

Final Report

Assessing Abundance and Age Distribution of Juvenile American Eel within the Delaware River: Upper Delaware Scenic and Recreational River-4870

Task Agreement Number P18AC01150 Chesapeake Watershed Cooperative Ecosystems Studies Unit (CW CESU) Cooperative Agreement

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Executive Summary

The primary objectives of this study were to: 1) determine the age distribution of American Eels (hereafter Eels) in the Upper Delaware Scenic and Recreational River (UPDE); 2) determine the size range of Eels at different ages, 3) developing a method to estimate or index Eel population size at the sampling site, using depletion sampling, mark-recapture or catch-per-unit-effort (CPUE) sampling; 4) investigate infection rates by the nematode *Anguillicola crassus*; 4) investigate trophic position of Eels of different sizes; 5) establish a baseline to which future studies and other rivers can be compared.

Age estimates were based on otolith analyses of 100 eels in each of the three study years 2018-2020. Eels were collected by boat and backpack electrofishing at different parts of UPDE, and samples for analyses were chosen to provide specimens in 10-cm bins of total length. Ages were estimated from thin sections of otoliths, based on apparent annuli read blindly with respect to individual specimen data.

Estimated ages ranged from 1 to 11 years, with no evidence of different age-length relationships among the three study years or among different parts of the study area. There was high variability in length of each age class. There was almost complete overlap in lengths among eels older than 6 years. Fitting von Bertalanffy growth curves to the age-length relationships found an L_{∞} of 59.3 cm. Precise estimates of the age distribution of eels in the river couldn't be made, because of differences in the size distribution of eels in boat and backpack electrofishing samples and the high variability in length within age groups, Age 2 to age 4 fish were estimated to comprise 70% of backpack electrofishing samples and 77% of the boat electrofishing samples. Age 2 was estimated to be the most abundant age group in the backpack samples, compared to age 3 in the boat electrofishing samples. Only three eels greater than 60 cm were found among the total capture of 1564 eels. Much larger eels have been reported in other studies, both in the drainage and in other areas. The smaller maximum sizes in the Delaware River could reflect lower growth rates due to high densities of eels.

Sex was assessed visually on 318 eels, including the 300 used for aging. Sex could be determined on 59% of eels, of which 98% were females. The four fish sexed as males were 2 or 3 years old; three of them were among the largest of their age group captured in each year.

Reach-wide sampling was used to provide both mark-recapture and depletion estimates of total population. Tagging and recapture of tagged eels was feasible. However, these techniques were ultimately infeasible, because of the inability to completely sample eels in deep water, the ability of eels to move into and out of the reaches between samples, and negative effects of repeat sampling on eels.

Estimates of population densities in 20 m x 5-m backpack electrofishing samples of shoreline habitats were developed as an abundance index. Different sampling protocols, using different configurations of block nets, were used to analyze sensitivity of population estimates to escapement of eels from the sampling area. Catchability of eels was high in these samples but was lower in samples from deep water. Population density was not clearly related to measures of sample depth or current velocity. However, density was higher in samples with boulder and cobble substrates. Population density was lower in the upper river (e.g., river km 517-531, near Hancock, Long Eddy and Lordville) than in the lower river (e.g., river km 273-277, around

Lackawaxen and Barryville). However, coarse substrates were more frequent in downstream samples, so it was difficult to separate the longitudinal and substrate relationships.

Different protocols for monitoring with CPUE indices are discussed. Depletion sampling of small areas, i.e., as in the shoreline sampling or this project, can provide good estimates for analyses of trends. Protocols could include single-pass sampling and/or multi-pass (depletion sampling), with different options for number of block nets for depletion sampling. Combinations of techniques could be used, with more accurate methods, such as multi-pass sampling with two blocks, used to calibrate single-pass or multi-pass samples with fewer blocks. Mixed techniques could be used to develop Bayesian models of abundance. The precise protocol will depend on consistent availability of staff and the total amount of effort that is feasible. The monitoring program would need to develop a sampling frame of suitable habitat for sampling. This frame would need to include areas suitable for precise estimates and sufficient good habitat to efficiently collect eels. However, the range of habitats included in the frame would need to account for bias produced by correlations of habitat use with total abundance of eels.

Anguillicola crassus was frequent and common in eel swim bladders, as evidenced by presence of nematodes and/or effects on the swim bladder. Prevalence was 0.48-0.55, average incidence was 1.4-1.6 nematodes per eel, and maximum incidence was 15-18 nematodes per eel in the three years, with no temporal trend. Large numbers of nematodes, especially larval nematodes, were found mainly in large eels, but there were few strong relationships among various measures of *A. crassus* abundance and damage. Swim bladder thickness (measured) and opacity (a visual index) were positively correlated.

The guts of 318 eels were examined, including the 300 used for otolith analyses. Insects, found in 91% of all non-empty guts, were the most frequent food item. Among identifiable insects, stoneflies, caddisflies and mayflies were the most frequent groups; beetles (especially water pennies) were also frequent. Other food items included crayfish, bivalves and gastropods. Only one food item identifiable as a fish (of unknown species) was found, although some unidentifiable, digested organic matter may have been fish tissue.

The patterns of $\delta^{13}\text{C}$ indicated two main sources of carbon to eels, likely corresponding to autochthonous (aquatic algae and plants) and allochthonous (detritus from riparian plants) sources. Values of $\delta^{13}\text{C}$ of eels suggested that the contribution of these sources to eels varied greatly among individual eels. Most 2018 eels linked to the less-depleted, putative autochthonous carbon source, while 2019 and 2020 eels showed links to both carbon sources. Values of $\delta^{15}\text{N}$ showed the anticipated pattern of higher values among secondary and primary consumers. As with $\delta^{13}\text{C}$, 2018 values differed from those in 2019 and 2020, with 2018 eels having higher $\delta^{15}\text{N}$ values, consistent with extensive piscivory. Eels from 2019 and 2020 showed lower $\delta^{15}\text{N}$ values. The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values did not change with size of the eel, consistent with finding insects as a frequent food item in eels of all sizes. Values of $\delta^{13}\text{C}$ were extremely variable among primary producers, especially aquatic plants.

The cause of these differences between 2018 and 2019-2020 cannot be determined. However, Delaware River flows were markedly higher in the summer and early fall of 2018 than in 2019-2020, which could have a variety of effects on nitrogen cycling, riverine ecology and eel feeding. The 2018 eel samples were taken a month later (in late October) than the 2019-2020 samples. There may have been different food types available for eels in October, and the contributions of nitrogen from spring, summer and fall could differ.

This study provides valuable information on eels in the upper Delaware River and suggests that coordinated studies in different parts of the drainage, analyses of feeding across the growing season, and further development and implementation of abundance monitoring would provide a more comprehensive understanding of eel ecology, demography and population trends in the Delaware Drainage.

This study could be expanded in future years to increase understanding of eel ecology in the Delaware River. Determining age-length relationships and densities of yellow eels of different sizes across the entire drainage would help understand relationships between settlement, movement, density and growth.

I Introduction

American Eel (*Anguilla rostrata*; hereafter eel) is recognized as one of the Outstandingly Remarkable Values (ORV) of the Upper Delaware Scenic and Recreational River (UPDE), the Middle Delaware National Scenic and Recreational River (MDSRR), and the Lower Delaware Scenic and Recreational River (LODE; National Park Service 2012). The American Eel has recently been identified as the primary host fish for the Eastern Elliptio (*Elliptio complanata*) mussel (Lellis et al. 2013), which can comprise the greatest animal biomass in the river and play an integral role in its ecological function through their filtering and nutrient processing capabilities. The American Eel may itself constitute the largest fish biomass in streams (Horwitz, et al. 2015). The American Eel is commercially important as a food fish. However, the rangewide population of American eel has exhibited sharp declines (Casselman 2003). The Delaware River and estuary are strongholds for the eel because of the undammed main stem. Despite this importance, American eel abundance, age distribution, and population trends are not well understood on the Delaware River, while a commercial fishery for them still exists in the river.

The Eel is a catadromous species that spawns in the Sargasso Sea of the North Atlantic Ocean. Its larval stage, the leptocephalus, is transported by ocean currents until reaching the coastal nearshore zone where it metamorphoses into the unpigmented glass eel phase. Glass eels actively migrate toward land and freshwater or coastal waters, where they transform into small yellow eels, informally referred to as elvers. Transformation involves change of body shape from laterally compressed to oval, development of pigmentation and resumption of feeding. Elvers either remain in estuaries or enter river systems and migrate upstream. Maturation occurs, often after decades in freshwater systems, and the eel enters the silver eel phase which is accompanied by sexual maturation, changes in body color and morphology and the outmigration from freshwater environments into the Atlantic Ocean to the spawning area within the Sargasso Sea (Facey and Van Den Avyle 1987). The life cycle and life history of the eel is complex with many aspects, such movements within freshwater and between freshwater and saltwater, which are not fully understood or documented. Recent work by the New York Power Authority on eel biology within the upper St. Lawrence River has documented an average age of 23 years for out-migrating adult eels (J. Casselman, Queen's University, personal comm.).

Due to growing concern about decreasing eel populations, a number of research projects have been conducted by state and federal agencies, academia, and private industry. These projects have included investigation of upstream and downstream migratory behavior, growth rates, eel distribution and abundance within waterbodies, mortality associated with turbine entrainment at hydroelectric projects, collection techniques, age analysis, and feeding characteristics. To date, little work has been conducted on the age distribution of juvenile eels residing in a large non-tidal river system such as the Delaware River.

The Delaware River is the last major river on the Atlantic Coast that has an undammed main stem, flowing 330 miles from Hancock, NY to the Atlantic Ocean. As such, it is the longest free flowing river in the range of the American Eel in the eastern United States.

This river provides a unique environment for research on the life history and population dynamics of the American Eel which are not impacted by physical instream barriers. American Eel is an indigenous species to the Delaware River and is present throughout the entire length of

the river and throughout most of its tributaries. The absence of instream barriers, other than in its headwaters and some tributaries, allows for eels to migrate from the ocean without impediments and fully access much of the available rearing habitat. Dams on some tributaries, such as the Schuylkill River (see Table I.1 for location information) and Lehigh River, impede or block upstream in sections of the basin. Dam removals on tributaries such the Musconetcong River and the Paulins Kill are expected to increase eel passage, as well.

Discharge on the upper Delaware River is partly controlled by releases from Cannonsville Dam on the West Branch Delaware River and Pepacton Dam on the East Branch Delaware. Cold-water releases from Cannonsville Dam create coldwater conditions in the far upper Delaware River. Depending on weather and release conditions, coldwater conditions may occur as far downstream as Callicoon (Figure I.1).

An active weir fishery for out-migrating silver eels has been established in the upper sections of the river and tributaries for over 100 years, although only a few weirs are still operated. Local weir operators have noted and reported an overall decrease in their annual harvest of silver eels (Don Hamilton, pers. comm.).

This study is designed to provide information on several aspects of Eel ecology in the upper Delaware River and to develop protocols for subsequent monitoring.

Project Objectives

The primary objectives of our project included:

- 1) determining the age distribution of Eels, based on sampling at two primary locations in the UPDE, with sampling at additional sites to better extrapolate conditions in the study area to the riverine population.
- 2) determining the relative abundance of eels of different age classes or lengths within these sites.
- 3) determining the size range of eels at different ages.
- 4) developing a method to estimate or index eel population size at the sampling site, using depletion sampling, mark-recapture and/or catch-per-unit-effort (CPUE) sampling.
- 5) investigate infection rates by the nematode *Anguillicola crassus*.
- 6) investigate trophic position of eels of different sizes.
- 7) establish a baseline to which future studies and other rivers can be compared.

This information is important as it allows for estimates of the age distribution and the relative abundance of eels of different ages and can be used to help resource agencies in the management or restoration of eels in this and other river systems.

II. Methods

II.A Sampling

Our field sampling was designed to meet several objectives:

- 1) Obtain specimens of eels for analyses of age, parasite load, stomach contents and relative abundance of C and N stable isotopes.
- 2) Obtain samples of other taxa, such as potential eel prey to provide context for eel isotope relationships.
- 3) Evaluate techniques to estimate abundance and indices of abundance of eel.

Eel Sample Sites

Collections of specimens for laboratory analyses were made in different sites (Figure I.I and Table I.I) than those used for abundance estimation, although a few specimens were used from the abundance samples, e.g., eels which died during processing. Specimens for laboratory analysis were collected by boat and backpack electrofishing at Buckingham, Callicoon Tower Road access, Westcolang, and Lackawaxen.

We evaluated pool depletion and mark-recapture analyses in 2019 in two pools, selected to provide discrete, relatively small sample sites. These sites were the main stem pool adjacent to the old Red Barn Campground along the left bank (PA bank; all designations based on facing downstream) at Hankins, NY, and the pool at and upstream of the Damascus Pennsylvania Fish and Boat Commission Launch.

We did shoreline abundance sampling at accessible, nearshore, shallow sites in 2019 and 2020. These included sites at Hancock Junction Pool, Lordville, near Long Eddy, Damascus, downstream of Lackawaxen and Barryville.

Primary Sampling equipment

Boat electrofishing: We used a 5.2-m electrofishing boat outfitted with a Smith Root 5.0 GPP Electrofisher system (control box, generator and SAA-6 electrode arrays) for collection of eels for laboratory analysis and for testing abundance protocols. The output used was DC pulse set at high voltage, producing 4-8 amps. The procedure for this sampling technique consisted of two netters using 1.8 m long, 3.18 mm (1/8 in) mesh dip nets and one boat operator. Electrofishing was typically done in an upstream direction. The boat was equipped with a high intensity overhead lighting system for night sampling. Upon collection, eels were placed into an aerated onboard holding tank with ambient water.

Backpack electrofishing: We used one or two Smith-Root model LR 20B backpack units to collect Eels and other taxa in shallower habitats along shorelines. Output settings were based on conductivity of the water and adjusted for effective capture of eels with minimal mortality.

Sampling protocols

Obtaining specimens for laboratory analysis

We obtained specimens for analysis by qualitative collections in which time and distance of sampling were not recorded (although start and stop time of each electrofishing period was recorded), and no block nets were used; these samples included both day, dusk, and night sampling. Only Eels and a few individuals of some other species were netted. Since the goal of this protocol was to obtain approximately equal numbers of Eels of different sizes, some smaller eels were not netted once a sufficient number of smaller eels were captured. Specimens were collected by boat electrofishing at Buckingham and Lackawaxen and by backpack electrofishing at Buckingham, Callicoon (Tower Road Access), Westcolang and Lackawaxen.

Place	Rkm	Rm Reach	Sampling for this study
Hancock	531	330 U1	Shoreline sample
Buckingham	521	324 U2	Specimen
Lordville	517	321 U3	Shoreline sample
Long Eddy	505	314 U5	Shoreline sample
Hankins	501	311 U6	Depletion and mark-recapture samples
Callicoon	489	304 U7	Specimen Depletion, mark-recapture and shoreline samples
Damascus	480	298 U8	Shoreline sample
Westcolang	451	280 U13	Specimen
Lackawaxen	446	277 U14	Specimen
bel Lackawaxen	451	280 U13	Shoreline sample
Barryville	439	273 U15	Shoreline sample
Montague	396	246	
Paulinskil mouth	333	207	
Lehigh River mouth	296	184	
Musconetcong River mouth	282	175	
Trenton	216	134	
Penns Manor	203	126	
Neshaminy Creek	187	116	
Schuylkill River mouth	150	93	
Tacony Creek mouth	169	105	
Ridley Creek mouth	135	84	
Marcus Hook Creek mouth	129	80	

Table I.1. Location of sampling sites and other places mentioned in the text. Reach designations refer to 4 km reaches designated in Horwitz, et al. 2008.

Dip nets, seines and hand collection were used to obtain some samples for stable isotope analyses. Surficial sediment samples were collected manually in depositional areas of Cherry Creek and Aquashicola Creek.

Reach-level sampling

Boat electrofishing was done in the pool at Damascus and Hankins to evaluate use of mark-recapture and depletion sampling. Sampling at Damascus was done at night, while sampling at Hankins was done during the day. Work crews typically consisted of three people on the boat, and 3-5 people handling fish on shore.

Portions of each pool were sampled in each electrofishing run, to avoid crowding of eels in the boat live well. Runs were typically made in an upstream direction. The high velocity area at the upstream end of the Hankins pool (i.e., the downstream end of the riffle) could not be effectively sampled in an upstream direction and was sampled by moving downstream. At the end of each run, eels were transferred to aerated holding tubs on shore, where they were processed (see below). All eels were held onshore until the end of sampling

Once the target area of the pool (entire pool at Hankins, downstream portion of the pool at Damascus), the areas were resampled for depletion analysis of abundance. For the mark-recapture analysis, eels were sampled and marked at Hankins on 27 August, resampled and marked on 29 August and sampled again on 25 October 2019. At Damascus, eels were sampled and marked on 28 August 2019 and resampled on 24 October.

Shoreline sampling

We electrofished 20-m by 5-m areas with one or two backpack operators and one-two netters. Eels were netted during the sampling pass and from the block net at the end of the sampling pass. All these samples were taken during the day. Several variations of this technique were evaluated:

1. Several passes over the area to allow a depletion estimate of abundance:
2. Use of no block nets.
3. Using a single 6.1 m, 1.5 m deep, extra weighted block net (3.18-mm mesh) with a bag, with ends fixed 5-m apart at the downstream end of a sampling area.
4. Using an additional 20-m long x 1.5 m deep extra-weighted flat seine net held parallel to the current at the outer edge of the sampling area. This net was either held at the upstream corner of the area by additional sampling crew or held in place with an additional weight on the upstream corner of the area.
5. Using an additional 5-m long net across the upstream end of the sampling area.

Sampling at the sites within each station typically proceeded from the downstream-most sample site to the upstream-most site and were set contiguous to each other separated by at least 5-10 m. Most of the sites bordered directly with the shoreline, with some being taken a distance offshore to avoid very shallow habitat. Within most individual samples, the direction of sampling proceeded upstream to avoid turbidity disturbance when capturing eels (e.g., edge of pool at Station U8), but sampling proceeded in a downstream direction in areas with fast flow (e.g., riffles at Station U15). The mesh size of all block nets was 3.18 mm (1/8 in).

We took a minimum of two sampling passes. Depending on the observed pattern of depletion and observation of eels evading capture, we took additional passes (up to four sampling passes). We noted non-target fish species affected by the sampling but made no attempt to catch or comprehensively tabulate species presence or abundance other than eels. We also noted non-target fishes when checking the block nets for eels at the end of each pass. Some of the non-target specimens were retained and frozen for stable isotope work.

For each sample, the first pass results and the depletion population estimates using all passes provided two types of indices, each of which could be evaluated with the five different combinations of block nets outlined above. Depletion estimates of abundance were made using Microfish software (Version 4.0, Van Deventer 2020). Habitat variables were recorded from nine points spread equally through the sampling area and included depth (cm), velocity (m/s) and substrate class (i.e., boulder, cobble, gravel, etc.). The maximum depth in each sampling areas was also noted. Notes on submerged aquatic vegetation and aquatic moss in sampling areas were recorded. Water temperature, pH, dissolved oxygen and conductivity at each sample location were measured with a YSI model Pro Plus water quality meter.

Fish crews typically consisted of two people operating backpack electrofishers, two or three people netting fish and transferring to holding containers, two people holding block nets at the downstream end. Where a side block net was used, an additional crew member held the net, or it was held in place with weights.

Specimen handling in the field

Specimens were held in containers filled with ambient water between collection and processing, and between processing and release. Containers were typically aerated. Four protocols were used, depending on use of the specimens:

- 1) Specimens were selected for laboratory analysis based on field estimates of total length to provide representative samples within 10-cm length bins (5 bins from 10-60 cm) and representing both upstream (Stations U1-U8) and downstream sites (Stations U13-U14). There were usually less than 20 eels in some size bins (usually larger sizes and 10-19.9 cm), and all eels in these classes were kept. More than 20 eels were selected from the remaining size classes to obtain the total sample (118 in 2018 and 100 in 2019 and 2020).
- 2) Specimens preserved for laboratory analysis were euthanized (immersion in a lethal dose of clove oil), weighed and measured, placed in labeled plastic bags and held on ice or dry ice until transferred to the laboratory.
- 3) Specimens to be released without tags (eels less than 25 cm total length and/or collected in the shoreline sampling) were digitally photographed for laboratory length estimation to reduce specimen handling. These eels were placed in shallow tubs with a ruler and sample identifier and photographed. We typically took multiple photographs of each specimen. We took most photographs using a camera held at a fixed position on a copy stand and the eel container on the base of the copy stand. This procedure ensured that the camera was parallel to the sample container. Some photographs were taken without a stand, with the ruler in each sample allowing calibration for length estimation.

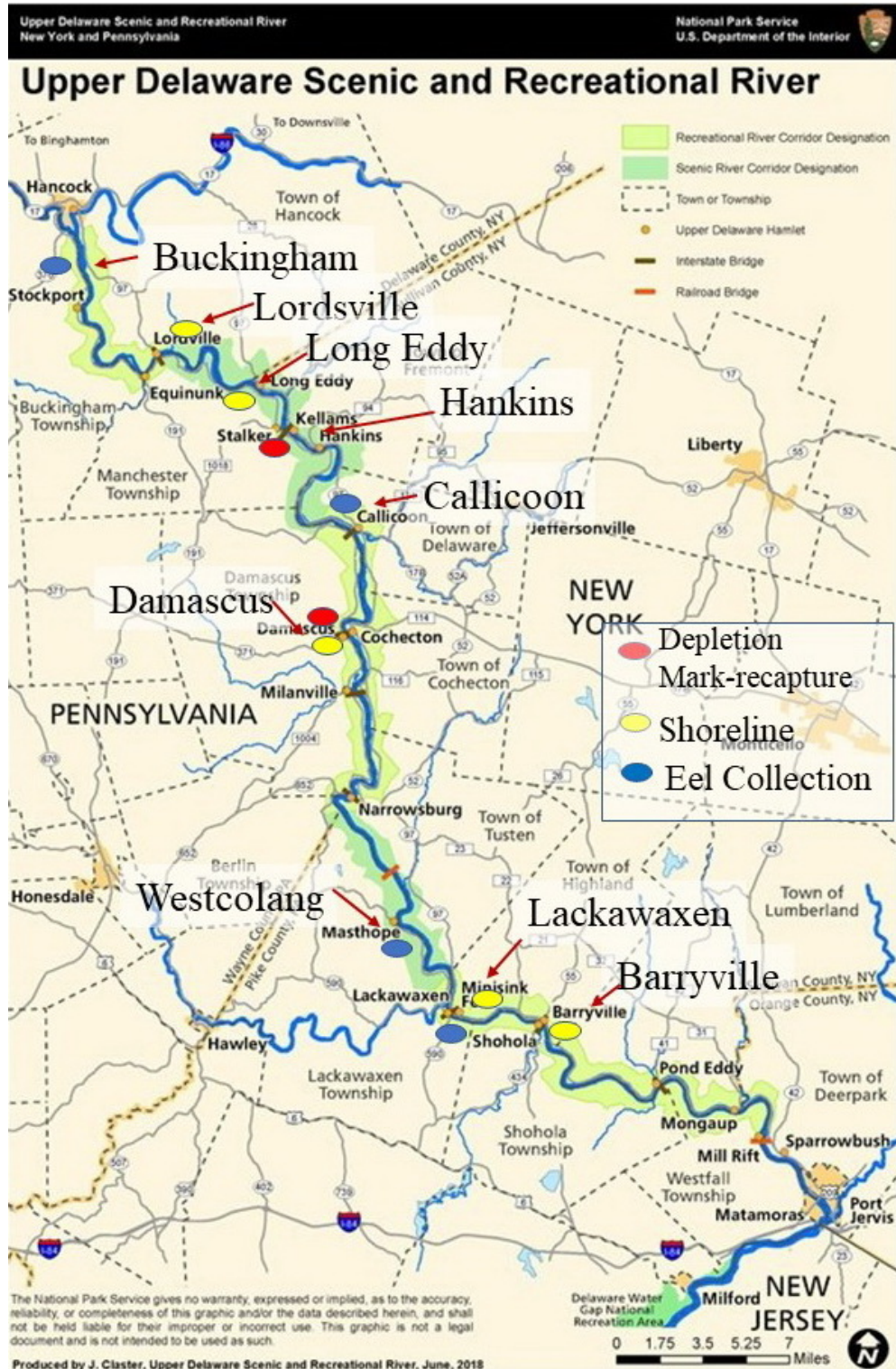


Figure I.1. Map of sampling sites for this study. Base map from Claster (2018)

- 4) For the mark-recapture study, specimens that were greater than 25 cm total length (a minimum size deemed suitable for PIT tagging) were anesthetized, measured, tagged, and released. We performed PIT tagging on 352 eels captured during the 2019 sampling, following procedures outlined in Drexel/NPS Institutional Animal Care and Use Committee (IACUC) protocol # 20726. We anesthetized eels in individual buckets with an appropriate concentration of AQUI-S 20E® (INAD 11-741), recorded time to loss of orientation, measured total length and weight and then implanted with a PIT tag (Biomark HPT9 tags,). PIT tags were inserted with single use sterile needles in a posterior direction into the abdominal cavity off the mid-ventral line to avoid spleen damage. We scanned the tagged eel with a PIT tag reader (Biomark HPR lite reader, 134.2 kHz) and recorded the unique code. We placed eels into individual buckets and until recovery, recording the recovery time. We placed recovered eels into aerated tubs or a 570-liter inflatable pool containing aerated ambient river water for observation and holding until release to the river at end of the sampling/ tagging procedure.

The protocols for individual holding of eels and measurement of times to sedation and subsequent recovery from sedation were requirements of the agreement between the Academy and the US Fish and Wildlife Service (USFWS) for use of AQUI-S. Fish treated with this drug are approved for immediate release to the stream. We also recorded general fish behavior and any adverse effects relating to treatment. The purpose of this protocol for Aqu-i-S®20E is to develop clinical efficacy field trial data that will be used to determine the most appropriate treatment regime for Aqu-i-S®20E for use as an anesthetic in a variety of fish species. These data will be used to support a new animal drug application (NADA) for Aqu-i-S®20E. These data on sedation times and effects were reported to USFWS.

II.B. Laboratory methods

Sample preparation and parasite analysis

We held eels in freezers and then thawed the eels for sample processing. We measured thawed eels in the laboratory for total length (cm), total weight (g), and sexed eels where possible. Both sagittal otoliths from each eel were removed, cleaned, and retained dry in small vials in lab until transfer to the Casselman lab. The gut contents of each eel were assessed and, where present, described qualitatively. Lastly, we examined the swim bladder of each eel to document the presence of the eel swim bladder parasite, *Anguillicola crassus*, and assess damage caused to the swim bladder by prior nematode infection. To do so, two techniques were adapted from the work of Lefebvre et al. (2002) and Palstra et al. (2007). First, the number of *A. crassus* present in the swim bladder was recorded and these were preserved. Larger *A. crassus* were noted without magnification; a dissecting scope was used to find smaller *A. crassus*. We measured (mm) the swim bladder length (SBL) and swim bladder thickness (SBW) with metric calipers and used these to calculate the length ratio index (LRI), a ratio of swim bladder length to fish total length ($LRI = SBL/TL$) (Palstra et al. 2007). Mechanical damage to the swim bladder by *A. crassus* infection leads to thickened walls, decreased volume, and shrinking of the swim bladder. Therefore, the LRI can be used to quantify damage caused by *A. crassus* infection. The swim bladder degenerative index (SDI) (Lefebvre et al. 2002) involved scoring the opacity, thickness, and amount of pigmentation/exudate of the swim bladder on a 0-2 scale. The swim bladder

degenerative index is the sum of the scores for these three categories. These three metrics can be macroscopically assessed and cover *A. crassus* impacts to both the swim bladder wall and lumen. This species has been variably placed in *Anguillicola* or *Anguilliculoides*; we use the former name in this report.

The laboratory procedure for the 2019 and 2020 specimens was like that used for the 2018 specimens, with the following exception: in 2019 and 2020, eels were refrozen after measurement and otolith dissection, with the remaining lab work (bladder parasite condition, sex and stomach contents, preparation of material for isotope analysis) done later after thawing a second time. We instituted this change based on John Casselman's examination of the dissected otoliths. The change was done to minimize the time eels and dissected otoliths were held in ambient condition, thereby decreasing the probability of erosion of the edge of the otoliths. In addition, otolith sample vials were not capped for two days after dissection, to allow drying.

Length measurement of released eels

We selected the best photograph of each specimen based on focus, absence of blur and visibility of the entire specimen. We measured the image length along the curvature of the eel using ImageJ software (<https://imagej.nih.gov/ij/>) and calibrated with the ruler in the photographs to estimate total length of each specimen. Where groups of photographs had been taken at the same distance between camera and subject, i.e., using the copy stand, calibrations were only done for a subset of the specimens.

Age estimation of eels

Sagittal otoliths were used to assess age. The preparations and interpretations were conducted at the CSAGES Laboratory at Queen's University at Kingston, Ontario. Various preparation procedures were used but quite generally involved making transverse thin sections. The sections were variously ground, polished, and treated using a procedure of acid-etching and cellulose acetate replication. All stages of the preparation procedures were documented with high-resolution photographs. These were taken sequentially during various stages of grinding and polishing and acid-etching. Various insights were acquired concerning otolith growth and multiple independent interpretations were conducted to provide a calcified-structure age, which was subsequently converted to calendar age and year-class. The preparations were numerous and detailed and will be documented in a separate report on the otolith age and growth interpretations and procedures. But for general purposes, the following reports and references apply (Casselman and Gunn 1992; Casselman and Scott 2000; Casselman 2008, 2015a, 2015b), although as will be indicated in the aforementioned report, in some cases methods were modified somewhat to obtain the best otolith preparations for these very small eel otoliths.

Other ancillary data were collected to document the samples for subsequent analysis such as whole-otolith photographs and otolith weight. The age estimates were done blindly: otoliths were assigned a laboratory number by the ANSDU laboratory, and specimen information (such as fish length) was not linked until after age estimates were completed.

It was apparent that many of the otoliths were very small and required very careful sectioning. In addition to the age estimates done for this project, some otolith measurements were made to estimate size of the leptocephali at metamorphosis, and in two samples (2018 and 2019), size of the elver at the first annulus was estimated.

Gut Contents

We enumerated stomach contents from the specimens used for aging for all three years. We also enumerated the intestinal contents from specimens from 2019 and 2020. We identified contents to the lowest practicable level and enumerated, without any estimates of volume or weight of different food types. It is possible that some food items were regurgitated during the sampling and holding procedure. No large food items were noted in holding containers, but no thorough search was made.

Stable Isotope Analyses

Values of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were obtained for 80 eels and 40 representatives of other taxa from the Delaware River. In addition, samples of eels and other components were analyzed from Cherry Creek, a direct tributary of the Delaware River downstream of the Delaware Water Gap, and Aquashicola Creek, a tributary of the Lehigh River. These additional samples provide comparison of trophic patterns at the different sites and components not sampled in the Delaware River (sediment, detritivorous-herbivorous macroinvertebrates). Delaware River samples were from all three years 2018-2020, while Cherry Creek and Aquashicola Creek samples were all from 2018.

We analyzed muscle tissue of larger fish without skin tissue. Subsamples of the entire muscle was used for larger fish, including most eels. We composited whole individuals of aquatic macroinvertebrates, and small fish were composited to obtain enough weight for analysis. Crayfish samples included only tail muscle tissue. In addition, some eel stomachs were analyzed.

Tissue and macroinvertebrate samples were freeze dried with a Labconco FreeZone Freeze Dry System for 24 h. Plant and sediment samples were dried at 30°C for 48 h. Subsequently, dried specimens were homogenized with a Retsch Mixer Mill MM400. Aliquots of between 0.40 and 1.0 mg were weighed into pre-cleaned tin capsules. Samples were analyzed with an Elementar Isoprime100 Isotope Ratio Mass Spectrometer with an Elementar Pyrocube located in the Stable Isotope Ecology Facility at the Academy of Natural Sciences of Drexel University. Stable isotope values were calculated as:

$$X\text{‰} = \left(\frac{X_{\text{sample}} - X_{\text{standard}}}{X_{\text{standard}}} \right) * 1000$$

Where X is the ratio of the heavy isotope to the light isotope (i.e., $^{15}\text{N}/^{14}\text{N}$, $^{13}\text{C}/^{12}\text{C}$). These values will be referred to as $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the remainder of the report. Isotope values were calculated based on three internal standards which were analyzed with samples at a rate of approximately 20%. Internal standards: i) B2150 (Elemental Microanalysis Ltd.): high organic sediment, ii) elk: sediment, and iii) dorm: dried fish, were calibrated with the international standards IAEA-N-1, IAEA-N-3 and USGS26 for N and NBS19, NBS22 and IAEA-CH-6 Sucrose for C. The internal

standards have a precision of $\pm 0.32\%$ (N) and $\pm 0.14\%$ (C) based on long term replication.

Statistical analyses

Simple regressions were done within Sigmaplot (Systat Software ND). ANCOVAs were done with Statistica (Tibco ND)

II.C Agreements and Permitting

A Chesapeake Watershed Cooperative Ecosystems Studies Unit (CW CESU) Cooperative Work and Joint Venture Agreement, Number P18AC00637, was entered into among CW CESU and the Academy of Natural Sciences of Drexel University (ANSDU). This project was conducted under Cooperative Agreement, Task Agreement # P18AC01150 (September 2018) among CW CESU, ANSDU, and the National Park Service (NPS)

ANSDU obtained appropriate sampling permits from PAFBC, NYSDEC and NPS. The animal handling protocols were approved by both the NPS IACUC and the Drexel University IACUC (Protocol # 20726). Year-end collection reports were compiled, sent and approved by both IACUC groups. ANSDU obtained approval for use of AQUI-ES as a fish anesthetic. Collection results were reported to PAFBC, NYSDEC and NPS, and the sedation information were reported to the USFWS.

III. Results

IIIA Measures of eel abundance

Pool samples

Depletion estimates

We conducted depletion sampling at the pool at Hankins (Reach U6) on 27 and 29 August 2019. Much of the pool could be effectively sampled, However, even when stunned eels were visible, capture of eels was difficult in the center channel of the pools at depths greater than about 3 m.

We made three passes at Hankins on 27 August, capturing 87, 67, and 34 eels on the three passes. This resulted in a depletion population estimate of 253 eels (confidence interval 200-306) and a capture probability of 0.36. With the estimate of 253 fish as the starting population, capture probabilities for the three passes were similar: 0.34, 0.40 and 0.34. We completed two passes on 29 August, capturing 67 and 23 eels. This resulted in a depletion estimate of 100 eels (confidence interval 86-114) and a capture probability of 0.68. The capture probability of the two passes were similar, 0.67 and 0.70, but much higher than those on 27 August. The difference between the two sampling patterns and population estimates suggests some problem with the procedure (i.e., violation of depletion estimate assumptions). It is also possible that each estimate was appropriate, with the difference between the dates due to emigration.

We conducted two sampling events at Damascus (Reach U8) on 28 August 2019 and 24 October 2019. Because of the size of the pool, we were unable to thoroughly sample the pool on one night or do more than one sample pass. Furthermore, we had low collection efficiency in some parts of the pool, especially deeper, high velocity areas in the center channel downstream of the bridge and deep pool areas upstream of the bridge. As a result, these samples were used only for mark-recapture study.

Mark-recapture estimates

We used the two depletion sampling events of 27 and 29 August 2019, and a third sampling on 25 October 2019 at Hankins (U6) and two sampling events at Damascus (U8) on 28 August 2019 and 24 October 2019, to assess the feasibility of mark-recapture population estimates. At U6, we marked 159 eels on August 27 (an additional 29 eels captured were too small to tag). We recaptured 13 of the tagged eels on 29 August (8.7% of marked eels) and tagged an additional 67 eels. We recaptured two marked eels at U6 on 25 October (0.82% of eels tagged on the two previous dates). At U8, we recaptured one eel on October 24 of the 126 (0.79% of marked eels) that were marked on Aug. 28.

The mark-recaptures on 27 and 29 August 2019, at U6 allow a total population estimate of 1027 (Lincoln population estimate) or 970 (Chapman population estimate, appropriate for small sample size). The recapture rate was relatively high over 2 days: 8.2% of marked fish were recaptured and 15.5% of the fish caught on the second date were marked. However, the difference in catch rates between the two dates (117 fish which could be marked and 154 total on the first two passes on 27 August and 84 fish which could be marked and 90 total on 29 August) and difference in depletion estimates on the two dates (253 on 27 August and 100 on 29 August) suggests that fish vulnerable to capture on 27 August were not vulnerable on 29 August, either due to emigration, mortality, or modified behavior. As a result, one of the major assumptions of the mark-recapture method was probably violated. Over the longer period (late August through late October), differences in catch rates and recapture rates between sample periods (especially at U6) and mortality of a few eels after capture at U6 indicates that assumptions of no mortality, immigration or emigration of simple mark-recapture models were not met over the entire sample period. While more complex models allowing mortality, emigration and or immigration are available, they require an intensity of sampling which is logistically difficult and would probably result in undesirable levels of eel mortality.

Thus, we were able to recapture marked eels over the entire period, and recapture rate was relatively high for the short term (two day) mark-recapture samples. However, the depletion and mark-recapture estimates give very different results, which is likely due to violation of assumptions of both methods.

Shoreline density estimates

Evaluation of protocols, especially use of block nets, was one of the goals of the study. When electrofishing was done in a downstream direction (usually in higher velocity sites), eels were frequently caught in the downstream net, either in the net or as eels attempted to escape upstream, i.e., out of the block net. Use of the side block net is expected to increase sampling

efficiency but involved extra staff to hold, position and move the second net. In some samples, the side net was anchored at the upstream end; this eliminated need for extra staff to hold the net, but still increased time to set up, take down and move nets. A third block net was placed along the upstream end of the sample area in a few samples where sampling was done in an upstream direction, as well as in some samples taken in a downstream direction. However, there was no evidence that eels attempted to escape by swimming upstream in these samples (excepting in the immediate vicinity of the downstream block net). As a result, the third net was not used in most riffle samples, and results are not differentiated in this report. In addition to requiring extra staff, use of the upstream net impeded entry into the sampling area by staff.

In 2019, we took 16 depletion samples using backpack electrofishing, with each sample consisting of 2-4 separate passes. We caught no eels in any of the four samples at Junction Pool (Reach U1), taken at the right bank of the island along the left bank of the channel of the Junction Pool. However, eel presence at the Junction Pool was confirmed by qualitative sampling farther from shore. In 2020, we took 26 sets of depletion samples. We caught no eels in nine depletion samples at U5, although eels were caught in two other samples at U5.

We made four passes at two sites, three passes at three sites and two passes at 39 sites, including the 14 samples in which no eels were caught. In five samples, all eels were caught in the first sample. No statistical population estimate can be made for these sites, but it is assumed that the catch represented the entire population in the sites. The total population estimate was equal to the total catch over all passes, except for one sample where the difference was one in. Resulting density estimates were 1-33 fish per 100 m², with an average of 9 (all samples) and 13 (excluding samples with no eels caught), and median of 6 (all samples) and 9.5 (excluding samples in which no eels caught). The estimated capture probability (per individual per pass) ranged from 0.64 to 0.92, with a median of 0.87. However, this estimate is presumably biased low, since it excludes the five sites where all fish were caught on the first pass, for which capture probability was probably high.

Over all samples (Figures IIIA.1 and IIIA.2), there was no relationship between catch probability and average or maximum sample depth. Samples taken with no blocks appeared to have lower catch probabilities than those taken using one or 2 blocks.

Eel density is expected to vary with habitat conditions and possibly with longitudinal river position. Longitudinal position could affect eels by coldwater conditions created in the upper river by upstream dams on the East and West Branches of the Delaware. However, patterns of immigration could also create longitudinal differences in density which might be hard to separate from temperature effects. There was no clear relationship between estimated density and average depth (Figure IIIA.3), maximum depth or average velocity. There was a relationship between eel density and substrate (Figure IIIA.4). Samples with coarse substrate (substantial cover with boulders and cobble) had moderate and high densities; most samples from U15 (Lackawaxen) had higher densities than samples from U8 (near Callicoon). Samples with fine substrates had low eel densities. Most samples from the uppermost stations, U1 (Junction Pool), U3 (Lordville) and U5 (Long Eddy), had no or a low density of eels. Most of these samples had relatively fine substrates. Densities in samples from downstream stations (Callicoon and Lackawaxen) with similar fine substrates had higher densities. However, only one sample, from U5 (Lordville) with

coarse substrates was located for sampling at accessible sites in U1, U3 and U5. The Lordville sample with coarse substrate had low density of eels compared to downstream samples, but higher density than the samples with finer substrate in U1, U3 and U5.

III.B. Age-length analyses

In each year, estimated ages ranged from 1 to 11 years (Table IIIB.1). Mean age at length increased approximately linearly through age 7 with little or no apparent increase in older fish (Table IIIB.2 and Figures IIIB.1 and IIIB.2). However, there were few samples at ages greater than seven, reducing precision in mean length for older fish. There was no consistent difference in mean lengths at age among the three sample years. However, variability within years was high—for example, there was almost complete overlap among ages four through six (as indicated by standard deviations and ranges).

Growth rates could differ among parts of the river, e.g., between fish in colder tailwaters at the upstream end of the sampling area and warmer areas at the downstream end. Age-length relationships were separated (Figure IIIB.3) for fish collected in the uppermost stations (U2, Buckingham), intermediate stations (U5-U8, near and upstream of Callicoon) and the lowermost stations (U13-U14, near and downstream of the Lackawaxen). There were relatively few fish in the intermediate stations. There was no apparent difference in size at age for eels from the different sections.

The age distribution among 10-cm length bins was calculated for the 300 aged eels. Both laboratory and field-measured lengths were tabulated (Tables IIIB3 and IIIB4). Eels were not separated by collection gear types since the distributions are not affected by the different selectivity of backpack and boat electrofishing. They would be affected if the gears selectively captured faster or more slowly growing eels.

In order to estimate the age distribution within the population, the distributions of field lengths of all eels measured in the studies was compiled, including the 300 specimens which were aged, and additional eels caught in the samples used to obtain specimens for aging or estimating abundance and catch per unit effort.

Estimation of age distribution within the population is affected by selectivity of sampling gear. Therefore, we compiled ages separately for backpack and boat electrofishing samples. Lengths estimated from photographs of live eels were included in the field lengths. While fish up to 59 cm total length were captured by both backpack and boat electrofishing, boat electrofishing tended to sample larger fish (Table IIIB5), including three fish greater than 60 cm total length. Within each gear type, the proportion of fish in different size classes did not vary much among years, but the two sample gears were used unequally in the sample years: boat electrofishing was used primarily in 2019 for obtaining specimens and for the pool depletion and mark-recapture studies, as a technique for obtaining specimens in 2018 and not at all in 2020. As a result, no estimate of differences in age or size composition among years is possible. Total lengths in boat and backpack electrofishing were compiled separately into 10 cm bins (Table IIIB5). The age

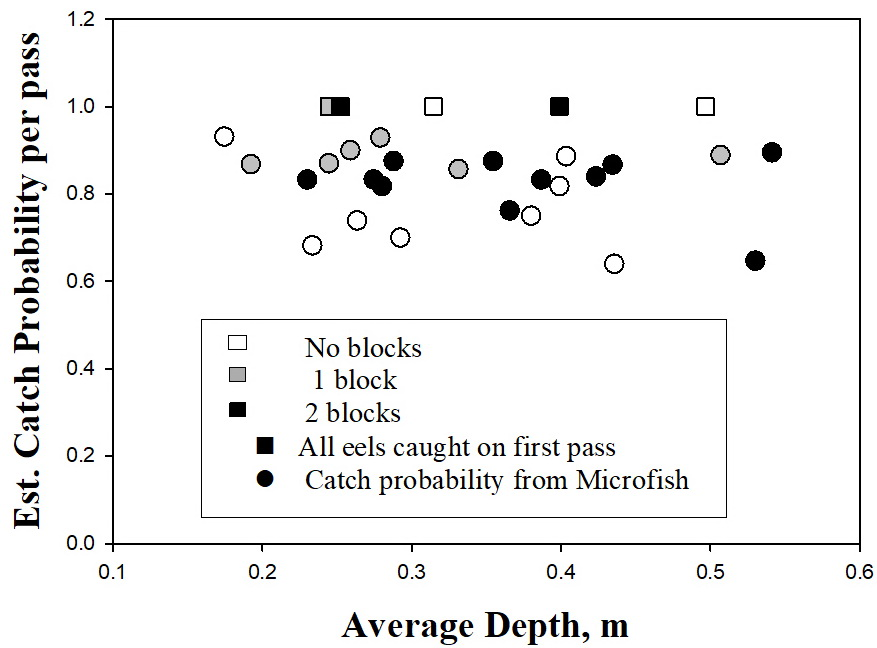


Figure IIIA.1 Relationship between estimated catch probability (i.e., per individual per pass) and average depth in shoreline depletion samples.

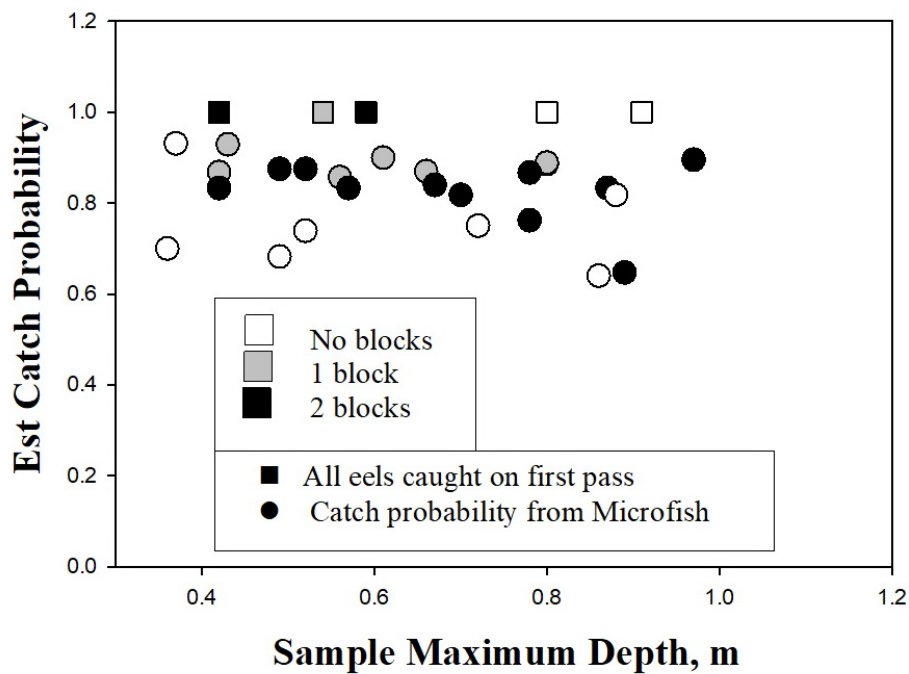


Figure IIIA.2. Relationship between estimated catch probability (i.e., per individual per pass) and maximum depth in shoreline depletion samples.

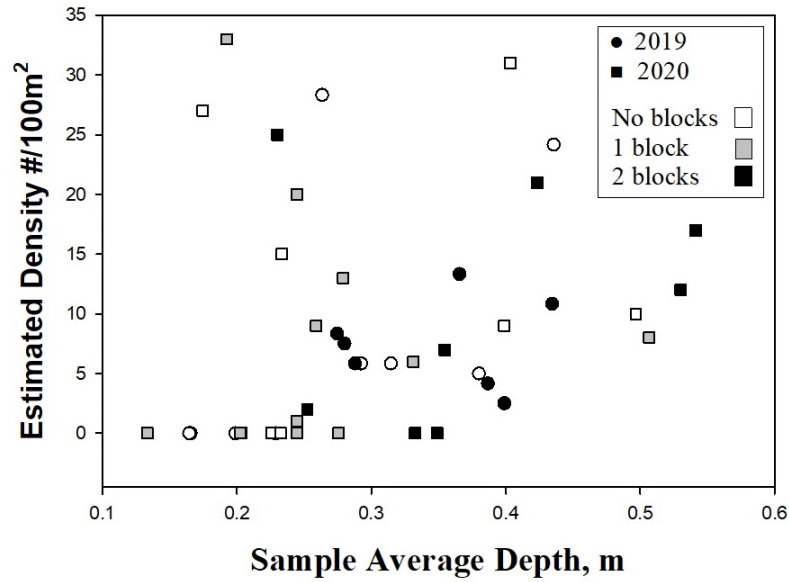


Figure IIIA.3. Relationship between estimated density of American Eel and average sample depth in shoreline depletion samples.

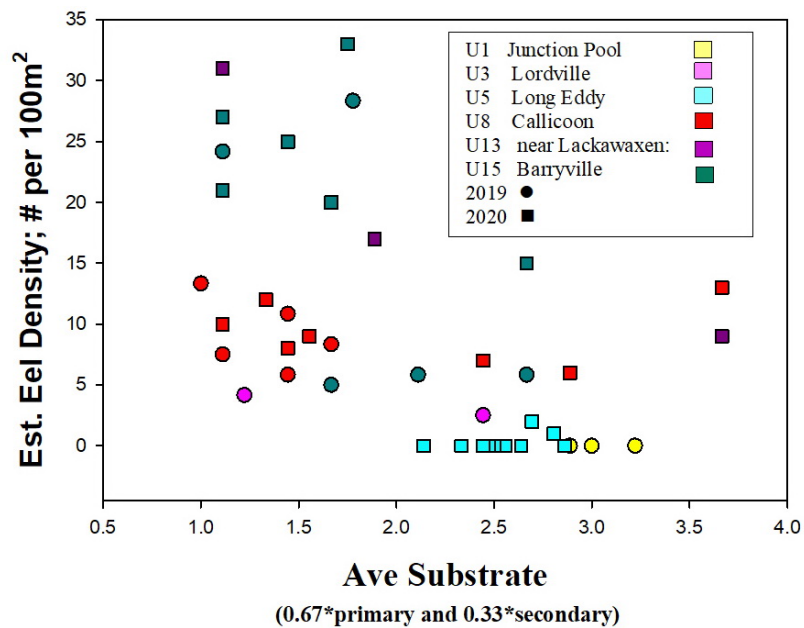


Figure IIIA.4. Relationship between estimated density of American Eel and a measure of substrate in shoreline depletion samples. The index combines ordinal values of primary and secondary substrates, with 1 representing boulders and 4 representing silt.

composition within bins was estimated for both sampling techniques (Table IIIB5) from these averages and the estimated distribution of ages among length bins (Table IIIB4). For this estimate, Eels greater than 60 cm in length were assumed to be evenly divided among age 6-11, although these could have included older age fish as well. This length class comprised a very small proportion of the total catch (3 fish out of 708), and the artificial age assignment was done only to note presence of larger, presumably older, eels. Age 2 eels were estimated to be most frequent class in backpack samples (28.5%), although age 3 eels were almost as frequent (27.5%). Age 3 Eels were estimated to be most abundant age class (26% of total) in boat electrofishing samples. Age 2 to age 4 eels were estimated to comprise 70% of backpack electrofishing samples and 77% of the boat electrofishing samples.

If a population is at stable age distribution without immigration, the ratio of adjacent age proportions provides an estimate of population retention, i.e., population not dying or emigrating (Table IIIB5). For the population estimates, this results in estimates of retention of 55% to 87% from ages 5 to 10. Since age 1 eels were estimated to form a lower proportion than age 2 and 3 eels in boat electrofishing samples and age 1 and age 2 eels were estimated to form a lower proportion than age 3 eels in backpack electrofishing samples, the assumption of no immigration within these age groups is not true, at least for younger fish. For older fish, retention is likely to involve decreasing mortality with increasing size and maturation and emigration of larger fish.

These estimates do not account for immigration, e.g., from tributaries upriver or downriver. Greater rates of immigration of younger eels would cause underestimation of true retention, while lower rates of immigration of younger eels would cause overestimation of true retention.

We estimated parameters of the Von Bertalanffy growth equations (Table IIIB.2):

$$L_a = L_{\infty} (1 - e^{-K(A-t)})$$

Where A is the age of the fish at the time of annulus formation, and L_a is the length of the fish at age A. Since fish were collected near the end of the growing season, A was taken to be the age in the year following capture. The parameters may be interpreted as:

L_{∞}	Asymptotic length of fish
K	Factor related to growth rate
t	Fitted parameter to adjust position along x-axis.

In addition, ω , estimated at $K * L_{\infty}$ was calculated, following Galucci and Quinn (1979), which can be interpreted as a growth rate of young (post-transformation) fish.

Table IIIB.1 Summary statistics for lengths within estimated age classes of American Eels. LTL is total length of fish measured in the laboratory.

Age	Median			Mean			Standard Deviation			Range						count		
	2018	2019	2020	2018	2019	2020	2018	2019	2020	min max 2018	min max 2019	min max 2020	2018	2019	2020	2018	2019	2020
1	21.8	21.0	19.75	22.9	21.6	20.0	3.6	2.9	1.8	19.1	28.8	17.7	27.7	17.7	22.5	8	20	6
2	25.4	28.1	25.4	25.9	27.4	25.5	4.4	4.2	3.5	19.1	34.0	18.6	34.1	18.7	32.8	29	22	25
3	34.9	31.8	29.3	34.1	32.6	29.1	3.4	4.1	3.8	24.7	39.8	25.5	44.1	22.4	35.8	19	23	29
4	37.0	36.3	34.2	38.0	39.4	34.3	3.8	6.3	4.6	33.0	44.2	32.4	51.6	26.1	41.5	10	12	17
5	42.1	39.5	40.15	41.9	41.5	39.5	5.5	5.3	6.1	33.4	49.3	36.0	51.6	29.1	49.3	11	8	10
6	43.3	44.7	47.3	44.4	43.3	47.5	2.3	4.1	0.8	42.1	47.3	36.6	46.6	46.5	48.4	7	5	5
7	47.9	51.2	48.8	47.7	49.7	48.8	0.6	2.5	0.8	47.0	48.5	46.8	51.2	48.2	49.4	6	3	2
8	48.7	49.0	56.9	49.2	49.0	56.9	4.9	2.5		43.8	57.0	47.2	50.8	56.9	56.9	5	2	1
9	49.3	48.8	49	50.3	48.8	48.6	7.3	-	1.9	43.6	58.1	48.8	48.8	46.6	50.3	3	1	3
10	58.5	47.9	50.0	58.5	47.9	50.0	-	5.5		58.5	58.5	44.0	51.8	50.0	50.0	1	2	1
11	47.3	49.7	57.3	47.3	49.7	57.3	-	6.4		47.3	47.3	45.2	54.2	57.3	57.3	1	2	1

Table IIIB2. Length at otolith age for Delaware eel samples, 2018, 2019, and 2020, collected late October 2018, mid-August 2019, and early September 2020, respectively. Lengths in mm, length at age at annulus 1 determined by back-calculation from the 2018 and 2019 samples, and with the means used for 2020 and combined sample for all three years. All other sizes at age determined from length at time of sampling. At time of sampling, growing season was not completed, therefore lengths somewhat underestimate length at age. Experience from other studies suggests that by mid-August, at least 80% of the growing season has been achieved. For eels sampled later, a higher percentage of the seasonal growth would have been achieved. Von Bertalanffy equations calculated using mean length at age over the age range indicated. Von Bertalanffy parameters are provided, including omega determined by $L_{\infty} \times K$. Otolith age determined by thin transverse sectioning. Age at annulus 2 and older considered to be length at beginning of next growing season for that age.

Age at Annulus	2018 Sample					2019 Sample					2020 Sample					Combined Sample				
	N	Upper 95% CL	Mean	Lower 95% CL	Von Bert.	N	Upper 95% CL	Mean	Lower 95% CL	Von Bert.	N	Upper 95% CL	Mean	Lower 95% CL	Von Bert.	N	Upper 95% CL	Mean	Lower 95% CL	Von Bert.
1		151	161	173	150		145	154	162	147		147	157	168	144		147	157	168	151
2	8	203	235	267	223	20	206	221	235	222	6	188	209	230	213	34	211	222	233	219
3	29	249	266	283	285	22	262	281	300	285	25	250	265	280	273	76	261	270	280	277
4	19	331	347	364	336	23	318	336	354	337	29	286	301	315	325	71	314	324	335	328
5	10	361	388	415	380	12	365	506	447	380	17	333	356	380	371	39	362	380	397	373
6	11	390	428	466	416	8	381	427	453	416	10	365	411	456	410	29	400	422	443	411
7	7	433	454	475	446	5	394	446	499	446	5	413	452	491	445	17	436	451	467	445
8	5	479	487	495	471	3	452	515	579	471	2		498		475	11	486	497	509	474
9	6	455	501	546	493	2		497		491	1		490		501	8	467	499	531	499
10	3	327	511	695	510	1		504		508	3	358	474	589	524	7	444	494	544	521
11	1		586		525	2		486		522	1		465		543	4	407	506	605	540
12	1		482		538	2		507		534	1		543		561	4	434	510	585	556
13					548					544					576					571
14					557					552					589					583
15					564					559					600					594
16					571					565					610					603
17					576					569					619					612
18					580					573					626					619
19					584					576					633					625
20					587					579					639					630
Von Bertalanffy relationship																				
N	100					100					100					300				
Ages used					1 to 10					1 to 7					1 to 8					1 to 9
L_{∞}					602					592					677					666
K					0.177					0.186					0.138					0.141
Omega					106.6					110.1					93.4					93.9

Table IIIB3. Estimated proportion of age classes in 10-cm bins of field total length. N is the number of aged Eels in each size class. Three Eels greater than 60 cm in length were captured in abundance surveys. No eels that size were aged, and the age distribution of these Eels was arbitrarily divided among age classes 6-11.

TL cm	N	1	2	3	4	5	6	7	8	9	10	11
15-19.9	15	0.67	0.33	0	0	0	0	0	0	0	0	0
20-29.9	98	0.23	0.55	0.18	0.020	0.010	0	0	0	0	0	0
30-39.9	102	0.010	0.17	0.48	0.25	0.088	0.010	0	0	0	0	0
49-49.9	63	0	0	0.063	0.16	0.24	0.25	0.13	0.063	0.048	0.016	0.032
50-59.9	22	0	0	0	0.09	0.18	0	0.14	0.18	0.18	0.14	0.091
60-69.9	0	0	0	0	0	0	0.17	0.17	0.17	0.17	0.17	0.17

Table IIIB.4. Proportions of 10 cm bins of field total length of Eels captured in this study.

	Backpack Electrofishing				Boat Electrofishing		
Field TL cm	2018	2019	2020	total	2018	2019	total
15.2-19.9	0.053	0.037	0.032	0.036	0.000	0.003	0.003
20-29.9	0.42	0.37	0.41	0.39	0.26	0.18	0.19
30-39.9	0.37	0.43	0.38	0.40	0.40	0.44	0.43
40-49.9	0.12	0.12	0.15	0.13	0.30	0.28	0.28
50-59.0	0.035	0.042	0.024	0.034	0.049	0.097	0.092
60-69.9					0.0	0.005	0.004
Number	57	430	371	858	81	627	708
median				31.7			37.3

Table IIIB.5. Estimated proportions of age classes in all electrofishing samples in 2018-2020.

	Age:	1	2	3	4	5	6	7	8	9	10	11
Backpack		0.12	0.30	0.27	0.13	0.078	0.038	0.022	0.015	0.013	0.0067	0.0073
Boat		0.050	0.17	0.26	0.16	0.12	0.077	0.049	0.035	0.031	0.018	0.018

Table IIIB.6. Size information on the only four individuals sexed as males in the study. Rank is the rank (from largest to smallest) of the length of the individuals with respect to other Eels (females or undetermined) of the same age from the same year.

Date	Field TL (cm)	Lab TL (cm)	Estimated Age	Rank	Total Number
16 August, 2019	40.4	38.8	3	3	42
23 October, 2018	35.6	34.9	3	7	19
23 October, 2018	34.4	33.4	2	2	29
23 October, 2018	35.2	34.0	2	1	29

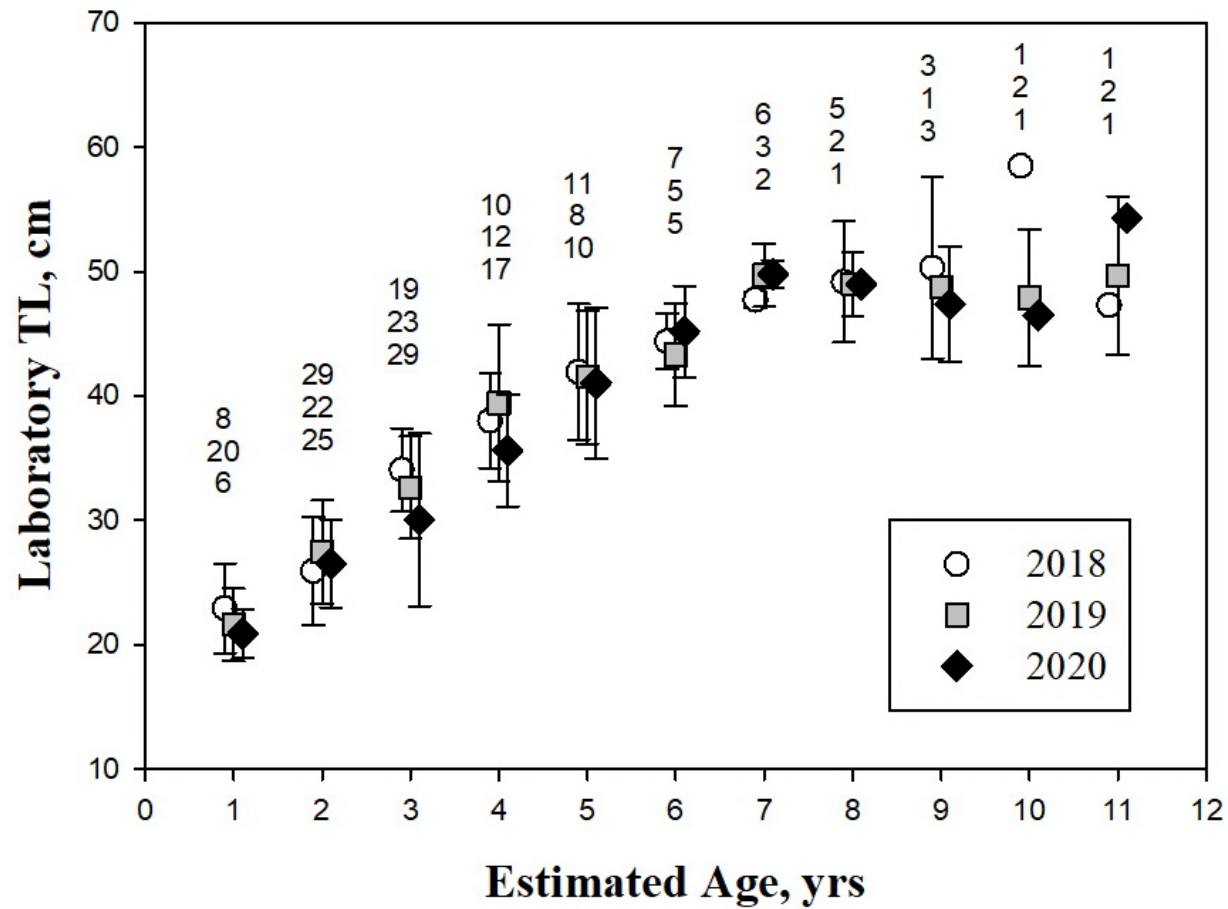


Figure IIIB.1. Mean and one-standard deviation of laboratory total length for each age group for Delaware River American Eels. Numbers above points indicate number of specimens (2018-2020 from top to bottom). Ages are offset for visibility

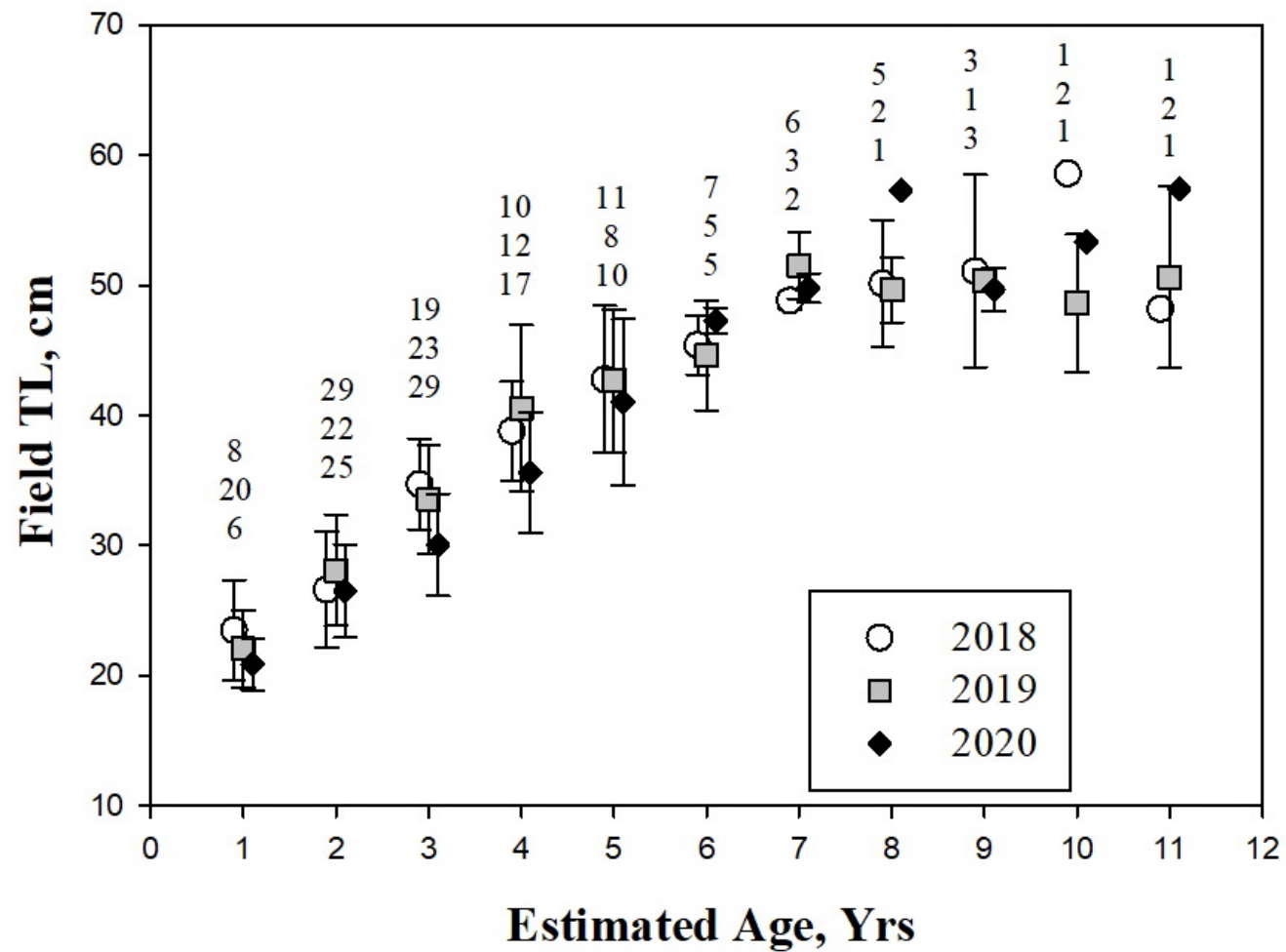


Figure IIIB.2. Mean and one-standard deviation of field total length for each age group for Delaware River American Eels. Numbers above points indicate number of specimens (2018-2020 top to bottom). Ages are offset for visibility.

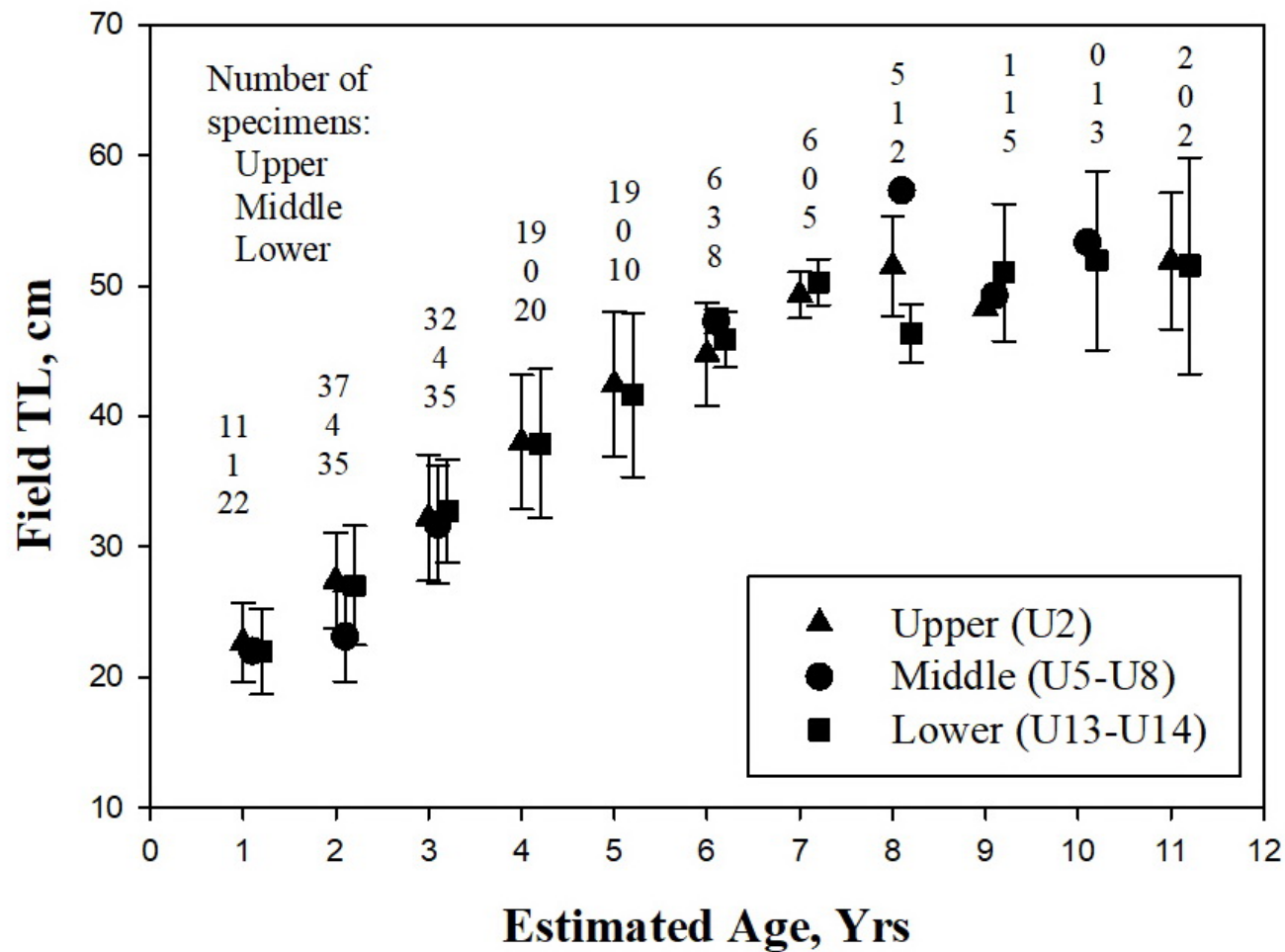


Table IIIB.3 Relationship between field TL and estimated age for Eels from different sections of the upper Delaware River (Upper: near Buckingham; middle: near Callicoon; lower: near Lackawaxen). Ages are offset for visibility

III.C Sex determination

Sex was assessed visually on 318 specimens, including the 300 used for other analyses and 18 preserved for other purposes. Of these, sex could not be determined for 131 fish, 183 eels were female (98% of eels which could be sexed) and four (2%) were males.

Because of the small number of males, length-age information was not tabulated by sex. The four males were all caught near the Zane Grey Launch (Table III.C.1). Three of the four were among the largest eels of that age class caught in that year (ranks one, two and three), while one was of more typical lengths (seventh out of 19 eels).

Table III.C.1 Summary of sample information on male eels collected in the upper Delaware River. Total number is the number collected in that age group in that year; rank is the rank (largest to smallest) of the male eel among the total number.

Date	Field TL (cm)	Lab TL (cm)	Estimated Age	Rank	Total Number
16 August 2019	40.4	38.8	3	3	42
23 October 2018	35.6	34.9	3	7	19
23 October 2018	34.4	33.4	2	2	29
23 October 2018	35.2	34.0	2	1	29

III.D Parasite Load

We analyzed the extent of infestation by *A. crassus* and its effects on swim bladder condition by laboratory examination of the eel specimens used for age analysis (Table IIID.1). These included measures of the length and thickness of the swim bladder, counts of larval and adult *A. crassus*, and indices of swim bladder opacity (0-2), swim bladder thickness (0-2), and swim bladder contents and calculated a swim bladder degeneration index as the sum of the three indices (0-6).

Swim bladder length increased with eel length (Fig. IIID.1). However, there was large variability around the relationship, possibly reflecting decrease in swim bladder length due to *A. crassus*. There was no difference in the regression among the three years. The proportion of eels with *A. crassus* was similar among years. The variance in the relationship between swim bladder length and TL appears to increase with TL. As a result, a logarithmic regression was done (Figure IIID.2). There was no difference in the relationship among years. However, in 2018, the relationship may have been nonlinear, with relatively higher swim bladder length for both small and large eels. There also appeared to be more dispersion below the regression line than above for 2019 and 2020. This might reflect small swim bladders resulting from *A. crassus* infection.

The highest numbers of *A. crassus* (more than 8) were only found in eels greater than 33 cm total length, but there was no obvious relationship between eel length and *A. crassus* load (Fig.

IIID.3). There was a relationship between eel length and the number of larval *A. crassus* ($r^2=0.12$; Fig. IIID.4). There was no clear relationship between the number of adult *A. crassus* and eel length, except the highest values (more than four nematodes) was found in large eels. The swim bladder opacity and contents indices were both zero in over half the specimens, with a value of 1 in most of the remaining specimens. The swim bladder thickness index was not informative since it was 1 in four specimens and 0 in all other specimens. As a result, variation in the degenerative index only reflected variation in the opacity and contents indices. The degenerative index was 0 and 1 in similar proportions of specimens (35% and 34%, respectively), 2 in 25% and 3 in 7%. No specimens had degenerative index of 4 (e.g., the maximum value of 2 in the opacity and contents indices). The two measures of swim bladder wall characteristics, thickness and opacity were correlated ($r^2 = 0.27$ Fig. IIIC.6). The measures of *A. crassus* load and effects and other measures of damage were weakly correlated (Table IIIC.2), e.g., swim bladder thickness and length (Fig. IIIC.7), swim bladder thickness and degenerative index (Fig. IIIC.8), swim bladder thickness and total number of *A. crassus* (Fig. IIIC.9) and SBI (ratio of swim bladder length to fish total length) and total number of *A. crassus* (Figure IIIC.10). However, the highest opacity indices and the most degenerate swim bladders occurred in the eels with the thickest swim bladders (> 0.9 mm).

Table IIID.1 Summary of *A. crassus* analyses for 318 specimens from the Delaware River.

Year	Prevalence	Mean Number of <i>A. crassus</i>			Maximum Proportion		Mean 100*SBL/TL
		Total	Larval	Adult	Total	Opacity>0	
2018	0.55	1.47	1.23	0.26	18	0.20	0.94
2019	0.48	1.43	0.95	0.48	18	0.49	0.91
2020	0.52	1.59	1.12	0.47	15	0.20	0.83

Table IIID.2. Correlations (r) among TL and measures of *A. crassus* abundance and swim bladder damage. The outlined correlations in italics are those between the SBI and the indices used to calculate the SBI.

Variable	# Larvae	# adults	SB thick	Opacity	SB cont	SGI	SBL/TL
TL (Laboratory)	0.35	0.17	0.37	0.01	0.12	0.11	0.48
Number of larval <i>A. crassus</i>		0.30	0.06	-0.11	0.26	0.07	0.35
Number of adults <i>A. crassus</i>			-0.05	-0.16	0.33	0.07	0.35
Swim bladder thickness				0.61	-0.08	0.49	-0.08
Swim bladder opacity					<i>-0.03</i>	<i>0.79</i>	-0.33
Swim bladder contents						<i>0.58</i>	0.19
Swim bladder degenerative index							-0.14
Ratio of swim bladder length to TL							

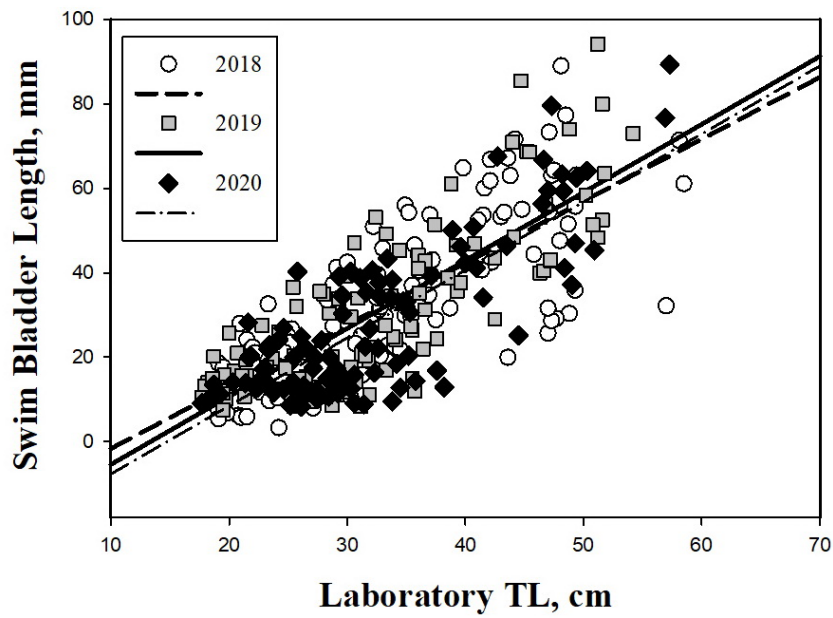
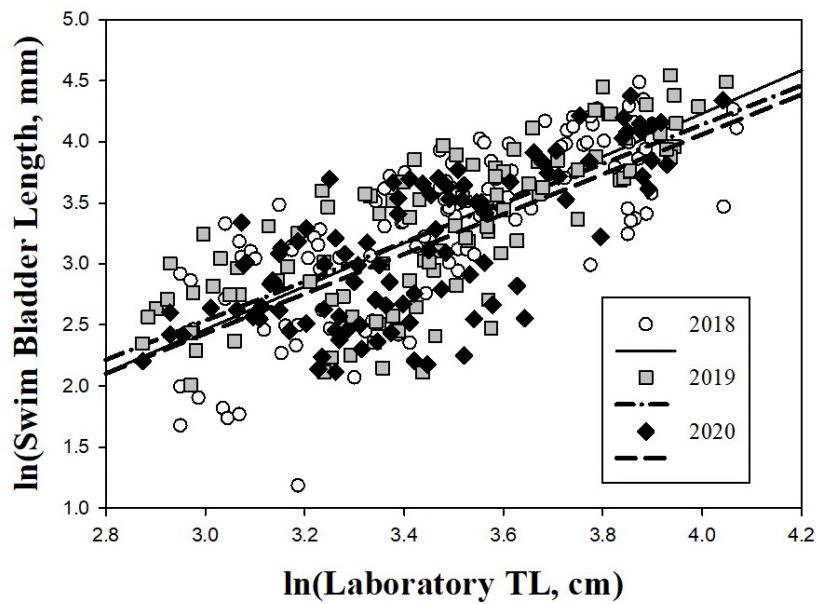


Figure IIID.1. Relationship between swim bladder length and total length for American Eels from the upper Delaware River year of collection.



IID.2. Relationship between the logarithm of swim bladder length and the logarithm of Laboratory total length for American Eels from the upper Delaware River.

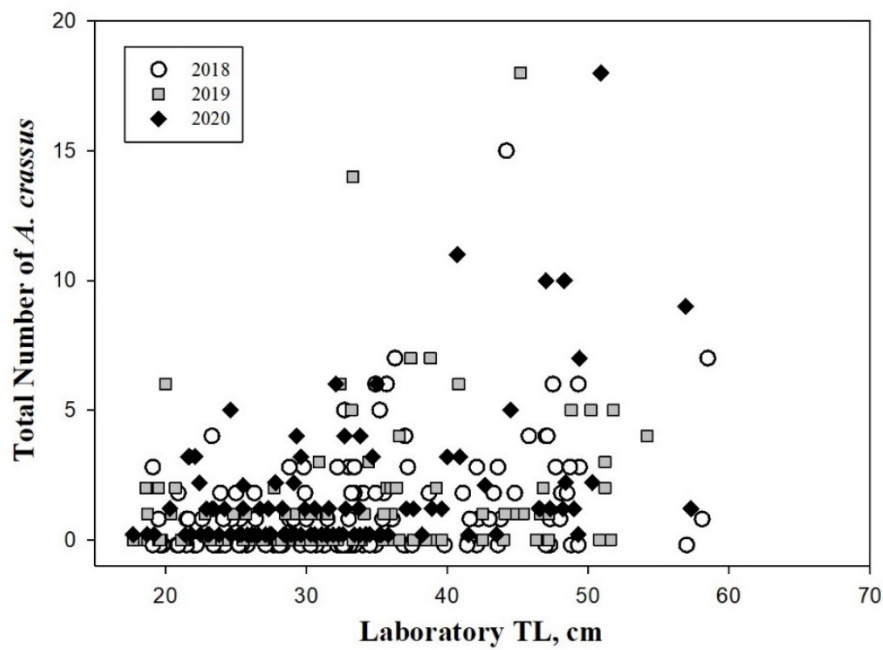


Figure IIID.3. Relationship between total number of *Anguillicola crassus* in American Eel swimbladders and total length. For values of total number of *A. crassus* of 0 to 3, data are offset by year to improve visibility. All specimens are from the Upper Delaware River.

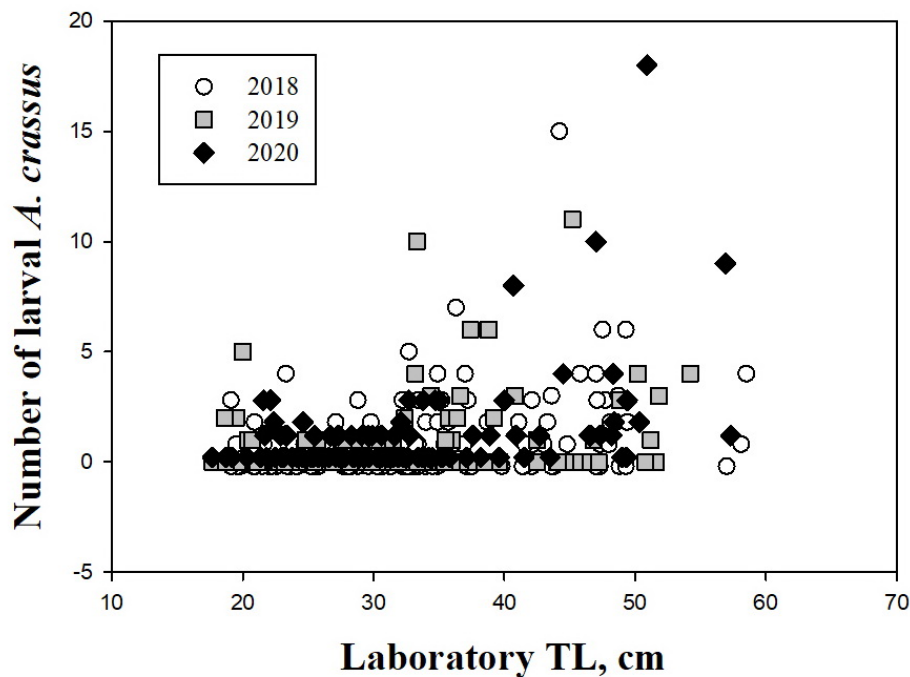


Figure IIID.4. Relationship between the number of larval *A. crassus* and eel TL in American Eels from the upper Delaware River. Values of number of larvae from 0 to 3 are offset for visibility.

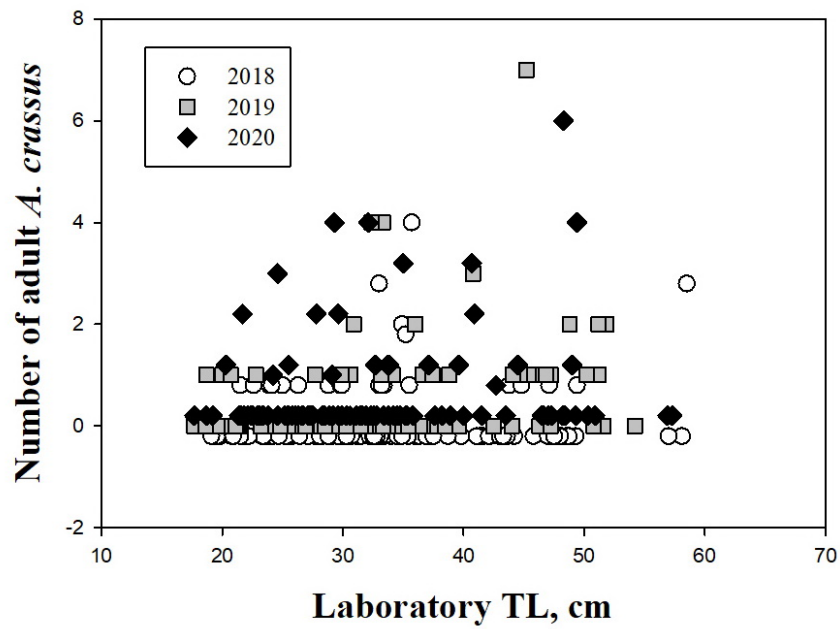


Figure IIID.5. Relationship between the number of adult *A. crassus* and eel TL in American Eels from the upper Delaware River. Values of number of larvae from 0 to 3 are offset for visibility.

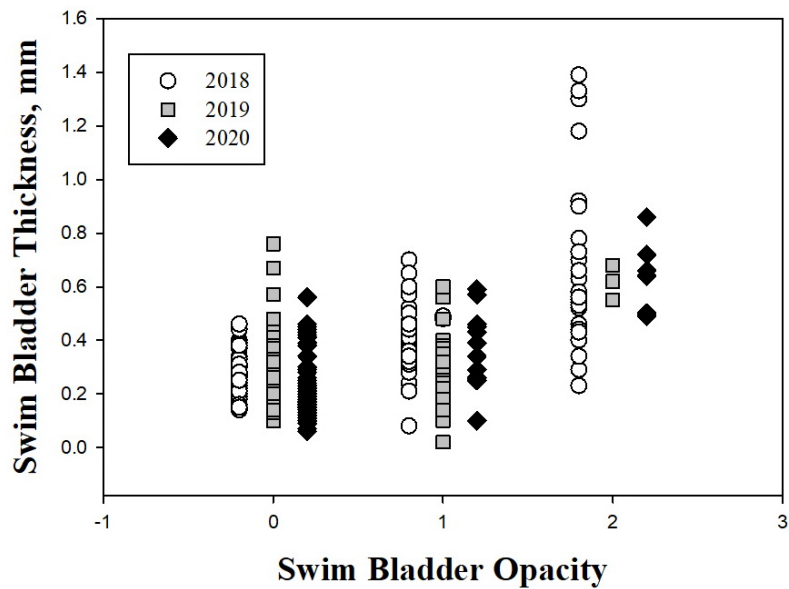


Figure IIID.6. Relationship between swim bladder thickness and opacity index in American Eels from the upper Delaware River. The swim bladder opacity index takes values of 0, 1 and 2; values are offset for visibility.

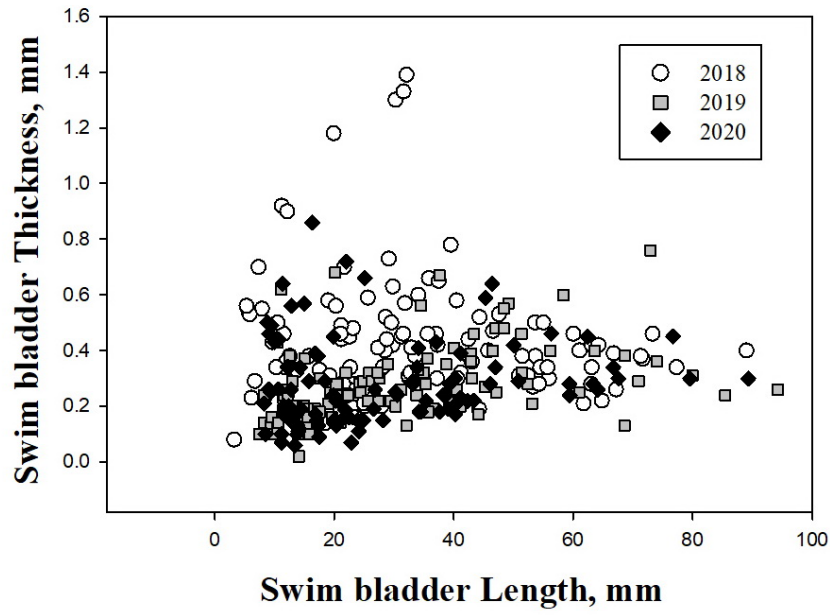


Figure IIID.7. Relationship between swim bladder thickness and length in American Eels from the Upper Delaware River.

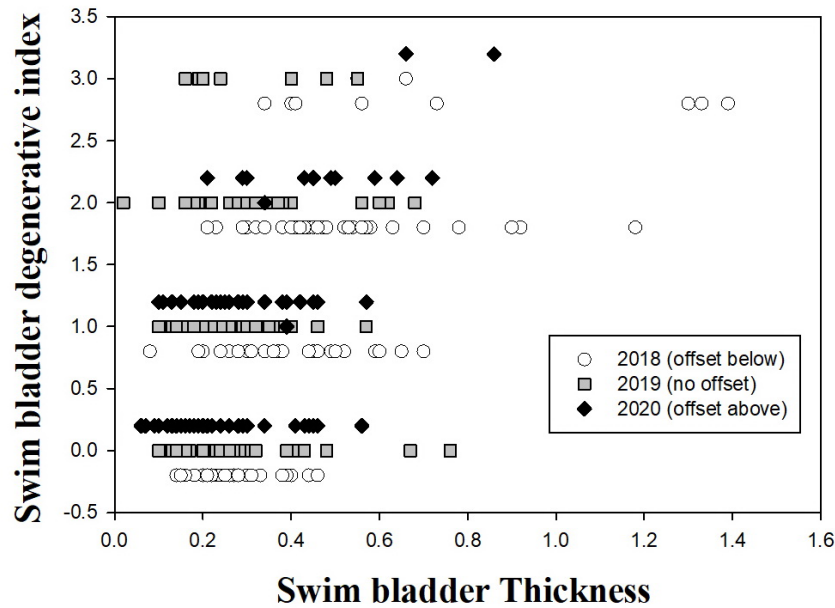


Figure IIID.8. Relationship between swim bladder degenerative index and swim bladder thickness (mm) in American Eels from the Upper Delaware River. The swim bladder degenerative index takes integral values of from 0 to 4; values are offset for visibility.

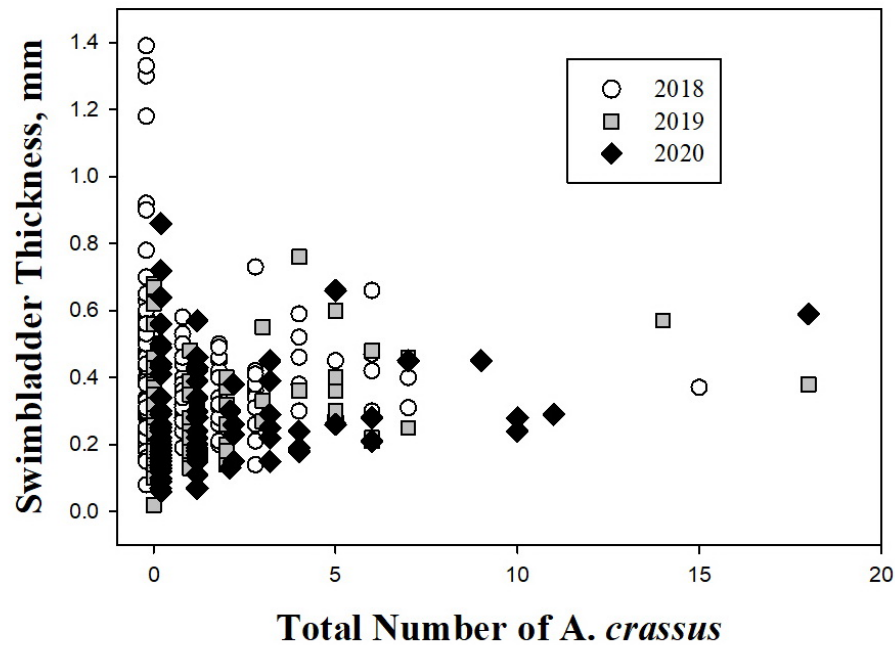


Figure IIID.9. Relationship between swim bladder thickness and total number of *A. crassus* in American Eels from the upper Delaware River.

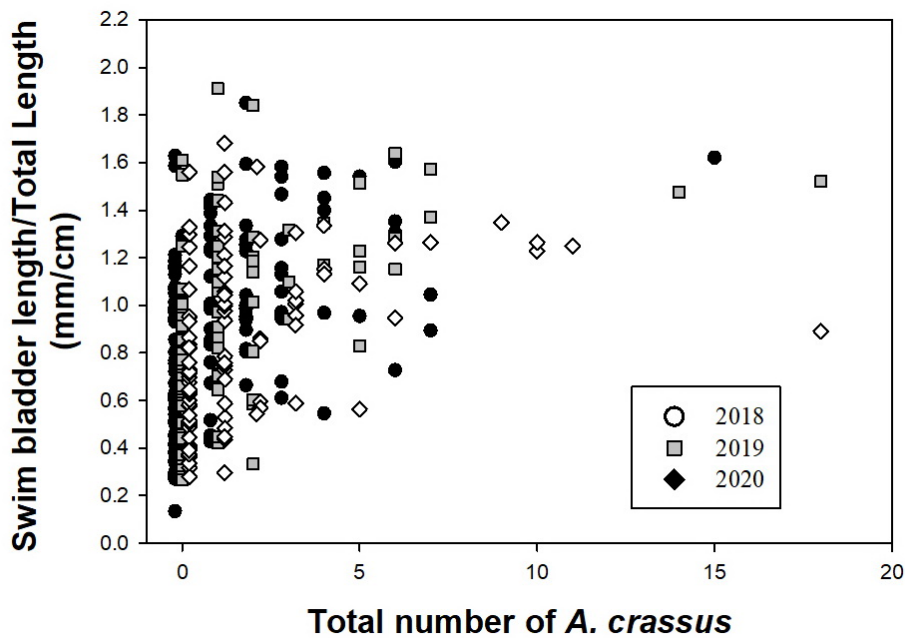


Figure IIID.10. Relationship between ratio of swim bladder length (mm) to fish total length (cm) and total number of *A. crassus* in American Eels from the Upper Delaware River. Numbers of *A. crassus* from 0-3 are offset slightly for visibility.

III.E Trophic ecology

IIIE.1 Gut contents

The 2019 and 2020 data provide the most complete information on occurrence of food items, since intestines, which frequently contained material even when the stomach is empty, were not examined in 2018. Of the 200 specimens analyzed in 2019 and 2020, 138 contained material identifiable at some level. The remainder were either empty or only contained unidentifiable digested organic material. Eels with empty stomachs tended to be captured later in the day than eels without food (Table IIIE.1), suggesting that digestion of food eaten the previous night affected the proportions of empty stomachs.

Among 2019 and 2020 stomachs containing food items, 91% contained insect remains (Table IIIE1). Of these, 63% were unidentifiable to lower taxonomic levels. The most common identifiable insects were stoneflies (Plecoptera), caddisflies (Trichoptera), and mayflies (Ephemeroptera), each order occurring in over 15% of stomachs with identifiable material and greater than 25% of stomachs with insects identifiable to order. (Table IIIE2) Less frequent insect orders were beetles (Coleoptera, almost all of which were water pennies Psephenidae), hellgrammites (Corydalidae, Megaloptera), and aquatic flies (Diptera), including both blackflies (Simuliidae) and midges (Chironomidae). In 2018, a specimen probably representing a dragonfly or damselfly (Odonata) was found. Mayflies found in one sample were identified as Baetidae and *Isonychia*.

Crayfish were found in 15 stomachs (11% of non-empty stomachs). Identifiable crayfish were all *Orconectes limosus*. A crayfish was found in 2018 samples as well. Mollusks, including both bivalves (fingernail clams, Sphaeriidae) and gastropods (some identified as *Physa*) were found in 23 stomachs (17% of non-empty stomachs). An unidentifiable fish was found in the stomach of a large eel (52.4 cm field TL). In 2018, annelid worms were found in one stomach and tentatively identified in another.

Small amounts of plant material and detritus were also found in 2018.

Table IIIE1. Numbers of samples of American Eel stomachs with and without food, and average time of start of sample for empty and non-empty stomachs.

Number of fish			
Total	118	100	100
Empty	89	18	59
Not empty	29	82	41
Proportion empty	0.75	0.18	0.59
Time of start of sample			
	2018	2019	2020
Empty	12:56	12:00	11:42
Not empty	12:39	9:41	10:18
Average time	12:51	10:06	11:07

Table IIIE2. Proportion of food items among eel guts with food. Proportions are calculated only among individuals with identifiable contents, except where indicated by *. The number of guts with no identifiable material is the sum of categories without identifiable material (empty, detritus, organic matter only).

	2019 and 2020 only			2018	
Number analyzed	200			118	
With Identifiable contents	138			11	
	Count	Proportion		Count	Proportion
		Among All	Among Insects		
Any insect	126	0.91		8	0.73
Identified insect	80		0.63	1	0.091
Plecoptera	33	0.24	0.41	0	
Trichoptera	25	0.18	0.31	0	
Ephemoptera	22	0.16	0.28	0	
All Coleoptera	13	0.094	0.16	0	
Pseniidae	11	0.080		0	
Corydalidae	5	0.036	0.06	0	
Odonata (cf)				1	0.091
Simulidae	2	0.014	0.03	0	
Chironomidae	3	0.022	0.04	0	
Unid Arthropoda	3			0	
Crayfish	15	0.11		1	0.091
<i>Orconectes limosus</i>	5			0	
Mollusk	23	0.17		0	
Unid mollusk	4			0	
Sphaeriidae	13	0.094	0.57	0	
Gastropoda, all	6	0.043		0	
<i>Physa</i>	1			0	
Annelid and detritus	0			2	0.18
Fish	1	0.0072		0	
Plant				2	0.18
No identifiable material	59	0.30*		107	0.91*
Unidentified animal				1	0.091
Only detritus				4	0.36
Detritus and unid organic matter				1	0.067
Organic matter in stomach a/o intestine	14	0.10		0	
Stomach only with organic matter				12	0.10*
Stomach and intestine empty	45	0.23*		na	
Stomach empty				89	0.75*

IIIE.2. Stable Isotope analyses

Samples of a variety of taxa were captured and analyzed (Table IIIE3). Data for each sample (individual specimen or composite of smaller specimens) are grouped into putative trophic groups based on species ecology for graphing:

Low-level predatory fish: juvenile Smallmouth Bass (*Micropterus dolomieu*), and juvenile and small adult Rock Bass (*Ambloplites rupestris*).

Large individuals of generalist invertivore fish: adult and large juvenile Redbreast Sunfish (*Lepomis auratus*) and adult Common Shiner (*Luxilis cornutus*).

Small individuals of generalist invertivore fish: juvenile Fallfish (*Semotilus corporalis*) and juvenile American Shad (*Alosa sapidissima*).

Large benthic invertivore fish: adult Cutlip Minnow (*Exoglossum maxillingua*), adult Blacknose Dace (*Rhinichthys atratulus*), adult White Sucker (*Catostomus commersoni*), large juvenile Northern Hog Sucker (*Hypentelium nigricans*), adult Shield Darter (*Percina peltata*), adult Tesselated Darter (*Etheostoma olmstedii*) and adult Margined Madtom (*Noturus insignis*).

Small benthic invertivore fish: juvenile White Sucker, YOY Cutlip Minnow, and YOY or small juvenile Blacknose Dace, Longnose Dace (*R. cataractae*), Bluntnose Minnow (*Pimephales notatus*), Margined Madtom and Tesselated Darter.

Detritivorous: Sea Lamprey (*Petromyzon marinus*).

Predatory aquatic macroinvertebrates: Water Strider (Gyrinidae), stonefly (Perlidae), larval dragonfly (Gomphidae), larval fishfly (*Nigronia*), larval alderfly (Sialidae), and larval dobsonfly (hellgrammite; Corydalidae).

Detritivorous/herbivorous macroinvertebrates: larval crane fly (Tipulidae), larval net-spinning caddisfly (Hydropsychidae), larval giant stonefly (Pteronycaridae), larval brush-legged mayfly (Isonychiidae), larval flatheaded mayfly (Heptageniidae), unionid mussel (Unionidae) and snail (Physidae).

Crayfish: Spiny-cheek Crayfish (*Faxonius limosus*) and Appalachian Brook Crayfish (*Cambarus bartonii*).

Aquatic plant: Riverweed (*Podostemum ceratophyllum*), Pondweed (*Potamogeton*), Waterweed (*Elodea*), Bur-reed (*Sparganium*), aquatic moss and unidentified submerged aquatic vegetation.

Riparian plant: Sycamore (*Platanus americanus*), Black Oak (*Quercus velutina*) and Japanese Knotweed (*Polygonum cuspidatum*).

Algae: *Spirogyra* and unidentified filamentous algae.

Sediment.

Table IIIE.3. Numbers of samples of different groups for which stable isotope analyses were done. Plants include submerged aquatic vegetation and leaves of riparian plants.

	Delaware River	Aquashicola Creek	Cherry Creek
American Eel	80	4	4
Other Fish	28	15	11
Invertebrates	4	8	10
American Eel gut contents	0	4	1
Plants, algae, moss	8	3	4
Sediment	0	4	4

The $\delta^{15}\text{N}$ values, which provide information on trophic level of taxa, varied greatly among eels (Figures IIIE.1- IIIE.3 and Tables IIIE.4 and IIIE.5). Eel values included the highest values in the sample set, indicating that some eels are high-level predators in the system. The $\delta^{15}\text{N}$ values for 2018, including samples from the Delaware River, Cherry Creek and Aquashicola Creek, were typically higher than those from 2019-2020 (Table IIIE.5 and Figure IIIE.3). The $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values were not correlated with eel length (Figures IIIE.4 and IIIE.5). An ANCOVA of $\delta^{15}\text{N}$ with year as a categorical factor and either TL or year x TL as continuous terms found a strong year difference and weak year x TL interaction. However, this interaction was created by two extreme datapoints with very low values (< 0). When these two data points were removed, there was a strong year difference ($p < 0.00001$, adjusted $r^2 = 0.47$). Linear contrasts found a highly significant difference between 2018 and the other two years, but no difference between 2019 and 2020. An analogous ANCOVA with $\delta^{13}\text{C}$ found no significant effects when all data were used. When two points with extreme values (> -15) were removed, there was no year difference ($p < 0.10$), but a significant year ($p < 0.009$) and weakly significant year-TL interaction (0.036) effects. The slope of the 2018 effect was slightly negative (-.08), while the slopes of the other two years were positive (1.1 in 2019, and .0.8 in 2020). Linear contrasts of least squares means found a weakly significant ($p < 0.052$) difference between 2018 and the other two years, and no significant difference between 2019 and 2020. The $\delta^{15}\text{N}$ results suggest that trophic level does not generally increase with fish size. The $\delta^{13}\text{C}$ results indicate that carbon sources to eel may change with eel size as mediated by other factors. Although there was variability in $\delta^{15}\text{N}$ within groups, values tended to follow expected trophic position: moderately high for low-level predatory fish and predatory aquatic macroinvertebrates, variable but typically lower for invertivore fishes, and lowest for detritivores, plants and sediment. The trophic groups were designed *a priori* and were not modified by the stable isotope results.

The $\delta^{13}\text{C}$ values provide information on carbon sources for different trophic levels. Values of $\delta^{13}\text{C}$ were not as variable among various consumer groups as values of $\delta^{15}\text{N}$ (Figures IIIE.1 and IIIE.2). However, $\delta^{13}\text{C}$ appear to form two clusters, one with concentrations more negative than -22 per mil and other less negative than -22 per mil. Almost all the 2018 eels were in the less depleted cluster, while 2019 and 2020 eels fell in both clusters (Figure IIIE.3 and Table IIIE.5). Plant samples included autochthonous sources such as filamentous algae and aquatic plants and allochthonous sources such as leaves of riparian trees. The $\delta^{13}\text{C}$ values were highly variable

within these plant groups, likely reflecting the taxonomic diversity of taxa analyzed. However, the $\delta^{13}\text{C}$ of riparian plants fell within the more depleted cluster (lower values), while the aquatic plants were mostly in the less depleted cluster (higher values). It is plausible that the two clusters represent two distinct carbon sources, e.g., an allochthonous one represented by the leaves of riparian trees, and the other autochthonous, represented by the aquatic plants. Eels with $\delta^{13}\text{C}$ values in the more depleted range tended to have lower $\delta^{15}\text{N}$ values, comparable to other invertivore fishes. Eel with $\delta^{13}\text{C}$ values in the less depleted range tended to have $\delta^{15}\text{N}$ values higher than or like other invertivore species.

The difference in stable isotope patterns between 2018 and the other two years could be due a variety of factors. Since the 2018 samples from Cherry Creek and Aquashicola Creek were like those from the Delaware River (Table IIIE.5), a regional factor is more likely than a river-specific factor, such as coldwater releases in the Delaware River. Differences in rainfall and discharge could affect trophic relationships. The three years differed in the amount of summer and early fall flow, i.e., the months preceding and including sampling (Table IIIE.6). At a number of USGS gaging stations, average June-September discharge was much higher in 2018 than in 2019 or 2020; the average 2019-2020 flows for this period were 0.32 to 0.63 that of 2018 flows for all but one station examined. The difference varied among months. June flows were typically higher in 2019 and 2020 than in 2018, while August and September flows were much lower in 2019 and 2020. At some stations, July flows in 2018 were lower than the 2019 and 2020 flows, while at most stations, 2018 flows were higher in 2018.

In addition, the samples were collected at somewhat different times of the year, which might also explain differences between $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in 2018 compared to 2019 and 2020 (Figure IIID.6 and IIID.7). The 2018 samples, which had the highest $\delta^{15}\text{N}$ values and almost entirely less-depleted concentrations of $\delta^{13}\text{C}$, were collected later in the year (late October) than the 2019 and 2020 samples (collected in early and mid- September).

Table III E.4. Summary of stable isotope results for taxonomic-trophic groups.

Taxon	$\delta^{15}\text{N}$			$\delta^{13}\text{C}$		Waterbodies		
Group	N	Mean	StDev	Mean	StDev	Delaware	Aquashicola	Cherry
Fish								
American Eel	85	8.80	-21.80	2.56	5.30	X	X	X
Trout	3	9.90	0.38	-25.38	0.54		X	X
Low-level Predatory	6	7.88	5.41	-22.05	3.70	X	X	X
Large generalist invertivore	10	7.70	3.82	-22.03	3.91	X	X	X
Large benthic invertivore	22	8.52	0.92	-20.92	4.15	X	X	X
Small benthic invertivore	9	8.96	0.96	-24.17	5.35		X	X
Detritivorous (Larval Sea Lamprey	3	4.67	2.12	-21.74	1.44	X	X	X
Macroinvertebrate								
Predatory	7	5.07	1.31	-25.30	5.59	X	X	X
Crayfish	4	5.59	2.43	-26.59	6.82	X	X	X
Detritivorous/herbivorous	11	3.34	2.46	-29.39	5.41		X	X
Primary Producer								
Aquatic Plant	8	3.18	2.25	-34.24	16.26	X	X	X
Riparian Plant	4	0.24	2.45	-41.35	22.33	X	X	
Algae	3	2.09	2.69	-34.47	14.61	X	X	X
Sediment	6	-0.20	2.90	-26.75	5.26		X	X
American Eel gut	6	3.89	2.12	-28.07	1.11		X	X

Table III.E.5. Comparison of stable isotope results among the three water bodies and the three study years. Only groups which were collected in the Delaware River and at least one of the other streams are shown.

Taxonomic-trophic Group	Year	N	dN		dC	
Water Body			Mean	Stdev	Mean	Stdev
American Eel						
Aquashicola Creek	2018	85	8.80	2.56	-21.80	5.30
Cherry Creek	2018	4	9.02	0.51	-25.60	0.26
Delaware River	2018	29	11.13	1.30	-20.44	2.08
Delaware River	2019	30	7.26	2.21	-22.61	6.47
Delaware River	2020	18	7.50	2.46	-21.10	7.06
Fish: Low-level predatory						
Aquashicola Creek	2018	1	8.69		-25.26	
Cherry Creek	2018	2	10.95	0.19	-25.50	0.00
Delaware River	2019	3	5.57	7.45	-18.69	0.44
Fish: Large generalist predatory						
Aquashicola Creek	2018	3	9.37	0.09	-26.93	0.80
Cherry Creek	2018	1	9.49		-25.01	
Delaware River	2020	1	10.44		-18.29	
Fish: Small generalist invertivore						
Aquashicola Creek	2018	3	8.73	0.75	-25.05	6.64
Delaware River	2019	3	8.24	0.50	-17.29	0.63
Fish: Large benthic invertivore						
Aquashicola Creek	2018	4	9.25	0.65	-26.37	1.25
Cherry Creek	2018	3	9.24	0.67	-27.00	0.64
Delaware River	2019	11	8.17	0.97	-18.55	0.98
Delaware River	2020	4	8.21	0.54	-17.41	1.34
Fish: detritivorous (larval Sea Lamprey)						
Aquashicola Creek	2018	1	5.96		-21.99	
Cherry Creek	2018	1	5.82		-23.03	
Delaware River	2019	1	2.23		-20.19	
Macroinvertebrate: Aquatic Predatory						
Cherry Creek	2018	3	4.64	0.94	-28.69	1.71
Delaware River	2019	1	7.27		-19.13	
Delaware River	2020	2	5.20	1.32	-19.88	1.75
Crayfish						
Aquashicola Creek	2018	1	2.68		-35.62	
Cherry Creek	2018	2	7.58	0.44	-25.79	1.18
Delaware River	2019	1	4.52		-19.15	
Plant: Aquatic						
Aquashicola Creek	2018	2	2.78	0.28	-47.64	0.87
Cherry Creek	2018	2	3.36	0.65	-49.31	14.60
Delaware River	2019	2	1.85	5.11	-21.12	0.18
Delaware River	2020	2	4.72	0.43	-18.87	2.68
Plant: Riparian						
Cherry Creek	2018	1	-1.31		-74.84	
Delaware River	2019	3	0.76	2.72	-30.19	0.26
Algae						
Aquashicola Creek	2018	1	0.68		-44.12	
Cherry Creek	2018	1	0.39		-41.64	
Delaware River	2019	1	5.19		-17.66	

Table IIIE.6. Average discharges (cfs) and comparisons of discharge at selected USGS stations in the Delaware River. The top half shows average discharges in the June-September period at each of the stations. The bottom half shows the ratio of 2019 and 2020 discharges to the corresponding 2018 discharge.

Waterbody	USGS Gage Number	Location	June-Sept Ave (cfs)				Monthly average (2019 and 2020)/2018				
			3-year	2018	2019	2020	June-Sept	June	July	August	Sept.
Brodhead Creek	01440400	near Anomalink	115	201	80	64	0.36	1.42	0.56	0.35	0.06
Little Lehigh Creek	01451500	Allentown	182	202	186	157	0.85	1.70	1.44	0.64	0.44
Lehigh River	01447500	at Stoddartsville	195	320	135	130	0.41	1.86	0.50	0.21	0.10
W Branch Delaware River	01425000	at Stilesville	681	891	681	472	0.41	1.45	1.25	0.33	0.51
Lackawaxen River	01432110	at Rowland	965	1279	859	756	0.63	2.65	1.02	0.51	0.13
E Branch Delaware River	01421000	near Fishs Eddy	1088	1988	642	634	0.32	1.88	0.52	0.18	0.13
Delaware River	01427519	at Callicoon	2586	4289	1855	1614	0.40	1.85	0.73	0.23	0.19
Delaware River	01438500	at Montague	4992	7478	3800	3698	0.50	1.86	0.84	0.32	0.19
Delaware River	01463500	at Trenton	10815	15599	8956	7888	0.54	1.65	0.84	0.38	0.19

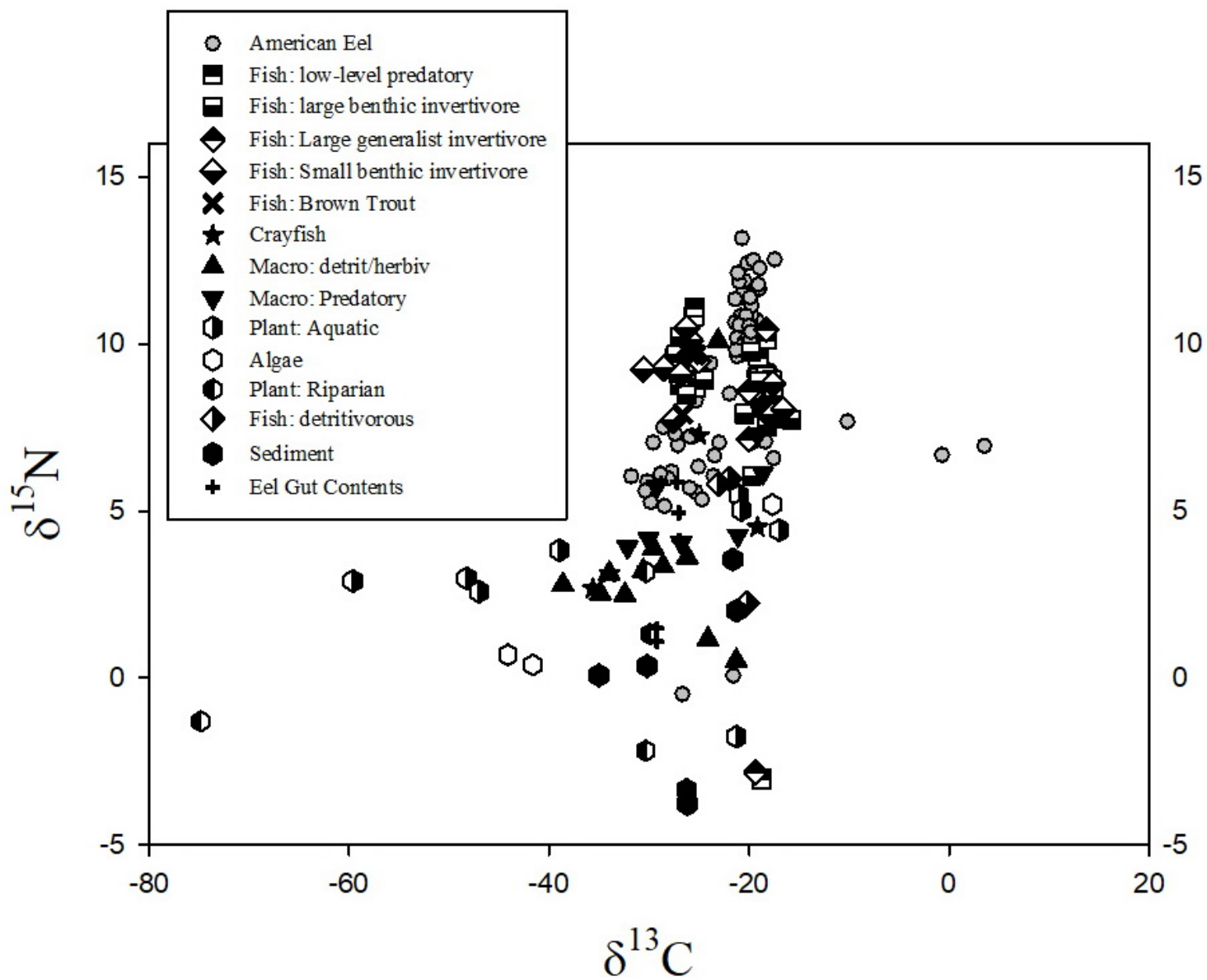


Figure III.E.1. Stable isotope ratios for taxa from Delaware River, Aquashicola Creek and Cherry Creek

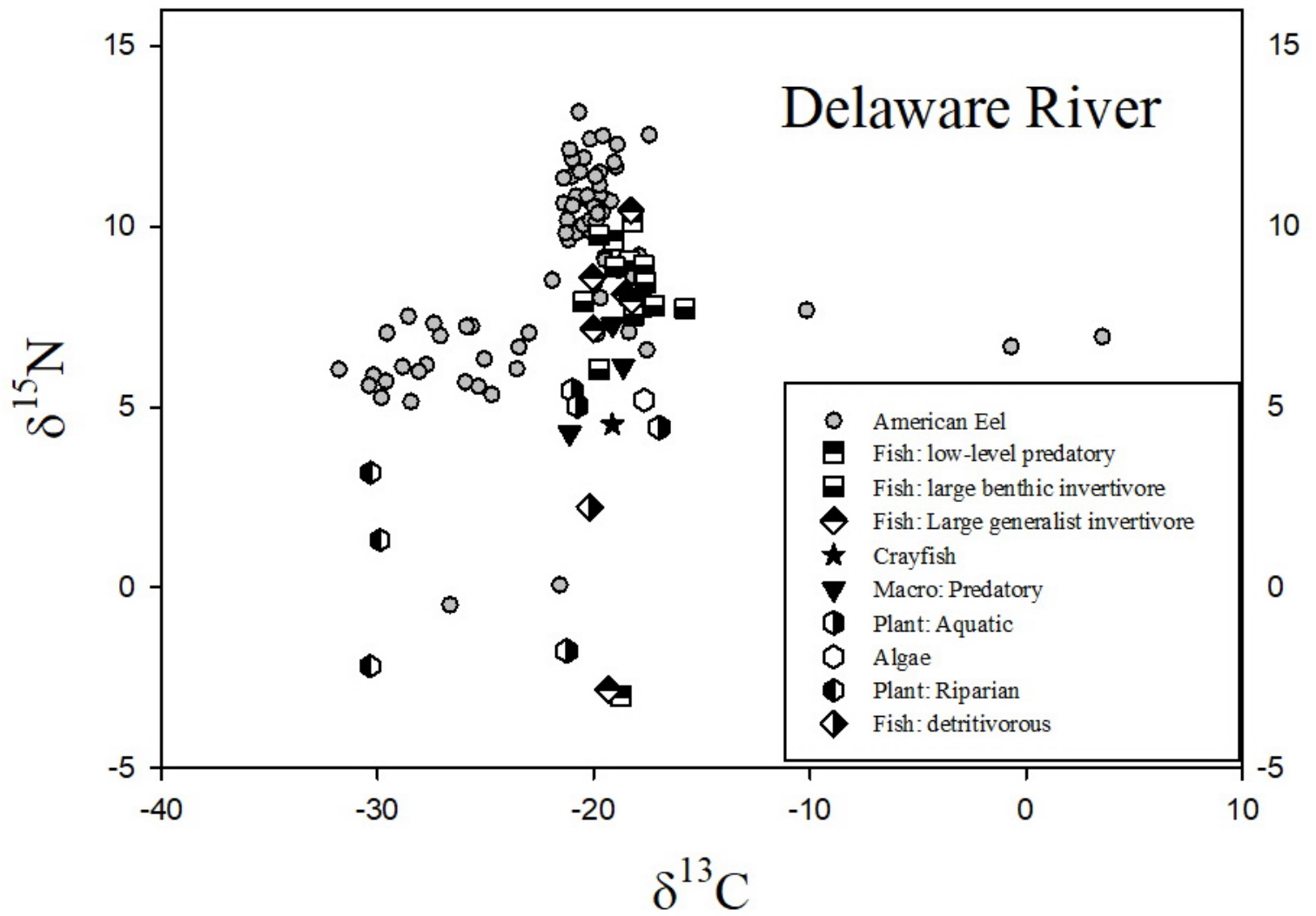


Figure IIIE.2. Stable isotope ratios for taxa from the Delaware River. Symbols and colors are defined in Table IIIE.4. Colored hexagons are eels, squares are fish and lamprey, stars are crayfish, diamonds are other aquatic macroinvertebrates, and circles are algae and plants.

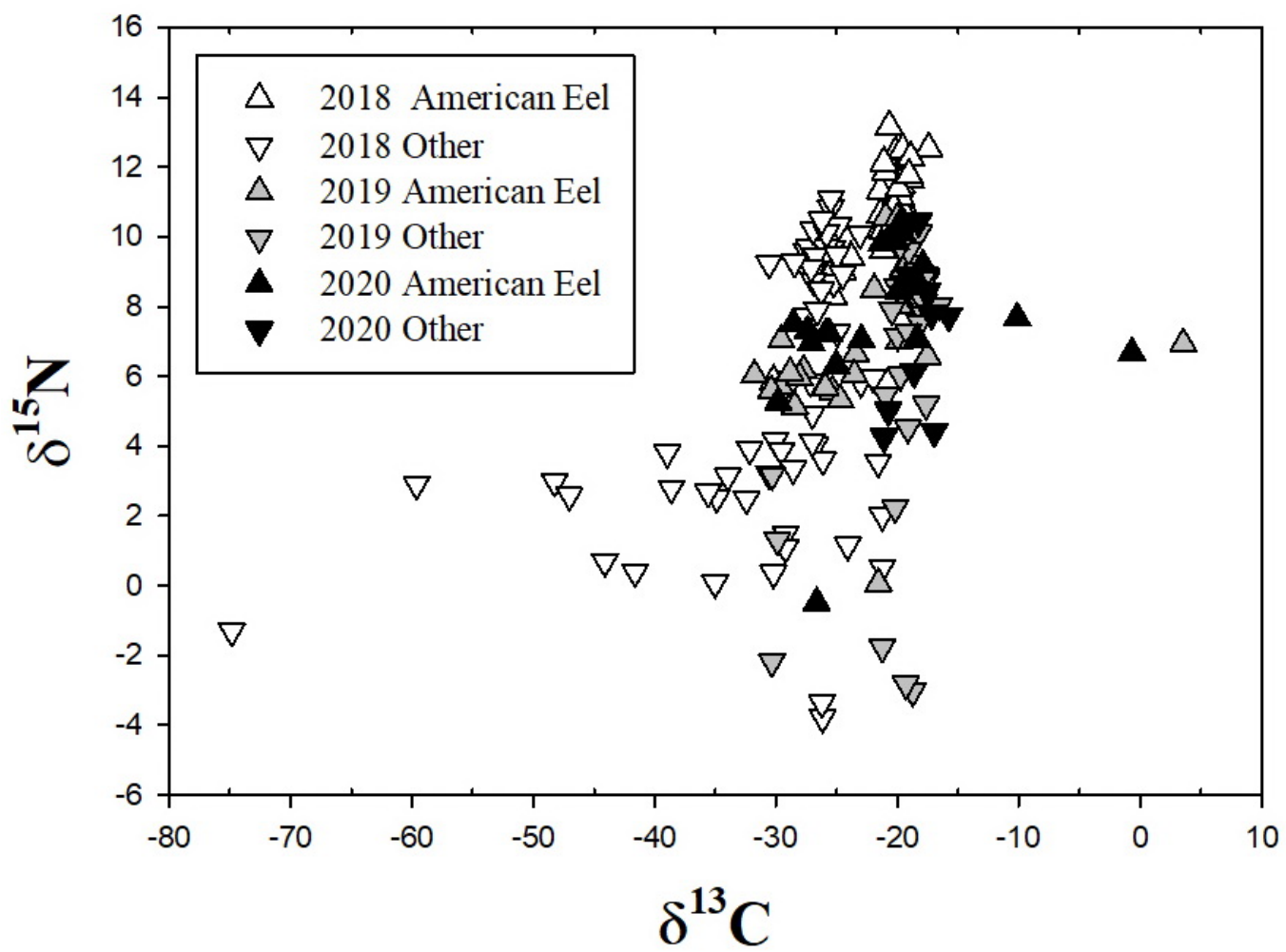


Figure IIIE.3. Stable isotope ratios for all samples, broken out by year and two groups (Eel and other taxa).

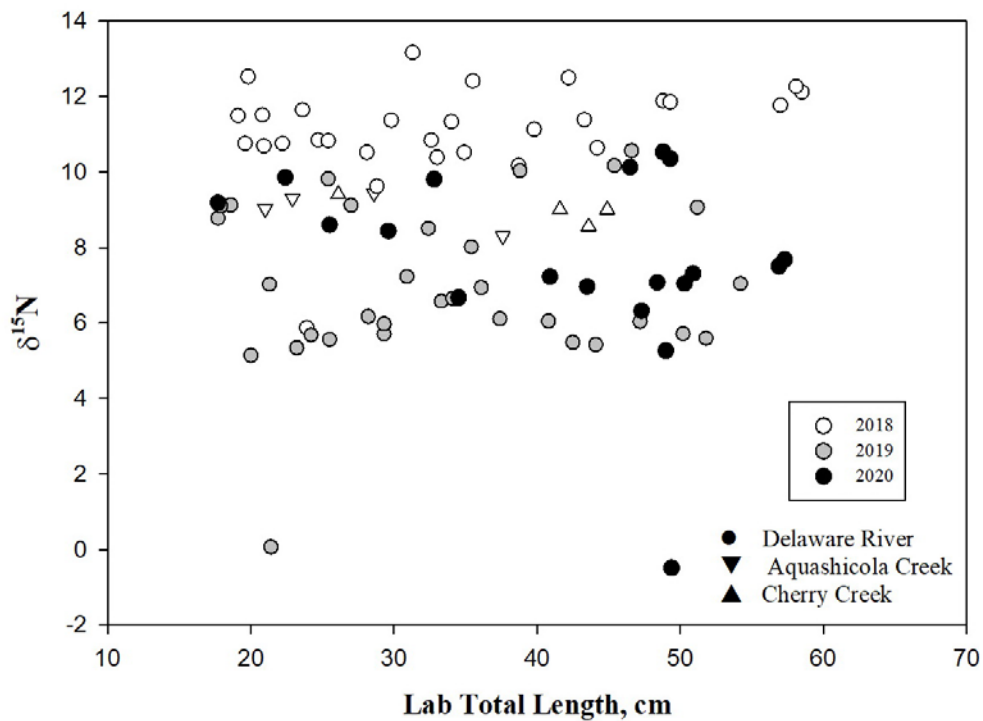


Figure III.E.4. Relationship between $\delta^{15}N$ values and total length for American Eels.

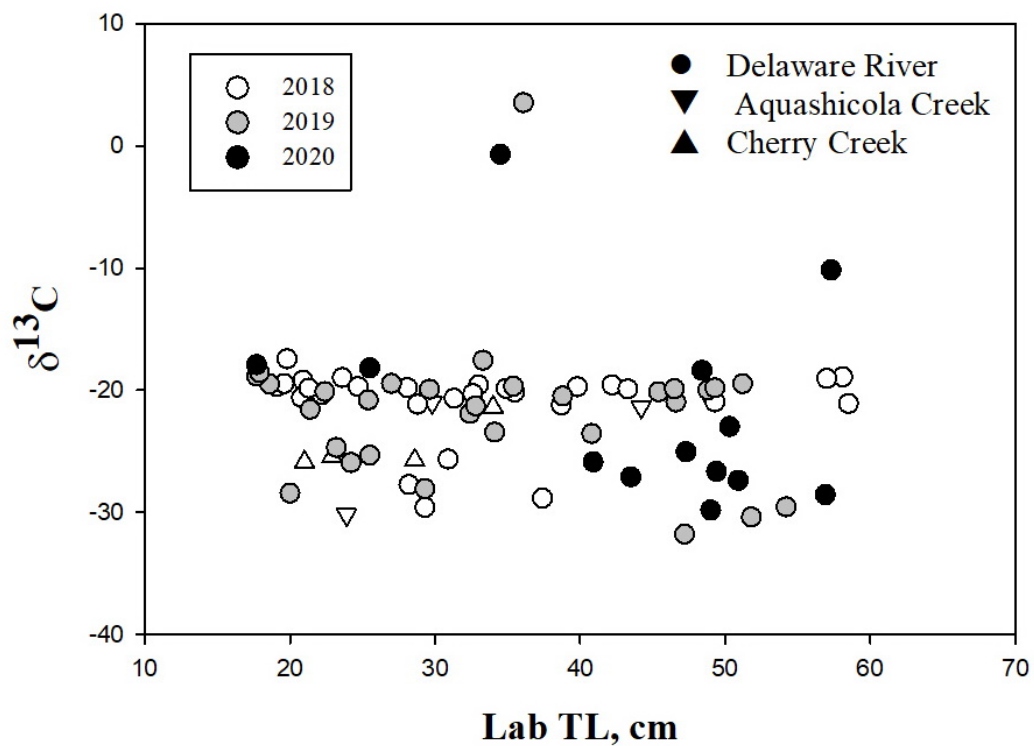


Figure III.E.5. Relationship between $\delta^{13}C$ values and total length for American Eels.

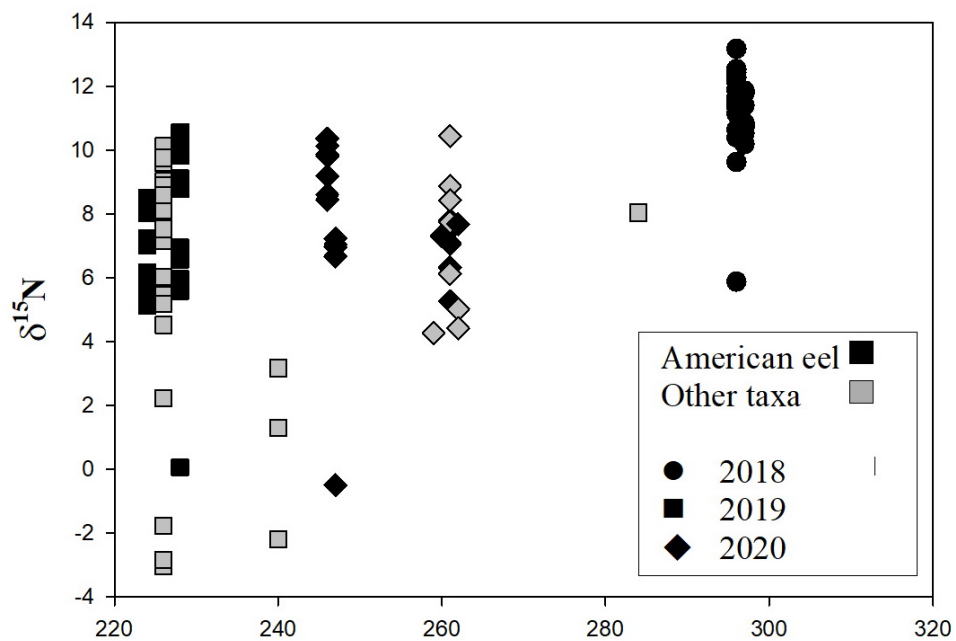


Figure III.E.6. Relationship between $\delta^{15}\text{N}$ values and day of year of collection for samples from the Delaware River.

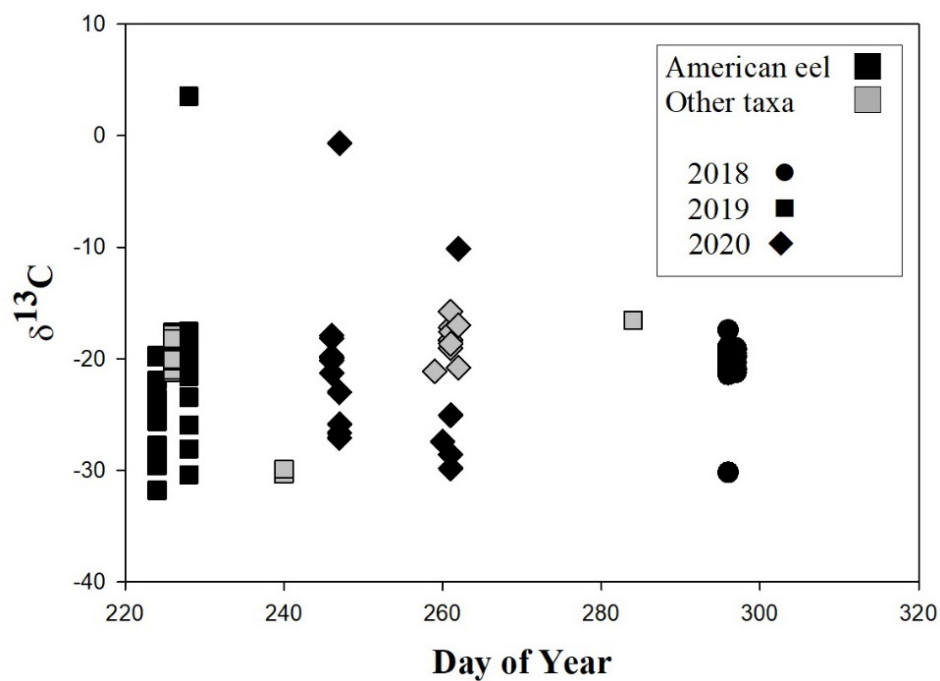


Figure III.E.7. Relationship between $\delta^{13}\text{C}$ values and day of year of collection for taxa from the Delaware River.

IV. Discussion

IVA. Estimates and indices of abundance

Estimates of actual population size are desirable but require additional sampling effort and depend on assumptions about the capture procedures. Bias in population estimates, i.e., tendency to under- or overestimate population size, are important considerations for interpreting population estimates. Two population estimation methods, mark-recapture and depletion were investigated in this study and are discussed below. In contrast, catch indices are not measurements of population size. For catch indices, relationships between the index and true population size are important. Both the shape (e.g., linear versus asymptotic) and variance of the relationships are important. Catch indices are discussed in the following sections.

The relationship between bias and variance differs in reach-level population estimation and small-area sampling and between population estimates and catch indices. In reach-level estimation, the population size in a given area is of primary interest. Bias may be introduced by violation of assumptions of the estimation technique or by inherent bias in the statistical measure. For small-area sampling, the population in the sampled area is a subsample of the larger population of primary interest. Bias may be introduced by estimation of the population in the subsample (as in reach-level sampling), as well as by bias in aggregation of subsample catches into estimates of the population of interest. Population estimates in small-area samples may be used to reduce or eliminate bias in the subsample estimates, but representativeness of the subsamples needs to be considered in the sampling design to address the bias in the population index. Alternately, the inherent bias of catch indices as estimates of population is of no interest if these are used for spatial or temporal comparisons. However, bias in sample aggregation is still important.

Literature reports may refer to European Eel as well as American Eels, since sampling issues are similar.

Pool samples

Mark-recapture methods

Mark-recapture methods provide a strong method of estimating population sizes. The simplest models depend on several assumptions, notably constant population (closed, no immigration-emigration) and identical probability of capturing unmarked eels and recapturing marked eels during any sample session (i.e., no capture-aversion due to stress, nor capture-disposition due to bait). As a result, mark-recapture is most easily done in small, confined areas over short time periods. Extensive research has been done to develop more complicated statistical models to incorporate violation of the model assumptions, although these require additional effort. Mark-recapture has been used to estimate eel (American Eel or European Eel) population size by traps (Morrison and Secor 2004), fyke nets (Poole 1990) and electrofishing (Carss et al. 1999, Feunteun et al. 2000). Morrison and Secor (2004) sampled eels using traps in the Hudson River,

with modeling to overcome the small home ranges of eels during the study, which led to spatial differences in capture probabilities of individual eels among pots in different locations.

The 2019 boat electrofishing sampling evaluated mark-recapture estimates of eel abundance in single pools. Although we were able to recapture marked eels, these techniques have several critical problems, making these methods impractical, at best: 1) Emigration of eels between sampling sessions; 2) Low sampling efficiency in deep or fast water, with almost no efficiency in the deep mid-channel habitats; 3) Many eels were immobilized by electrofishing but couldn't be captured, because of depth, water velocity and substrate; as a result, eels would be shocked a number of times during depletion passes or mark-recapture operations, with detrimental effects; 4) large effort in terms of personnel, sampling dates and equipment.

Depletion estimates

We attempted reach-level depletion sampling along with mark-recapture sampling, but these were affected by the same issues affecting the mark-recapture analyses listed above. An alternate approach of estimating or indexing population size over small areas was used in 2019 and 2020. Because of the difficulties with the reach-level sampling, the small-area sampling was the only technique used in 2020.

Small-area samples

We define small-area sampling techniques that use a set of individual samples to develop a population estimate or index of larger population. A variety of small area sampling techniques has been used to index eel populations, e.g., open-water boat-electrofishing transects producing catch per area indices (Cairns, et al. 2021), trap and fyke net samples producing catch per soak night (Cairns, et al. 2021), visual surveys in glass-bottomed boats (Cairns, et al. 2021) and frequency of attacks by eels on small fish caught in gill nets (Prchalova et al. 2013). Presumably, analogous catch indices could be formed using other gear used to catch eels, such as spearing (MacKenzie 1992, Znamierowska-Pruffer 1966). Point samples of density (PASE) have been obtained by capturing eels in 1-m diameter circles with electrofishing by a stationary sampler; these point estimates were correlated with density estimated from depletion sampling of larger areas (Laffaille, et al. 2005). All such indices are subject to variation in catch rate created by sampling conditions (e.g., depth, water clarity and surface smoothness) and fish behavior. For example, night-time electrofishing frequently produces higher catch rates of eels than daylight sampling (Cairns et al. 2021). These factors increase variance in the index-population relationship. Typically, a range of sampling conditions (e.g., water depth and clarity) and fish characteristics (e.g., size) in which the protocol is useful need to be defined.

Estimation of population sizes within small areas can reduce or eliminate the sampling variation in catch indices in the small areas, e.g., due to different catchability in different places or under different sampling conditions. Effects of gear selectivity (e.g., related to fish size) can be reduced by forming different estimates for classes (e.g., sizes) with differing catch efficiency, excepting absolute selectivity (i.e., inability of the gear to capture any fish of a given class).

We effectively sampled eels using shoreline electrofishing at sites throughout UPDE. These samples can be used to develop population estimates within the individual sampling sites or used as catch indices within the individual sites. Since these samples are limited to some habitats (shallow, nearshore habitats), it is expected that both local population estimates and catch indices would provide a biased estimate of eel abundance, but both types could be valuable for evaluating trends in eel abundance. The local population estimates could reduce variation in the overall population indices.

We evaluated both local population estimates and catch indices for the Delaware River eel sampling. There are several considerations in designing a shoreline monitoring program for eels, relating to precise sampling protocol, range of habitats sampled and fixed versus variable site location. These decisions will usually involve tradeoffs between 1) more precise, but labor-intensive sampling versus less precise, simpler sampling, potentially allowing greater sample sizes; 2) more extensive, but more variable, sampling providing statistical information across a range of habitats versus less extensive, less variable sampling which requires assumptions about relationships between eel abundance in sampled and un-sampled habitats. These tradeoffs are inter-related since decisions on sampling sites affect differences among protocols.

Estimating abundance is analogous to estimating concentrations in quantitative chemistry; estimation needs to deal with incomplete recovery. The typical approach is calibration using reference material or spiked samples. These data can be used in two ways: 1) to calculate and use average % recovery to correct sample estimates; or 2) defining an acceptable range of % recovery and discarding sample data outside the range. While introduction of marked fish into sample areas, directly analogous to spiked samples, has been used for population estimation, it is typically unpractical for sampling small areas, where retention and normal behavior could not be guaranteed. However, intensive sampling in some sites could be used to produce accurate population estimates which could be used for correction or to assess data acceptability

Sampling protocols could use single-pass or depletion sampling, producing catch index or population estimates. Depletion sampling is less biased and less sensitive to differences in catchability among sampling locations and dates. This ability to estimate catchability for each sample is useful for the shoreline sampling, where differences in sample habitat among samples requires different protocols. For example, in areas of low current velocity, sampling in an upstream direction was often most effective, since sediment raised during sampling would obscure the sample area were sampling done in a downstream direction. In these habitats, many eels were detected and captured by netters as stunned eels drift slowly. In areas with high current velocity where fine sediment is not a problem, sampling in a downstream direction was more effective. In these situations, electrofisher operators and netters could follow stunned or partly stunned eels as they drifted downstream. If sampling were done in an upstream direction, eels would frequently move and drift downstream too quickly to be captured. However, depletion sampling takes more time, reducing the total possible sample size for a given amount of total effort. The actual sampling for depletion sampling took about twice (two passes) to four times (four passes) the time of single-pass sampling. However, the difference in total sampling time is much lower when tasks with fixed time, such as setting up gear, recording data, measuring habitat and moving to and between sites, are considered. Catchability of eels appeared to be high in all samples, so that single-pass estimates provide a reliable index of total population.

However, catchability did vary among sites, reducing precision of single-pass abundance estimates. By limiting sampling to sites with high catchability, e.g., shallower sites, single-pass precision may be increased, although that incurs problems with inferring river trends from sample data (see next section).

Depletion estimates (Zippin 1958) rely on the pattern of decrease of capture of previously uncaptured individuals (unmarked if fish are not removed between passes; all fish if fish are removed) on consecutive passes through an area. The assumptions of these methods are analogous to those of mark-recapture: no change in population size between passes and equal probability of capture of individual fish on each pass. Estimates are usually made over naturally or artificially blocked areas over short time periods to meet the first assumption. Violation of the second assumption has been attributed to decreasing vulnerability of individual fish on consecutive passes (Cowx 1983), resulting in underestimates of population size. Underestimation due to decreasing capture probability has been documented (Riley and Fausch 1992, Hedger et al 2013), although Hedger et al. found increasing capture probability in a number of samples. The most critical form of decreasing vulnerability is caused by escapement of fish from the sample area and occurrence of fish in or movement into inaccessible habitats, e.g., under rocks and in root wads. In contrast, two factors increase probability of capture between consecutive passes: as the number of fish in an area decrease, fewer fish are missed because of attention to other individuals; and individuals may be more likely to be caught if chased into vulnerable habitats. If more than two passes are made, changes in capture probability among passes (first to second versus second to third, etc.) can be assessed. Population estimates can be modified to account for these changes, e.g., using the second and subsequent passes to estimate population at the end of the first pass and adding the fish pass catch total to estimate initial population size. While the Zippin method may underestimate population size in many situations, it can overestimate population size where there is little decrease in catch rate across different samples.

Depletion estimates of eels within blocked stream reaches has been done in numerous studies (e.g., Kwak et al. 2007, Roghair et al. 2014, Horwitz, et al. 2008). The sampling protocol of the SUDOANG program (SUDANG 2019), a regional European Eel monitoring project in Southwest Europe (SUDOE, including Spain, France and Portugal) is like that used here, except that it does not use stream blocks. The protocol prescribes two-pass electrofishing samples, covering the entire stream width where feasible and only the shoreline in deeper channels. The protocol does not describe data analysis.

The maximum likelihood Zippin estimator is commonly used to estimate capture probability and population size. Carle and Strub (1978) proposed a modified estimator, a weighted maximum likelihood estimator. The Carle and Strub estimator (hereafter CSE) can use informative prior information to increase accuracy of population estimates. The CSE is more robust and can be used where the Zippin estimator is infeasible, e.g., with non-decreasing catch pattern. However, the CSE may fail if only two passes are used (Pisces 2022).

The capture probability could be affected by habitat conditions, especially depth, and by the number of blocks used. Empirical estimates of the relationship between capture probability and other factors can be used to improve the accuracy of population estimates. Hedger, et al. (2013) found that total number of fish captured was the best predictor of capture probability, and other

habitat parameters were not useful. Riley and Fausch (1992) did not find correlations between habitat factors and capture probability.

This study looked at sampling with different numbers of blocks. If significant numbers of eels escape when no side block is used, the capture probability will be biased high, since fewer eels are present in the site on subsequent passes. The use of blocks was not randomized with respect to habitat, so results are not strictly comparable. Samples with no blocks had lower estimated catch probabilities than samples with one or two blocks. There was no obvious difference in catch probabilities for one versus two blocks. Two blocks may be more reliable for depletion sampling, since it reduces undetected escapement of eels, which leads to a violation of a primary assumption of depletion estimation. We used seines with extra weights on the bottom for both side and downstream blocks. Weight could be reduced on the side net, making it easier to set up samples.

Another approach is to use information from prior studies or from the entire survey (the set of all individual samples) to improve estimation. For example, an estimate of population size can be developed for single-pass samples, using an estimate of capture probability from other studies. Hedger et al. (2013) found higher variance in this technique than in other estimators. However, this approach may be successful with eels in shoreline or small stream samples, because of the high capture probability. This approach would be analogous to chemical quantitation, as discussed above. For example, on each sampling day at each location, multi-pass depletion sampling of enclosed areas could be used as calibration samples. The primary sampling at the site could be single-pass or multi-pass with fewer blocks. Bayesian analyses provide a more formal approach to using prior information. For example, the CSE can use informative priors to improve accuracy and precision of depletion estimates (Hedger, et al. 2013). Hedger et al. used depletion sampling in open and closed river situations. They found the CSE with informative priors provided little or no improvement in population estimates in the closed systems, but the CSE was superior to other methods in the open systems. They attributed the difference in efficiency of the CSE to absence of escapement in the closed situations, so that other estimators performed adequately. Wyatt (2002) has developed hierarchical Bayesian models which can use both single and multiple pass samples from a survey to provide a total population estimate.

Considerations for selection of individual samples sites for estimation of population characteristics are like those in other statistical surveys (e.g., Kalton 1983, Milliken and Johnson 1989, Thompson and Seber 1996). These involve considerations of variance and bias. Techniques to reduce variance include stratification and inversely weighting estimates by sample variance. Reduction of bias depends on representative sample designs or weighting systems for non-representative samples (Thompson and Seber 1996). We expect that without the ability to sample eels in the center of riffles and deep pools, any estimate will be biased, but an index over habitats which can be sampled can still be useful for trend detection and spatial comparisons. The selection of sampling sites with respect to river position and specific habitat affects precision. Sampling a wide range of habitats is likely to lead to more variable abundance, greater problems with sampling efficiency, and potential inefficiencies in sampling relatively uninformative, low-density sites. However, sampling a narrow range of habitats means that assumptions about density in those sites compared to the river are necessary. For example, it is possible that eels may reach high densities in preferred habitats and move into less preferred

habitats in years with high total eel abundance. If so, sampling of preferred habitats could be relatively insensitive to river-wide trends in abundance. The same reasoning applies to sampling stations—downstream stations appear to have higher densities, but densities in these stations may fluctuate less than the total eel population.

The decision to use fixed sampling sites, i.e., to sample the same locations over different surveys, versus randomly selecting different specific sampling sites for each survey involves similar issues of representativeness. The densities in fixed sites could vary less than river densities, as discussed above. Densities in fixed sites could vary more than river densities, e.g., if habitat suitability within each varies with river level or with changes in channel form. There is finite shallow, accessible nearshore habitat in the river, which could limit the ability to find enough suitable sites to allow a random selection of a suitable number of sites.

This study cannot provide definitive specifications for an eel monitoring survey, especially since the protocol will depend on availability of staff. We make the following recommendations:

- 1) We think that depletion sampling is preferable if sufficient staff are likely to be available across a number of surveys. If not, single-pass sampling would still provide useful data.
- 2) The use of two blocks did not obviously improve sampling efficiency, but the second net may reduce undetected escape of eels. The second block either increases set up times for the same number of staff or requires an additional person to set up the side block. Therefore, one block at the lower end of the survey station is recommended, but use of some two-block samples would allow assessment of escapement. If staff is limited and single-pass sampling used, use of no blocks may be acceptable if sampling is limited to relatively shallow sites.
- 3) Sampling should be limited to sites with a maximum depth; based on the data from this study, a maximum depth of less than 0.8 m is reasonable.
- 4) Sampling should be limited to avoid sites with expected low densities of eels but should not target a “best-available” habitat. Habitats with at least moderate amounts of cobble and boulders and without large amounts of gravel, sand and silts should be sampled. Habitat measurements should be taken, allowing analysis and adjustment for habitat variability.
- 5) Sampling should be done at different longitudinal positions in the river.
- 6) Sampling should be avoided in areas which were recently exposed (e.g., within one month) and should be done only at times of moderate to high water clarity.
- 7) A list of suitable sampling sites (the sampling frame) should be developed, from which a sampling list can be generated each year. If the frame includes a range of potentially suitable sites, the frame should be usable across years. If selected sites are not suitable in a given year, e.g., due to water level, an alternate can be sampled. This study only used sites with public access or where landowner access was obtained. Additional sampling areas may be located if sample access is done by boat or if additional private access is available.

The use of a mixed sample design, e.g., with some multiple pass samples with two blocks and some single pass samples could provide a more efficient sampling program.

Another possible approach for monitoring eels would be measurement of density at the mouths of tributaries which enter the Delaware River within DEWA. We have done depletion sampling of tributary reaches in the past (Horwitz et al 2008), so this is a feasible sampling technique. To the extent that tributary populations are correlated with river populations, tributary sampling could be used as an index for trend monitoring. As with the shoreline sampling, a hybrid program using both tributary mouths and shoreline samples could also be developed.

IVB. Age-length relationships

The estimated age of eels in this study ranged from 1 to 11 years, and younger eels, age 2-4, were estimated to comprise most of the population. Much older eels are documented in many freshwater systems, especially in northern drainages (e.g., greater than 16 years, Facey and LaBar 1981, Goodwin 1999, Oliveira and McCleave 2000, Machut et al. 2007, Jessop et al. 2009, COSEWIC 2012). On average, the length of eels did not increase much after age 7, and age of maturation may reflect decreased opportunity for future growth (and higher fecundity) relative to risks of mortality.

Age is calculated relative to settlement and transformation into the yellow eel phase. Eels estimated to be Age 1 were moderately frequent in samples. These eels ranged in length from 17.7-28.8 cm. The absence of age 0 eels indicates that it takes at least a year for eels to migrate from the lower Delaware River/Delaware Bay. Observations of glass eel movement into tributaries of the lower Delaware River (Horwitz, et al. 2007 and unpublished data) found glass eels as far upstream as Pennsbury Manor (river mile 125), 245 km (152 miles) downstream of the lowermost station in the current study. In this study, the relative abundance of age-1 eels was lower than that of age-2 eels, suggesting some eels take more than 1 year to move upstream into the sample. Collections of small yellow eels in downstream tributaries (Horwitz, et al., 2007) in summer (July 5-August 4, 2006; June 8-29, 2007) consistently found modal lengths of 6-7 cm and 10-12 cm (Figure Appendix 1.1-1.3), in addition to less evident modes at larger sizes. The mode of 6-7 cm is consistent with the length of glass eels and newly transformed yellow eels (Horwitz, et al. 2007). In the early-June 2007 sample (Ridley Creek), there was an evident shape difference in girth between smaller eels (confirmed by length-weight analysis), consistent with the smaller eels being recently transformed from the glass eel stage. Based on these observations, the mode of 6-7 cm was interpreted as newly transformed yellow eels, and the mode of 10-12 cm eel was interpreted as age 1 eels. Tuckey and Fabrizio (2020) found a similar pattern in spring sampling in Virginia using eel ramps. They found two length modes, and interpreted eels less than 8.5 cm as YOY and greater than 8.5 cm as elvers, consistent with earlier studies by Geer (2001).

The sizes of age 1 eels in the present study are much larger than those collected farther downstream in spring. This difference suggests significant growth during age 0 and age 1 (through early fall), despite energetic demands of upstream movement. It is possible that larger eels are more likely to migrate upstream, so eels started moving upstream at lengths greater than 10 cm. It would be valuable to integrate length and age data from different parts of the drainage at different seasons.

Only three eels greater than 60 cm were caught in this study, all in boat electrofishing samples, comprising 0.35% of eels caught with that technique. This is like proportions in samples for the NPS inventory (Horwitz, et al. 2014), where 0.30% eel caught by boat electrofishing eels and 0.20% of eels caught in tow-barge electrofishing were greater than 60 cm.

Larger eels have regularly been caught in ANSDU samples from other parts of the Delaware Drainage. Eels greater than 70 cm have been caught in Piedmont tributaries, Coastal Plain tributaries and lakes of the tidal Delaware River and Bay, and tributaries of the Delaware River downstream of the Delaware Water Gap. Eels of 71 and 77 cm were caught in the mainstem at the mouth of the Lehigh River and in the freshwater estuary. In the northern part of the drainage, eels of 60-74 cm total length were caught in Swartswood and Steenykill Lakes, in Dingmans Creek, the Raymondskill and the Neversink River. There are many literature reports of yellow or silver eels greater than 75 cm long (78 cm Strickland 2002; 85 cm (Machut et al 2007), 88 cm (Olivera and McCleave 2000), 93 cm (Jessop et al. 2009), 93 cm (Denny et al. 2013), 98 cm (Goodwin and Angermeier 2003), with extreme sizes of 1.3 m (Goodwin 1999; Kahn 2019).

Given the high variability in length and age, these large eels could represent older or faster-growing fish. Many of the larger eels were in parts of the drainage likely to have higher nutrient concentrations, which may generate more food. Larger eels also occur in situations such as lakes and above dams which are partial passage blocks. In these sites, eel densities are often low, possibly permitting higher growth rates. For example, the largest eel encountered, 115 cm, was from Manatawny Creek, a Schuylkill River tributary, in which only a few eels were caught in extensive collecting (Horwitz et al. 2001).

IVC Parasite Load

There was only a weak relationship between eel size and number of *A. crassus* in the swim bladder, although very high number of nematodes were only found in large eels. If *A. crassus* infection created high mortality, lower numbers of nematodes in larger eels would be expected, i.e., only uninfected or weakly infected eels survived and grew. This pattern was not seen in this study.

Surveys of *A. crassus* have been done in Europe, North America and Asia, with studies looking at European, American, and Japanese Eels (e.g., Nagasawa et al. 1994, Genc, et al. 2005, Kennedy and Fitch 1990, Audenaert et al. 2003 Kangur et al. 2002, Warshafsky et al 2019). Studies have been done in wild fish as well as in cultured fish. The rapid increase in prevalence in short periods of time (30% in three years in the Chesapeake Bay; Warshafsky et al. 2019), size/age related differences in infection and variability among sites at any given time makes it hard to compare patterns of prevalence, incidence and damage. In general, correlations between nematode abundance and swim bladder damage are weak, but significant differences in growth or survival have been noted in infected eels in some studies.

For North America, Aieta and Oliviera (2005) sampled Eels from a number of streams throughout Eastern Canada and New England in 2005-2007. They found relatively high prevalence in eels from Rhode Island, Massachusetts, and Maine north to the St. George River. Prevalence ranged from 0.07 to .76, with a median of 0.53. The median incidence was 3.1, with a

maximum of 11 *A. crassus*. There was a sharp decrease in prevalence north of the St. George River, with *A. crassus* found in only two (Sheepscot River and Penobscot River, ME) out of 20 streams from Maine to Quebec. *A. crassus* was found in Eels from the commercial catch from New Brunswick and Nova Scotia, but not from Prince Edward Island or Newfoundland. However, in 2009-2010, Kenny et al. (2013) found higher incidence in a northern site, with incidence of 0.46 in the Bras d'Or Lakes, Nova Scotia. Among infected Eels, incidence varied from one to 75 *A. crassus*. Prevalence and intensity of infection increased in tributaries of the St. Lawrence River between 2010 and 2018; Machut and Limburg (2008) investigated occurrence in Eels along longitudinal gradients in six tributaries of the Hudson River. Prevalence ranged from 0 to 0.60 at individual sites and 0 to 0.52 across the six tributaries. They found higher incidence in eels downstream of barriers and in more urban drainages. For example, prevalence was 0.43 in the more downstream sites across all streams, compared to 0.10 for the more upstream sites. Across all streams, incidence varied from 1.7-2.8. There have been several studies in the Chesapeake Bay Drainage (Fenske et al. 2010, Zimmerman and Welsh 2012, Warshafsky et al. 2019). In tributaries of Chesapeake Bay, Fenske et al. found highly variable rates of prevalence across tributaries, ranging from 0.18 to 0.72, with higher prevalence in up bay tributaries than in those farther south. They found no relationship between prevalence, swim bladder damage and growth rate. Warshafsky et al. found a correlation between swim bladder damage and nematode abundance. They also found lower survival in Eels with *A. crassus*. Low prevalence (0.02) was found in Eels from an Eel Ladder in the Shenandoah River, Potomac River drainage (Zimmerman and Welsh 2012). They also found a relationship between length-at-age and infection. Hein et al. (2014) studies infection in 2011-2012 in Eels from South Carolina. They found a prevalence of 0.45 of parasites in the lumen and 0.58 for any stage. The mean intensity was 3.0 with a maximum of 15.

IVD. Trophic ecology

Eels are frequently cited as having a wide dietary range, including aquatic macroinvertebrates and fish (Denoncourt and Stauffer 1993). Fish are often more frequent in larger eels (Ogden 1970, Lookabaugh and Angermeier 1992, Waldt et al. 2013). In this study, only one fish was found in the guts of the 318 eels analyzed, although some unidentifiable organic matter may have been fish. Insects were the most frequent group in the gut contents. Crayfish were also frequent. Given the relatively large size of crayfish, they form an important part of eel diet in the study area.

The stable isotope analyses found that the $\delta^{15}\text{N}$ values of some eels caught in 2019 and 2020 were higher than those of smaller fish, which in turn had higher $\delta^{15}\text{N}$ values than many macroinvertebrates. These isotope values suggest that fish form part of the diet of many eels. However, $\delta^{15}\text{N}$ values in eels from the Delaware River and two tributaries in 2018 were higher than the 2019-2020 values, suggesting a lower importance of fish in the two later years. The $\delta^{15}\text{N}$ were also related to $\delta^{15}\text{C}$ values in the 2019-2020 eels, with higher $\delta^{15}\text{N}$ in fish with less-depleted (higher) $\delta^{15}\text{C}$ values. The $\delta^{15}\text{N}$ did not increase with increasing fish size, consistent with finding insects as a frequent food item in eels of all sizes.

As noted in the results section, the difference between 2018 and 2019-2020 samples could be explained by differences in flow among the years, with higher discharge in 2018, especially in

August and September, than in 2019 and 2020. While the causal link between discharge and isotope patterns cannot be determined, differences in flow could affect many aspects of river ecology, related to both watershed and in-stream processes. Higher flows might contribute more runoff from the drainage, including movement of fertilizer from agricultural areas. The amount of wetted area changes with discharge, so algal production might vary among years. In a Virginia study, the contribution of autochthonous carbon (from phytoplankton) to growth of larval American Shad decreased with increasing discharge (Hoffman et al. 2007).

It is possible that differences in collection procedures created differences among years. The eel samples for stable isotope analyses were taken in similar locations across the three study years. Most samples were from Buckingham and Lackawaxen in all three years, so location does not seem a factor in the among-year differences. The 2018 samples were taken later in the year than the 2019-2020 samples, so it is possible that the observed differences reflect seasonal changes in diet. The 2018 samples would have contributions from feeding in October. Thus, types of food more available in October would be evident only in the 2018 samples. The 2018 samples might have a higher proportion of summer food and lower proportion of winter food than the 2019-2020 samples. The differences in flow between 2018 and 2019-2020 were most prominent in August and September, and later-collected fish would have a lower contribution from earlier feeding.

Many aquatic macroinvertebrates overwinter and grow as nymphs and emerge in late winter-early spring. Other taxa occur as nymphs in the spring and summer and emerge at various times from spring through fall. It is possible that eels utilize the high densities of aquatic macroinvertebrates in late winter and early spring. While some small fish are available year-round, the main pulses of availability occur in spring through fall, with appearance of young-of-year fish. Movements of small fish in the fall, such as out-migration of juvenile American Shad, might also provide a food source for eels. Another possible source of enriched $\delta^{15}\text{N}$ is carrion, analogous to nutrient subsidies to streams by Pacific salmon (Wipfli et al. 2003). In the Columbia River Basin, introduced American Shad import marine nitrogen, to some extent replacing historical inputs from salmonids (Haskell 2017). All adult Sea Lamprey and many adult American Shad die after spawning, and their carcasses or organisms feeding on their carcasses could be food sources of American Eel. Most growth of American Shad and Sea Lamprey occurs in estuaries or the ocean, and marine organisms often have higher $\delta^{15}\text{N}$ values than analogous freshwater organisms (France 1994). American Shad feed on invertebrates and Sea Lamprey is parasitic on a variety of fishes, so that $\delta^{15}\text{N}$ may be relatively high in these taxa, especially Sea Lamprey. Shad and Sea Lamprey carcasses are present mainly in late spring and early summer, so feeding on these carcasses or the carrion-based food web could also create seasonal patterns in stable isotopes.

The contribution of food from different times depends on turnover time of fish tissue. In a laboratory setting, Eberhardt (2019) analyzed the rate of response of tissue $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ following a switch in diet from 10% to 108%. Muscle tissue turned over relatively slowly; at 35 days, muscle $\delta^{15}\text{N}$ was less than 50%, i.e., about half the dietary $\delta^{15}\text{N}$. However, the eels lost weight during the study. Wild fish would likely show faster change in isotopes because of growth dilution.

Seasonal physiological cycles could also create differences in isotopic patterns. Lipids are more depleted in ^{13}C than muscle tissue. Therefore, changes in lipid content, such as increased lipid content before winter, would create difference in isotopic patterns between the October (2018) and September (2019-2020) samples could create the observed differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$.

There were small differences in preparation among the years, although these differences seem less important than the differences in river conditions and collecting timing. The 2018 samples were analyzed in 2019, along with the 2019 samples, so the difference is not obviously an analytical artifact. The 2018 eel samples were fileted by a different individual than those from 2019 and 2020, but the preparer of 2018 samples was trained by and under supervision of the preparer of the other samples. It is possible that slight differences in preparation, e.g., amount of subdermal fat included in the filet, affected isotope concentrations. The 2018 samples were stored frozen for over a year, while the 2019 and 2020 samples were stored for shorter periods. Barrow et al. (2008) reported no effect of freezing on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for storage up to 30 days but found statistically significant depletion of both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ at 60 days. However, they reported another study which found enrichment of $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ by freezing, and nine other studies which found no effect. There was a change in specimen handling after 2018. In 2018, laboratory procedures (swim bladder and gut analyses, preparation of filets) were done at the same time as otolith removal. In 2019-2020, after discussion with J. Casselman, specimens were refrozen after otolith removal, to facilitate otolith preparation. The laboratory analyses were done after thawing a second time. We know of no reasons that this difference would affect stable isotope values.

In all years, there was no relationship between $\delta^{15}\text{N}$ values and eel size, suggesting that the importance of fish did not increase with fish size. Thus, a range of sizes of eels were represented in fish with both higher $\delta^{15}\text{N}$ values (higher trophic level) and intermediate $\delta^{15}\text{N}$ values.

V. Additional data needs

The results of this investigation provide important baseline data for age distribution and abundance not previously established for the Delaware, as well other rivers in the mid-Atlantic and Northeast. This study can promote new research on eel demography and ecology of eels, particularly in the study area and other parts of the Delaware drainage.

This study provides a basis for an abundance monitoring program. The most practical form of this program would be developing an abundance index based on abundance in accessible shoreline habitats or in tributary mouths. The sampling protocol would depend on likely availability of staff, since more staff would allow more robust protocols, i.e., less sensitive to variable catchability and eel escapement from sampling areas.

The age-length analyses provide intensive information about eel growth. As is typical of eels, there is great variability in length within age classes, precluding accurate estimation of age structure from length distributions. An important need is linking observed growth patterns in the study area with migration and growth in other parts of the basin. These linkages would assess patterns and timing of movement of young yellow eels and potential trade-offs between eel density, food availability and energetic costs of movement.

The information on prevalence and incidence of *A. crassus* provides a baseline for future analyses

and comparisons with other regions. As with many studies, there were relatively weak relationships between measures of incidence and fish size, and between measures of incidence and swim bladder damage. More work linking incidence, swim bladder damage and effects on eel growth or survival would be important. The complexity of these relationships stems from the dynamics of *A. crassus* within eels and transmission among eels.

The stable isotope analyses showed several intriguing patterns related to possible among-year differences in carbon sources and the amount of piscivory of eels. This study did not find evidence of increase in trophic level with increasing eel size, which has been noted from other studies based on gut analyses. Furthermore, trophic level of eels was lower in 2019 and 2020 than in 2018, when many eels had the highest $\delta^{15}\text{N}$ values among taxa analyzed. Based on $\delta^{13}\text{C}$, there were two main sources, consistent with algal- and detritus-based food web. In 2019 and 2020, eels showed evidence of food from both sources, but 2018 eels appeared to feed primarily on one of the webs. These differences could be related to among-year differences in flow and/or among-season difference in feeding. Higher resolution sampling over different seasons and across more potential food sources would be important to resolve these differences. Eberhardt (2019) found a more rapid response of mucus isotope patterns than muscle tissue following a change in diet. Analysis of mucus could provide more information on feeding over shorter time periods, as well as allowing sampling without killing eels.

Co-ordinated studies in different parts of the drainage, analyses of feeding across the growing season, and further development and implementation of abundance monitoring would provide a more comprehensive understanding of eel ecology, demography and population trends in the Delaware Drainage.

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Appendix 1. Figures from Horwitz, et al. 2007.

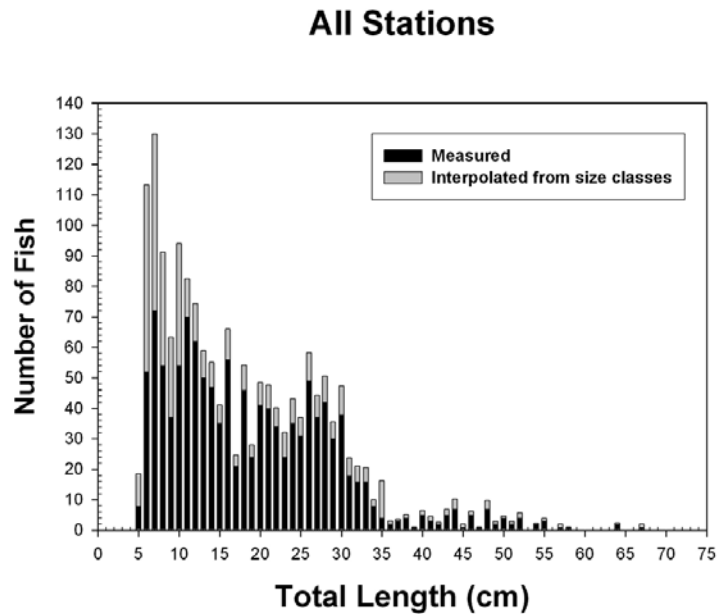


Figure A.1. Histogram of measured and interpolated lengths of yellow eels caught in 2006 in tributaries of the lower Delaware River in Pennsylvania, Neshaminy Creek downstream to Marcus Hook Creek. Figure is taken from Horwitz, et al 2007.

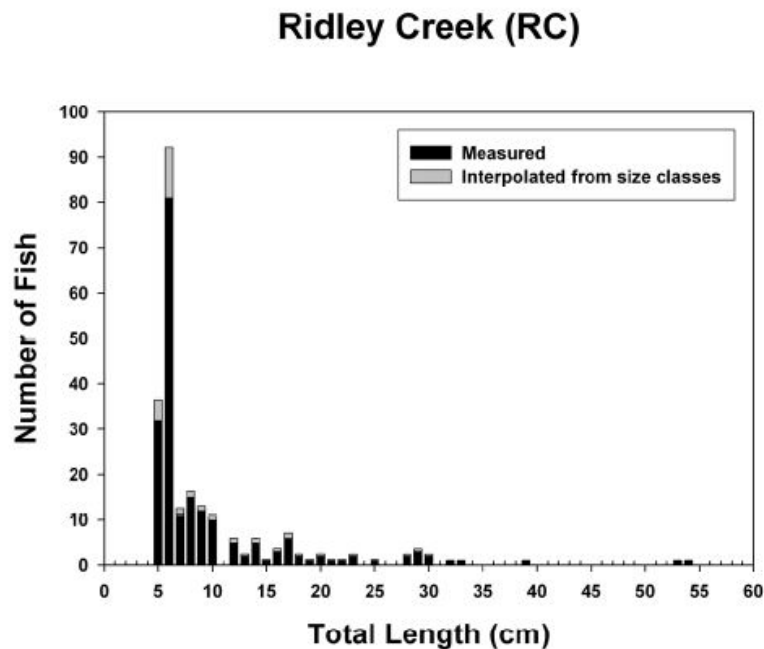


Figure A.2. Histogram of lengths of yellow eels caught in Ridley Creek, PA, on June 8, 2007. Figure is taken from Horwitz, et al 2007.

Tacony Creek (TCJ)

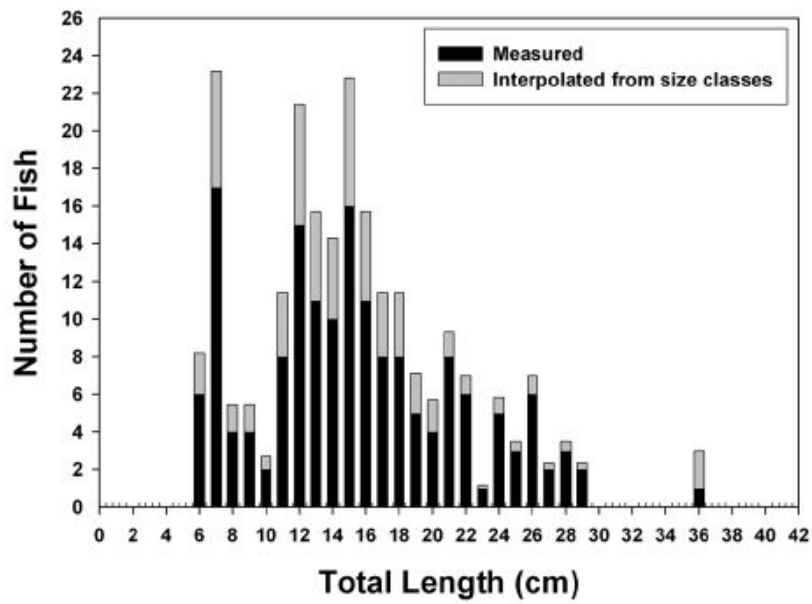


Figure A.3. Histogram of lengths of yellow eels caught in Tacony Creek, PA, on June 28, 2007. Figure is taken from Horwitz, et al 2007.