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BLOOFINZ - Gulf of Mexico

Active prey selection in developing larvae of Atlantic bluefin tuna (*Thunnus thynnus*) in spawning grounds of the Gulf of Mexico

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Bluefin tuna spawn in restricted areas of subtropical oligotrophic seas. Here, we investigate the zooplankton prey and feeding selectivity of early larval stages of Atlantic bluefin tuna (ABT, *Thunnus thynnus*) in larval rearing habitat of the Gulf of Mexico. Larvae and zooplankton were collected during two multi-day Lagrangian experiments during peak spawning in May 2017 and 2018. Larvae were categorized by flexion stage and standard length. We identified, enumerated and sized zooplankton from larval gut contents and in the ambient community. Ciliates were quantitatively important (up to 9%) in carbon-based diets of early larvae. As larvae grew, diet composition and prey selection shifted from small copepod nauplii and calanoid copepodites to larger podonid cladocerans, which accounted for up to 70% of ingested carbon. Even when cladoceran abundances were $<0.2\ \mathrm{m}^{-3}$, they comprised 23% of postflexion stage diet. Feeding behaviors of larvae at different development stages were more specialized, and prey selection narrowed to appendicularians and primarily cladocerans when these taxa were more abundant. Our findings suggest that ABT larvae have the capacity to switch from passive selection, regulated by physical factors, to active selection of presumably energetically optimal prey.

KEYWORDS: ichthyoplankton; prey preference; cladocera; oligotrophic; zooplankton

INTRODUCTION

Atlantic bluefin tuna (ABT, *Thunnus thynnus*, Linnaeus 1758) is both an ecologically and economically important top predator, exploited by 53 fishing nations (Scheffer *et al.*, 2005; International Commission for the Conservation of Atlantic Tunas, 2020). Like other widely migrating bluefin tuna species, the pelagic habitat of ABT ranges broadly from rich juvenile and adult feeding grounds in the temperate to sub-polar North Atlantic to primary spawning grounds in the subtropical Gulf of Mexico (GoM) and Mediterranean Sea (MED) (Fromentin and Fonteneau, 2001; Block *et al.*, 2005; Fromentin and Powers, 2005). Spawning in warm oligotrophic seas occurs mainly during late April to June in the GoM and June to August in the MED (Mather *et al.*, 1995; Fromentin and Powers, 2005; Scheffer *et al.*, 2005). Bluefin tuna recruitment is strongly influenced by survivorship during the larval period, which is particularly vulnerable to both starvation and predation mortality (Hjort, 1914). Thus, although areas of higher prey abundance might promote faster growth, ABT spawn in very low productivity seas with fewer prey as a presumptive tradeoff to minimize predation risk (Bakun and Broad, 2003; Shropshire *et al.*, this issue). Even within these spatially restricted spawning habitats, however, ABT larvae are observed to be more abundant in association with anticyclonic eddies in the GoM (Bakun, 2006, 2013; Lindo-Atichati *et al.*, 2012) and frontal zones in the MED (Alemany *et al.*, 2010; Muhling *et al.*, 2017). Specific characteristics of such mesoscale features that might make them more favorable for ABT survival are not known, but presumably involve prey resources promoting faster growth and development.

Opening of the mouth occurs 2–4 days post hatch, and when the yolk sac has been absorbed, larval ABT switch to exogenous feeding on small catchable prey. Sensory ability and locomotion is limited at first feeding; as visual predators (Morote *et al.*, 2008), the ability of larvae to perceive, catch and handle prey improves greatly as they grow and transition through three stages of development: preflexion, flexion, and postflexion (Ahlstrom and Mosser, 1976; Kendall *et al.*, 1984; Richards, 2005). Prey size and taxa are known to change with ABT larval ontogeny (Catalán *et al.*, 2011; Llopiz *et al.*, 2015; Tilley *et al.*, 2016; Uriarte *et al.*, 2019), though gut content analyses results can be quite variable and are poorly understood on a mechanistic basis. Along with advances in food capture capabilities during larval development, dietary composition is influenced by prey size, visibility, behavior, encounter rates and avoidance characteristics (Buskey *et al.*, 1993; Heath, 1993; Buskey, 1994). Turbidity and turbulence further affect encounter rates and capture success (Kiørboe and Mackenzie, 1995; Meager and Batty, 2007). The intersection of predator, prey and environmental characteristics results in the selection of certain prey over others. Of the few studies that have looked at larval prey selection by bluefin tuna species (Young and Davis, 1990; Catalán *et al.*, 2011; Kodama *et al.*, 2017, 2020), none have evaluated whether or how selection changes with ontogeny and prey availability. Nonetheless, as experiments with other species have shown (Connaughton and Epifanio, 1993; Meeren and Næss, 1993; Einfalt and Wahl, 1997; Reiriz *et al.*, 1998), larval ABT likely select certain prey actively based on familiarity or nutritional value when those prey become relatively more available as larvae hone their hunting skills.

As part of the BLOOFINZ-GoM project (Bluefin Larvae in Oligotrophic Ocean Foodwebs, Investigation of Nutrients to Zooplankton in the GoM), we studied the availability of zooplankton prey, the consumed diet and the prey selection patterns of larval ABT in two water parcels sampled in GoM nursery areas during peak spawning in May 2017 and 2018. Our study goals were to determine: (i) how the selection of prey by size and taxon changes as larvae grow and (ii) if larvae feed preferentially on the prey taxa that are most abundant within appropriate size classes in their environment. Using natural differences in the relative abundances of prey in two water parcels as a basis of comparison, we also evaluate the null hypothesis that prey selectivity remains constant regardless of prey composition.

METHOD

Larval sampling

Larval ABT were collected during the peak spawning period in the GoM during May of 2017 (NF1704) and 2018 (NF1802). Samples were taken with a dual 90-cm diameter bongo frame (bongo-90) equipped with 500-μm synthetic nylon mesh nets by oblique hauls in the upper 25 m of the water column and towed at an average over-ground speed of 2.22 knots for 10 minutes (Laiz-Carrion *et al.*, 2013, 2015; Habtes *et al.*, 2014). Filtered volumes were calculated with a mechanical flowmeter (2030R, General Oceanics Inc.) centered in the net mouths. Larval patches were located by transect net sampling and real-time sample sorting (every ∼10 nautical miles) across favorable habitat with higher probability of containing ABT larvae according to the BFT Index (Domingues *et al.*, 2016; Gerard *et al.*, this issue). Once larvae were detected, the core of the patch was located and marked with a drogued satellite-tracked drifter equipped

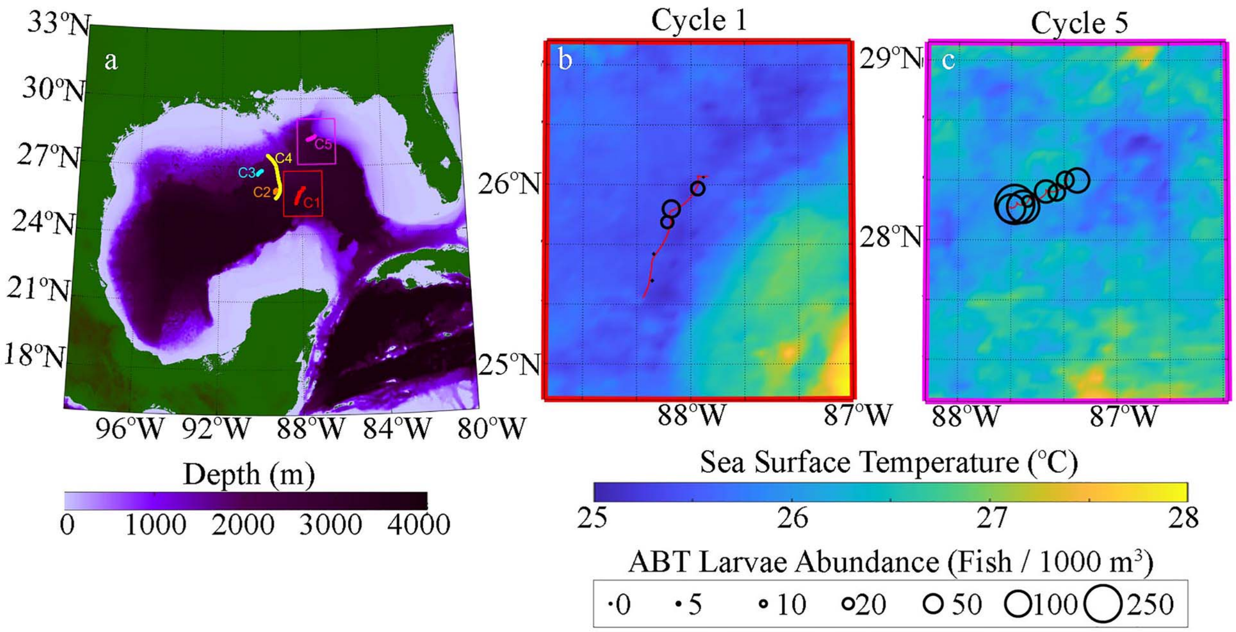

Fig. 1. Map of study area with drift tracks of cycle experiments C1–C3 from NF1704 and C4–C5 from NF1802 overlying bathymetry (**a** on left). ABT larval densities (individuals 1000 m^{-3}) from ABT positive experimental cycles overlying sea surface temperature (**b**) and (**c**). Average density for nursery area C1 (b, center, red border) and C5 (right, magenta border) are 13.1 and 97.7 larvae per 1000 m^3, respectively. Densities are estimated from six tows at C1 and eight tows at C5 that are ~12 hours apart carried out around 2:00 and 14:00 ± 1 hour.

with a strobe light, beginning a 3–4 day experimental cycle during which the ABT larvae were sampled close to the drifter every 3 hours. Two cycles, NF1704-C1 and NF1802-C5, were carried out in ABT larval patches, and only results from these cycles are presented in this study (Fig. 1). Another three cycles (NF1704-C2, C3 and NF1802-C4) were carried out in favorable habitat (Domingues *et al.*, 2016), but where extensive searching did not produce any larvae. For more information on protozoans and mesozooplankton in cycles without ABT larvae, the reader is directed to Landry *et al.* (this issue) and Landry and Swalethorp (this issue).

Upon collection, left bongo-90 samples were immediately fixed in 95% ethanol (EtOH), and the right bongo-90 samples were immediately sorted for ABT larvae that were then fixed in EtOH. Contents were concentrated on a sieve using 50-μm filtered cold seawater and placed in a large Petri dish in a Styrofoam box with ice while sorting. Using a dissecting microscope (MZ12, Leica Microsystems), ABT larvae were removed from small portions of the wet sample that could be sorted within 10 minutes and placed in small Petri dishes in the cooler. The remaining zooplankton in the sample were immediately fixed with 95% EtOH. The collected zooplankton volume was generally small (<250 mL during the day), but some night samples exceeded 1 L. For larger samples, sorting was halted within 1 hour and fixed to prevent sample degradation; sorting was resumed back in the lab. Fixative was refreshed after 24 hours to prevent sample degradation from dilution.

Zooplankton sampling

Every 12 hours, the bongo-90 collections were augmented with a 20-cm diameter bongo frame (bongo-20) tow with 200 and 50-μm mesh nets, each with a flowmeter, for sampling of the ABT larval prey field. Upon collection, the 200-μm net sample was split using a Folsom plankton splitter, and half was immediately preserved in 95% EtOH, with 24-hour postcollection EtOH change. Smaller animals collected with the 50-μm net were screened through a 200-μm sieve to remove larger mesozooplankton already captured by the 200-μm mesh net and concentrated on a 50-μm sieve. During the NF1802 cruise, 50% of this small 50–200 μm mesozooplankton fraction was preserved in 10% formalin in filtered seawater to better quantify copepod nauplii undersampled with the 200-μm net.

Protozooplankton samples were taken once a day in 12 L Niskin bottles mounted on a Conductivity Temperature Depth profiler (CTD) rosette. Mixed-layer samples (150 mL) were collected at 5 and 20 m during C1 and at 5 and 12 m during C5. The samples were preserved with 5% acid Lugols in dark polyethylene bottles and stored at room temperature for up to 6 months. They were then filtered onto 8-μm polycarbonate membranes, mounted on glass slides with Cargille Series A immersion oil to clear the filter, and analyzed at 200X by bright-field inverted microscopy (Freibott *et al.*, 2014).

Larval identification and selection

In the lab, ABT larvae were handpicked from bongo-90 samples under a dissecting microscope (M205C, Leica Microsystems) and identified to either preflexion (between yolk exhaustion and before upward flexing of the notochord), flexion (0° < notochord tip angle < 45°) or postflexion (urostyle ∼45° from the notochord axis) stage (Ahlstrom and Mosser, 1976; Kendall *et al.*, 1984; Richards, 2005). On C1, all samples were sorted for larvae because of low catches. C5, however, had the highest larval density recorded in over four decades of sampling in the GoM; hence, only some samples were sorted for larvae. Larvae were photographed (EC3, Leica Microsystems) and measured for standard length (SL), lower jaw length (LJL) and upper jaw length (UJL)—following Llopiz and Cowen (2009)—using image analysis software (Leica Application Suite (LAS) v.4.3, live measurement module). Not all larvae were fixed with their mouth completely open. The gape (hypotenuse) was calculated from UJL and LJL measurements as height and base of a right triangle. Only larvae with intact jaws were considered. For gut content analyses, larvae were selected only from daytime sampling. From all stations, starting with the smallest individuals and excluding damaged fish, larvae of each stage were chosen in increments of 0.1 mm SL to ensure full coverage of their size range.

Gut contents analysis

Care was taken to minimize damage to the larvae during gut content examination. The alimentary canal from pharynx to anus was dissected using tweezers and scalpel by carefully tracing and cutting along the dorsal margin of the gut from anus to operculum, just deep enough to allow access to the organs. Another deeper cut was made under the operculum and gills to separate the pharynx from the head, and tweezers were used to open the lateral muscular membrane and pick out the internal organs. The digestive tract was carefully opened from anus to stomach using sharpened tips of insect pins, and contents were isolated and imaged. We recorded the location of each prey item in three sections of the alimentary canal: foregut (from pharynx to stomach), midgut (anterior intestine, a large ventral pouch) and hindgut (posterior intestine, a narrow tube connecting dorsally over the right side of the stomach to the anus) (Govoni *et al.*, 1968).

Morphologically distinguishing features of ingested prey had generally degraded from digestion and compaction. Even crustaceans with hard exoskeletons were often missing appendages crucial for identification to lower taxonomic level. Appendicularian trunks were mostly digested, and the tails compacted into somewhat distinguishable "packets" (Llopiz *et al.*, 2010). Hence, the taxonomic resolution of the gut contents (nine categories, further explained below) was lower than for *in situ* collected prey categories.

Zooplankton identification

Daytime *in situ* mesozooplankton were identified using regional taxonomic literature (Owre and Foyo, 1967; Campos-Hernandez and Suárez-Morales, 1994; Boltovskoy, 1999; Dahms *et al.*, 2006; Conway, 2012) under a dissecting microscope to general categories of Class or Order, with the exception of mollusks and echinoderms, which were identified to Phylum. For Class Copepoda, adult male and females (AFM) were identified to Genus, and copepodites were identified to Order. Copepoda nauplii were counted as a separate category. Enumeration and sizing of mixed-layer ciliates was done by Image Pro software (Landry *et al.*, this issue). Cell biovolumes and carbon contents were estimated from appropriate geometric shapes and a carbon conversion of 0.19 μg C μm^{-3} (Putt and Stoecker, 1989). Weighted average C biomass of ciliates was calculated for the upper 25 m assuming the upper samples to represent the surface and the deeper sample to represent the 25 m strata.

Large mesozooplankton *in situ* samples (>200 μm) were partitioned twice using a Folsom plankton splitter and each of three aliquots (50, 25 and 25%) were analyzed in succession. Mesozooplankton categories that had >50 individuals after completion of an aliquot were not counted in the subsequent aliquot(s). Small mesozooplankton samples (50–200 μm) were split until there were at least 300 organisms in an aliquot and counted following the same protocol as the larger mesozooplankton samples. To facilitate identification, small mesozooplankton samples were dyed with Bengal rose pigment. The first 50 individuals of *in situ* mesozooplankton categories and all ingested zooplankton were imaged and measured for body lengths and widths using the LAS measurement bundle.

In situ densities of zooplankton were estimated from the filtered water volume of the net tows and the number of counted individuals in the aliquots. *In situ* and ingested mesozooplankton carbon (C) weights were estimated using length—weight conversion factors from the literature (Supplementary Table SI). In a few cases (6%), when ingested zooplankton were too digested or fragmented to be measured, an average measurement was taken from other individual prey of the same taxa in the gut of the larvae or the guts of similarly sized larvae. For Podonidae, we generated our own body length (along the longest axis, from the eye to end of the brood pouch, excluding the spine) to C weight conversion factor. Ten different size groups (range: 400–825 μm) each containing 20–40 individuals of a mix of *Evadne spinifera* and *Pseudevadne tergestina* (see discussion) were picked from 4% formaldehyde preserved samples. Individuals' lengths were each measured to the nearest 20 μm, rinsed in distilled water, placed directly in 4 × 6 mm tin capsules, dried at 60°C for 24 hours and stored in a desiccator. Dry weights were measured to the nearest 1 μg and C content analyzed on CE Instruments NC2500 Elemental Analyzer coupled via a Thermo-Finnigan Conflo III interface to a Thermo Electron Delta + XP Isotope Ratio Mass Spectrometer at the Stable Isotope Laboratory facility at University of California, Santa Cruz. The length (L)—C weight regression was: $\log_{10}(\mu g\ C) = 2.8844\ (\pm 0.1856) \times \log_{10}(L_{\mu m}) - 8.0767\ (\pm 0.5172)$, $P < 0.001$, $r^2 = 0.97$.

Data analyses

Larval ABT dietary indices, prey niche and preferences were evaluated from the Index of Relative Importance (%IRI) (Pinkas *et al.*, 1970; Morote *et al.*, 2008) based on ingested C, Levins' standardized niche breadth ($\hat{B}_A$) (Hurlbert, 1978) and Chesson's selectivity index (α) (Chesson, 1978). Diet indices and prey niches were estimated for each larval development stage and included the nine prey taxonomic categories: Ciliophora, Podonidae, Copepoda nauplii, Calanoida, Corycaeidae, Other Copepoda, Appendicularia, Acanthopterygii larvae and Other prey—which included other crustacean zooplankton. Ciliophora were excluded from the preference estimation because of abundant smaller ciliates *in situ* that were mostly outside the prey size spectra of the larvae. Acanthopterygii larvae and Other prey were also excluded from the IRI because of lack of reliable C estimates, and from the prey preference estimation because of low number of cases and/or inability to quantify their abundances from the daytime bongo-20 samples.

IRI was calculated from the percentage of each prey taxa C contribution over total prey C ingested (%C) and the percentage of larvae with non-empty guts that had ingested that taxa %FO by equation: IRI = %C× %FO, and presented as: %IRI = IRI/σIRI, where σIRI is sum of all IRIs from all seven prey categories. Levins' standard niche breadth $\hat{B}_A$ was calculated from: $\hat{B} = \frac{1}{\sum \hat{p}_j^2}$ and $\hat{B}_A = \frac{\hat{B}-1}{n-1}$, where $\hat{B}$ is Levins' measure of niche breadth, and $\hat{p}_j$ is the proportion of individuals with prey category j and n is number of prey categories observed. High $\hat{B}_A$ values indicate wide feeding niche (generalist feeding behavior), and low values indicate a narrow niche (specialist feeding behavior).

Chesson's α-selectivity index was calculated for the six taxonomic prey categories indicated above and/or eight logarithmic length classes of prey with the midpoints of 84, 119, 168, 237, 335, 473, 668 and 1122 μm (corresponding size ranges: 71–100, 101–141, 142–200, 201–282, 283–400, 401–562, 563–800 and 801–1585 μm) from:

$$\propto_i = \frac{d_i/z_i}{\sum \left(d_j/z_j\right)}, \text{for } j = 1, \ldots, , N$$

where d_i and z_i is the abundance of prey item i in the gut and environment, respectively, and N is the number of prey items considered. We used average estimates of prey abundance in the environment from daytime bongo-20 tows at C1 ($n = 3$) and C5 ($n = 4$). The index was calculated for individual larvae and averaged for each development stage, where a high α_{i} value indicates high preference. For C1, where only a 200-μm mesh-sized bongo-20 net was used to sample the daytime prey, preferences were only calculated for taxonomic prey categories. Furthermore, we restricted the gut content and *in situ* data to include only prey sizes effectively captured by the 200-μm net by comparison of abundance estimates of different prey size groups in the small (50–200 μm) and large (>200 μm) mesozooplankton from C5 (data not shown). We found that mesozooplankton in the 283–400 μm size class were more abundant in the large mesozooplankton samples and thus only included prey > 283 μm in length. Because we did not resolve prey size preferences, we also limited the *in situ* data to include only prey sizes below the maximum size that could be ingested by the larvae, assuming that to be the largest prey recorded in stomach contents of each larval development stage.

We modeled the theoretical prey size spectra for 2018 by fitting a Gaussian distribution function to the relative preference for each prey length class and prey length relative to larval length (dividing prey length class midpoint by larval SL) for each larva in each development stage (Swalethorp *et al.*, 2014, 2015). We assumed the distribution of α (from the previous equation) to be normal over the prey length classes. The relative preference (p), prey length of maximum preference (prey_{max}) and width of the prey size spectra (b) could then be determined from the following equation:

$$p_i = q_i / \sum_{j=1}^{N} q_i, \text{for } j = 1, \ldots, , N$$

where $q_i = \exp\left(-0.5 \times \left(\frac{\log_{10}(i) - \log_{10}(\text{prey}_{\max})}{\text{b}} \right) \right)^2$, and i is the length interval, and N is the number of prey length classes considered.

Differences in ABT larval gut content of different flexion stages and water parcels in prey numbers and carbon weight of identifiable prey were tested by analysis of variance (ANOVA) using R (R Core Team, 2020). Data were log transformed and inspected for the normality of distribution and homogeneity of variance. Changes in diet composition and selection were tested in Primer v.6.1.7 (Primer-E, Ltd) by permutational multivariate ANOVA on a Bray–Curtis similarity matrix followed by PAIR-WISE testing of cycle experiment and larval development stage. All tests were carried out using 999 permutations. Before testing, prey numbers were square root transformed and prey carbon weights were log transformed. Analysis of similarities in *in situ* mesozooplankton community composition (ANOSIM) was carried out by pairwise testing between C1 and C5 on abundance data. Similarity percentage (SIMPER) analysis was carried out to identify which prey taxa contributed most to the dissimilarity among groups (SIMilarity PERcentage, Clarke and Warwick, 2001). All plots were done in R and Sigmaplot v.12 (Systat Software, Inc.).

RESULTS

Hydrography and zooplankton abundance

ABT larvae were only recorded in abundance during two of the experimental cycles, NF1704-C1 and NF1802-C5. For C1, the experiment drifted 89 km in an south-southwest direction (Fig. 1), and no larvae were collected during the last 24 hours, indicating a gradual slip of the drifter out of the larval patch or larvae decline because of dispersal or mortality. For C5, the experiment drifted 65 km southwest and remained in the larval patch. The average temperatures and salinities [(± Standard deviation (SD)] recorded around noon for the upper 25 m during the experiments were 24.54 ± 0.15°C and 36.43 ± 0.01 Practical Salinity Unit (PSU) during C1 and 25.17 ± 0.60°C and 36.06 ± 0.23 PSU during C5. More information on the hydrographical context during the experiments can be found in Gerard *et al.* (this issue).

From the seven daytime (three from C1, four from C5) bongo-20 net tows targeting the prey-field, total zooplankton ranged from 86 to 249 ind. m^{-3} (Supplementary Table SII). Class Copepoda of all development stages were the most dominant groups (contributing 62.0–81.2%, Supplementary Table SII). The groups comprising the top 95%: Class Sagittoidea (5.4–10.2%), Order Cladocera Family Podonidae (0–12.2%), Class Appendicularia (1.9–8.1%), Class Scyphozoa (1.2–6.5%), Phylum Mollusca (1.3–3.5%) and Phylum Echinodermata (0.3–4.3%). ANOSIM analysis showed significant differences in mesozooplankton community structure between C1 and C5 ($R = 1$, $P = 0.029$). Taxa contributing >3.5% to the dissimilarities under one-way SIMPER test were: Podonidae (10.5%), Cyclopoida copepodites (7.5%), *Oncaea* spp. (AFM) (7.1%), *Clausocalanus* spp. (AFM) (6.2%), Calanoida copepodites (4.9%), *Paracalanus* spp. (AFM) (3.8%) and Appendicularia (3.7%). The *in situ* biomass of prey categories important in ABT larval diets (see below) are displayed in Fig. 2. Of the ciliates and the large mesozooplankton fraction (>200 μm) sampled at both larval rearing sites, biomasses of Podonidae, Calanoida and Corycaeidae were much higher during C5.

ABT larval spatial and size distribution

In total, 280 and 1847 larval ABT were collected from the right bongo-90 net during C1 (27 tows) and C5 (36 tows), respectively, with larval densities averaging (± SD) 13.1 ± 14.5 and 97.7 ± 86.1 individuals per 1000 m^3 (Fig. 1). From both left and right bongo-90 samples, 293 daytime-caught larvae were examined during C1, and 18, 220 and 55 were in preflexion, flexion and postflexion stages, respectively (Fig. 3). From C5, 326 daytime caught larvae were examined, with 224, 54 and 48 from the three stages, respectively. In total, 115 of the larvae were intact from C1 (39%, 4.14–7.90 mm SL) and 133 from C5 (41%, 2.72–8.51 mm SL; Fig. 3). Only intact larvae were selected for gut content analysis, and an effort was made to distribute the selected larvae evenly across the size range of each stage.

ABT larval diet

A total of 2210 prey items were identified from 248 larvae (1218 from C1, 992 from C5; Table I). Feeding incidence was 98.4%, where at least one prey item was observed in all but four preflexion larvae from C5 (2.72–3.21 mm SL). Of those four, the digestive tract of the smallest larvae appeared underdeveloped. Almost all identified prey were located in the midgut and hindgut (53.7 and 48.0% from midgut, and 44.7 and 50.4% from hindgut, for C1 and C5, respectively).

Larval ABT were more capable of ingesting prey of increasing size as they grew and their mouth gape widened (Figs 4 and 5). Small larvae consumed the longest

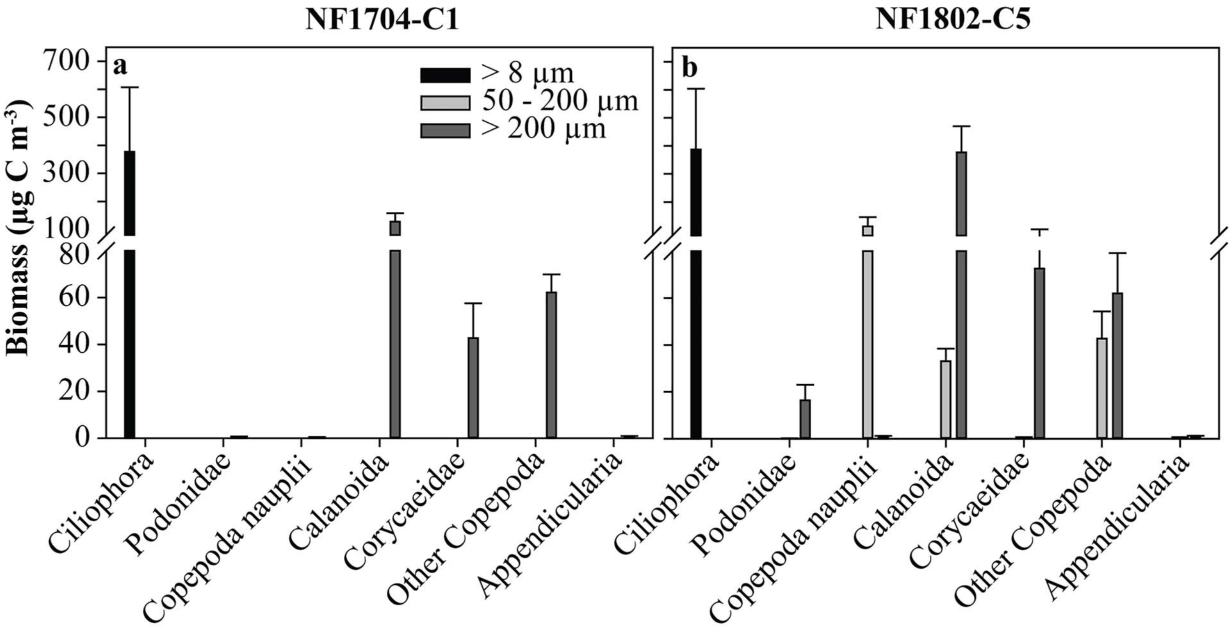

Fig. 2. Bongo-20 *in situ* zooplankton biomass availability of dominant prey taxonomic groups of ABT for rearing site (**a**) NF1704-C1 and (**b**) NF1802-C5 in the GoM. Zooplankton in the 50–200 µm size fraction were only sampled on C5, and the > 8 µm fraction only considers Ciliophora.

prey relative to gape (Fig. 4), suggesting they fed closer to the upper end of their prey size spectra compared with larger larvae. We observed a gradual change in prey taxa with increasing larval size (Fig. 5). Copepoda nauplii, Calanoida and Podonidae were the most widely ingested items across all larval sizes. Only five instances of piscivory were observed, all in large postflexion larvae (>6 mm SL).

There were significant effects of larval development stage on number (*n*; df = 2, F = 12.14, $P < 0.001$) and carbon mass (C; df = 2, F = 58.31, $P < 0.001$) of ingested prey (Table I). Diet composition (*n*, C) also differed significantly among larval development stages ($P < 0.032$; Table I, Figs 6 and 7). Podonidae, Copepoda nauplii and Calanoida accounted for 56–86% of the dissimilarity (*n*, C) among stages. For C1, ontogenetic changes in diet occurred as a gradual transition from small-sized Ciliophora, Copepoda nauplii and Appendicularia to larger Podonidae, Calanoida and Corycaeidae (Figs 6 and 7). For C5, preflexion larvae fed on Copepoda nauplii, Podonidae and Calanoida, and diet gradually shifted toward near-total reliance on Podonidae as the larvae developed (Figs 6 and 7).

We also observed significant effects of year/rearing site on number (df = 1, F = 14.46, $P < 0.001$) and mass (df = 1, F = 39.15, $P < 0.001$) of prey ingested and diet composition (*n*, C; $P = 0.001$) (Table I, Figs 6 and 7). Podonidae, Ciliophora and Appendicularia accounted for 60% of the abundance dissimilarity (*n*) between C1 and C5 experiments, and Podonidae, Calanoida and Copepoda nauplii accounted for 66% of diet C dissimilarity. Larvae from C1 generally had a more diverse diet than those from C5 where Copepoda nauplii and Podonidae accounted for almost all the ingested prey (Figs 6 and 7).

ABT larval feeding niche and preferences

Ontogenetic changes in feeding niche were observed for ABT larvae, but these changes were not consistent between the two rearing sites. Prey size niche did not change markedly with larval development (Fig. 8a). C1 larvae generally displayed a broader size and taxonomic feeding niche than C5 larvae, but ontogenetic patterns were largely opposite (Fig. 8a and b). Interestingly, C5 postflexion larvae showed narrower taxonomic and size niches than C1—indicating that they were feeding on fewer prey taxa of less variable size.

Prey selection also changed during larval development, particularly with respect to taxonomic preference (Table II; $P = 0.001$), with Podonidae accounting for 36–90% of this change. For C5, postflexion larvae greatly narrowed their preferences to only Podonidae and large Appendicularia, whereas Copepoda nauplii and Calanoida were also preferred by preflexion and flexion stages. Overall, Podonidae were the most preferred taxon, by far, for all larval tuna, but Copepoda nauplii were almost as important to preflexion larvae. Although all development stages for C5 showed the highest preference

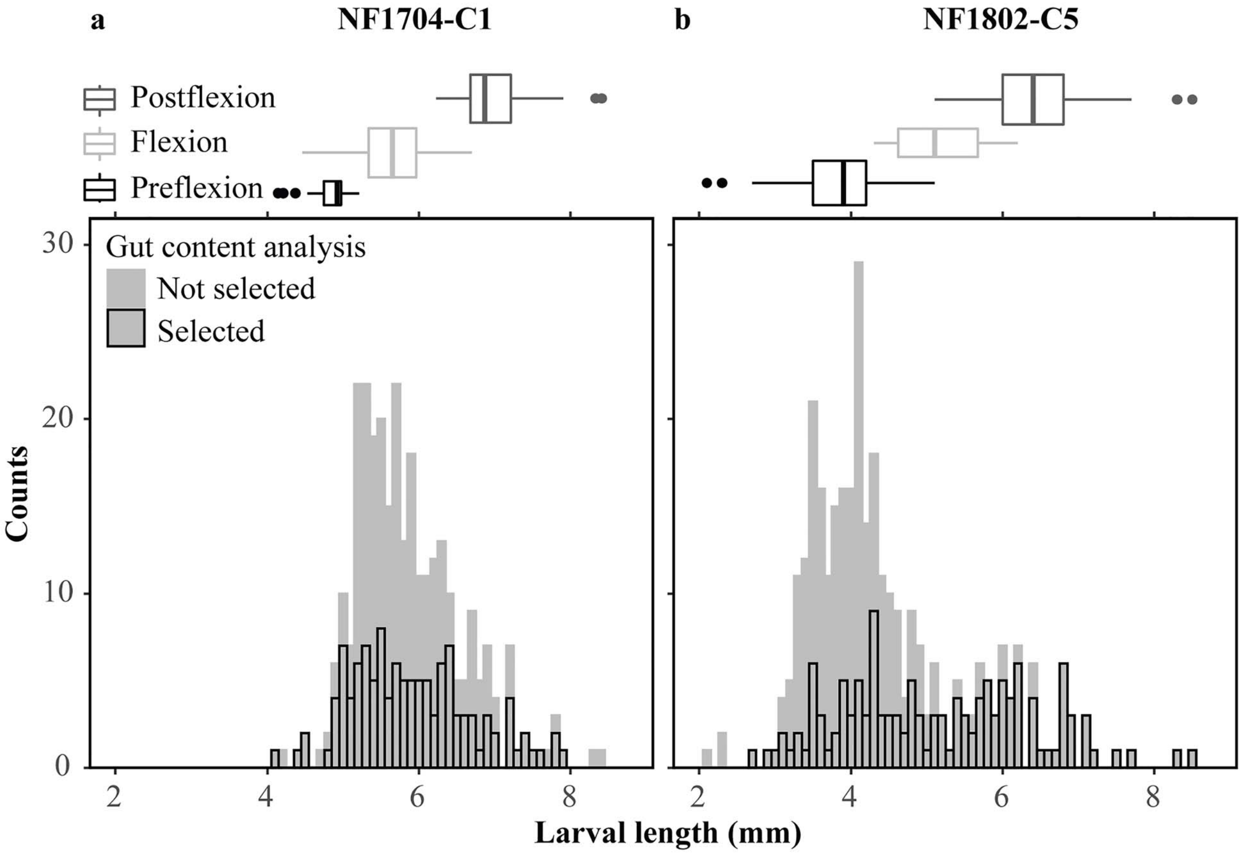

Fig. 3. Histogram showing the daytime ABT larval catch for (**a**) NF1704-C1 ($n = 293$) and (**b**) NF1802-C5 ($n = 326$) in the GoM. The marginal box plot above shows size ranges of flexion stages. Only intact larvae were analyzed for gut contents ($n = 115$ and 133 for C1 and C5, respectively), covering the entire size range in 0.1-mm increments. For C1, flexion larvae were abundant. For C5, preflexion larvae were abundant, whereas flexion larvae were sparse.

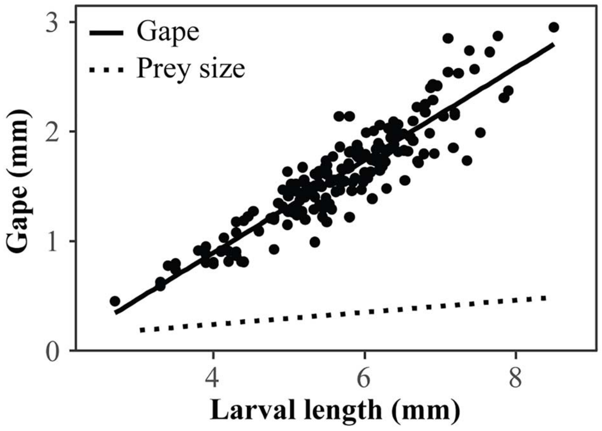

Fig. 4. Relationship between pooled gape height and prey length relative to lengths of ABT larvae in the GoM. Solid line indicates the linear regression line for gape measurements ($r^2 = 0.83$, df = 176, $t = 29.401$, $P < 0.001$). Dotted line is regression line for pooled prey lengths identified in gut contents ($r^2 = 0.09$, df = 1461, $t = 11.74$, $P < 0.001$; see Fig. 5).

for prey in the 283–400 μm size range (Table II), size selection did change with ontogeny toward larger prey (Fig. 9a), especially for Appendicularia, Copepoda nauplii and Calanoida (Table II). Flexion larvae were the most taxonomically diverse in their preferences, selecting the largest prey relative to their own size (Table II, Fig. 9b).

Taxonomic prey preferences of ABT larvae were not static but changed in response to prey availability in the environment. Selection changed significantly between C1 and C5 (df = 1, F = 10.73, $P = 0.001$), with Podonidae accounting for 37% of the difference. Flexion and postflexion larvae were more diverse in their prey selection, with higher preferences for Copepoda nauplii, Calanoida and Corycaeidae on C1 compared with C5 (Fig. 10). For C5, prey selection by all larval stages focused on Podonidae and Appendicularia, which were more available (Figs 5 and 10). This narrowing of the prey taxa spectra accentuates the importance of Podonidae in larval ABT diet. However, rearing site differences should be viewed cautiously as the prey size range of the *in situ* community was incompletely resolved for

Table I: ABT larvae and gut content metrics by flexion stage and rearing site

Rearing site			NF1704-C1				NF1802-C5	
	n	*n* larvae^{-1}	Mean length (μm)	Weight (μg C) larvae^{-1}	*n*	*n* larvae^{-1}	Mean length (μm)	Weight (μg C) larvae^{-1}
Preflexion	9		4710 ± 299		45		3800 ± 466	
Non-empty guts	9		4710 ± 299		41		3880 ± 403	
Ciliophora	30	3.33 ± 3.67	137 ± 31	0.08 ± 0.11	0			
Podonidae	1	0.11 ± 0.33	440	0.04 ± 0.12	38	0.93 ± 1.23	343 ± 82	0.19 ± 0.32
Copepoda nauplii	27	3.00 ± 3.67	237 ± 64	0.58 ± 0.54	104	2.54 ± 1.95	195 ± 59	0.31 ± 0.31
Calanoida	5	0.56 ± 0.53	405 ± 49	0.27 ± 0.28	23	0.56 ± 0.63	287 ± 87	0.13 ± 0.19
Corycaeidae	0				0			
Other copepods	0				6	0.15 ± 0.42	252 ± 37	0.03 ± 0.09
Appendicularia	15	1.67 ± 2.00	n/a	0.18 ± 0.21	4	0.10 ± 0.37	207 ± 97	0.01 ± 0.03
Acanthopterygii larvae	0			n/a	0			n/a
Other	0			n/a	1	0.02 ± 0.16	172 ± 53	n/a
Preflexion total	78	8.67 ± 4.95	1220 ± 670	1.14 ± 0.46	195	4.76 ± 2.41	1115 ± 576	0.66 ± 0.47
Flexion	78		5700 ± 473		41		5100 ± 556	
Non-empty guts	78		5700 ± 473		41		5100 ± 556	
Ciliophora	425	5.25 ± 5.71	144 ± 33	0.23 ± 0.34	0			
Podonidae	81	1.00 ± 1.14	369 ± 102	0.26 ± 0.34	132	3.14 ± 2.50	350 ± 78	0.65 ± 0.49
Copepoda nauplii	127	1.57 ± 2.09	266 ± 56	0.37 ± 0.56	60	1.43 ± 1.64	246 ± 63	0.29 ± 0.33
Calanoida	95	1.17 ± 1.38	445 ± 111	0.81 ± 0.95	36	0.86 ± 1.12	385 ± 113	0.42 ± 0.63
Corycaeidae	39	0.48 ± 0.91	430 ± 78	0.57 ± 1.07	1	0.02 ± 0.15	327	0.01 ± 0.08
Other copepods	10	0.12 ± 0.43	313 ± 90	0.04 ± 0.16	8	0.19 ± 0.63	231 ± 82	0.04 ± 0.17
Appendicularia	194	2.40 ± 2.26	275 ± 117$*$	0.28 ± 0.31	18	0.43 ± 0.80	255 ± 66	0.04 ± 0.08
Acanthopterygii larvae	0			n/a	0			n/a
Other	3	0.04 ± 0.19	212 ± 73	n/a	4	0.10 ± 0.37	196 ± 82	n/a
Flexion total	974	12.36 ± 7.15	2411 ± 1369	2.55 ± 156	303	7.39 ± 3.36	2219 ± 905	1.46 ± 0.80
Postflexion	28		7000 ± 488		47		6440 ± 715	
Non-empty guts	28		7000 ± 488		47		6440 ± 715	
Ciliophora	21	0.75 ± 1.94	n/a	0.02 ± 0.06	1	0.02 ± 0.15	125	0.00 ± 0.00
Podonidae	53	1.89 ± 2.45	452 ± 122	0.82 ± 1.25	355	7.55 ± 4.10	376 ± 97	2.02 ± 1.38
Copepoda nauplii	22	0.79 ± 2.02	264 ± 50	0.18 ± 0.45	42	0.89 ± 1.86	236 ± 69	0.17 ± 0.36
Calanoida	47	1.68 ± 1.70	484 ± 141	1.56 ± 1.76	38	0.81 ± 1.28	364 ± 179	0.48 ± 1.24
Corycaeidae	14	0.50 ± 0.69	507 ± 144	1.05 ± 1.94	1	0.02 ± 0.15	380	0.02 ± 0.11
Other copepods	2	0.07 ± 0.26	365 ± 0	0.04 ± 0.14	9	0.19 ± 0.54	273 ± 56	0.05 ± 0.17
Appendicularia	6	0.21 ± 0.63	313 ± 29$^{\#}$	0.03 ± 0.09	31	0.66 ± 1.26	358 ± 92	0.15 ± 0.26
Acanthopterygii larvae	1	0.04 ± 0.19	1387	n/a	4	0.09 ± 0.28	2860 ± 1520	n/a
Other	0			n/a	1	0.02 ± 0.15	440 ± 396	n/a
Postflexion total	166	5.93 ± 3.80	1908 ± 1217	3.70 ± 2.63	494	10.51 ± 4.05	3964 ± 1501	2.89 ± 2.07
ABT total	115		5900 ± 748		133		5190 ± 1210	
Prey total	1218	10.50 ± 6.89	2191 ± 1332	2.72 ± 1.93	992	7.69 ± 4.05	2504 ± 1501	1.72 ± 1.64

Larval ABT prey categories showing numeric frequency (n; $*n = 9$, $^{\#}n = 2$), average number of prey categories per larvae, mean prey length, and mean carbon weight of prey per larvae. All data are displayed ± SD of the mean. Unidentified prey are not included in the Other category as they are usually fragments rather than whole prey. Carbon weights for Acanthopterygii larvae and Other prey were not assessed because of lack of reliable C estimates and low number of cases.

C1 because only large copepod nauplii, copepods and appendicularians were considered in Fig. 9. For the same reason, we did not estimate larval size preferences for C1. Nevertheless, our preference analysis did not show ABT larvae selecting for Corycaeidae or other non-calanoid copepod taxa during any point in their development (Table II, Fig. 10).

Comparing results from the prey preference analysis with the tuna larval diet, larvae demonstrate feeding close to their theoretical optima. For preflexion, flexion and postflexion stages, average lengths of ingested prey were 262, 323 and 454 μm for C1 and 245, 335 and 473 μm for C5, respectively. For preflexion and postflexion larvae, these are not far from optimum prey lengths (length of max preference, Fig. 9a) of 265 and 432 μm, respectively (flexion optimum = 408 μm). However, in terms of the optimal prey taxa, Podonidae and Copepoda nauplii for preflexion, and Podonidae for flexion and postflexion

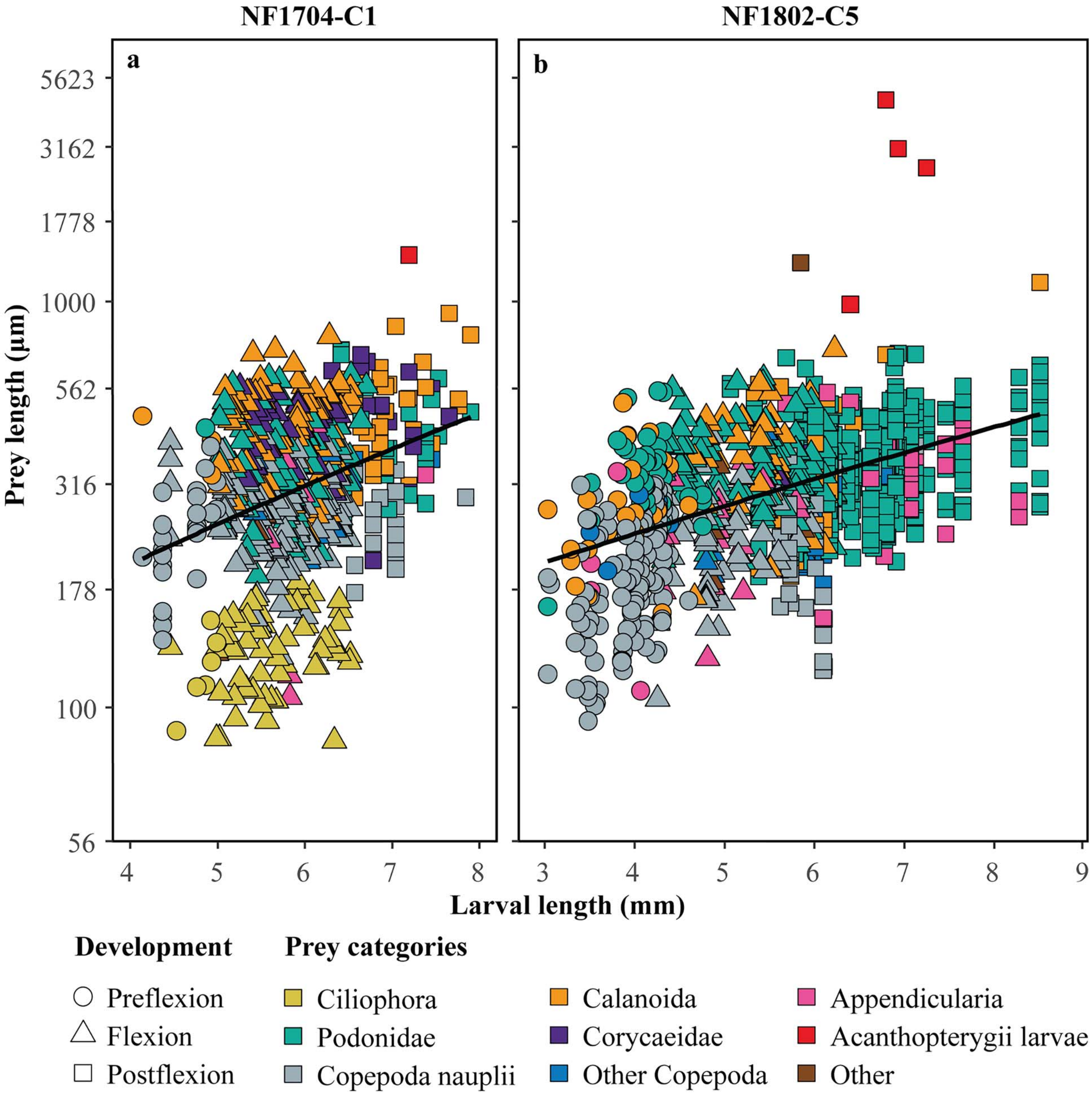

Fig. 5. Relationships between lengths of identified prey on a $\log_{10}$ scale (y-axis) and ABT larval length from (**a**) NF1704-C1 and (**b**) NF1804-C5. Symbol shapes correspond to flexion stages (preflexion, circle; flexion, triangle; postflexion, square). The r^2 values for NF1704-C1 and NF1802-C5 were 0.11 (df = 550, $t = 8.362$, $P < 0.001$) and 0.23 (df = 909, $t = 16.420$, $P < 0.001$), respectively.

larvae did comprise substantially higher proportions of larval diets during C5 (Figs 5 and 10, Tables I and II).

DISCUSSION

This study presents information on the abundance, prey availability, diet, feeding niche and prey selection of developing ABT larvae in the GoM. We identified ontogenetic changes in diet and prey selection, and specific zooplankton prey taxa of key importance to larval feeding. By contrasting two rearing sites with markedly different proportions of zooplankton taxa, we also observed prey selection to be an adaptive mechanism modified with the availability of preferred prey. In the sections below, we discuss these findings in relation to environmental conditions and in the context of other studies from the GoM and elsewhere.

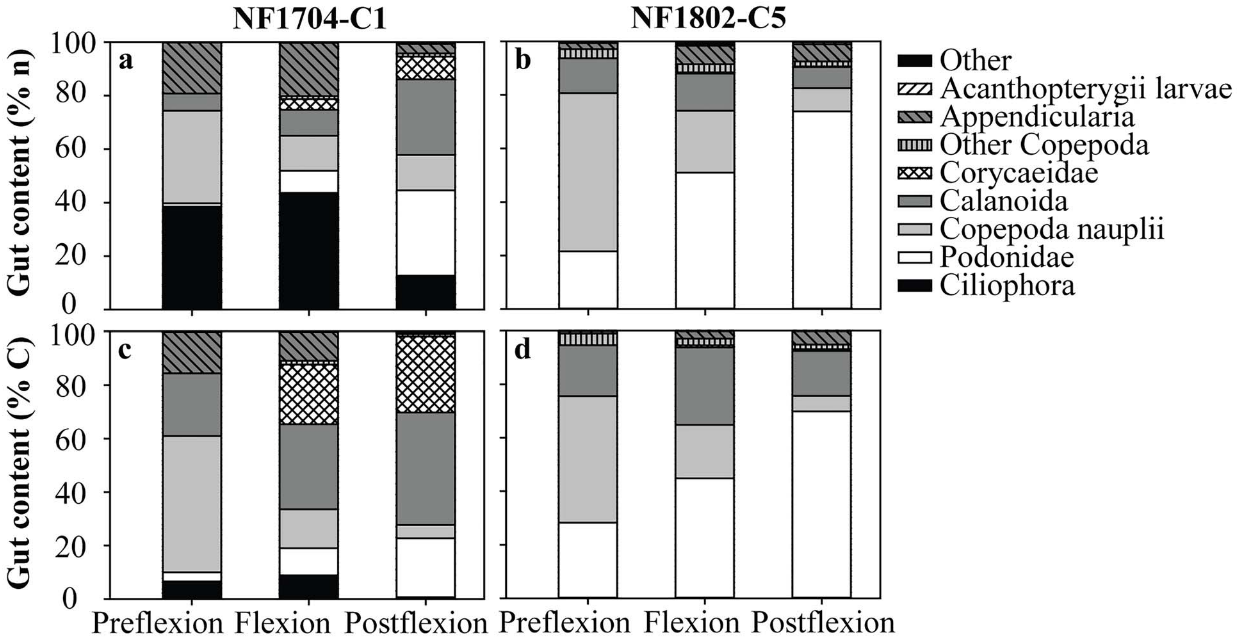

Fig. 6. Relative taxonomic compositions of diet in preflexion, flexion and postflexion stages of ABT larvae in the GoM. Panels (**a**) and (**b**) are numbers and (**c**) and (**d**) are carbon biomass estimated from prey length and conversion factors (Supplementary Table SI). Larvae collected during (a, c) NF1704-C1 and (b, d) NF1802-C5. Carbon weight was not estimated for Acanthopterygii larvae and other prey.

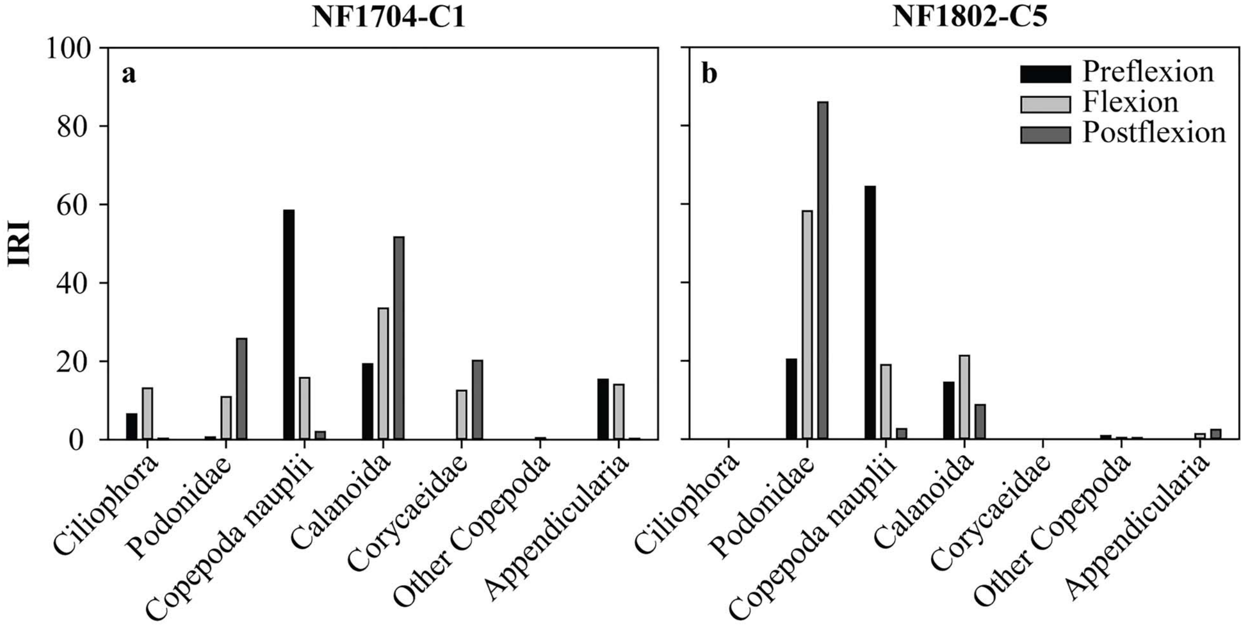

Fig. 7. Relative carbon contributions of seven prey groups to the diet of preflexion, flexion and postflexion stages of ABT larvae in the GoM. Indices of relative importance (IRI, %) are shown for larvae collected during (**a**) NF1704-C1 and (**b**) NF1802-C5.

Communities and distributions

Zooplankton assemblage composition differed markedly between the two ABT rearing sites. *Oithona* spp. were notably abundant in the offshore water sampled during C1, whereas Podonidae, *Oncaea* spp., Corycaeidae, *Paracalanus* spp. and *Clausocalanus* spp. were abundant in the near-continental shelf waters sampled during C5. The differences reflected hydrographic differences of the two

Table II: Prey size and taxonomic preferences of ABT larvae

		Prey length interval (μm)							
Flexion stage	Prey taxa	84	119	168	237	335	473	668	1122
Preflexion	Podonidae				2.5 ± 1.0	35.3 ± 7.0	3.6 ± 2.5		
	Copepoda nauplii	0.3 ± 0 0.3	2.2 ± 1.1	9.1 ± 3.7	17.1 ± 4.5	6.3 ± 3.2			
	Calanoida			4.6 ± 3.1	7.3 ± 3.6	4.1 ± 2.1	2.5 ± 2.4		
	Corycaeidae								
	Other Copepoda			0.4 ± 0.4	1.8 ± 1.3	0.8 ± 0.8			
	Appendicularia		0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	1.7 ± 1.7			
Flexion	Podonidae				9.0 ± 3.4	63.8 ± 6.4	8.0 ± 3.4	1.4 ± 1.4	
	Copepoda nauplii		<0.1 ± < 0.1	0.2 ± 0.2	0.4 ± 0.2	2.6 ± 2.4			
	Calanoida			2.0 ± 1.9	<0.1 ± < 0.1	0.4 ± 0.3	5.9 ± 3.2	<0.1 ± < 0.1	
	Corycaeidae					0.8 ± 0.8			
	Other Copepoda			0.1 ± 0.1	0.1 ± 0.1		<0.1 ± < 0.1		
	Appendicularia		<0.1 ± < 0.1	2.2 ± 2.0	1.2 ± 0.9	1.8 ± 0.9			
Postflexion	Podonidae				7.9 ± 1.8	76.8 ± 3.5	5.7 ± 1.0	0.3 ± 0.1	
	Copepoda nauplii		<0.1 ± < 0.1	0.2 ± 0.2	1.7 ± 1.7	0.4 ± 0.4			
	Calanoida			0.2 ± 0.1	0.1 ± 0.1	<0.1 ± < 0.1	0.1 ± 0.0	<0.1 ± < 0.1	<0.1 ± < 0.1
	Corycaeidae					0.9 ± 0.9			
	Other Copepoda			<0.1 ± < 0.1	<0.1 ± < 0.1	<0.1 ± < 0.1			
	Appendicularia			<0.1 ± < 0.1	<0.1 ± < 0.1	1.5 ± 0.5	4.1 ± 1.9		

Data from Chesson's α-selectivity index for 2018 for six taxonomic groups and eight logarithmic size classes. Values are average preference in percentage ± standard error of the mean. Shaded bold values denote prey positively selected by the larvae. Neutral preference is 2.1%. Blank areas indicate no cases.

sites, with C5 showing a lower-salinity river influence in surface waters, a shallower mixed layer, and a shallower depth of the chlorophyll *a* maximum, but higher chlorophyll *a* concentrations (Landry *et al.*, this issue). Ortner *et al.* (1989) also observed similar differences in copepod community structure, with *Oithona* spp. dominant in the open-ocean GoM and *Clausocalanus* spp. prevalent in the Mississippi River plume. Backtracking experiments of water parcels revealed lateral advection of surface water, and likely zooplankton, from the productive northeastern shelf region to both C1 and C5 (Gerard *et al.*, this issue). The closer proximity of C5 to the shelf may explain the abundance of podonids, which are generally recognized as neritic species (Onbé, 1999). Closer examination of mesozooplankton samples from C5 revealed that ~66% of Podonidae were *E. spinifera* and the rest *P. tergestina*. Both taxa are oceanic (Longhurst and Seibert, 1972) and have similar upper thermal ranges and salinity tolerances (28°C, 37 PSU), but *E. spinifera* also occur in temperate brackish waters (3°C, 6 PSU, Della Croce and Angelino, 1987; Onbé, 1999). There are no other records of podonids in the north-central GoM; only from southeastern coastal areas (Della Croce and Angelino, 1987), northwestern coastal areas (*P. tergestina*, Mullin and Onbé, 1992) and from Loop Current regions of the central GoM (*E. spinifera*, Tilley *et al.*, 2016).

ABT larval abundances and size distributions also differed between the two rearing sites. Most notably, larval abundance was higher during C5 than C1 (Fig. 1). Tuna larvae have been observed previously in both areas, and in association with specific mesoscale features (Richards and Potthoff, 1980; Muhling *et al.*, 2010; Domingues *et al.*, 2016; Laiz-Carrión *et al.*, 2019). Furthermore, few preflexion larvae were caught during C1, whereas relatively few flexion stage larvae were caught during C5 compared with postflexion (Fig. 3). The differences in total larval abundance between rearing sites is partly explained by the differences in preflexion larval abundance, which could reflect slight discrepancies in timing between ABT spawning and our sampling. Spawning had likely stopped during C1, but was still ongoing during C5. The bimodal distribution of larval sizes for C5, with few larvae in the flexion stage, could be the result of asynchronous spawning or sampling overlapping patches. Half of the bongo-90 tows on C5 contained both preflexion and postflexion stages, although mostly during the first half of the cycle. Higher abundances of flexion and postflexion stage larvae during C5 may also stem from greater prey availability.

Predation induced mortality could have affected larval abundance and size distribution but was not assessed in this study. Cnidarians, chaetognaths and other piscivorous tunas such as *Auxis* spp. and *Katsuwonus pelamis* that are potential ABT larval predators (Llopiz *et al.*, 2010, 2015; Laiz-Carrion *et al.*, 2019) were not notably abundant in our samples at either site or cohabitated the same strata, but this might be due to our focus on daytime mixed-layer net tows (data not shown). For the full euphotic zone, Landry and Swalethorp (this issue) found that carnivorous taxa, dominated by chaetognaths, comprised ~ 70% of the daytime biomass of > 1 mm mesozooplankton during both C1 and C5. Cannibalism

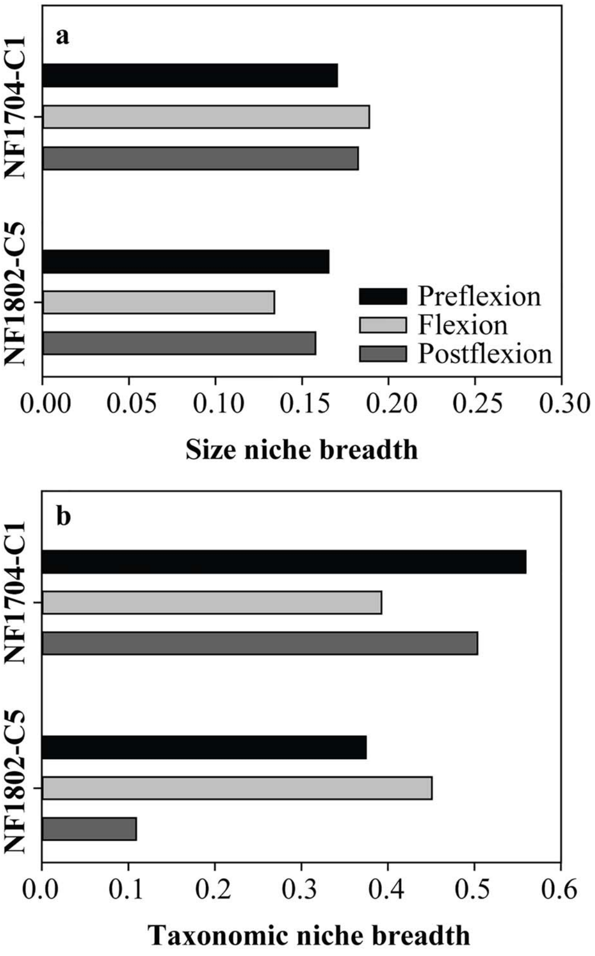

Fig. 8. Prey size (**a**) and taxonomic niche breadth (**b**) for ABT larval stages collected during NF1704-C1 and NF1802-C5. Size niche breadth is estimated as the SD of the $\log_{10}$(length) of all ingested prey, and taxonomic niche breadth is based on Levin's index.

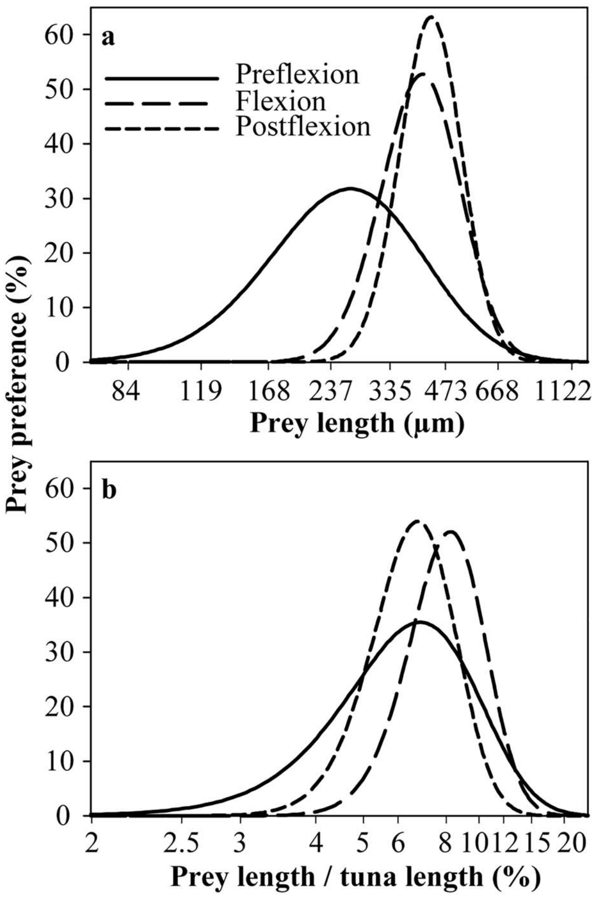

Fig. 9. Theoretical prey size spectra for three development stages displayed of ABT larvae. Data fit by Gaussian 3 parameter normal distribution functions with $\log_{10}$-scaled x-axes. (**a**) Prey length of maximal preference (prey_{max}) and b coefficients were 265 μm and 0.19 for preflexion ($r^2 = 0.22$), 408 μm and 0.11 for flexion ($r^2 = 0.47$), and 432 μm and 0.09 for postflexion larvae ($r^2 = 0.68$), respectively. (**b**) Prey length to larval length ratios of maximal preference (prey_{max}) and b coefficients were 6.8% and 0.17 for preflexion ($r^2 = 0.27$), 8.2% and 0.11 for flexion ($r^2 = 0.46$), and 6.7% and 0.11 for postflexion larvae ($r^2 = 0.59$), respectively.

by older cohorts of postflexion ABT larvae (>6 mm SL) has been observed in other studies (Llopiz *et al.*, 2015; Uriarte *et al.*, 2019). We observed five cases of piscivory in 6–8.5 mm larvae, possibly due to low larval densities, minimizing encounters (Uriarte *et al.*, 2019). In addition, larger individuals were not effectively collected with our bongo-90 net. Other factors such as adult ABT spawning density, food competition and growth rates could have affected larval abundance and size distribution, but were not assessed in this study.

Ontogenetic changes in diet

We observed marked changes in diet size and taxa selected as larval ABT grew and developed. Preflexion stage larvae fed mainly on small prey, particularly copepod nauplii, and also on small appendicularians and ciliates. These taxa persisted in larval diet throughout ontogeny, although their contributions gradually decreased. Copepod nauplii are generally important in the diets of early and later ABT development stages (Catalán *et al.*, 2011; Llopiz *et al.*, 2015; Uriarte *et al.*, 2019), other scombrids (Llopiz and Hobday, 2015), and many other species of larval fish (e.g. Pepin and Penney; 1997; Llopiz, 2013; Swalethorp *et al.*, 2015). Appendicularians have previously been highlighted as important in ABT larval diets and are fed on by other scombrids as well (Morote *et al.*, 2008; Llopiz *et al.*, 2010, 2015; Kodama *et al.*, 2017). Ciliates have been recorded in the stomachs of bluefin larvae in other areas (Kodama *et al.*, 2020), but this is the first time that they were observed to be

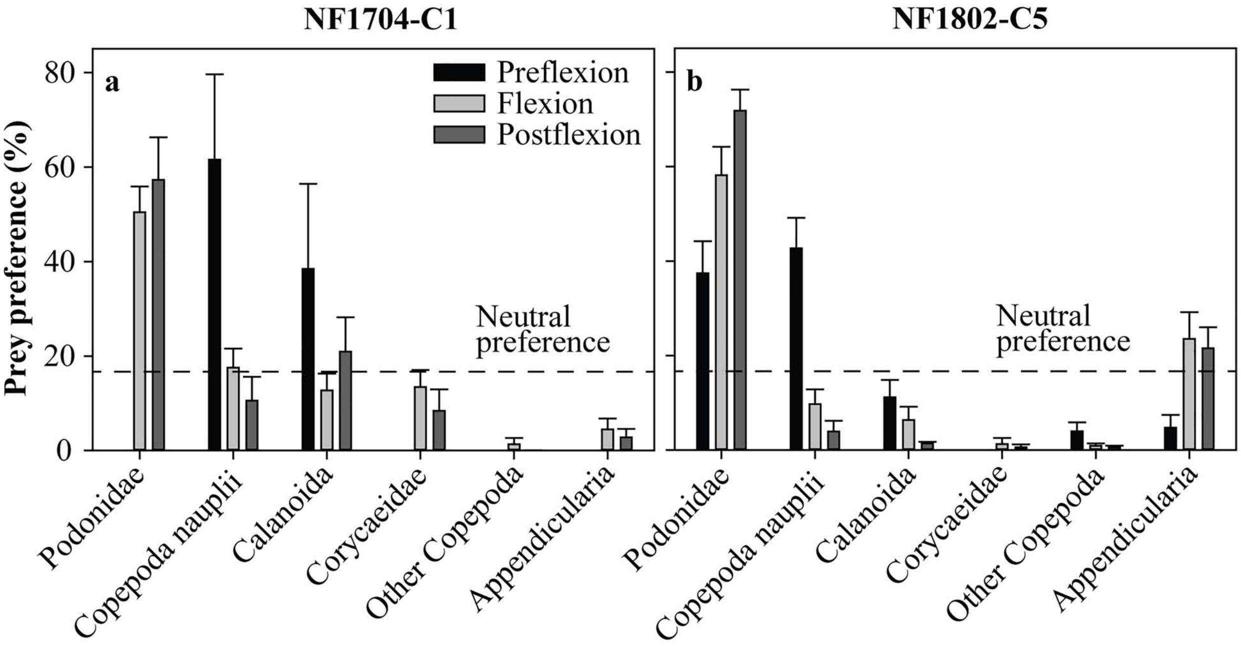

Fig. 10. Prey taxonomic preferences (Chesson's α index in % ± SE) calculated for important prey in the diet of tuna larvae development stages sampled during (**a**) NF1704-C1 and (**b**) NF1802-C5. Only prey sampled with a 200-μm mesh net are considered (prey lengths > 282 μm). The analysis includes prey up to the maximum lengths ingested by preflexion (562 μm), flexion (800 μm), and postflexion (4662 μm) stages, assuming these to be the theoretical maxima that can be ingested.

significant contributors to diet (up to 9% of ingested C). Protozoans likely provide important opportunities for first feeding larvae to gain energy and improve hunting capabilities (Scura and Jerde, 1977; Van Der Meeren and Moksness, 2003; Overton *et al.*, 2010), but have long been ignored as they are rapidly digested and thus generally underestimated in diet studies (De Figueiredo *et al.*, 2007; Montagnes *et al.*, 2010; Landry *et al.*, 2017). Likely, other soft-bodied prey are also underestimated as suggested by metagenetic analyses—e.g. chaetognaths, annelids, and cnidarians as prey—although at much lower frequency than appendicularians (Kodama *et al.*, 2017, 2020).

Larger flexion and postflexion larvae gradually switched to larger prey, predominantly corycaeids (mostly *Farranula spp.*), and calanoid copepods, and particularly podonids. Calanoid copepodites are common in the diets of larval ABT (Llopiz *et al.*, 2015; Tilley *et al.*, 2016) and many other species of larval fish (Llopiz, 2013). Previous studies have also reported corycaeids as significant contributors to diet in the GoM and MED (Tilley *et al.*, 2016; Uriarte *et al.*, 2019), and to other scombrids (Young and Davis, 1990; Govoni *et al.*, 2003; Llopiz and Cowen, 2008). In the present study, podonid contributions were exceptional for larvae of all size, present in 64–96% of the guts of postflexion larvae and up to 70% of ingested C. Even when Podonidae abundance was < 0.2 individuals m^{-3} during C1, they accounted for a significant fraction of ingested prey. Cladocerans, being highly catchable, are often consumed at high rates by tuna and other fish larvae (Pepin and Penney, 1997; Llopiz and Cowen, 2008; Catalán *et al.*, 2011; Swalethorp *et al.*, 2014, 2015; Uriarte *et al.*, 2019). Other studies in the GoM have also found cladocerans in the guts of ABT larvae (Llopiz *et al.*, 2015; Tilley *et al.*, 2016), though not to the extent shown here. Thus, marine cladocerans are an essential link between phytoplankton and higher trophic levels (Miyashita *et al.*, 2011). Our results showing low importance of cyclopoids (other than Corycaeidae) and harpacticoid copepods are in line with most studies, as larvae seldom prey extensively on these taxa. Although piscivory was observed in 6–8.5 mm postflexion larvae—particularly in C5 (12% of larvae), which was comparable to Llopiz *et al.* (2015) and Uriarte *et al.* (2019)—this strategy is more pronounced in larger larvae than those analyzed here.

Ontogenetic changes in prey selection

Larval feeding niche and prey selection changed substantially with ontogeny, both in terms of prey size and taxa consumed. Prey size niche was largely constant, but as the larvae grew, size preferences shifted toward larger prey and preferences shifted from copepod nauplii and calanoids toward appendicularians and particularly podonids. Catalán *et al.* (2011) also observed positive selection for cladocerans and negative selection for copepod nauplii and copepodites in 3–9 mm SL ABT but did not investigate ontogenetic changes or differences in copepod taxonomic orders. Studies on other bluefin tuna species also reported positive selection for appendicularians and podonids (Young and Davis, 1990; Kodama *et al.*, 2017, 2020). Greater preference for larger copepod nauplii in preflexion larvae may be the result of preferential feeding on later development stages. Late nauplii stages are typically more active swimmers, as they have commenced feeding activities, which increase the chance of encounters with ABT larvae (Buskey *et al.*, 1993; Titelman and Kiørboe, 2003a, b; Kiørboe *et al.*, 2014). Landry (1978) and Landry and Fagerness (1988) also observed that predatory copepods strongly select larger nauplii, which they attributed to a sharp increase in escape ability after metamorphosis to copepodid stages. For young ABT larvae, the preference for calanoid copepodites is likely a function of encounter rate and visibility, as calanoids tend to swim more continuously compared with the jump-sink motility of many poecilostomatoids and other cyclopoids (e.g. Hwang and Turner, 1995; Kiørboe *et al.*, 2014). Furthermore, the association of many harpacticoid and cyclopoid copepodites (e.g. *Oncaea* spp.) with suspended particles (Green and Dagg, 1997) may make them more difficult to detect.

As the eyes, caudal fins and mouth gapes develop, increases in prey perception, swimming speed and prey handling ability make ABT larvae more proficient hunters (Morote *et al.*, 2008). Increasing capabilities are particularly obvious during the flexion stage where ABT undergo rapid metamorphosis. Flexion larvae had the most taxonomically diverse feeding preferences and handled the largest prey relative to size, illustrating an especially significant transition in hunting capabilities during this development stage. This enables larvae to catch larger and more energetic prey to satisfy increasing metabolic and growth demands while expending less energy on prey search and handling. The ontogenetic shift in taxonomic preferences could be due to the relatively poor escape capabilities of podonids (Verity and Smetacek, 1996) compared with similarly sized taxa, and the attractive undulating motility pattern of free swimming appendicularians resembling ichthyoplankton (Purcell *et al.*, 2005). The large pigmented compound eye also renders podonids more visible (Zaret and Kerfoot, 1975; Wong *et al.*, 2008), and the tendency of many podonid taxa to form dense near-surface patches (Onbe and Ikeda, 1995; Andersen and Nielsen, 2002; Saito and Hattori, 2000) could make them particularly vulnerable to visual predators (Young *et al.*, 2009). Although fine-scale vertical patchiness was not assessed in the present study, the high contribution of podonids during C1 despite low *in situ* abundance suggests a patchy distribution. Podonidae was the only prey taxon where larval ABT of all development stages preferred the same size group (283–400 μm; Table II). This size group may have comprised a particular Podonidae taxon that was easiest to catch.

A noteworthy observation was the apparent decrease in prey length relative to gape height with larval size (Fig. 4). Although this trend was partially due to prey length being used instead of width (not measured, but important in limiting ingested prey sizes), esophagus diameter, which tends to increase slower than gape height relative to SL as the larvae grows, could be more important in regulating prey size (e.g. Busch, 1996).

Feeding habits modified by prey availability

Larval ABT feeding niche and prey selection changed in response to prey availability and relative proportions of prey taxa at the two rearing sites. C5 larvae, especially the postflexion stage, displayed a more specialist feeding behavior, with narrower size and taxonomic diet niches. If prey selection was entirely passive, it should have remained constant regardless of prey abundance and community composition, since all relevant prey groups were represented at both rearing sites. However, when podonids and appendicularians were more available in C5, larvae of all development stages adjusted their preferences to focus more on them—even selecting against calanoids, an otherwise preferred prey—despite higher calanoid abundance. These observations suggest that selection for podonids and appendicularians was an active mechanism. Our findings also suggest that thresholds in abundance and/or patchiness of key prey taxa could exist and determine the points at which larval ABT switch from passive to active selection of energetically optimal prey. It has long been debated whether young fish are capable of active selection (Juanes and Conover, 1994). However, laboratory studies have shown other species to feed selectively on certain prey taxa when relative concentrations increased (e.g. Meeren and Næss, 1993; Einfalt and Wahl, 1997), indicating active selection. Laboratory studies have also shown that young fish favor the prey that they have prior experiences with (Connaughton and Epifanio, 1993; Reiriz and Brañta, 1998), indicating that cognition is a factor in prey selection. It is possible, however, that slight differences in prey appearance, behavior or capabilities in response to environmental turbidity or other environmental conditions could have existed and contributed to the differences in prey preferences between study sites.

Active prey selection may occur where it becomes energetically advantageous to pursue specific types of prey high in nutrition or catchability (Meeren and Næss, 1993; Juanes and Conover, 1994). Kodama *et al.* (2017) estimated that the podonids and appendicularians ingested by Pacific bluefin tuna were less nutritious than calanoids and argued that they must be passively selected. However, we found that podonids (8 ± 1% N, 39 ± 5% C of dry weight, ± SD, $n = 11$; unpublished data) and appendicularians (9 ± 1% N, 35 ± 5% C, $n = 6$) could be just as nutritious as calanoids (9 ± 2% N, 35 ± 8% C of *Clausocalanus* spp. dry weight, $n = 6$). Appendicularians reproduce and grow faster than crustaceans and are often highly abundant in oligotrophic ecosystems (Landry *et al.*, 1994; Catalan *et al.*, 2011; Llopiz *et al.*, 2010). Under favorable environmental conditions, parthenogenesis by podonids can also quickly increase population size to comprise a significant fraction of the mesozooplankton community (Marazzo and Valentin, 2001; Wong *et al.*, 2004, 2008). Both taxa are filter feeders capturing cells down to just a few microns in size (Sommer and Stibor, 2002). *E. spinifera* also feeds preferentially on autotrophic cells < 5 μm (Broglio *et al.*, 2004). High catchability coupled with availability, high production, nutrition and efficient energy transfer from the base of the food web in oligotrophic ABT nursery grounds would make podonids and appendicularians attractive prey. Ultimately, smaller larval fishes will be actively selected when ABT larvae switch to piscivory (Govoni *et al.*, 2003; Reglero *et al.*, 2009; Catalán *et al.*, 2011; Uriarte *et al.*, 2019).

CONCLUSION

In the present study, we explored the feeding habits of developing ABT larvae at two rearing sites, on two GoM cruises, that differed in prey availability and community composition. As the larvae grew, diet composition and prey selection shifted from small Copepoda nauplii and Calanoida copepods toward larger Podonidae. In waters sampled in May 2017 (C1), with low abundance of podonids, the larvae exhibited a more generalist feeding behavior that included Ciliophora and Corycaeidae; however, podonids remained a significant prey of postflexion larvae. For the first time, these results also documented the quantitative importance of ciliate carbon in the diet of early bluefin tuna larvae. In the May 2018 (C5) study of waters with higher Podonidae availability, larvae of all developmental stages were more specialized in their feeding behavior, exhibiting narrow selection for podonids. These findings underline the importance of podonids in ABT larval diets and suggest that larvae have the capacity to modify feeding behavior throughout ontogeny, switching from passive selection based on physical factors (larval capabilities, prey visibility, avoidance capability and encounter) to active prey selection presumed to optimize tradeoffs in prey catchability and nutrition. As projected for other regions (Johns *et al.*, 2005; Atienza *et al.*, 2016), climate-related changes in precipitation, stratification, and temperature in the GoM could become favorable to podonids. Given the vulnerability of early larvae to starvation in the oligotrophic rearing habitats (Shropshire *et al.*, this issue) and importance of cladocerans to their diet, future studies need to address how variability in habitat structure in the GoM influences the availability of specific prey types and sizes that are preferentially consumed by Atlantic Bluefin tuna larvae.

SUPPLEMENTARY DATA

Supplenetary data can be found at *Journal of Plankton Research* online.

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DATA ARCHIVING

Data presented here have been submitted to the NOAA's National Centers for Environmental Information data repository and will also be archived at Biological and Chemical Oceanography Data Management Office site https://www.bco-dmo.org/program/819631.

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