Bioaccumulation of PCBs by a seaweed bloom (Ulva rigida) and transfer to higher trophic levels in an estuarine food web

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ABSTRACT: Many urban estuaries worldwide contain both eutrophication-induced macroalgal blooms and persistent organic pollutants (POPs) in their sediments. Between 2007 and 2012, we studied an annually occurring green tide of Ulva rigida in the PCB-contaminated Superfund site of New Bedford Harbor, MA, USA. Ulva PCB concentrations were highest (95–99 mg kg⁻¹ dry weight) near the contamination source and decreased to only 2–4 mg kg⁻¹ at the site’s southern end, approximately 2.5 km away. To assess the bloom’s potential role as a PCB contributor to higher trophic levels, we performed stomach content analysis on the system’s primary mid-trophic level predator, the salt marsh mummichog Fundulus heteroclitus, and stable isotope analysis on associated potential energy sources. In addition to detritus and macroinvertebrate prey, Ulva was a major component of mummichog stomach contents, and a ¹⁵N-labeled feeding experiment demonstrated that mummichogs can assimilate ingested Ulva. Among invertebrate prey, stable isotope mixing models showed Ulva as the main diet source for amphipods Gammarus spp., while grass shrimp Palaemonetes spp. and sandworms Nereis spp. relied on a mix of Ulva and Spartina or particulate organic matter, respectively. Sandworms and grass shrimp were the main mummichog energy sources, suggesting that this estuarine keystone species is linked to the Ulva bloom mainly through predation on Ulva-consuming grazers rather than direct ingestion. Our data provide evidence for a potentially overlooked impact of macroalgal blooms—namely, their potential role in the trophic transfer of PCBs and other bioaccumulated pollutants.

KEY WORDS: Stable isotopes · Fundulus · New Bedford Harbor · Macroalgal blooms · Persistent organic pollutants

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1. INTRODUCTION

Most estuaries around the world have been affected by anthropogenic nutrient enrichment resulting in some degree of eutrophication (Kennish 2002). In the most recent assessment of eutrophication in estuaries in the USA, Bricker et al. (2008) reported the majority of estuaries assessed (64 of 99 systems) had eutrophic conditions rated as ‘moderate’ to ‘high.’ Eutrophication of estuaries is predicted to increase in the 21st century due to the consequences of climate change and increased precipitation (Gao et al. 2017, Sinha et al. 2017). One of the most common symptoms of excess nutrients in estuaries is the production of algal blooms, which consist of 2 general types. The most common type of algal bloom consists of planktonic, single-celled microscopic algae. The second type of bloom consists of multicellular, opportunistic macroalgae (or seaweed) which typically grow attached but can grow unattached, forming large,
floating mats. Because the 2 types of algae compete for the same nutrient and light resources, estuaries are typically dominated by one or the other type (Valiela et al. 1997). Which type of algae becomes dominant in an eutrophic estuary depends upon several factors, including the level and persistence of nutrient loading, the amount of salt marsh fringing the estuary, and the water residence time (Valiela et al. 1997). Estuaries with a relatively high nitrogen load, small amount of fringing salt marsh, and low water residence time favor macroalgal blooms.

The most common contributor to macroalgal blooms in temperate and sub-tropical coastal waters is the opportunistic green algal genus Ulva (Teichberg et al. 2010). Members of this large genus, which consists of approximately 100 species (Guiry 2017), have several bloom-forming adaptations, including fast growth rates, broad tolerances to high and low temperature, light, and salinity, as well as rapid uptake rates of both nitrate and ammonia, and simple constructions consisting of 1- or 2-cell thick, sheet-like blades or tubular forms, capable of growing unattached (Fujita 1985). Ulva spp. (hereafter, Ulva) blooms are common in temperate, shallow water embayments on both the east and west coasts of the USA (Lapointe et al. 2018), as well as around the world (Teichberg et al. 2010), including the UK (Taylor 1999), the Brittany coast of France (Charlier et al. 2007), and the Baltic (Blomster et al. 2002) and Mediterranean Seas (Srifo et al. 1992). The largest seaweed bloom ever recorded was an Ulva bloom that occurred along the coast of Qingdao, China in 2008; it measured 30,000 km² and weighed one million metric tons (t) (Liu et al. 2009, Ye et al. 2011). Because of its ability to regrow from fragments and germlings, Ulva blooms can reappear annually for a decade or longer (Zhang et al. 2011, Rinehart et al. 2014). The distribution of Ulva blooms is predicted to become even more widespread in the future due to climate change (Gao et al. 2017).

In addition to eutrophication, many urbanized estuaries and coastal lagoons also contain sediments polluted with persistent organic pollutants (POPs), particularly polychlorinated biphenyls (PCBs) (Skei et al. 2000, Kennish 2002). PCB contamination has been reported from estuaries around the world, including New Bedford Harbor, Narragansett Bay, and the Hudson and Delaware Rivers in the USA (Lake et al. 1995, Ashley et al. 2003, Morgan & Lohmann 2010), the Mersey Estuary in the UK (Vane et al. 2007), the Seine Estuary in France (Loizeau et al. 2001), the Minjiang River Estuary in China (Zhang et al. 2003), the Lagoon of Venice in Italy (Micheletti et al. 2007), Mar Chiquita Lagoon in Argentina (Menone et al. 2001), and the Laguna de Terminus in Mexico (Carvalho et al. 2009). According to the most recent US National Coastal Condition Report, PCBs are the most widespread organic pollutant responsible for a ‘poor’ rating being given to estuaries along the eastern coast of the USA (US EPA 2012). PCBs are also a major contributor to US fish consumption advisories (US EPA 2012).

PCBs are of particular environmental concern because of their persistence in the environment and their bioaccumulation in higher trophic levels due to their lipophilic nature (Islam & Tanaka 2004, Loganathan 2011). Because fish consumption is the major source of PCBs in humans, and PCBs can cause immunotoxicity, and neurodevelopmental and reproductive damage in animals (Fernández-González et al. 2015, Hens & Hens 2018), the US EPA has banned the consumption of fish from many rivers and estuaries in the Northeast USA (US EPA 2012). PCB accumulation has been implicated in the deaths of marine mammal top predators, including harbor seals Phoca vitulina and killer whales Orcinus orca (Van Loveren et al. 2000, Desforges et al. 2018).

One of the most studied PCB-contaminated estuaries is the upper portion of the Acushnet River–New Bedford Harbor system (MA, USA), which contains the New Bedford Harbor (NBH) Superfund site. From 1947 to 1977, approximately 18,000 acres (73 km²) of the upper NBH estuary were contaminated with PCB Aroclors 1016, 1242, and 1254 used in the production of electrical capacitors by Aerovox Corporation (Weaver 1982, Farrington et al. 1985). Upper harbor sediment PCB levels at the time of its Superfund designation in 1982 ranged from 50–4000 μg g⁻¹, with ‘hot spots’ as high as 100,000 μg g⁻¹ (Nelson et al. 1996, Nelson & Bergen 2012). Lake et al. (1995) examined PCB levels and compositions of biota in NBH’s upper harbor, including water, surface sediment, ribbed mussels Modiolus demissus, grass shrimp Paleomonetes pugio, mummichogs Fundulus heteroclitus, and eels Anguilla rostrata. They discovered that mummichogs, a mid-trophic level omnivorous fish species, had the highest PCB levels, which ranged between 198 and 2340 mg kg⁻¹. These values were significantly higher than what has been reported for 2 of the estuary’s top predator species, striped bass Morone saxatilis (3–58 mg kg⁻¹) and bluefish Pomatomus saltatrix (35–98 mg kg⁻¹) (Rusek 1989, Deshpande et al. 2013), and are among the highest levels reported for any fish species (Lake et al. 1995). In addition, Lake et al. (1995) showed high correlation coefficients between the 2 environmental
PCB sources (water and sediment) and mussels and mummichogs, but not with shrimp and eels. In an earlier study, Connolly (1991) described a PCB transfer model for flounder *Pseudopleuronectes americanus* and lobster *Homarus americanus* in NBH’s less contaminated lower harbor. In his model, flounder and polychaetes had the highest total PCB values (ca. 10 μg g⁻¹), followed by mussel (ca. 3 μg g⁻¹), spider crab *Libinia emarginata* (ca. 2.0 μg g⁻¹), and lobster (ca. 0.8 μg g⁻¹). The majority of PCBs (75–94%, depending upon degree of chlorination) found in flounder came from individuals whose PCB burden was derived from the sediment.

While the sources and transfer of PCBs have been examined extensively in freshwater and marine food webs around the world (e.g. Hargrave et al. 1992, Berglund et al. 2000, Romero-Romero et al. 2017), few studies have examined PCB source and transfer in estuarine and shallow coastal food webs (but see Bodin et al. 2008, Morgan & Lohmann 2010, Kobayashi et al. 2015). Morgan & Lohmann (2010) used stable isotope analysis (SIA) in their study of Narragansett Bay, RI, and found that their study species fell into either a more benthic group (composed of lobsters, scup *Stenotomus chrysops* and crabs *Cancer* spp.) or a more planktonic group (composed of butterfish *Peprilus triacanthus* and squid *Loligo pealei*), and that the majority of both groups’ PCB body burdens came from sediments. They concluded that the water column made little contribution to the bioconcentration of PCBs in fish and invertebrates. Bodin et al. (2008), Matsuo et al. (2009), and Kobayashi et al. (2015) used SIA to analyze the source and trophic transfer of PCBs in French and Japanese estuarine and shallow coastal food webs. Although they did not use isotopic mixing models to specifically quantify primary production sources, in general, they found that PCB concentrations were related to trophic level. Thus, despite SIA having been used to track the sources and transfer of PCBs in a number of estuarine and shallow coastal food webs, it has not been used to our knowledge to examine the transfer of PCBs, or any POP, in an estuarine food web with a macroalgal bloom. Our discovery of an *Ulva* bloom in NBH’s Superfund site in 2007 suggested it would make a good model system for such an investigation.

The objectives of this study were to determine the level to which the *Ulva* bloom located in NBH’s Superfund site was capable of concentrating PCBs, and whether the bloom could be a contributing source of PCBs to higher trophic levels. Here, we report on the PCB levels found in the NBH *Ulva* bloom and assess its potential transfer to higher trophic levels by examining the stomach contents of the system’s primary mid-trophic level predator, the mummichog, and by using SIA techniques to examine the contributions of *Ulva* to the principal consumers in this system.

### 2. MATERIALS AND METHODS

#### 2.1. Study site

With the exception of one seaweed sample collected from the Hurricane Barrier at the entrance of NBH, all samples were collected from the ‘upper harbor’ (as defined in Nelson et al. 1996) of NBH, between the Coggeshall and Wood Street bridges (Fig. 1; sampling details in Table S1 in the Supplement at [www.int-res.com/articles/suppl/m611p075_supp.pdf](http://www.int-res.com/articles/suppl/m611p075_supp.pdf)). Most field studies occurred at our primary study site, north of the Coggeshall Street Bridge on the western shoreline, hereafter referred to as ‘Coggeshall Street.’ This location was selected because of its previously reported high total PCB concentrations in mummichogs *Fundulus heteroclitus* (Lake et al. 1995), its reoccurring large mats of *Ulva*, and site accessibility. A secondary study site located on the opposite bank and slightly northeast of the Coggeshall Street site, hereafter referred to as ‘Eastern Shore,’ was selected for geographic comparison within the upper harbor (Fig. 1). All seasonal data comparisons were performed at the Coggeshall Street site.

![Fig. 1. New Bedford Harbor sampling sites](http://www.int-res.com/articles/suppl/m611p075_supp.pdf)
2.2. Bloom size and species composition

In July 2008, we measured the standing crop (total biomass) of the seaweed bloom along the western shoreline of the upper portion of NBH. We divided its distance into 3 sections. The northern section was the shortest (450 m) but included the site of the Aerovox Corporation facility, the primary source of PCB contamination in NBH's upper harbor. The middle section (~1330 m) and southern section (~1230 m) were similar lengths, with the latter section terminating at the Coggeshall Street site.

The width and length of the band of seaweed in each section were measured from aerial photographs available on Google Earth. A total of 20 random 0.25 m² quadrats were collected to determine the average biomass per m² in each area, and total biomass was estimated by multiplying the quadrat biomass per unit area by the length and width of the bloom area. Each quadrat was returned to our laboratory where the *Ulva* plants were washed with distilled water, scrubbed, blotted dry with paper towels, and weighed to determine fresh weight.

The dominant species of the bloom consisted of sheet-shaped forms of *Ulva* of varying sizes and lengths, which are difficult to identify to species morphologically in New England (Guidone et al. 2013). Molecular techniques were employed for species identification of 6 random samples collected from several locations along the upper harbor's western shore during peak biomass in July 2012. DNA extraction, amplification, and sequencing were performed as described in Guidone et al. (2013).

2.3. PCB concentrations

The congener-specific PCB content was determined from 9 samples of *Ulva* collected from 6 sites along the western shoreline of NBH’s upper harbor, and one site outside of it, the Hurricane Barrier at the entrance to New Bedford Harbor (Fig. 1). Samples were collected in July and August 2007 and 2008, and consisted of approximately 90 g (fresh weight) of unattached plants, 20 to 30 cm long. Immediately after collection, samples were washed repeatedly in the laboratory with distilled water to remove any sediment, air dried in a flow hood, and ground to a powder for PCB analysis.

In addition, Aroclor 1016 PCB analyses were conducted on several potential primary PCB sources and food web species from the Belleville Road site in NBH’s upper harbor during the fall of 2011 and 2012. Specifically, samples of *Ulva*, sediment (top 5 cm), grass shrimp *Palaemonetes* spp. (2.4–3.7 cm total rostral length), and mummichogs (7.4–10.6 cm total length) were collected from the Belleville Road site. In addition, striped bass *Morone saxatilis* (48–64 cm straight fork length) and surface water were collected adjacent to the Coggeshall Street Bridge (Fig. 1). Other sentinel species included in the food web analyses (amphipods *Gammarus* spp. and sandworms *Nereis* spp.) were not included in PCB analyses as they were unavailable during collection efforts.

All PCB analyses were conducted using the gas chromatography/mass spectrometry (GC-MS) facilities at the University of New Hampshire. Samples were extracted with an ASE 200 accelerated solvent extractor and concentrated on a SE 400 solvent evaporator (both from Dionex Corporation), cleaned up as required, and the extracts analyzed for congener-specific PCB analysis using a Varian CP3800 gas chromatograph/ Saturn 2200 ion trap mass spectrometer with a CP8400 autosampler. Algal samples were first dried and ground using liquid nitrogen methods similar to those described for *Spartina alterniflora* in Watts et al. (2006). Our PCB analyses provide the total PCB congener composition in each sample, from which we were able to determine the concentration of specific, especially harmful monortho and coplanar congeners, such as congeners (BZ no.) 77, 105, 118, 126, 157, 167, and 189 (Ballschmiter & Zell 1980).

2.4. *Ulva* bloom seasonal abundance

Seasonal abundance of the *Ulva* bloom was estimated by collecting 0.25 m² quadrats from the Coggeshall Street site in NBH’s upper harbor in 2012. Before May and after October, ‘presence or absence’ observations were made without the use of quadrats. Between May and October, sampling occurred approximately every 2 wk as representative ‘early’ and ‘mid’ month measurements. *Ulva* abundance was quantified by collecting the contents of four 0.25 m² quadrats within the bloom. Each quadrat was processed to remove debris and sediment, after which isolated *Ulva* was firmly patted dry with paper towels and weighed to the nearest 0.1 g. *Ulva* abundance was calculated as averages (x ± SE) per square meter based on individual 0.25 m² quadrat measurements. Significance of peaks in *Ulva* abundance with an alpha value of 0.05 and presumed homoscedasticity
was determined using 1-way ANOVA with further post hoc grouping analysis performed in JMP v.10.0 for Mac. Ulva abundance data were square-root transformed to meet the assumption of normal distribution.

### 2.5. Mid-trophic level species composition and seasonal abundance

Seine sets were made at the Eastern Shore and Coggeshall Street sites to characterize the nearshore fish and invertebrate community using a 5.3 m long seine with 16 mm\(^2\) mesh in July 2012. For each seine set, one end of the net was held at the shoreline while the outer end was extended perpendicular to the shoreline to a water depth of approximately 1 m. The outer end of the seine was then hauled towards shore in an arc motion until the net was parallel to the shoreline, at which point all collected species were identified and counted.

Amphipods and grass shrimp were collected from the Coggeshall Street site in 2012 once per month in May and October and approximately every 2 wk between June and September. Amphipod abundance was quantified from the same 0.25 m\(^2\) quadrat contents used to estimate Ulva abundance. Each piece of Ulva and the other contents were carefully inspected and any amphipods found were removed and counted. Grass shrimp abundance was quantified using a 3-dimensional 0.25 m\(^2\) quadrat (50 × 50 × 46 cm) constructed as a 4-walled mesh cube using 0.64 cm galvanized hardware cloth overlaid with window screen mesh. For each of 4 measurements, the mesh cube was rapidly placed into the water and pushed into the top layer of soft sediment. Grass shrimp were immediately removed using a small aquarium dip net, divided into juvenile (<2.3 cm) and adult (>2.4 cm) size classes, and counted.

Amphipod and grass shrimp abundance were calculated as averages (\(\bar{x} \pm SE\)) per square meter based on individual 0.25 m\(^2\) quadrat measurements. Four 0.25 m\(^2\) quadrats were collected per sampling date per species. Significance of peaks in invertebrate abundance was determined using 1-way ANOVA with further post hoc grouping analysis performed in JMP v.10.0 for Mac. Amphipod and grass shrimp abundance data were log transformed to meet the assumption of normal distribution.

Mummichogs were collected twice per month from the Coggeshall Street site between May and October 2012. Mummichog abundance was quantified by deploying non-baited wire minnow traps (total trap length 41.2 cm, mesh size 9 mm, outer opening diameter 18.7 cm, tapered opening diameter 1.5 cm) for 20 min intervals and counting the number of mummichogs collected. Traps were thrown into the water as pairs during the daytime on falling tides in multiple locations within the study site. All traps remained submerged in the water during the entire 20 min period. Mummichogs were transported on ice to the laboratory and immediately placed into −20°C freezer storage. Specimens were later thawed, measured (±0.1 cm), and counted. Averages per sampling date (\(\bar{x} \pm SE\)) included any retrieved empty cages and were determined for both individual size classes and total counted mummichogs.

### 2.6. Mummichog stomach content analysis

Samples for stomach content analysis (SCA) were collected once in May and October and twice per month from June to September 2012 at the Coggeshall Street site and during mid-July 2012 at the Eastern Shore site using the same methods (non-baited minnow traps). Prior to performing SCA, bulk frozen samples were thawed, and individual mummichogs were patted dry with paper towels, weighed to the nearest 0.001 g using an analytical balance, measured to the nearest 0.1 cm total length, and classified by size class. Every effort was made to represent all size classes per sampling period but overall reported data reflect availability on a given collection date. The sample size of mummichogs analyzed per sampling period was \(\geq 15\). Mummichogs were dissected by making a ventral incision with a razor blade and removing the digestive tract from the body cavity. Using forceps or a spatula, the stomach contents were gently extruded onto a glass Petri dish lined with blocked grid paper (1 block = 0.5 cm\(^2\)) and spread evenly on the plate surface. Stomach contents were initially classified into the categories of Ulva, detritus/sediment, invertebrate, and other, based on identification using a dissecting microscope (Leica Zoom 2000). The ‘invertebrate’ stomach contents were further differentiated as amphipod, grass shrimp, sandworm, insect, snail, or unknown invertebrate. The ‘other’ stomach contents were also further differentiated as eggs, Spartina alterniflora, non-Ulva algae, unknown plant material, and other unknown. The percentage of each category was calculated as the number of counted blocks covered by each food item as an estimation of total contents biomass. Non-food items (e.g. pebbles) and empty stomach samples were excluded from further analysis.
2.7. Labeled $^{15}$N Ulva feeding/assimilation experiment

To determine whether Ulva consumed by mummichogs was being digested and assimilated, $^{15}$N labeled Ulva was presented to mummichogs in a laboratory. Ulva rigida was collected from the shoreline adjacent to Northeastern University’s Marine Center, Nahant, MA, USA. The samples were cultured in aerated 250 ml glass volumetric flasks containing a $^{15}$N labeled ammonium chloride solution for 24 h in an artificially lighted culture chamber prior to the feeding experiment. Labeled ammonium chloride (99.9% $^{15}$N) was serially diluted through addition of unlabeled ammonium chloride to produce a final solution with a $\delta^{\text{15N}}$ value ~100‰ (99.7 ± 18.1‰; n = 3). Mummichogs (n = 36; mean ± SD straight fork length = 62.8 ± 14.2 mm) were collected from the Coggeshall Street site in NBH and kept in a 95 l aquarium without food for 4 d prior to the Ulva feeding experiment. To estimate tissue $\delta^{\text{15N}}$ prior to ingestion of labeled Ulva, a subset of mummichogs were killed (n = 9), and their livers were removed for SIA. Liver was selected for analysis since the rapid nitrogen isotope turnover for this tissue would allow any assimilated labeled Ulva rigida to be detected in the short timescale of the experiment (Logan et al. 2006).

At the beginning of the feeding experiment, $^{15}$N-enriched Ulva was offered to the mummichogs ad libitum. Fish were then sampled 3 (n = 1), 4 (n = 5), 5 (n = 6), 6 (n = 7), and 7 (n = 8) d after the addition of labeled Ulva. Livers were removed from each fish and dried in glass scintillation vials at ~60°C for at least 48 h. Whole dried liver samples were then weighed (0.4−1.3 mg) and packed in tin capsules. Samples of Ulva were dried and homogenized using a mortar and pestle, then weighed (3.9−4.3 mg) and packed into tin capsules.

Samples were analyzed by $\delta^{15}$N by continuous flow using a Costech ECS4010 elemental analyzer (Costech Analytical Technologies) coupled with a DELTAPlus XP isotope ratio mass spectrometer (Thermo Scientific) at the University of New Hampshire Stable Isotope Laboratory (UNH). All nitrogen isotope data are reported in $\delta^{15}$N notation according to the equation $\delta^{15}$N = [(Rsample / Rstandard) − 1] × 1000, where R is the ratio $^{15}$N/$^{14}$N. The standard material for nitrogen was atmospheric N$_2$ (AIR). An internal standard (tuna white muscle) that was calibrated relative to ammonium sulfate standards was analyzed throughout the run and was both accurate and precise (12.2 ± 0.1‰; n = 7). Additional reference materials were also both accurate and precise (NIST 1515 [apple leaves]: 0.6 ± 0.1‰; n = 5; NIST 1575a [pine needles]: −3.6 ± 0.1‰; n = 6). All $\delta^{15}$N values were normalized on the AIR scale with IAEA N1 (0.4‰) and N2 (20.3‰).

2.8. Natural abundance SIA sampling and preparation

SIA was used to examine Ulva’s dietary contribution to NBH’s primary and secondary consumers. Representative source, primary, and secondary consumers were sampled from NBH’s upper harbor from the Coggeshall Street and Eastern Shore sites (Fig. 1) for carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) SIA. The Coggeshall Street site was sampled during fall 2011, summer 2012, and fall 2012. The Eastern Shore site was also sampled during summer 2012. Striped bass were sampled during fall 2011 from NBH’s upper harbor using rod and reel. Remaining fish and invertebrate samples were collected using a combination of seining, dip netting, and trapping. Suspended particulate organic matter (SPOM) was collected by forcing surface water through a 60 cc syringe attached to a 47 mm diameter ashed GF/F filter until the filter was clogged. Sheets of Ulva spp., living blades of S. alterniflora (hereafter, Spartina), and benthic POM (BPOM; approximately 1−2 cm of surface layer sediment) were each collected from 3 separate locations within the Coggeshall Street and Eastern Shore sites, at the same time in the summer of 2012. All samples were placed on ice during transport to the laboratory and subsequently stored frozen at ~−20°C until analysis.

All samples were prepared for SIA by first thawing the frozen sample and isolating the desired material/tissue. Amphipods and grass shrimp were prepared whole while mummichogs were dissected to obtain left-side muscle fillet and, when possible, whole liver. BPOM, Ulva spp., and S. alterniflora were prepared by removing debris and SPOM samples were prepared by cutting a ¼ size piece from collected filter paper. Striped bass samples were also dissected to obtain a sub-sample of dorsal muscle and liver. All samples were rinsed with deionized water, wrapped in tin foil, and placed in a drying oven set between 70 and 80°C for a minimum of 48 h. After drying, all samples except SPOM filters were crushed using a mortar and pestle and/or pellet grinder (Retsch MM400; 1/30 s frequency, 90 s pulses). Lipids were not removed from any samples. Fish and invertebrate samples were adjusted for lipid content using C:N-based correction equations (Logan et al. 2008). Carbonates were removed from BPOM and SPOM sam-
amples through treatment with 1 N HCl until bubbling ceased (Jacob et al. 2005) and exposure to HCl fumes in a glass desiccator, respectively. Approximately 4.0–4.5 mg of plant material, 20.0–21.0 mg sediment (BPOM), and 0.6–1.2 mg of animal material were weighed on an analytical balance to the nearest 0.001 mg (Mettler Toledo MX5) and packed into tin capsules. SPOM filters were not weighed but instead packed as approximately 4.34 cm² sized pieces.

Prepared samples were analyzed using a EuroVector elemental analyzer (EA3028-HT) and a GV IsoPrime isotope ratio mass spectrometer at the University of Massachusetts School for Marine Science and Technology to obtain δ¹³C (%o), δ¹⁵N (%o), %C, and %N values. Equipment was calibrated and sample testing range was corrected using the laboratory internal standard ε-amino-n-caproyl acid (Sigma #A-2504) and a matrix standard of tuna white muscle. Fish and invertebrate samples were normalized based on repeated analyses of an internal standard of tuna white muscle.

Contributions of suspended and benthic particulate organic matter (S-BPOM), *S. alterniflora* detritus, and *Ulva* spp. to amphipods, grass shrimp, sandworms, and mummichogs were assessed using the Bayesian stable isotope mixing model ‘MixSIAR’ (Stock & Semmens 2013) in R (R Core Team 2016). Multiplicative error was included in all runs to reflect the distinct predation events reflective of all consumers considered in our analysis (Stock & Semmens 2016). For grass shrimp and mummichogs at the Coggeshall Street site, sampling year was included as a random effect. For summer grass shrimp, size class (juveniles and adults) was included as a fixed effect. All grass shrimp collected in fall samples were adults. While diet sources varied in elemental composition, concentration dependence was not included in mixing model analyses, as much of the variability was likely due to indigestible material (e.g. cellulose, lignin) and so would not reflect assimilated food sources (Phillips et al. 2014). For cases where individual sources had similar stable isotope values and reflected similar functional groups, such sources were combined into single prey guilds in the mixing model analyses (i.e. SPOM and BPOM for all summer data sets, *Ulva* and amphipods for summer mummichog data sets) to reduce model uncertainty (Phillips et al. 2005, 2014).

Different diet-tissue discrimination factors (DTDFs) were used for each consumer species based on controlled laboratory feeding studies. For amphipods, mean (±SD) DTDFs were −0.7 ± 1‰ (δ¹³C) and 0.5 ± 1.5‰ (δ¹⁵N) based on observed DTDFs and associated variability for amphipods (Macko et al. 1982). For grass shrimp and sandworms, DTDFs were 0.8 ± 0.3‰ (δ¹³C) and 2.5 ± 1.5‰ (δ¹⁵N) based on primarily herbivorous marine invertebrates (Bosley et al. 2017). For mummichogs, DTDFs were 1.4 ± 1‰ (δ¹³C) and 2.0 ± 1‰ (δ¹⁵N) based on liver data for a variety of teleost fishes (Sweeting et al. 2007a,b). Liver tissue was used for mummichogs since the isotopic turnover rates of this tissue match the timescale (months) of dietary analysis for this study (Logan et al. 2006). For all MixSIAR runs, *S. alterniflora* δ¹⁵N values from standing live blades were decreased by 2.5‰ to better reflect the δ¹⁵N value of *S. alterniflora* detritus that would be available to the NBH food web (Currin et al. 1995, Baker et al. 2013).

Sentinel species trophic levels (TLs) were estimated based on tissue δ¹⁵N using the equation:

\[
TL = \lambda + \frac{\delta^{15}N_{\text{secondary consumer}} - \delta^{15}N_{\text{base}}}{\Delta n}
\]

(Phillips et al. 2002), where secondary consumer is grass shrimp (whole), sandworm (whole), mummichog (muscle), or striped bass (muscle), base is a lower TL reference organism, λ is the TL of the reference organism, and Δn is the DTDF for δ¹⁵N. Amphipods were used as the reference organism with a presumed TL of 2.0 based on herbivory. Grass shrimp and sandworm Δn for TL calculations were 2.5‰. For mummichog and striped bass, a higher Δn value of 3.2‰ was used to reflect DTDFs for fish white muscle (Sweeting et al. 2007a). Sentinel species TL estimates were calculated separately for Coggeshall Street and Eastern Shore sites and separately for summer and fall at the Coggeshall Street site. Site-specific amphipod δ¹⁵N values were used for all TL calculations except striped bass, which used an average of the 2 sites based on presumed feeding across sites for this migratory predator.

### 3. RESULTS

#### 3.1. Bloom size, species composition, and seasonality

When we measured the *Ulva*-dominated seaweed bloom in upper NBH in 2008, the bloom occurred on both the east and west sides between the Coggeshall and Wood Street bridges (Fig. 1). Along just the western shore, the bloom was approximately 3 km long by 5–12 m wide for a total area of approximately 0.024 km², and weighed approximately 21,850 kg or 24 tons (US). We estimate the total bloom size across both shores to be approximately double in size. Its average biomass was highest in the southernmost section (930 g m⁻²),
intermediate in the middle section (570 g m⁻²), and lowest in the northern section (360 g m⁻²).

The *Ulva* bloom appeared annually during the study from 2007 to 2012, but showed marked seasonality. The bloom generally first appeared in early May, increased in biomass until July, and then declined and disappeared in late October (Fig. 2). Prior to the bloom, small (1–2 cm) juvenile blades of *Ulva* appeared on the surfaces of small rocks in the lower intertidal zone in April, after the winter ice cover disappeared. By late May, these blades had become large, oblong, unattached sheets (0.25–0.5 m long), which covered the lower intertidal zone with a substantial biomass (229 ± 19.34 g m⁻²). The bloom reached its peak abundance (805.8 ± 243 g m⁻²) in early July, remained high during the summer months, and then decreased between September and October to a seasonal low (19.5 ± 3.87 g m⁻²) before dying and disappearing in November (Fig. 2).

Based on molecular analyses, the bloom consisted primarily of *Ulva rigida* and to a lesser extent *U. compressa*. Both are distromatic, sheet-forming forms of *Ulva* that have been responsible for other blooms in New England (Hofmann et al. 2010, Guidone et al. 2013). Of the 6 specimens tested, 5 matched in both its *rbcl* (across 1292 positions) and ITS rDNA sequences across 822 positions (including 300 bp of the 18S region, ITS1, 5.8S, ITS2), the phylogenetic placement (clade E) in Guidone et al. (2013) of *U. rigida*. The sixth specimen differed from the others by ca. 2.5% (33 of 1292 positions) in *rbcl* sequence, while its ITS sequence differed much more, matching the phylogenetic placement (clade I) of Guidone et al. (2013) of *U. compressa*. During the summer months, a small number of other seaweed species could also be found in the bloom, including *Agardhiella subulata* and several filamentous red algae. However, these species never contributed to more than 2% of the bloom’s total biomass.

### 3.2. *Ulva* PCB concentrations and composition

*Ulva* PCB concentrations in NBH’s upper harbor were highest at the Aerovox factory site with a mean of 97 ± 2 mg kg⁻¹, followed by the Sylvia Street site just to its north, at 84 mg kg⁻¹, and the Hadley Street site just to the south at 53 mg kg⁻¹ (Table 1). PCB concentrations in the 3 southernmost sites were considerably lower and decreased with distance from the Aerovox facility (23, 10, 2–4 mg kg⁻¹, respectively). Table 2 shows the relative abundance (% of total) of congeners found in the *Ulva* sample collected from the Aerovox factory site that had a 94.5 mg kg⁻¹ total PCB concentration. In general, lesser chlorinated PCB congeners between BZ #28 and #153 made up the majority of PCBs. Included in its congener composition were the non-ortho substituted dioxin-like congener #77 (0.173 mg kg⁻¹), the mono-ortho substituted dioxin-like congeners #105 (0.348 mg kg⁻¹) and #118 (3.540 mg kg⁻¹), and the non-ortho substituted coplanar congener #126 (0.356 mg kg⁻¹).

Aroclor 1016 PCB measurements were made in 2011 and 2012 at the Belleville Road site (Table 3). The water column samples had a mean concentration of <0.001 mg kg⁻¹. The sediment samples were highly variable (39 and 233 mg kg⁻¹), with a mean of 135.6 mg kg⁻¹. The *Ulva* samples ranged from 6.3 to 10.0 mg kg⁻¹ with a mean of 8.1 mg kg⁻¹. Among the higher TL species, mummichogs...
**3.3. Mid-TL species composition and seasonal abundance**

Based on our seine surveys, the Coggeshall Street and Eastern Shore sites had similar compositions of fish and invertebrate species with some variability in relative abundances (Table S2 in the Supplement). The most abundant nekton and epifauna were grass shrimp, silversides *Menidia menidia*, mummichogs, nine-spine sticklebacks *Pungitius pungitius*, and mud snails *Ilyanassa obsoleta*. We chose grass shrimp, mummichogs, sandworms *Nereis* spp., and amphipods *Gammarus* spp. (which were not collected in the seine) for our SIA studies because of their abundance and previously reported importance in NBH’s upper harbor and other estuaries.

Amphipods first appeared in abundance in our quadrat samples in early May and remained relatively constant at densities between $10^2 \pm 25.5$ and $146 \pm 36.5$ m$^{-2}$ through late June (Fig. 2). In early July, amphipods reached their peak density ($506 \pm 126.5$ m$^{-2}$), coinciding with peak *Ulva* abundance. Grass shrimp first appeared in abundance in early May (Fig. 2) and remained lower than amphipod abundance until July. Grass shrimp abundance peaked in early September ($362 \pm 137.02$ m$^{-2}$), and again in early October ($321 \pm 48.20$ m$^{-2}$) (Fig. 2).

**3.4. Mummichog stomach content analysis**

A total of 940 mummichogs were analyzed for stomach content analysis in this study. The number ranged between 15 and 200 sample$^{-1}$. Individuals with empty stomachs ($n = 40$) were excluded from analysis. The dominant stomach content for the first...
part of summer (early June to mid-July) was invertebrates (Fig. 3), and shifted to a higher percentage of Ulva and detritus in late summer and fall. Peak consumption of Ulva (42.3 ± 8.4 mg dry weight) occurred in August (Fig. 3). Within the invertebrate category, amphipods were generally the dominant food source between early May and early July (Fig. 3). Polychaete worms were dominant in mid-summer and early fall with contents shifting to grass shrimp in late October (Fig. 3).

3.5. Labeled ¹⁵N Ulva feeding/assimilation experiment

Liver δ¹⁵N values increased during the labeled-feeding experiment for most individuals (Fig. 4). Mean values increased from 13.0 ± 1.1‰ (n = 9) at the start of the experiment to 21.5 ± 2.7‰ (n = 8) at Day 7, showing only partial incorporation of the ¹⁵N label (~100‰). Some individuals sampled during Days 4 to 6 had δ¹⁵N values that overlapped with the pre-diet switch group and probably did not ingest Ulva during the experiment.

3.6. Natural abundance SIA

Ulva contributions to carbon and nitrogen pools varied among sentinel species (Table 4). According to Bayesian mixing model estimates, amphipods from both Eastern Shore and Coggeshall Street sites fed mainly on Ulva, with median contributions of 80 and 82%, respectively, with Spartina and S-BPOM making smaller contributions (Table 4). Grass shrimp contributions were more complicated and varied seasonally, as well as between size classes and sites (Figs. S1–S3 in the Supplement, Table 4). At the Coggeshall Street site during summer, the highest median contributions for both adults and juvenile grass shrimp were from Ulva (68 and 70% respectively), with far lesser contributions from Spartina (19 and 9%, respectively) and S-BPOM (12 and 19%, respectively). At the Coggeshall Street site during summer, Ulva had the highest contribution in adults (47%) with S-POM second with 31% and Spartina third with 21%. However, in juveniles, Spartina detritus had the highest contribution (70%), followed by Ulva (21%) and S-BPOM (9%). In our 2011 and 2012 fall adult grass shrimp samples from Coggeshall Street, Spartina detritus was the main diet source in 2011 (44%) and 2012 (67%) while Ulva was second (32 and 18%), and BPOM third (16 and 10%). In sandworms, Ulva and S-BPOM made nearly equal contributions (40
and 44%, respectively), while Spartina detritus was 16%. Our mixing model results for mummichogs for both summer and fall at both sites revealed a primarily invertebrate diet, which accounted for the majority of their dietary carbon and nitrogen. Their dominant prey for both sites and seasons were grass shrimp (33 and 36%) and sandworms (46 and 43%) (Table 4). Ulva was a minor contributor in the summer (2 to 5%), and larger contributor in the fall (20 to 21%) (Table 4).

Sentinel species relative TLs were consistent across sites and seasons. Sentinel fish and invertebrates all had higher average TLs than the amphipods used as the baseline reference (Fig. 5). For the Eastern Shore site, sandworms occupied the next lowest TL (2.2), followed by juvenile grass shrimp (2.4), adult grass shrimp (2.6), and mummichogs (3.0). Shrimp and mummichogs had similar relative TLs at the Coggeshall Street site with summer average values of 2.8 (juvenile grass shrimp), 3.3 (adult grass shrimp), and 3.5 (mummichogs), and fall average values of 2.9 (adult grass shrimp) and 3.4 (mummichogs). Striped bass had the highest estimated TL (4.3) (Fig. 5).

4. DISCUSSION

4.1. Ulva bloom and PCB bioaccumulation

The presence of an Ulva bloom in NBH's upper harbor was first observed in 2007, however, it likely occurred as far back as the mid-1990s based upon past reports of Ulva's presence (Absher-Mitchell 1995, Normandeau Associates 1999, J. L. Lake pers. comm.). The bloom's growth is the result of nutrient inputs into the upper harbor from the Acushnet River, urban runoff, sewer overflows, and the Fairhaven Wastewater Treatment Plant outfall, which have occurred for decades (Ho et al. 2012). During the time of our study, water column nutrients (0.66−0.78 ppm...
total N) and phytoplankton concentrations (14−30 μg l⁻¹ total chl a + Pheo) were relatively low in the upper harbor (The Coalition for Buzzards Bay 2009), the total fringing Spartina area was relatively small, and tidal exchange was intermediate (Kim et al. 2003); i.e. conditions that fit the model for an estuary dominated by macroalgae (Valiela et al. 1997).

At its peak biomass, the Ulva bloom covered an area of approximately 0.024 km², and weighed approximately 21 850 kg (24 tons [US]) just along the western shore of the upper harbor. While unremarkable in size compared to other Ulva blooms, NBH’s bloom is notable because of its high concentrations of PCBs. Our highest PCB values (94.5−98.9 mg kg⁻¹), derived from Ulva collected in front of the Aerovox factory site, are nearly 800 times higher than the highest PCB concentration previously reported for a sea-weed, namely the Ulva rigida bloom in the Lagoon of Venice (Sfriso et al. 1992, Micheli et al. 1995). Even our most distant sample site, 2500 m south of Aerovox, had PCB concentrations of 2−4 mg kg⁻¹, which equals or exceeds the current FDA tolerance levels for PCBs in food (0.2−3 mg kg⁻¹) (ATSDR 2018).

The Ulva PCB concentrations at our 6 sample sites decreased with distance from Aerovox, however very large differences in concentration occurred over short distances. For example, the PCB concentration of Ulva samples collected at Aerovox was almost twice the concentration of the Ulva sample collected just 250 m to its south at Hadley Street (53 mg kg⁻¹), which in turn, was over twice the concentration of the sample collected 230 m below it at Belleville Road (23 mg kg⁻¹). Based upon the low PCB concentration in our Aroclor water column sample (Table 3) and

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Fig. 5. Food web diagram showing linkages to Ulva spp. Solid lines: linkages based on stable isotope mixing model output; dashed lines: linkages based on stomach content analyses averaged across sites and seasons. Line widths are scaled to reflect proportional contributions relative to the highest proportional estimate (Ulva to amphipods Gammarus spp.: 81 %). ( *) Higher trophic level (TL) linkages to mummichogs Fundulus heteroclitus are based on literature stomach contents data (Grant 1962, Meredith & Lotrich 1979, Abraham 1985, Tupper & Able 2000, Nemerson & Able 2003, Ferry & Mather 2012) while amphipod and grass shrimp Palaemonetes spp. stomach content linkages are based on literature grazing experiments (Guidone et al. 2015). Line thicknesses for literature-based linkages are not scaled to show proportional contributions. PCB concentrations (mg kg⁻¹) for Ulva, grass shrimp, and mummichogs are average Aroclor 1016 values from the Belleville Road site while the higher TL PCB value is the striped bass Morona saxatilis average Aroclor 1016 value measured at the Coggeshall Street Bridge. TLs are averages estimated from δ¹⁵N values from all sampling sites. Sandworm Nereis spp. and shrimp images: Dieter Tracey, IAN Image Library (http://ian.umecs.edu/imagelibrary/). Amphipod, mummichog, bluefish Pomatomus saltatrix, and American eel Anguilla rostrata images: Tracey Saxby, IAN Image Library. Striped bass and blue crab Callinectes sapidus images: Jane Thomas, IAN Image Library.
what was reported at the time of our study (<1.0 μg l⁻¹; Battelle 2008), absorption of PCBs from the water column could not explain the large differences in concentration over short distances. Therefore, we hypothesize that Ulva plants took up most of their PCBs from the porewater of the underlying sediments as a consequence of their close contact with the sediment surface, and to a lesser extent from the water column. Consistent with this hypothesis, Ulva has been shown to rapidly take up both ammonia and PCBs from sediment in past microcosm laboratory experiments (Ho et al. 1999, Cheney et al. 2014). This rapid PCB uptake could potentially be utilized as part of a sediment remediation mat system in which uptake could be grown in contaminated systems, removed, dried down and disposed of in a manner similar to contaminated sediments (Cheney et al. 2014).

The PCB congener composition profile for our Ulva sample from Aerovox (Table 2) showed a high concentration of congeners between BZ #18 and #153, and with an octanol–water partitioning coefficient, Kow, of less than 7.0. Ulva’s congener profile is similar to the NBH ‘hot spot’ sediment profile in Lake et al. (1995). It should be noted, however, that since our method of detection did not allow the same degree of congener separation as Lake et al. (1995), in some cases, we had more than one congener co-elute at the same time. Nevertheless, the 12 most abundant congeners in Ulva are similar in ranking to those in the sediment sample of Lake et al. (1995), and include the following (as a % of total PCB): (1) BZ #91 and #95 (9.70%), (2) #28 and #31 (9.34%), (3) #42 and #44 (8.20%), (4) #52 (7.76%), (5) #25 and #26 (7.23%), (6) #49 (6.23%), (7) #18 (5.73%), (8) #110 (4.06%), (9) #118 (3.72%), (10) #47 and #48 (3.18%), (11) #153 and #165 (2.30%), and (12) #138 (1.78%). Similarities between the PCBs taken up by Ulva and the sediment beneath it have also been reported in both laboratory and short-term field experiments by Cheney et al. (2014).

4.2. Food web analyses

Mummichogs Fundulus heteroclitus are a keystone species in this and other estuaries due to their abundance and the pivotal role they play in transferring estuarine production to higher TL predators (Kneib 1986). According to our stomach content analysis, Ulva contributed between 20 and 75% to the diet of mummichogs between mid-June and mid-September (Fig. 3). Peak Ulva consumption occurred in early August and again in mid-September, when the density of Ulva was high (>607 g m⁻²) but plants had stopped growing. Our diet evaluations using the MixSIAR mixing model also suggest an Ulva contribution to mummichogs (Table 4); however, stable isotope data suggest that the main long-term trophic link for mummichogs to Ulva is through secondary consumption of invertebrate grazers, namely amphipods, sandworms Nereis spp. and grass shrimp Palaemonetes spp.

While Ulva was observed in mummichog stomach contents in this and other east coast estuaries (McMahon et al. 2005, Schein et al. 2013), it is probably not a major direct energy source and may be ingested only incidentally, when mummichogs are feeding in Ulva mats on co-occurring fish and invertebrate prey (Merchant et al. 2001). Both stomach contents and stable isotope mixing models showed a strong reliance of mummichogs on sandworm and grass shrimp prey. Mixing models further showed that these 2 prey groups were directly linked to Ulva as a food source. Since the mummichog liver stable isotope values reflect assimilated diets integrated over a seasonal timescale (Logan et al. 2006), these isotope data provide stronger support for predation on invertebrate grazers, in particular grass shrimp and sandworms, as the primary trophic link between mummichogs and Ulva in NBH.

Amphipods Gammarus spp. were seasonally important diet items for mummichogs in NBH based upon stomach contents, but were not a major energy source based on isotope mixing models. Amphipods, however, did have the most direct trophic link to Ulva based upon our isotope mixing model (82%). Amphipod grazing on Ulva is not unique to NBH; gammarid amphipods consume Ulva in eutrophic estuaries worldwide (Balducci et al. 2001, Zheng et al. 2014, Guidone et al. 2015). The lack of isotope-based support for amphipods as an important energy source likely relates to the short duration of their peak abundance in NBH.

Sandworms in NBH consumed similar proportions of both Ulva and S-BPOM (40 and 44%, respectively) based upon our mixing models. Ulva and amphipods were included in the mixing model as a single source, so the Ulva component may have been derived by predation of amphipods rather than direct grazing on Ulva. Previous studies have demonstrated Ulva grazing by Nereis succinea (Levin et al. 2006) and N. diversicolor (Engelsen & Pihl 2008), as well as predation of amphipods and other invertebrates (Wilson & Ruff 1988, Caron et al. 2004). Thus, sandworms in NBH are likely linked to Ulva via both direct consumption as well as predation on Ulva’s grazers.
Grass shrimp connectivity to *Ulva* varied spatially and temporally in NBH in a pattern that appears to be correlated to the bloom’s abundance. In particular, when the bloom reached its peak abundance in the summer, *Ulva* was the primary diet source for adult grass shrimp at both the Eastern Shore and Coggeshall Street sites. Juveniles at Eastern Shore also preferred *Ulva*, however juveniles at Coggeshall Street preferred a *Spartina*-based diet. This may have been due to differences in accessibility to *Spartina alterniflora* between the 2 sites at flood tide, as described by Deegan & Garritt (1997) and McClelland & Valiela (1998). Furthermore, when the bloom declined in the fall, the proportion of *Ulva* in grass shrimp diet declined and that of *Spartina* increased. The differences between the 2011 and 2012 results reflect differences in the bloom’s abundance; specifically, in 2011, *Ulva* remained abundant through September, but only until August in 2012 (Sly 2013). Our mixing model assumed herbivory, but elevated δ¹⁵N values for adults could have also been due to predation on juvenile grass shrimp or other invertebrate prey. However, grass shrimp are more herbivorous in eutrophic estuaries relative to non-eutrophic systems (Fox et al. 2008).

*Ulva* may have been more prevalent in grass shrimp diet overall relative to other systems due to the eutrophic conditions and low phytoplankton abundance in NBH. Although we did not measure the phytoplankton concentration, measurements made in the upper harbor at the time of our study by the Coalition for Buzzards Bay were between 14 and 30 μg l⁻¹ total chl a + Pheo (The Coalition for Buzzards Bay 2009). Adult grass shrimp δ¹³C values also closely matched *Spartina* source values, which further suggests that adults were also relying on *Spartina* detritus through secondary consumption. *Spartina* was the main energy source for adults sampled in the fall, consistent with the reduced abundance of *Ulva* during this season, which suggests that the trophic connection between grass shrimp and *Ulva* may be constrained to the spring and summer period when the bloom is most abundant.

Although our study focused on the food web dynamics of mid- and lower TL species, studies in other systems have documented mummichogs as prey for a variety of fish, avian, and invertebrate predators (see review in Abraham 1985). Juvenile striped bass *Morone saxatilis*, an economically important species, are among the many teleost predators that feed on mummichogs in US east coast estuaries (Tupper & Able 2000, Nemerson & Able 2003, Ferry & Mather 2012). Our striped bass liver stable isotope values, which would be more reflective of local diet than muscle for a highly migratory species (Thomas & Crowther 2015), matched closely with mummichog values following corrections for trophic enrichment. However, striped bass are generalist predators (Walter et al. 2003), and while consistently present, mummichogs were only a relatively minor component of juvenile striped bass diets in other Massachusetts estuaries (Ferry & Mather 2012). There are several other fish and invertebrate predators that we did not examine but which are present in NBH (Table S2) (Lake et al. 1995, Deshpande et al. 2013) and could also consume mummichogs (e.g. American eel *Anguilla rostrata*, bluefish *Pomatomus saltatrix*, blue crab *Callinectes sapidus*) (Grant 1962, Meredith & Lotrich 1979), thereby also deriving PCBs through the *Ulva* trophic transfer pathway quantified in our study (Fig. 5).

Taken together, our stomach contents and stable isotope results show that the *Ulva* bloom in NBH’s upper harbor is an important source of primary production and a major contributor to the food chain of mummichogs (Fig. 5). This is consistent with previous stable isotope tracer studies of New England estuarine food webs showing that primary and secondary consumer diets reflected the dominant form of primary production (Deegan & Garritt 1997, McClelland & Valiela 1998, McMahon et al. 2005). For example, in McClelland & Valiela’s (1998) study of Child’s River, the estuary with the highest nitrogen load in Waquoit Bay, MA, their isotopic results showed that primary production was dominated by the macroalgal *Cladophora vagabond* and *Gracilaria tikvahiae* (73% combined), compared to 26% for phytoplankton, and that the 4 primary consumers of the system (Cymadusa, *Gammarus*, Erichsonella, and Cyprinodon) consumed a mixture of these 2 macroalgae. However, in Deegan & Garritt’s (1997) study of the Plum Island Sound estuary, MA, an estuary with low nitrogen loading and only incidental macroalgae, the isotope values of their primary consumers reflected a mixture of *Spartina spp.*, benthi microalgae, and marine phytoplankton. Similarly, McMahon et al. (2005) showed in their stomach contents and isotopic results of the Sprague River estuary, ME, that while the diets of mummichogs were predominantly composed of primary producers (78%), they differed in the degree of *Spartina ssp.*, *Ulva*, and particulate organic carbon (POC; consisting primarily of phytoplankton) depending upon their relative abundance in different parts of the estuary. These patterns are also apparent in estuaries outside of New England. For example, Park et al. (2016) provided stable isotope-based evidence for the importance of *Ulva*-dominated macroalgal blooms as primary production sources in coastal food webs of Korea.
4.3. PCB transfer

Based upon our Aroclor 1016 data (Table 3) and our stomach contents and isotopic analyses, we propose a food web for NBH with Ulva at its base, being consumed by amphipods, sandworms, grass shrimp, and mummichogs (Fig. 5). The PCB concentrations Lake et al. (1995) report for their biota resemble our findings. For example, their mummichog PCB values are also the highest of all species they examined, including shrimp. In a comparison of correlation coefficients, they show that mummichogs had a 0.89 coefficient with sediment at their ‘hot spot’ site, which, as pointed out above, has a very similar congomer composition to that of Ulva. In particular, our Ulva sample and Lake et al. (1995)’s mummichog sample share high concentrations for many of the same congeners (e.g. BZ #26, 28, 31, 49, 52, 95 and 110).

Although this study was not designed to directly trace PCB transfer from Ulva to higher TLs, we think this is a reasonable assumption based upon several lines of evidence. First, Cheney et al. (2014) showed that living Ulva samples were significantly higher in both their uptake rate and quantity of PCBs than dead Ulva plants. This suggests that the uptake process in Ulva must involve more than simple adsorption to the cell wall and that PCBs are accumulated internally by Ulva. This hypothesis is supported by the report of PCBs being accumulated inside the cells and becoming associated with the lipid-rich thylakoid membranes of the unicellular green alga, Chlamydomonas reinhardtii, which is distantly related to Ulva (Jabusch & Swackhamer 2004). In addition, there is evidence that another POP, 2,4,6-trinitrotoluene (TNT), is not only taken up and stored internally by Ulva lactuca (which is similar in structure and composition to U. rigida) had a significantly higher uptake rate by the brittle star Amphipura filiformis than the control and other sediment enrichments tested, including Ceratium, Ascophyllum, and Zostera. This was believed to be due to the greater nutritional value of Ulva, which is rich in amino and fatty acids (Gunnarsson et al. 1999). The results of our study support the suggestion of Gunnarsson et al. (2000), that eutrophication may lead to an increase in the bioavailability of hydrophobic organic contaminants to benthic, and through trophic transfer, pelagic fish and invertebrates.

Based on our results, we think it is reasonable to assume that organisms that consume Ulva in our NBH food web also consume the PCBs inside its cells. We are not the first to suggest green algal-based PCB trophic transfer. Based on observations of rapid PCB uptake and long-term storage by the green alga Cladophora sp., Larsson (1987) theorized that it may be a mode of PCB transfer to grazing fish and detriti-vores. Although we have not shown that PCBs found in Ulva are assimilated by its consumers, we have shown that mummichogs are able to assimilate nitrogen from Ulva (Fig. 4). In addition, evidence of assimilation and in some cases assimilation efficiency (AE) rates (on a dry weight basis) have been reported for the consumption of Ulva by 2 other small, omnivorous fishes (Cebidichthys violaceus, with an AE of 43 %, Edwards & Horn 1982; and Salpa salpa with an AE of 61 %, Gerking 1984), as well as by amphipods (with an AE of 73 %) (Sotka & Giddens 2009), and shrimp (Gamboa-Delgado et al. 2011). Finally, the transfer of PCBs has been traced from a fungal food source to the amphipod Gammarus tigerinus by Pinkney et al. (1985); 24 h after they fed 14C-Aroclor 1254-labelled Fusarium oxysporum (with a mean of 9.29 μg g−1) to amphipods, the mean PCB concentration in the amphipods was 23 μg g−1. Based on its digestibility, lipid content (1−2.5 %; Gunnarsson et al. 1999, Kumari et al. 2010), and simple construction consisting of a 2-cell thick sheet, we think Ulva rigida, like F. oxysporum, is capable of transferring PCBs to amphipods and other consumers. Subsequent PCB transfer from macroinvertebrates (e.g. amphipods, polychaete worms) to higher TL fish predators is well documented (e.g. Pizza & O’Connor 1983, Rubinstein et al. 1984).

Finally, even after the Ulva bloom’s death and decompose in NBH every fall, it may still be contributing to the bioavailability of PCBs in its food web by enriching the organic content of the sediment. As the studies of Gunnarsson et al. (1999, 2000) have shown, sediments enriched with 14C-tetrachloro- biphenyl labelled Ulva lactuca (which is similar in amino and fatty acids (Gunnarsson et al. 1999). The results of our study support the suggestion of Gunnarsson et al. (2000), that eutrophication may lead to an increase in the bioavailability of hydrophobic organic contaminants to benthic, and through trophic transfer, pelagic fish and invertebrates.

5. CONCLUSIONS

The findings of this study provide evidence that the Ulva bloom in NBH’s upper harbor contains high concentrations of PCBs and is the principal source of primary production for primary consumers. Stable isotope and stomach content analyses support the hypothesis that PCBs taken up by the NBH Ulva bloom could be a source of PCB contamination in the system’s mid-trophic species (e.g. amphipods, grass shrimp, mummichogs) and to the higher TL fish predators that consume them. Given the increasing prevalence of Ulva and other macroalgal blooms
worldwide, this potential source of contaminant transfer and its consequences need to be further studied.

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