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Volume 9, 2022 Urban Naturalist No. 56

Urban Naturalist

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Cover Photograph: Top - Pequot Pond and electrofishing crew preparing for a day of sampling. Bottom - *Esox niger* (Chain Pickerel) sampled from the pond. Photographs © Dave Christensen.

The ***Urban Naturalist*** (ISSN # 2328-8965) is published by the Eagle Hill Institute, PO Box 9, 59 Eagle Hill Road, Steuben, ME 04680-0009. Phone 207-546-2821 Ext. 4, FAX 207-546-3042. E-mail: office@eaglehill.us. Webpage: http://www.eaglehill.us/urna. Published on an article by article basis. **Special issue proposals are welcome**. The *Urban Naturalist* is an open access journal. **Authors**: Submission guidelines are available at http://www.eaglehill.us/urna. **Co-published journals:** The *Northeastern Naturalist*, *Southeastern Naturalist*, *Caribbean Naturalist*, and *Eastern Paleontologist*, each with a separate Board of Editors. The Eagle Hill Institute is a tax exempt 501(c)(3) nonprofit corporation of the State of Maine (Federal ID # 010379899).

Isotopic Analysis of *Esox niger* (Chain Pickerel) Diet Contributions in an Urban Pond

David R. Christensen[1*], Carl A. Favata[2], and Raymond J. Bressette[3]

Abstract - Stable isotope analysis (SIA) of $\delta^{15}N$ and $\delta^{13}C$, and a multiple-source mixing model were used to evaluate diet variability of *Esox niger* Lesueur (Chain Pickerel) in Pequot Pond, an urban waterbody in Westfield, MA. Chain Pickerel diet contributions in urban aquatic ecosystems are not well documented, particularly using SIA. There was a positive relationship between $\delta^{15}N$ and the length of Chain Pickerel, indicating dietary shifts and an increase in trophic positioning as the fish grew. There was a negative relationship between Chain Pickerel length and $\delta^{13}C$, suggesting a possible shift from shallow littoral areas to deeper habitats. Model results indicated that *Lepomis macrochirus* Rafinesque (Bluegill) and crayfish were the principal prey items in the diet of Chain Pickerel. Smaller invertebrates and other fish species had a more diffuse contribution to chain pickerel diets. However, bluegill and *Lepomis gibbosus* Linnaeus (Pumpkinseed) had a greater contribution to the diets of Chain Pickerel ≥300mm while the contribution of crayfish had decreased. Despite an increasingly piscivorous diet among larger pickerel, crayfish remained an important dietary contribution, establishing the importance of large invertebrates in the diet of predatory Chain Pickerel. SIA and the use of multiple-source mixing models appear to be useful tools in evaluating dietary ecology of Chain Pickerel in urban ponds.

Introduction

It is well documented that freshwater piscivorous (feeding on fish) fishes can have a profound influence on prey fish density, size distribution, and behavior (Carpenter and Kitchell 1993). Structuring of prey fish demographics due to predation can also have a cascading effect on subsequent trophic levels, giving piscivorous fish the potential to influence an entire food web (Carpenter and Kitchell 1993, Potthoff et al. 2008). Large piscivorous fishes are also popular amongst recreational anglers due to their size and aggressive behavior. Inland recreational fisheries, often including piscivorous fishes, such as *Micropterus salmoides* Lacepede (Largemouth Bass) and *Esox Lucius* Linnaeus (Northern Pike), contributed $25.9 billion to the U.S. economy in 2011 (USDOI 2011). Due to their popularity, piscivorous fish have been intentionally stocked outside of their endemic ranges for decades (Fuller et al. 1999). Taken together, the trophic influence and recreational economic contribution of piscivorous fishes often make these fish a focal point for state and federal management authorities (Lathrop et al. 2002, Potthoff et al. 2008).

As communities in the United States expand, the proximity of aquatic ecosystems to urban areas is growing considerably. Many of these water bodies contain piscivorous fishes and are under increasing pressure by local communities for recreational angling purposes. Specifically, *Esox niger* Lesueur (Chain Pickerel) are abundant in lakes and ponds from

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Associate Editor: Hal Brundage, Environmental Research and Consulting, Inc.

western Texas through the northeastern United States, often in urban areas (Page and Burr 2011). Although much smaller in size (rarely exceeding 600 mm), Chain Pickerel share the Esocidae family with large piscivores, such as Northern Pike and *Esox masquinongy* Mitchill (Muskellunge). Despite their smaller size, Chain Pickerel are considered piscivorous and provide recreational angling opportunities and possible food web structuring through predation. Therefore, understanding the autoecology of piscivores, such as Chain Pickerel, is essential in managing any urban lake or pond. Despite the piscivorous nature and the broad geographical distribution and abundance of Chain Pickerel, few studies have addressed their feeding behavior, especially in urban aquatic ecosystems (Broderson et al. 2015, Hunter and Rankin 1939, Raney 1942).

In this study, we evaluated the feeding behavior of Chain Pickerel in an urban water body using the stable isotopes $\delta^{15}N$ and $\delta^{13}C$. Stable isotope analysis (SIA) has become a powerful tool in assessing trophic ecological interactions in aquatic ecosystems. In particular, $\delta^{15}N$ is fractionated and enriched in muscle tissue from prey to predator at a rate of about 3–4 ‰, indicating the trophic position (TP) of a consumer (Post 2002, Vander Zanden and Rasmussen 1999), while $\delta^{13}C$ fractionates differently based on the type of algae or plant in which the carbon was processed. Therefore, $\delta^{13}C$ can indicate where the organism may have been feeding such as the littoral zone ($\delta^{13}C$ processed by benthic algae) versus the pelagic zone ($\delta^{13}C$ processed by phytoplankton) of a lake (Post 2002, Vander Zanden and Rasmussen 1999). Because carbon and nitrogen in muscle tissue has a relatively slow turnover (weeks in younger fish to months in older fish) (Busst and Britton 2018, Weidel et al. 2011), the SIA gives a time-integrated estimate of fish diets. Therefore, SIA can give a temporal and spatial dietary estimate and the trophic position of a predator (Post 2002, Vander Zanden and Rasmussen 1999), eliminating the need for an extensive number of samples (Clark et al. 2005).

The objective of this study was to use SIA to evaluate the diet of Chain Pickerel during mid to late summer in an urban pond. We also wished to identify possible dietary shifts with increasing Chain Pickerel length. Diet estimates were made using the multiple source-mixing model, IsoSource, that estimates a range of diet possibilities of a consumer based on the isotopic signatures of potential prey species (Phillips and Gregg 2003). Dietary information on piscivores such as the Chain Pickerel is critically important in understanding and managing fish populations in urban aquatic ecosystems.

Site Description

Pequot Pond is an 80-hectare, dimictic, mesotrophic water body located in Westfield, MA. The pond is highly urbanized with residential areas surrounding the pond and homes constructed near the shoreline. Urban development comprises approximately 75% of the three miles of shoreline. The pond also contains a state park that is highly utilized by residents of Westfield, Holyoke, West Springfield, and Springfield, MA. Primary recreational uses of the pond include fishing, boating, swimming, and bird watching. The pond is characterized by moderately clear water, littoral macrophyte growth, mean depth of 3.6 m, and max depth of 9.1 m. The pond contains warmwater fishes such as Largemouth Bass, Chain Pickerel, *Lepomis macrochirus* Rafinesque (Bluegill), *Lepomis gibbosus* Linnaeus (Pumpkinseed), *Pomoxis nigromaculatus* Lesueur (Black Crappie), *Ameiurus nebulosus* Lesueur (Brown Bullhead), *Perca flavescens* Mitchill (Yellow Perch) and *Anguilla rostrata* Lesueur (American Eel). *Oncorhynchus mykiss* Walbaum (Rainbow Trout) and *Salmo trutta* Linnaeus (Brown Trout) are stocked biannually for recreational angling purposes. Information regarding the pond was obtained from the Massachusetts Division of Fisheries and Wildlife at https://www.mass.gov/doc/hampton-ponds/download and through personal communication.

Methods

Fish and Invertebrate Sampling

Chain Pickerel and other fish species were collected randomly from the littoral regions of Pequot Pond in mid-September using an electrofishing jon boat with a 5000-watt generator. Electrofishing was conducted during the day for approximately 60 min of generator on-time. Captured fish were netted and placed in a live well for further analysis. Chain Pickerel were measured to the nearest mm (total length, TL). We separated Chain Pickerel into functional feeding groups defined by ≤299mm and ≥300mm to identify size-related shifts in feeding behavior. Studies on other warm-water piscivorous freshwater fishes, such as Largemouth Bass, have indicated dietary shifts around 300mm, a size often associated with sexual maturity and a shift to a principally piscivorous diet (Christensen and Moore 2010, Garcia-Berthou 2002, Ward and Neumann 1998), although little has been documented in Chain Pickerel. Whole Chain Pickerel and other fishes were sacrificed by pithing (IACUC approved method) for SIA and placed on ice until we returned to the lab. Invertebrates used in the SIA were sampled from the littoral region using dip nets, while zooplankton were collected with an 80 µm Wisconsin style zooplankton net with a 30 cm opening and pooled from multiple net tows from three locations within the pond. Invertebrates were stored in small plastic vials and placed on ice. On return to the lab, invertebrates were frozen at -10 C until SIA was performed.

Stable Isotope Analysis

A small muscle sample of about 1–4 grams (wet) was removed from the left dorsal side of each fish just anterior to the dorsal fin (Pinnegar and Polunin 1999). Tissue samples were rinsed with deionized water and placed separately into small plastic vials, labeled and frozen at -10 C. Because most invertebrates were relatively small, the entire organism was used for SIA. All fish and invertebrate samples were eventually thawed, re-rinsed, and placed in a drying oven for 48 hr at 75 C. After drying, a mortar and pestle were used to homogenize each individual sample separately until it reached a very fine consistency. Subsamples of approximately 0.4–0.7 mg were taken from the dried, homogenized tissues and placed in small tin capsules. Due to the small size of many invertebrates, multiple samples of the same species were pooled in order to obtain adequate dry weight (Vander Zanden and Rasmussen 1999). The individual samples were shipped to the Washington State University (WSU) Biology Department Isotope Core Laboratory for processing in Pullman, WA. Stable isotope analysis of ^{13}C and ^{15}N were performed using a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan, Bremen; Brenna et al. 1997).

Delta (δ) notation was used to express the ratios of $^{13}C/^{12}C$ or $^{15}N/^{14}N$ deviation from standard reference material. Values were expressed in parts per thousand (‰) using the following equation where R represents the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$:

$$^{13}C \text{ or } ^{15}N = (\mathbf{R}_{sample}/\ \mathbf{R}_{standard} - 1) * 1000$$

Vienna Pee Dee Belemite (VPDB) limestone and atmospheric nitrogen are international standards for ^{13}C and ^{15}N, respectively, to which our samples were referenced. Keratin and corn were used as in-lab normalization standards. Standard deviation for an in-lab quality control test of standards was 0.07 for ^{13}C and 0.10 for ^{15}N. Lipid correction equations were used to normalize our ^{13}C values for samples that exceeded a C:N ratio of 3.5% using the formula (Post et al. 2007):

$$^{13}C_{normalized} = {}^{13}C_{untreated} - 3.32 + 0.99 \text{ x C:N}$$

To account for variability, the trophic position (TP) for each species was calculated using the formula (Post 2002):

$$TP = \lambda + ({}^{15}N_{sc} - [{}^{15}N_{base} \text{ x } \alpha + {}^{15}N_{base}{}^{2} \text{ x } (1 - \alpha)] / 3.4$$

Where:

$$\alpha = ({}^{13}C_{sc} - {}^{13}C_{base2}) / ({}^{13}C_{base1} - {}^{13}C_{base2})$$

The freshwater snail was used as the baseline, which had the lowest recorded ^{15}N value as a primary heterotroph in our samples due to its feeding behavior on littoral benthic algae (Post 2002). The rate of muscle turnover in fish can be relatively slow (MacAvoy et al. 2001), so isotopic ratios of our fish were assumed to represent feeding behavior during mid to late summer.

The data analysis software, SPSS (IBM SPSS Statistics) was used to conduct a simple linear regression to evaluate the relationship between $\delta^{15}N$ and $\delta^{13}C$ values with chain pickerel length.

Stable Isotope Mixing Model

The model IsoSource (Phillips and Gregg 2003) was used to determine the range of diet proportions (0-100%) for Chain Pickerel ≤299mm and ≥300mm. The model uses an algorithmic mass-balance approach from the mixture of prey source isotopic signatures to determine the range of all possible diet proportions that sum to the isotopic signature of the consumer. To account for trophic fractionation, 3.4 ‰ was subtracted from the ^{15}N signature of each functional feeding group of Chain Pickerel before entered into the model (Herlevi et al. 2018, Phillips and Gregg 2003, Roach et al. 2009). We subtracted 0.2 ‰ from the $\boldsymbol{\delta}^{13}C$ signature for each functional feeding group to account for carbon fractionation (Bunn et al. 2003). Since multiple combinations of the prey sources can have the same probability, it is encouraged to present the entire range of possibilities rather than the mean (Phillips and Gregg 2003). The model mass balance error tolerance was set at the minimum of 0.1 and presented at 10% increments. Model results for smaller invertebrates (leech, dragonfly, damselfly, and snail) were combined a posteriori to improve model interpretation of Chain Pickerel dietary contributions (Phillips et al. 2005). Grouping similar organisms can provide a more constrained outcome that is easier to interpret than numerous diffuse contributions. Further, this a posteriori combination can help solve the problem of numerous possible sources while retaining all possible dietary contributions (Phillips et al. 2005).

Mixing-polygons were created around the plotted Chain Pickerel isotopic signature by connecting the plotted isotopic signatures of the prey species with a line. All the prey signatures were situated around the signature of the Chain Pickerel when the trophic fractionation was removed, confirming the possibility of the sources contributing to the diet of the consumer (Phillips and Gregg 2003, Smith et al. 2013). When the isotopic signature of the

Chain Pickerel was near the line connecting two prey sources, the range of diet probabilities was constrained. This indicated a greater possible diet contribution to the Chain Pickerel and was displayed by a bell-shaped curve (Phillips and Gregg 2003). Diffuse diet contributions were indicated by incomplete curves. We used dietary information from the literature (Broderson et al. 2015, Hartel et al. 2002, Hunter and Rankin 1939, Page and Burr 2011, Raney 1942) to confirm possible dietary contributions and appropriate mixing polygons in our study.

Results

We sampled a total of 41 fishes from Pequot Pond, MA and a variety of invertebrates (Table 1). Fifteen Chain Pickerel were used to evaluate relationships between fish length and $\delta^{13}C$ and $\delta^{15}N$ as well as to estimate dietary contributions. There was a positive relationship between Chain Pickerel length and $\delta^{15}N$ values (y=0.008x+11.995, $F_{1,14=}139.16$, R^2=0.915, p<0.001) and a negative relationship with $\delta^{13}C$ values (y=-0.009x-21.271, $F_{1,14=}5.249$, R^2=0.288, p=0.039, Fig. 1). Because Largemouth Bass and Yellow Perch had $\delta^{15}N$ signatures greater than those of Chain Pickerel, they were left out of the diet contribution estimate (Phillips and Gregg 2003). The mean $\delta^{15}N$ value for Chain Pickerel ≤299mm was 13.54 ± 0.19 1 SE and 14.45 ± 0.09 1 SE for fish ≥300mm (df=14, t=4.098, p=0.001). The mean $\delta^{13}C$ value for Chain Pickerel ≤299mm was -22.86 ± 0.43 1 SE and -24.49 ± 0.34 1 SE for fish ≥300mm (df=14, t=-2.944, p=0.011). IsoSource model dietary estimates for Chain Pickerel ≤299mm were comprised primarily of crayfish and Bluegill with a diffuse contribution of Pumpkinseed and smaller invertebrates (Fig. 2). However, estimates for the dietary contribution of Bluegill and Pumpkinseed increased in Chain

Table 1. Taxa, common name, N (sample size), mean isotope values ± 1 SE, C:N range and trophic position (TP) of fishes and invertebrates collected from Pequot Pond, MA. Taxa with no SE were either pooled or small sample size.

Taxa	Common Name	N	$\delta^{13}C$ ± 1 SE	$\delta^{15}N$ ± 1 SE	C:N Range	TP
Micropterus salmoides Lacepede	Largemouth Bass	15	-24.23 ± 0.29	15.28 ± 0.19	3.30 – 3.54	4.5
Perca flavescens Mitchell	Yellow Perch	1	-26.38	15.60	3.22	4.4
Esox niger Lesueur	Chain Pickerel (≥300mm)	7	-24.49 ± 0.34	14.45 ± 0.09	3.21 – 3.60	4.3
	Chain Pickerel (≤299 mm)	8	-22.86 ± 0.43	13.54 ± 0.19	3.19 – 3.46	4.2
Anguilla rostrata Lesueur	American Eel	3	-26.38 ± 0.48	13.77 ± 0.24	4.52 – 6.05	3.9
Lepomis macrochirus Rafinesque	Bluegill	5	-26.11 ± 1.68	13.37 ± 0.44	3.29 – 3.64	3.8
Lepomis gibbosus Linnaeus	Pumpkinseed	1	-24.54	10.80	3.19	3.3
Ameiurus nebulosus Lesueur	Brown Bullhead	1	-29.88	13.15	3.35	3.3
Decapoda	Crayfish	5	-20.09	8.49	3.61	3.0
Hirudinea	Leech	5	-23.59	8.60	4.7	2.7
Anisoptera	Dragonfly	5	-24.85 ± 0.37	7.51 ± 0.39	3.92 – 4.07	2.2
Zygoptera	Damselfly	10	-23.70	6.50	4.25	2.1
Cladocera/Copepoda	Zooplankton	3*	-30.45	9.14	4.83	2.0
Gastropoda	Snail	10	-23.26 ± 0.71	6.26 ± 0.15	3.38 – 4.94	2.0

*Number of integrated sample tows using a Wisconsin style zooplankton net.

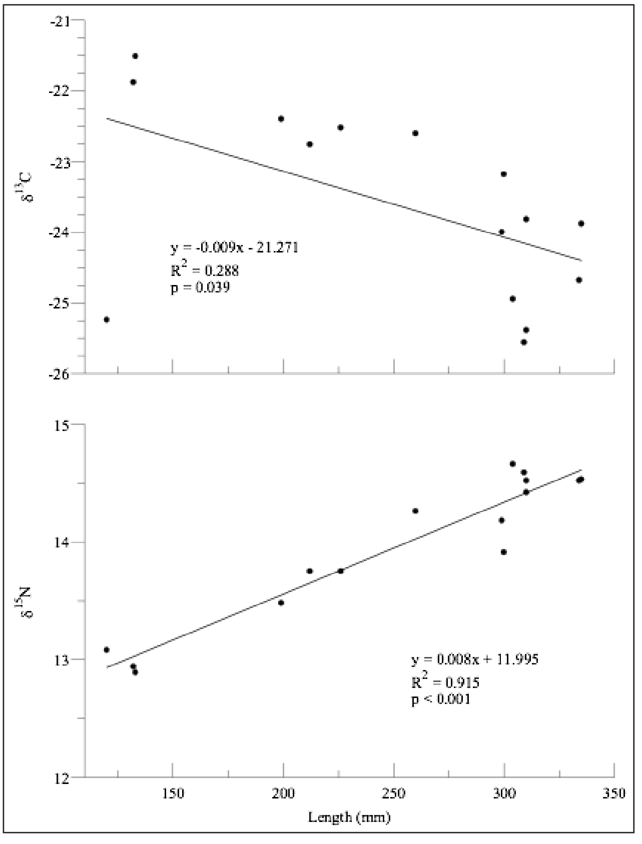

Figure 1. Relationship of $\boldsymbol{\delta}^{13}$C and $\boldsymbol{\delta}^{15}$N with increasing Chain Pickerel length collected from Pequot Pond, MA.

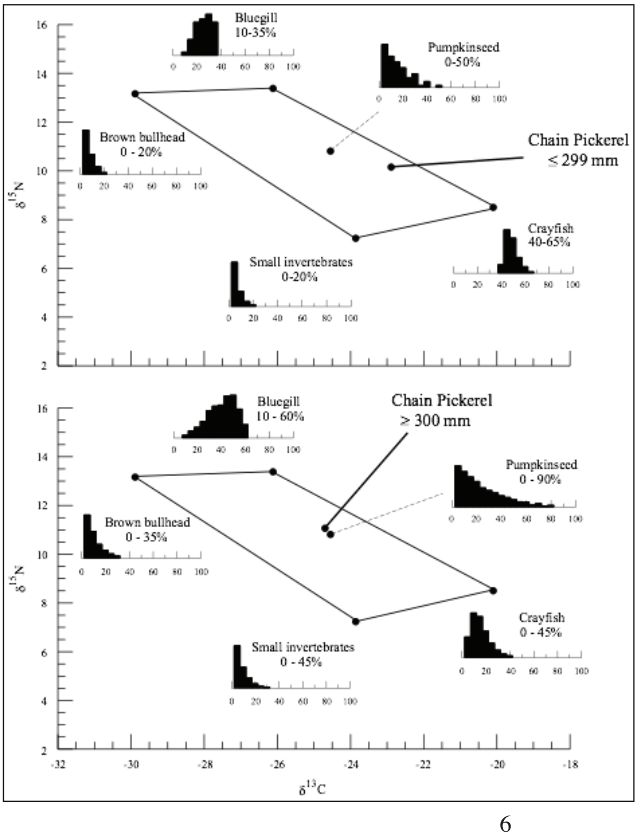

Figure 2. Mixing polygons for $\boldsymbol{\delta}^{13}$C and δ^{15}N signatures of principal prey organisms of Chain Pickerel ≤299mm and ≥300mm from Pequot Pond, MA. Corrections were made for Chain Pickerel by subtracting 3.4 ‰ from the δ^{15}N value and 0.2 ‰ from the δ^{13}C value of each feeding group to account for trophic fractionation from prey to predator. Each histogram represents the range of possible diet proportions for each prey organism of the Chain Pickerel on a 1-99 percentile scale. The range of diet contributions was estimated using the model IsoSource (Phillips and Gregg 2003).

Pickerel ≥300mm, while crayfish contributions decreased but remained constrained. The diffuse contributions of small invertebrates to the diet of Chain Pickerel ≥300mm remained similar to that of the smaller pickerel, although the percent contributions were marginally greater.

Discussion

Size-related variation in the diet of our sampled Chain Pickerel was evident, with a directional shift towards greater piscivory in pickerel ≥300mm. Further, the importance of Bluegill in the diet of Chain Pickerel ≤299mm, suggested an early onset to piscivory with an increasing reliance on fish consumption as the pickerel grew. However, the prevalence of crayfish in the diet of both size classes of Chain Pickerel illustrated that large invertebrate prey transcended ontogenetic dietary niches and remained an important contribution to the diet of Chain Pickerel. The importance of crayfish throughout ontogenetic diet shifts of other piscivores such as *Esox americanus* Gmelin (Grass Pickerel) in streams and Largemouth Bass in lakes has also been documented (Christensen and Moore 2010, Weinman and Lauer 2007). Beaudoin et al. (1999) found that large invertebrates can remain an important contribution to the diet of adult Northern Pike in Alberta Lakes, despite a principal consumption of other fish species. The Chain Pickerel ≥300mm diets in our study also indicated a possible increase in opportunistic feeding behavior among diffuse diet contributors such as smaller invertebrates, but this needs to be explored further.

Although it is generally understood that Chain Pickerel undergo ontogenetic dietary shifts like many other piscivores (Hartel et al. 2002), few studies have quantified this relationship in urban environments, and even fewer have used SIA (Broderson et al. 2015). Other studies in non-urban settings have found similar relationships between $\delta^{15}N$ values and length with other piscivorous species. For example, Grey (2001) found that piscivorous Brown Trout $\delta^{15}N$ signatures increased with length in a lake study, while Christensen and Moore (2009) identified a similar relationship with Largemouth Bass in lakes. The onset to piscivory in Largemouth Bass was well documented by Post (2003) using $\delta^{15}N$ values, and it was concluded that an early onset to piscivory increased growth rates and survival among the study population. Clark et al. (2005) found a distinct increase of $\delta^{15}N$ values with piscivorous *Oncorhynchus mykiss kamloops* Jordan (Kamloops Rainbow Trout) and *Ptychocheilus oregonensis* Richardson (Northern Pikeminnow) length in a large lake.

Some studies have found very little relationship between length and $\delta^{13}C$ values among piscivorous fish (Christensen and Moore 2009, Grey 2001), suggesting that the carbon source for those predators did not change throughout ontogenetic feeding shifts and these fish were all feeding in similar habitat types throughout their life cycle. Shifts in $\delta^{13}C$ values, however, would depend largely on the piscivore, prey species, and lake conditions. In lakes, it has been documented that $\delta^{13}C$ is often more depleted in pelagic food webs than in deeper benthic or littoral oriented systems (Post 2002, Vander Zanden and Rasmussen 1999). Therefore, a dietary shift from prey in the littoral zone, for example, to prey in a deeper benthic or pelagic zone could change the $\delta^{13}C$ value in the muscle tissue of that fish to express the shift in spatial feeding patterns. These types of shifts could occur daily, seasonally, and/or ontogenetically. A daily shift in spatial feeding behavior from littoral to pelagic zones would likely give a fish an intermediate $\delta^{13}C$ signature. For example, Christensen and Moore (2009) observed diel migrations in *Notemigonus crysoleucas* Rafinesque (Golden Shiner) from the vegetated littoral zone of a lake during the day to pelagic waters at night. Golden Shiner $\delta^{13}C$ signatures in that study were intermediate of the two spatial feeding zones (Christensen and Moore 2009). However, if spatial feeding behavior between major lake zones shifted ontogenetically, it could be hypothesized that $\delta^{13}C$ would also change as the predator grew. Clark et al. (2005) found that $\delta^{13}C$ values became more depleted among piscivorous Kamloops Rainbow Trout in a large lake, suggesting that juvenile fish had a more littoral diet than pelagic oriented adults. In our study, there was a negative relationship between Chain Pickerel length and $\delta^{13}C$ values, suggesting a shift from near shore littoral zones at smaller sizes to deeper waters as the fish grew.

Stable isotope analysis and isotope mixing-models can be an effective tool for fishery managers of urban lakes similar to Pequot Pond. In particular, SIA can give valuable insight into the feeding ecology of piscivores, such as Chain Pickerel. Understanding the dietary ontogeny, onset to piscivory, and prey selection of Chain Pickerel and/or other piscivores is critical in the management of any fish community (Carpenter and Kitchell 1993, Clarke et al. 2005, Lathrop et al. 2002). In our study, Chain Pickerel diets shifted both ontogenetically and spatially. Further, we found Chain Pickerel to rely on prey fish, such as Bluegill, at a relatively small size, suggesting an early onset to piscivory, while crayfish importance transcended ontogenetic shifts, establishing the importance of large invertebrates in the diets of Chain Pickerel. Documented shifts in feeding ecology can directly aid managers in determining angling restriction in order to maintain a healthy predator-prey relationship in heavily utilized urban water bodies (Lathrop et al. 2002). Knowledge of piscivore feeding behavior may also reduce unnecessary mortality of stocked fishes provided to support angling in urban ecosystems, and could reduce trophic disruptions through the unintended introduction of piscivores into other lakes and streams (Carpenter and Kitchell 1993, Christensen and Moore 2010, Potthoff et al. 2008).

Acknowledgments

We thank D. Basler with the Massachusetts Division of Fish and Wildlife and numerous students for their assistance with field sampling, tissue collection, and preparation. We also thank Washington State University Isotope Core Laboratory for stable isotope analysis of fish and invertebrate tissues. We thank Hampton Ponds State Park for access to Pequot Pond.

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