Conversely, for summer flounder ≥ 50 mm TL_f , reduced predation rates in May likely reflect the size-dependent vulnerability of winter flounder (Taylor 2003). Predatory summer flounder rely on vision to employ raptorial attack strategies (Olla et al. 1972, Staudinger & Juanes 2010b), and in the absence of morphological constraints, winter flounder vulnerability to summer flounder predation increases linearly with prey body sizes ranging from 20–90 mm TL (Manderson et al. 2000). The low predator-induced mortality of small winter flounder in late spring may be due to their relative inconspicuousness to visual predators (Man-

derson et al. 1999, 2000, Taylor 2003). After May, summer flounder predation on winter flounder increased considerably owing to interspecific growth differences. First, the rapid growth of summer flounder augmented the number individuals that effectively prey on winter flounder. For example, from June through mid-July, 83% of summer flounder exceeded the predatory minimum-size threshold of 50 mm TL_f. Moreover, a substantial discrepancy occurred between predator and prey body sizes at this time, hence intensifying the size-dependent predation mortality of winter flounder. Second, over time, the modest growth of winter flounder led to larger individuals that were likely more conspicuous to visually reliant, piscivorous summer flounder. It follows that winter flounder vulnerability to summer flounder predation would increase beyond mid-July, due to the aforementioned interspecific growth differences, yet there was no evidence of predation during these later months. The absence of predatory events in August/September may be due to reduced winter flounder densities, and thus decreased predator-prey encounters (Manderson et al. 2000). Indeed, in this study, occurrences of winter flounder in summer flounder stomachs was positively related to prey density from June to August/September (logistic regression; chi-squared = 5.51, $p < 0.05$), noting that May data were excluded because the majority of summer flounder were too small to prey on winter flounder at this time.

Spatial variations in flounder predator-prey interactions may similarly reflect riverine and site-specific differences in winter flounder densities. Summer flounder predation on winter flounder was most frequent in the oligohaline waters of the Seekonk River (site SR1), but not directly observed in the Taunton River. Mean winter flounder densities across years were ~5 times greater at SR1 than other Seekonk River sites, and moreover, winter flounder densities were ~12 times greater in the Seekonk River than in the Taunton River. The elevated densities of winter flounder in the Seekonk River, particularly at SR1, presumably increase interspecific encounters (Manderson et al. 2000), which result in more predation events. The presence of winter flounder in summer flounder stomachs may also vary according to the availability of alternative prey. In a concurrent investigation, Taylor & Gervasi (2017) documented that mysid shrimp, amphipods, and sand shrimp were preferred prey of summer flounder in the Seekonk and Taunton Rivers, and the heterogeneous distribution of these prey items possibly affects predation dynamics on winter flounder (Manderson et al. 2000). Finally,

interspecific predator-prey interactions may respond to spatial variations in habitat structure and complexity. Sediment grain size influences the burial capabilities of post-settlement flatfish, including winter flounder (Phelan et al. 2001, Stoner & Ottmar 2003), and burial behavior is a concealment strategy to avoid visual predators (Gibson & Robb 1992, Keefe & Able 1994, Ryer et al. 2008). Previous laboratory experiments further revealed that age-0 winter flounder experienced lower predation rates by age-1+ summer flounder in the presence of eelgrass *Zostera marina* and sea lettuce *Ulva lactuca* (Manderson et al. 2000). These habitat-related factors, however, do not explain spatial differences in the flounder predator-prey interaction in our study because sediments are remarkably consistent between the Seekonk and Taunton Rivers (% silt-clay = 47.6 and 48.0%, respectively; Taylor et al. 2016), and anecdotal observations suggest negligible differences in macrophyte densities among riverine sites (D. Taylor pers. obs.).

4.4. Winter flounder mortality owing to summer flounder predation

Predator-induced mortality during the early juvenile life stage may determine year-class strength and recruitment success of flatfish (Bailey 1994, Gibson 1994). Model simulations from this study estimated that predation by age-0 summer flounder, on average, accounted for 0.7% of the daily mortality of age-0 winter flounder in the Seekonk and Taunton Rivers (range = 0.0–2.9%), and consumed 3.0% of the total winter flounder year-class annually (range = 0.0–12.8%). Moreover, Taylor et al. (2016) documented that age-0 summer flounder population densities were significantly greater in the Seekonk and Taunton Rivers relative to adjacent systems, including the Narragansett Bay proper and Rhode Island coastal lagoons (mean monthly densities: rivers = 0.01–0.07; Bay = 0.0–0.001; lagoons = 0.001–0.003 ind. m⁻²). Age-0 summer flounder therefore ostensibly contribute even less to winter flounder mortality in these other areas because of low predator densities.

Relative to other predatory threats, e.g. other demersal fishes and decapod crustaceans, age-0 summer flounder likely have a nominal effect on winter flounder populations in tidal river, shallow-water habitats. For example, striped searobins *Prionotus evolans* 180–370 mm TL frequently preyed on age-0 winter flounder in the Sandy Hook Bay (%*F* in June ~69%; $n = 36$), and winter flounder contributed ~18% by weight to the overall searobin diet (Manderson et al.

1999). Through the use of PCR-amplification techniques, winter flounder DNA was evident in the diet of ~29 % of blue crabs sampled in the Shinnecock Bay (New York) from July through September (size >54 mm carapace width; $n = 42$), suggesting that crabs are an important mortality factor for early-stage flounder (Collier et al. 2014). Finally, by implementing a similar modeling approach, Taylor (2005a,b) predicted that green crabs *Carcinus maenas* from the Niantic River (Connecticut) and sand shrimp from Narragansett Bay contributed ~2 and 25 % to the daily mortality of age-0 winter flounder and ~10 and 44 % loss of the total year-class, respectively. The substantial mortality risk imposed by crangonid shrimp, but lesser for portunid crabs, resulted from the common occurrence of winter flounder in predator stomachs (% $F = 8.5$ and 4.8%; $n = 1270$ and 313, respectively) and extremely high densities of shrimp (>15 ind. m^{-2}), and moderate densities of crabs (~0.2 ind. m^{-2}), in the estuaries (Taylor 2005a,b).

It is noteworthy that the cumulative effect of multiple age-classes of summer flounder (age-classes ≥ 0) on winter flounder mortality may be equivalent or exceed the abovementioned predatory threats. First, in this study, winter flounder mortality calculations were based exclusively on age-0 summer flounder predation. As previously described, however, multiple age-classes of summer flounder prey on post-settlement winter flounder, with occurrences of predation by older conspecifics comparable or surpassing those observed in this study (% $F \sim 5\text{--}41\%$) (Festa 1979, Manderson et al. 2000, 2006, Sagarese et al. 2011). Second, the number of distinct prey items that age-0 summer flounder ingest per foraging event is constrained by their stomach volume. The mean number of winter flounder occurring in age-0 summer flounder stomachs in this study, for example, was 1.2 (maximum = 2). Comparatively, previous investigations in the Navesink River/Sandy Hook Bay reported that age-1+ summer flounder consumed, on average, 2.7 winter flounder per feeding episode, with as many as 9 and 11 winter flounder recovered from individual predator stomachs (Manderson et al. 2000, 2004, 2006). Lastly, the modeling exercise executed in this study revealed that under certain circumstances, age-0 summer flounder predation contributes substantially to winter flounder cumulative mortality. Taylor et al. (2016) reported a maximum age-0 summer flounder density of 1.5 ind. m^{-2} in the oligohaline waters of the Seekonk River. At this elevated density, age-0 summer flounder are projected to consume ~60 % of the winter flounder cohort in the immediate area. Moreover, winter flounder mortality owing to age-0

summer flounder predation is highly responsive to variations in predator-to-prey size ratios. To this end, spatiotemporally distinct physiochemical conditions that affect interspecific growth rates may alter the relative size-structure of age-0 summer flounder and winter flounder (e.g. differing growth responses to warmer water temperatures; Taylor et al. 2016), thus leading to an intensification of the predator–prey interaction. These collective results suggest that predator-induced mortality of winter flounder owing to summer flounder predation may be substantial when multiple age-classes of the predator are considered. Further, under certain conditions, age-0 summer flounder may elicit a strong effect on winter flounder recruitment, albeit at relatively small spatiotemporal scales.

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