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DIET AND PREY AVAILABILITY OF STURGEONS IN THE PENOBSCOT RIVER,
MAINE

by

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A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Marine Science)

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Abstract

Although vital to the protection and conservation of species listed under the U.S. Endangered Species Act, critical habitat of shortnose sturgeon and Atlantic sturgeon in the Penobscot River, Maine have not yet been described. Critical habitat includes food availability as well as the physical characteristics of foraging habitat. To characterize seasonal availability of benthic prey, a ponar grab was used to collect over 125 benthic samples between 21 May and 8 October 2012. Samples were stratified throughout the river and broadly categorized by sediment type. All organisms within samples were identified to the family level. To characterize diet, stomach contents were collected from eight Atlantic sturgeon and sixteen shortnose sturgeon using gastric lavage. Fifty-six percent of shortnose sturgeon and 33% of Atlantic sturgeon had empty stomachs. In the upper river, characterized by a freshwater environment, all of the lavaged sturgeon had empty stomachs. No successful ponar grabs were taken in the upper river because of compacted sediment and cobbles obstructing the grab. In the middle river there were no sturgeon caught and the benthic community had more freshwater benthic organisms than marine organisms. In the lower river, characterized by a brackish water environment and brackish water benthic community, only 7% of the lavaged sturgeon had empty stomachs. In the lower river 81% of ponar grabs were successful and no seasonal differences in species diversity were apparent. Spionid polychaetes were not only the most available prey in substrate samples (over 75% by abundance) but also in the diet (over 75% for both species). The distribution and abundance of spionid worms may provide an indication of critical foraging habitat for these species.

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Introduction

The United States Endangered Species Act (ESA) of 1973 was designed to protect at risk species from extinction as a result of human growth and development. A primary objective of the ESA is to preserve critical habitat, defined as “physical or biological features essential to the conservation of the species, which may require special management considerations or protection” (Endangered Species Act of 1973). The complex interactions of organisms with their environment are greatly influenced by changes to physical and biological habitat components (Polis *et al.* 1997). For example, benthic invertebrate communities often display patchiness related to substrate type with different habitats containing distinct fauna at different densities (Morrisey *et al.* 1992). The benthos of rivers and estuaries often contain annelids, crustaceans, insects and insect larvae, mollusks, and many other potential foraging resources for benthic fishes (Dadswell 1979, Jackson *et al.* 2002, Nilo *et al.* 2006). The benthic community is part of the biological component of critical habitat. Endangered benthic predators relying on the benthic community could be especially susceptible to any negative changes to this environment. Prey availability (and their associated relationships with substrate type, salinity and season), in such varied environments could help determine the presence or absence of such species.

The composition of benthic communities, especially in riverine and estuarine environments, is often influenced by extensive human use. The Penobscot River and its tributaries are no exception (Haefner 1967). Chemicals and toxins have been dumped into the water by wastewater treatment plants, pulp and paper mills and factories near the river (National Marine Fisheries Service 1998, Haefner 1967). Coal tar deposits and

mercury hot spots are still being discovered (Metcalf and Eddy 1994). All of these practices may have impacted benthic fauna and their predators (Metcalf and Eddy 1994). To this day the predominant substrate types of the Penobscot River and estuary reflect its industrial past. Woodchips, sawdust/silt, and *Mytilus* beds make up much of the substrate from south of Bucksport to Brewer (Metcalf and Eddy 1994). The depth of organic matter from wood (woodchips, sawdust, etc.) is as thick as three meters in some areas (Metcalf and Eddy 1994).

Improvements in wastewater treatment, curtailment of point source pollution and active logging practices, and the introduction of the Clean Water Act of 1972 brought about a new era for the Penobscot River (Atlantic Sturgeon Status Review Team 1997). With the elimination of these practices water quality improved substantially from the 1970s and continues to improve today. Efforts, such as the Penobscot River Restoration Project, are currently underway to further restore the river, remove dams, and return sea-run fish to their native habitats. This project will likely lead to further changes in the riverine and estuarine habitats, including changes to the benthic fauna.

Currently, the Penobscot River supports populations of shortnose sturgeon (*Acipenser brevirostrum*) and Atlantic sturgeon (*Acipenser oxyrinchus*). Both of these fish species are listed under the ESA and there are ongoing efforts to assess the population status of each species. Since 2006, research conducted by the University of Maine using acoustic telemetry elucidated the movements of shortnose sturgeon and Atlantic sturgeon in the Penobscot River and coastal Maine (Fernandes *et al.* 2010, Zydlewski *et al.* 2011, Dionne *et al.* 2013). Seasonal movements of both species within the Penobscot River are now well defined and predictable. In the spring shortnose

sturgeon move down river from their freshwater overwintering habitat and Atlantic sturgeon enter the river from the ocean. During this period, both species tend to reside in the lower river, near river km 21 (Fernandes *et al.* 2010). Throughout the spring and summer Atlantic sturgeon generally stay near this lower river location then leave the river entirely in the fall (Fernandes *et al.* 2010). Shortnose sturgeon move up river in the summer and aggregate near river km 37 in the fall and into the winter (Fernandes *et al.* 2010). The middle river generally serves as a corridor that the sturgeon use to move between the upper and lower locations, i.e., both species spend very little time in this section of river throughout the year. While acoustic telemetry has been extremely helpful in determining spatial and temporal patterns in sturgeon movements within the Penobscot River, understanding why these habitats are important is just as vital. The physical and biological features of these areas may play a role in why sturgeons are found in these locations. Both shortnose and Atlantic sturgeon diets and associations with prey, key components in understanding habitat use, have not been studied in the Penobscot River.

Sturgeons are benthic fishes and primarily feed on benthic organisms. A sturgeon mouth is in the inferior position and can protrude several inches, making it ideal for ingesting prey from the benthos (Brosse *et al.* 2000). In the St. John River estuary in New Brunswick, Canada, juvenile shortnose sturgeon are nonselective feeders (Dadswell 1979). Commonly observed stomach contents of juvenile shortnose sturgeon include muddy substrates, insect larvae (Ephemerae, Chaoboridae, Chironomidae) and occasionally small crustaceans (Gammaridae, Assellidae, Anthuridae) (Dadswell 1979). Nilo *et al.* (2006) reported that although juvenile shortnose sturgeon are opportunistic feeders, they do show some level of selection for amphipods.

Adult sturgeons are more selective in their prey choice than juveniles. Stomach contents of adult shortnose sturgeon captured in the St. John River estuary indicated selection of small mollusks and gastropods as prey, even if mollusks were not the most abundant prey item in the area (Dadswell 1979). Selection of prey items is common among adults of other sturgeon species, such as lake sturgeon and shovelnose sturgeon (Jackson *et al.* 2002, Rapp *et al.* 2011). Recently, invasive zebra mussels have been identified in the diet of lake sturgeon, leading to interesting questions about prey selection and changes from historical prey items (Jackson 2002). The diets of adult Atlantic sturgeon are less studied. However, polychaetes and isopods are dominant prey items from sturgeon in marine habitats off the New Jersey coast (Johnson *et al.* 1997).

Gastric lavage is a common, non-lethal technique for collecting stomach contents from sturgeon (Brosse *et al.* 2000, Nilo *et al.* 2006, Rapp *et al.* 2011, Usvyatsov *et al.* 2012). Gastric lavage is efficient at recovering stomach contents in the Siberian sturgeon (*Acipenser baeri*), suggesting it may be a good technique for collecting diet samples of other sturgeon species (Brosse *et al.* 2002). Wanner (2006), likewise, found that gastric lavage was a safe and efficient method for collecting stomach contents from hatchery raised pallid sturgeon (*Scaphirhynchus albus*). Pallid sturgeon were fed different and distinct diets, and food items were recovered from 100% of individuals that had food items in their stomach, however the recovery rate varied by prey item. The average recovery rate was about 74% by weight and soft bodied organisms had a higher recovery rate than organisms with exoskeletons. Brosse *et al.* (2002) examined the efficiency of gastric lavage on the Siberian sturgeon (*Acipenser baeri*) and found that the average recovery rate was 67.5% but varied depending on organism type. Unlike Wanner (2006),

Brosse *et al.* (2002) found vermiform organisms had significantly lower recovery rates than larger prey. According to both studies (Brosse *et al.* 2002, Wanner 2006), high recovery rates via gastric lavage are possible up to two hours after the sturgeon have last fed.

Benthic communities, i.e. potential sturgeon prey, vary spatially and temporally. In the Penobscot River little is known about prey availability in the areas where sturgeon spend most of their time (between river kms 21 and 42). Most of the available data on benthic communities in the Penobscot River are for river reaches south of river km 20 (Larsen and Johnson 1985; Haefner 1967; Metcalf and Eddy 1992; Metcalf and Eddy 1994). Metcalf and Eddy (1994) reported that pollution tolerant species were common and that the dominant invertebrate species able to colonize the wood chips and sawdust was the spionid polychaete *Marenzelleria (Scolecoides) viridis*. Dadswell (1979) reported that although spionids were present in the benthos in the St. John River estuary they were negatively selected for by adult shortnose sturgeon. In the lower Penobscot River, crustaceans and *Mytilus* beds are also common (Metcalf and Eddy 1994). However, Metcalf and Eddy (1994) also noted several species which were noticeably absent such as the bivalves *Mya arenaria* and *Macoma baltica* as well as aquatic insects, which are common prey items for sturgeon in other rivers and estuaries (Dadswell 1979). They noted that aquatic insects may increase in abundance upstream of their study sections, in reaches with fresher water (Metcalf and Eddy 1994). Overall, Metcalf and Eddy (1994) described that prey availability is low due to the large amount of wood chips in the lower river and that this may inhibit feeding for sturgeon, limiting critical habitat. Substrate composition could limit the presence of prey items and in turn limit the

availability of energetic components necessary for growth and survival (Metcalf and Eddy 1994). Studies have shown that sturgeon adjust their diet seasonally and with age so sturgeon may have an ability to cope with species changes or shifts in benthic fauna (Dadswell 1979, Chiasson *et al.* 1997, Brosse *et al.* 2000).

This research was conducted to provide a greater understanding of potential foraging areas for shortnose sturgeon and Atlantic sturgeon and to begin to characterize critical habitat that needs to be protected to aid in the recovery of these fishes. Data collected were used to assess temporal and spatial differences in the benthic communities of the Penobscot River and to characterize any sturgeon-prey associations, both of which were unknown. This study provides a greater understanding of potential foraging areas for shortnose sturgeon and Atlantic sturgeon and helps characterize critical habitat that needs to be protected.

Methods

Sturgeon Presence

Annually, since 2004, an array of acoustic receivers (Vemco) were deployed in the Penobscot River from upstream of river km 47 to downstream into Penobscot Bay, with receivers spaced at regular intervals throughout the range. Twenty-one shortnose sturgeon and 24 Atlantic sturgeon previously implanted with active acoustic tags were available for detection by Penobscot River receivers throughout the spring, summer and fall of 2012. The acoustic tags ping about every two minutes and every tag has a unique code that is decoded and recorded (with a date-time stamp) by any receiver within approximately 1 km of the tag. The number of detections a receiver decodes can be used as a proxy for the length of time a sturgeon spends near a receiver. The number of detections and the number of tags recorded by a receiver in each stretch of the upper river (river km 34-43), middle river (river km 26-31), and lower river (river km 21-24; Figure 1) was used to estimate the residence time each species spent in these respective reaches. The percent of time sturgeons spent in each reach of the river was calculated by first dividing the total number of detections in a single reach during a single season by the number of tags decoded, this gave the number of detections per sturgeon. Then the total number of detections per sturgeon in all three reaches was enumerated over an entire season. Finally, the percent of detections in each reach was calculated by dividing the number of detections in a single reach by the total number of detections from all three reaches combined.

Prey Availability

Prey availability was assessed in three study reaches of the Penobscot River; the upper river (river km 34-43), middle river (river km 26-31), and lower river (river km 21-24; Figure 1). Based on sturgeon movements in previous years (Fernandes *et al.* 2010; Dionne *et al.* 2013), the upper and lower river sections are most frequently used by sturgeon whereas the middle section simply serves as a corridor. Benthic samples were taken in the spring (May and June), summer (July and August) and fall (September and October) from all three study reaches except the middle river where no benthic samples were taken in the summer (Table 1). Throughout each reach, multiple benthic samples were taken using an 8.2 L ponar grab, from which a 0.6 L subsample was analyzed for abundance and diversity of benthic marine fauna. The ponar grab consisted of two semicircular jaws held open by a trigger that snapped shut when it came into contact with the bottom, collecting a sample. Water depth (meters), bottom temperature (°C) and salinity (parts per thousand) were measured where ponar grabs were taken. Substrate was visually categorized into three broad groups: mud, sand and gravel. The amount of organic matter (such as wood chips or sawdust) was also noted. Fauna were sieved from the substrate and collected. Invertebrate samples were fixed in 5% formalin and later identified to family level in the laboratory.

Multiple samples from the same reach, during the same season, were compared to determine if pooling was appropriate. If species diversity (Simpson's diversity index) was not significantly different (ANOVA or t-test) between separate sites within the same reach the data were pooled. The Simpson's Diversity index ranges from 0-1 with 1 having no diversity and 0 being samples where all individuals are different. Only

successful grabs were used in the data analysis. A successful grab was one where the jaws were completely closed upon retrieval. The abundance and diversity of the organisms in the benthic grab samples was compared temporally and spatially. Relative abundance was determined as the percent occurrence per subsample and species density (individuals per m^2). Species density was calculated by dividing the number of individuals collected per sub sample by the length-wise area (m^2) of the sub-sampling core. If two subsamples were collected, the area was doubled. Percent similarity and Morisita's index of similarity were used to qualitatively assess spatial and temporal similarity in the benthic communities of the Penobscot River. Morisita's index of similarity takes into account diversity whereas percent similarity does not. A t-test was run to quantitatively compare diversity (using Simpson's Diversity index) between the middle river and lower river in the spring as well as between the spring and summer diversity of the lower river.

Diet Composition

Gastric lavage was performed (according to the protocol given by the ESA permit numbers 16306 and 16526) on eighteen shortnose sturgeon and eight Atlantic sturgeon caught in the upper and lower Penobscot River (Table 1). Sturgeons were captured using six-inch stretch-mesh multifilament gill nets, which fished anywhere from fifteen minutes to an hour depending on the water temperature. No fishing effort was made in the middle river. Gastric lavage is a non-traumatic procedure used to collect stomach contents from fish. A small tube connected to a pump (hand pumped garden sprayer) is inserted into the mouth to the stomach and water is pumped into the stomach, flushing any stomach contents. All gastric lavage collections were performed on adult and subadult shortnose

sturgeon and subadult Atlantic sturgeon as those were the only developmental stages captured. Collected stomach content samples were placed in 95% ethanol solution to be later identified to family level in the laboratory. Once in the laboratory, diet samples were transferred to a 70% ethanol, 5% glycerol solution for preservation, sorting and identification. The number of each prey item in the diet was enumerated for each family and the percent occurrence of each prey family in the diet was normalized for each sturgeon and averaged for all sturgeon caught in the same area and season to qualitatively describe differences among season and species. Empty stomachs were excluded from diet calculations. The diets of shortnose sturgeon and Atlantic sturgeon were qualitatively compared spatially, temporally, and between species when possible. The mean number and standard deviation of spionid worms was calculated for shortnose and Atlantic sturgeon and compared using a t-test. Additionally, diet composition was compared to organism composition in the benthic samples. Ivlev's (1961) electivity index ($E = (r_i - p_i) / (r_i + p_i)$) was used to quantify prey selection, where r_i is relative abundance of prey in the gut and p_i is relative abundance of the prey item in the environment.

Results

Sturgeon Presence

Shortnose sturgeon were detected in all three reaches of the river (upper, middle, lower) in the spring, summer and fall (Figure 2). In the spring they spent most of their time (72.5%, 44,263 detections) in the lower river vs. in the summer and fall when they spent most of their time in the upper river (81.7%, 92,141 detections; 95.7%, 102,792 detections, respectively; Figure 2). The numbers of detections in the upper river in the spring and in the lower river in the fall were 151 detections and 368 detections, respectively. The largest percent of time shortnose sturgeon spent in the middle river was 25.1% (9,693 detections) in the spring.

Atlantic sturgeon spent the majority of their time in the lower river, regardless of season (Figure 3). In the spring they spent 99.9% (87,456 detections) of their time in the lower river. Similarly, in the summer and fall they spent 90.4% (78,324 detections) and 73.9% (32,276 detections) of their time, respectively, in the lower river (Figure 3). Most Atlantic sturgeon detections and tag codes were recorded in spring in the lower river (87,456 detections, 15 tags). The numbers of detections in the middle river in the spring, summer and fall were 3 detections, 105 detections, and 1,402 detections, respectively. The numbers of detections in the upper river in the spring, summer and fall were 0 detections, 659 detections, and 1,082 detections, respectively.

Prey Availability

In total, 69 benthic samples were attempted in the upper river. The upper river had no successful benthic samples regardless of season or location (Table 2). The average

water depth of collection for the upper river samples was 6 m (± 1.8 m) and the salinity never exceeded 0.0 ppt during the periods sampled. Depth ranged from 2.5m to 9m and was dependent on tide and position in the river. More than 65% of the attempted grabs were empty (i.e., hit a compacted bottom and did not close). Cobbles and gravel were the most common substrate collected by the grab, however these substrates generally prevented the ponar grab from closing properly and thus small particles (sand, pebbles, organisms) may have been lost. Caddis fly larvae tubes (Trichoptera) and stonefly larvae (Plecoptera) were observed on some of the cobbles, however these were not considered in any analyses because the grab was obstructed and the sample size was unknown.

Fifteen benthic samples were attempted in the middle river, with a success rate of 80%. These were primarily sampled in the spring ($n=12$). The average water depth for the middle river samples was 7.5m (± 2.2 m; 3.5m - 9.5m). The maximum salinity recorded was 2.5ppt in June. All samples taken in over 9m of water were unsuccessful. The majority, 87.5%, of the benthic samples taken in shallower water were successful. Oligochaetes were the most abundant organism in the middle river, constituting 71.6% of the benthic community sampled (Table 2). These oligochaetes were most abundant in muddy substrate with high levels of organic matter (mostly decaying wood chips and sawdust) and had a density of up to 4,900 individuals per m^2 . Bivalves (10% by abundance) and chironomid larvae (9% by abundance) were the next two most abundant species in the middle river. The remaining organisms comprised relatively small percentages of the overall composition, 2-6% (Table 2). The diversity of this reach in the spring was 0.416 (Simpson's Diversity Index).

Forty-two benthic samples were attempted in the lower river with an overall 81%

success rate. Each season (spring, summer, fall) had a similar sampling effort (13 samples, 18 samples, and 11 samples, respectively; Table 1). The average water depth for the lower river samples was 9.6m (SD= \pm 4.1m; 1.5m - 22m). The deepest three samples (17.9m, 18.3m and 22m) contained no benthic invertebrates, however there was no correlation between depth and species diversity or depth and species abundance ($R^2=0.1334$ and $R^2=0.0571$, respectively). In May the lower river had a salinity of 0.0 ppt at depth and salinity reached a maximum of 15.5 ppt at depth at the end of July. In the spring, summer and fall spionid worms were the most abundant benthic organism, constituting 65%, 88% and 99%, respectively, of the total number of organisms in the benthic samples (Table 3). The fall sample only had two grabs that contained any organisms, one contained only spionids and the other contained only anthurid isopods. Mean spionid densities were 4,150 individuals per m² (SD= \pm 3,700 individuals) in the spring, 3,075 individuals per m² (SD= \pm 3,925 individuals) in the summer and 4,700 individuals per m² in the fall. Spionids occurred most frequently and in the highest abundance in sand (Table 4). The next most abundant organisms in the spring were gastropods at 18%, and in the summer ampharetid worms constituted 7% of the organisms. The diversity of benthic species in the lower river, given by Simpson's Diversity Index, was 0.468 in the spring, 0.774 in the summer and 1.000 in the fall.

In the summer, benthic community diversity, measured by Simpson's Index, was not significantly different among the three collection sites in the lower river (one-way ANOVA; $p=0.957$, f ratio=0.0435). In the spring, benthic community diversity at the two lower river sites in common with the spring samples were not different (two-tailed t-test, $p=0.538$). For these two reasons the lower river data were pooled by season. In the

spring, benthic species diversity in the lower river was significantly higher than in the summer (two tailed t-test, $p=0.0241$). However, there were more species present in the benthic community during the summer. This difference in species diversity is due to the greater evenness in the spring sample.

Benthic communities in the lower river were similar in spring and summer (70.5% similarity and the similarity given by Morisita's Index = 0.924; Table 5). Benthic communities in the middle river and lower river in the spring were dissimilar (11.1% percent similarity and Morisita's Index of similarity = 0.0967; Table 6). However, species diversity did not differ between these two reaches (two-tailed t-test, $p=0.135$).

Diet Composition

In total, 18 shortnose sturgeon and eight Atlantic sturgeon were examined for stomach contents. Seven Atlantic sturgeon were caught in the lower river in the spring and summer, one had an empty stomach (Table 2). Spionids composed 100% of the diet of Atlantic sturgeon in the spring and 99% of the diet in the summer (Table 7). The only other organisms found in stomach contents of Atlantic sturgeon were anthurid isopods (0.18% of the diet) and mysid shrimp (0.18% of the diet). Atlantic sturgeon had a slightly positive to neutral selection towards spionids in the spring and summer based on Ivlev's electivity index (Table 8). Atlantic sturgeon showed negative selection towards both mysid shrimp and anthurid isopods. Eighty-three percent of Atlantic sturgeon had greater than 230 spionids in their stomach contents and one Atlantic sturgeon had over 3,330 spionids in its stomach contents.

Shortnose sturgeon ($n=6$) were captured only in the lower river during the spring and summer. Their diet was 100% spionids in the spring and 75% spionids in the

summer (Table 7). In the summer there was one diet sample from a shortnose sturgeon that contained only anthurid isopods and if this were excluded from the calculation, spionids consisted of over 90% of shortnose sturgeon's diet. There was not a difference between the numbers of spionids in shortnose sturgeon diet compared with Atlantic sturgeon when all stomach samples were pooled (two-tailed t-test, $p=0.149$) (Figure 4). There were not enough data points to confidently run a test for outliers, however even when the one Atlantic sturgeon with over 3,330 spionids in its stomach contents was removed (lowering variation), Atlantic sturgeon had significantly more spionids in their stomach contents than shortnose sturgeon (two-tailed t-test, $p=0.038$) (Figure 4). Other organisms found in the stomach contents of shortnose sturgeon were nereidid worms (7.6% of the diet) and anthurid isopods (16.9% of the diet; Table 7). There was slightly positive selection towards spionids in the spring and slightly negative selection towards spionids in the summer based on Ivlev's Electivity index and this coincided with the large abundance of spionids in the environment (Table 8). There was positive selection towards both nereidid worms and anthuridae isopods in the summer. There was a large amount of very digested polychaete bits (left unidentified) in all of the stomach content samples of both shortnose and Atlantic sturgeon. In the fall, ten shortnose sturgeon and one Atlantic sturgeon were caught and examined for stomach contents in the upper river, all had empty stomachs.

Discussion

Sturgeons are found most frequently in the upper (river km 34-43) and lower river (river km 21-24) reaches of the Penobscot River. Shortnose sturgeon spend more time in the upper river than Atlantic sturgeon but feed mostly in the lower river. In the Penobscot River, spionid polychaetes are an important prey item for both sturgeon species, especially in the lower river. Sturgeons do not appear to be feeding in the upper river. There is a clear distinction between the benthic communities of the middle river and the lower river, which may be driving sturgeon presence in those areas. Due to the presence of both shortnose sturgeon and Atlantic sturgeon and the availability of prey, the lower river is clearly important foraging habitat for sturgeon.

Movement patterns exhibited by the shortnose sturgeon and Atlantic sturgeon in 2012 were very similar to the movement patterns explained in Fernandes *et al.* (2010). Atlantic sturgeon subadults enter the river system in the spring and stay in the lower river through summer and fall. In the spring shortnose sturgeon move to the lower river and spend much of their time there. In the summer and fall shortnose sturgeon spend most time in the upper river and neither species spends much time in the middle river.

Metcalf and Eddy (1994) conducted the most recent study of the benthic communities of the Penobscot River until this study. Acoustic telemetry data gathered over the past six years suggests that sturgeons are most often found between river km 20 and 45 (Fernandes *et al.* 2010, Zydlewski *et al.* 2011, Dionne *et al.* 2013). However, all of the study sites from Metcalf and Eddy (1994) were south of river km 20 and there have not been any studies describing the benthic communities between river km 20 and river km 45. Metcalf and Eddy (1994) found high abundances of *Mytilus* beds, however, no

Mytilus were identified in this study. There were juvenile bivalves found in the middle river (n=9) and lower river (n=6), however, because of their small size (<2.0mm) and indistinct morphology, positive identifications were not possible. Sampling limitations and different sampling locations from Metcalf and Eddy (1994) may have played a role in the difference in *Mytilus* abundance between studies. *Mytilus* often occur on hard or rocky substrate, i.e., not surfaces that a ponar grab can adequately sample.

Metcalf and Eddy (1994) described low prey availability in the Penobscot River south of river km 20, however they did report spionids (specifically *Marenzelleria viridis*) were common and the most abundant invertebrate in the substrate. They noted that the poor substrate quality from the high pollution levels would limit the diversity and abundance of invertebrates able to colonize the benthos and in turn limit the availability of sturgeon prey. Bivalves such as *Mya arenaria* and *Macoma baltica*, which are common prey items for shortnose sturgeon in the St. John River (Dadswell 1979), were noticeably absent in the Penobscot and this absence may have led to the claim of low prey availability for sturgeon. However, there may have been a shift in prey availability since their study. Since that time efforts have been made to clean up the Penobscot River. These efforts may be helping to increase the abundance of the invertebrates already present. In either case, sturgeons are feeding in the lower river and at least one prey item (spionid worms) is highly abundant.

The lower river had the highest preferred prey availability and prey densities. This is a brackish water reach of the estuary with 0.0 ppt at depth in May and a maximum salinity of 15.5 ppt at the end of July. The organisms composing the benthic community in the lower river are able to withstand large salinity changes (Dauer *et al.* 18981, Zettler

1997). The benthic community species diversity was significantly different in spring and summer, however the species composition was very similar.

In spring and summer spionid worms were the most abundant organism and were found in the highest densities of all the organisms collected. The species of spionid found in the substrate samples was *Marenzelleria viridis*. These spionids are deposit and filter feeding polychaete worms that live in branched burrows up to 30cm deep in muddy and sandy substrate (Dauer *et al.* 1981, Zettler 1997). *Marenzelleria viridis* are commonly found in estuaries and areas of salinity lower than 5ppt and are highly motile (Dauer *et al.* 1981, Bochert *et al.* 1996). In the Penobscot the maximum density of *Marenzelleria viridis* in a benthic sample was 17,500 individuals per m². This is a little below the maximum densities found in estuaries in the Baltic Sea which had abundances of up to 28,000 individuals per m² (Zettler 1997). Zettler (1997) reported the substrate type for all of the sites was sand and the average densities of spionids were between 2,000 and 4,000 individuals per m² and was site specific (Zettler 1997). The substrate preference of *M. viridis* allows for relatively easy foraging by sturgeons. Sandy substrate is important foraging ground for sturgeon species (Peake 1999) and sturgeon's chemosensory barbels allow for the detection of prey items in the substrate (Rapp *et al.* 2011).

The diets of both shortnose sturgeon and Atlantic sturgeon were not diverse and reflected the benthic community where they were feeding. Of the fourteen families found in the benthic samples in the lower river, only four were found in the stomach contents. Of the four families of organisms identified in the stomach contents only spionids were present in any large numbers. Isopods, mysid shrimp, and nereidid worms were also present in the stomach contents but in very low numbers. Spionids occurred in greater

than 83% of shortnose sturgeon stomach contents and occurred in 100% of Atlantic sturgeon stomach contents.

In the Penobscot River, spionids are the most common benthic invertebrate and the most available prey item. However, this may not be the ideal food source, as Dadswell (1979) reported that spionids (*Marenzelleria viridis*) were negatively selected for in the St. John River in Canada. In contrast to the Penobscot River, spionids only occurred in 3% of the benthic samples taken in the St. John River (Dadswell 1979). Diet studies in the St. John River estuary found mollusks tend to be a dominant prey item of adult shortnose sturgeon (Dadswell 1979, Usvyatsov *et al.* 2012). Unlike the Penobscot River, mollusks are a very abundant food item in the benthos in the St. John River estuary. Johnson *et al.* (1997) reported that polychaetes were the most important prey item for Atlantic sturgeon off of the New Jersey coast. Johnson *et al.* (1997) also reported that sand and organic debris were a major component of the diet, this was not the case in the Penobscot River. While organic matter was present in the stomach content of 50% of the Atlantic sturgeon, it did not make up a major percentage of the diet.

The middle river had the lowest effort in terms of the number of times the benthos was sampled, also no sturgeon were captured in this section of the river so no diet analyses were performed. The telemetry data show that sturgeons do not linger in the middle river, as they do in the upper river and lower river. The sediment composition of the middle river was similar to the lower river and most sampled organisms were found in the sandy and muddy substrates. Oligochaete worms were the most abundant organism and empty caddis fly tubes were present in every grab. The middle river had a maximum salinity of 2.5 ppt and the benthic community reflected a more freshwater environment

than the lower river community. The overall abundance of organisms was less than that of the lower river. The relatively high abundance of benthic invertebrates (compared with the upper river) and lack of sturgeon presence may indicate the middle river is not preferred sturgeon habitat. Papers have listed oligochaetes as a food source for both shortnose sturgeon and Atlantic sturgeon (Dadswell 1979, Mohler 2003, Usvyatsov *et al.* 2012). Although oligochaete worms are present in abundances of up to 4,900 individuals per m² they may not be preferred prey for sturgeon in the Penobscot River.

No successful ponar grabs were taken in the upper river because the substrate was too compacted or cobbles obstructed the grab allowing any material in the grab to fall out. All sturgeons lavaged in the upper river had empty stomachs. The compact cobble sediment and empty sturgeon stomachs may indicate that the upper river is not critical foraging habitat. However, shortnose sturgeon spend at least a month or two of the summer, and overwinter for five to six months annually (Fernandes *et al.* 2010) in the upper river, so this section of the river has some other feature that is preferred by sturgeon.

Gastric lavage is a common technique used to collect stomach contents from fishes. Studies indicate a 70% recovery rate of stomach contents and success is dependent on the prey item (Brosse *et al.* 2002, Wanner 2006). The most common prey item in the stomachs of shortnose sturgeon and Atlantic sturgeon in the Penobscot River were spionid worms, which are vermiform organisms, so according to Brosse *et al.* (2002) may have a lower recovery rate than other organisms. Worms also digest faster than some other types of prey, which may limit successful identifications (Hyslop 1980). Given these two limitations, spionids may be more abundant in the diet than recorded in this

study. Likewise, stomach sampling biases could contribute somewhat to interpreting diet contents of sturgeon in the upper river. There are three possible reasons for empty stomachs: sturgeon are not feeding in the upper river at all, sturgeon had not fed in the two hours prior to being lavaged, or sturgeon are feeding on prey items that have very low recovery rates (i.e. an organism with a hard exoskeleton). Wanner (2006) suggested a hard exoskeleton might impede the expulsion of an organism during gastric lavage. Although the data indicate that sturgeons are not feeding in the upper river, it is possible they are feeding on organisms with hard exoskeletons such as stoneflies, which were observed on cobbles, and gastric lavage did not work.

Critical habitat as defined by the ESA is the “physical or biological features essential to the conservation of the species, which may require special management considerations or protection”. Shortnose sturgeon and Atlantic sturgeon are both listed under the ESA, so critical habitat has to be assessed for each of these species. The results from this research indicate the lower river reach in the Penobscot River is important foraging habitat for both sturgeon species and should be included in a critical habitat designation. Shortnose sturgeon spend approximately three months in the lower river (May, June and part of July) and Atlantic sturgeon spend over six months (May – Oct) in the lower river. The prey availability in the upper river is limited, however, there may be other components of habitat that make this reach desirable habitat for shortnose sturgeon since they use it for more than six months of the year (Fernandes *et al.* 2010).

Based on the benthic community data, substrate data, and salinity data gathered from the lower river the designation of critical habitat may be extended to other areas. Sturgeons’ preferred prey in the Penobscot River, spionid polychaetes, are geographically

widely distributed and live in sandy substrates in brackish water in very high densities at a large range of depths (Zettler 1997). Given these findings, environments similar to this in other river systems may also provide sturgeon habitat and warrant further examination. Side scan sonar produces high resolution echograms of underwater surfaces and can be used to map out the physical features and substrate of a river bottom (Kaeser and Litts 2010). Side scan sonar data of the entire Penobscot River is available. These data could be used to identify other prospective reaches with sturgeon habitat based on substrate type in a completely non-invasive way. Another area to explore is bioenergetics and the efficiency of spionid polychaetes as the main prey item for sturgeons in the Penobscot River. This may give insight as to why the sturgeons travel to the lower river to feed. Critical habitat designation is important for endangered species recovery and this study is a step closer to defining critical habitat for shortnose sturgeon and Atlantic sturgeon in the Penobscot River.

Figures

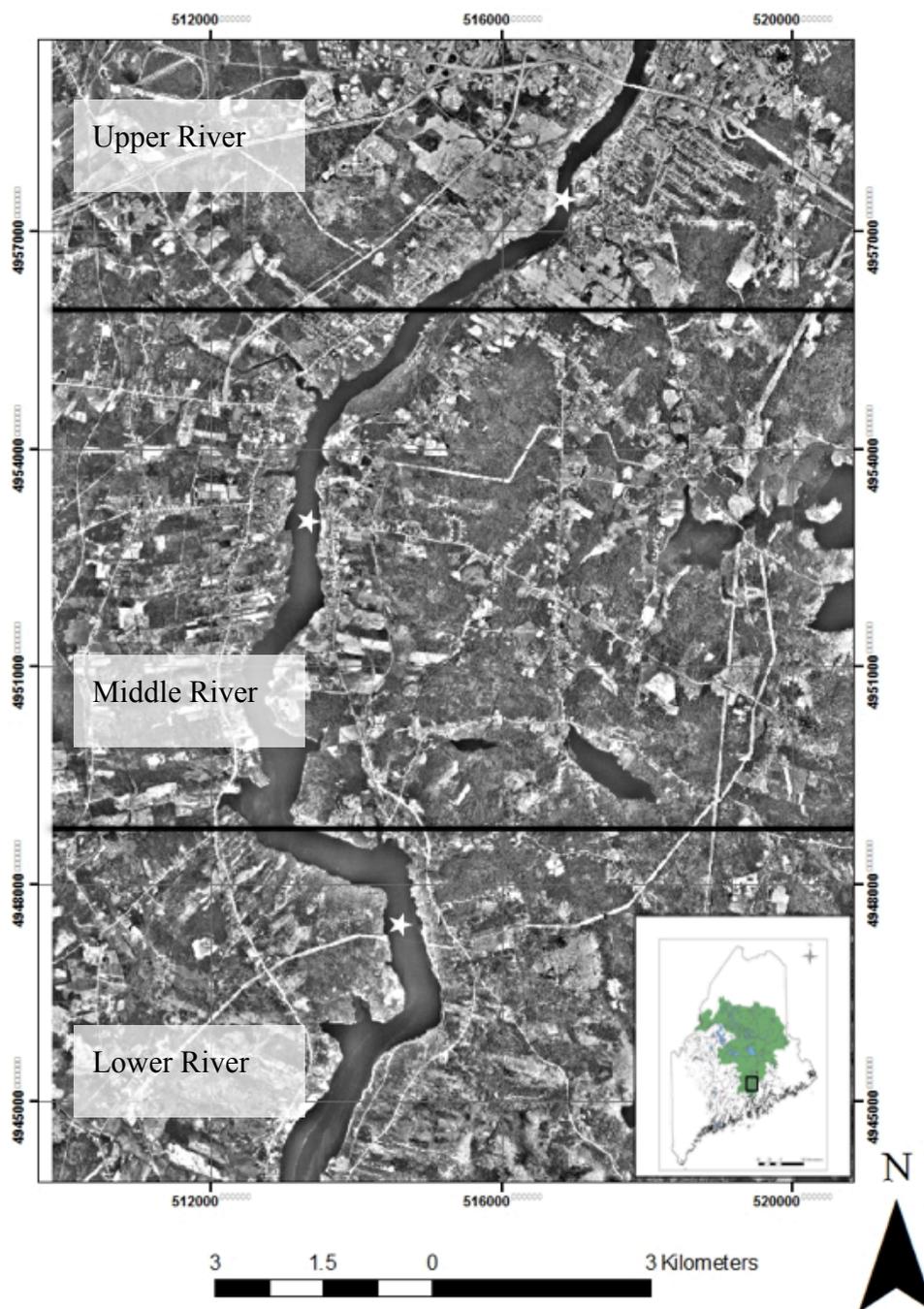


Figure 1. The Penobscot River from river km 21 to river km 38. The upper river (river km 34-43), middle river (river km 26-31), and lower river (river km 21-24) are each designated by horizontal lines and the upper river section extends above the image. The stars indicate the location of the receivers used for detecting tags implanted in sturgeon.

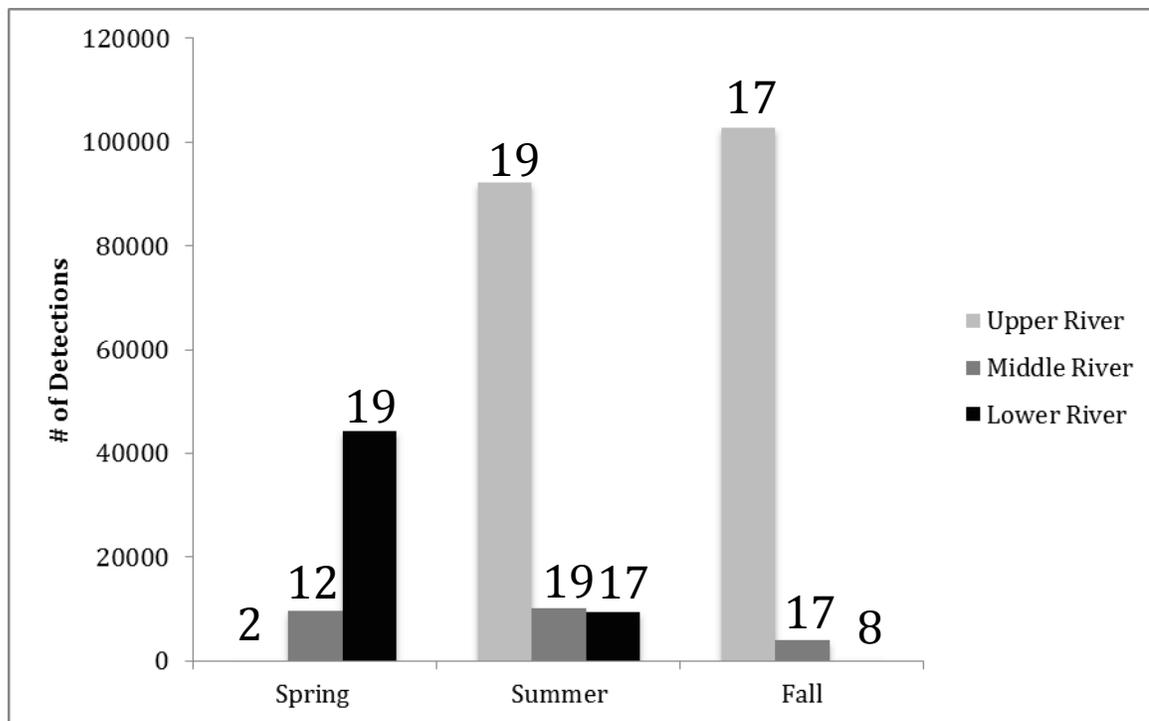


Figure 2. The total number of shortnose sturgeon detections decoded by receivers in the upper river, middle river and lower river reached of the Penobscot River in each season sampled. The total number of tags recorded is shown at the top of each column.

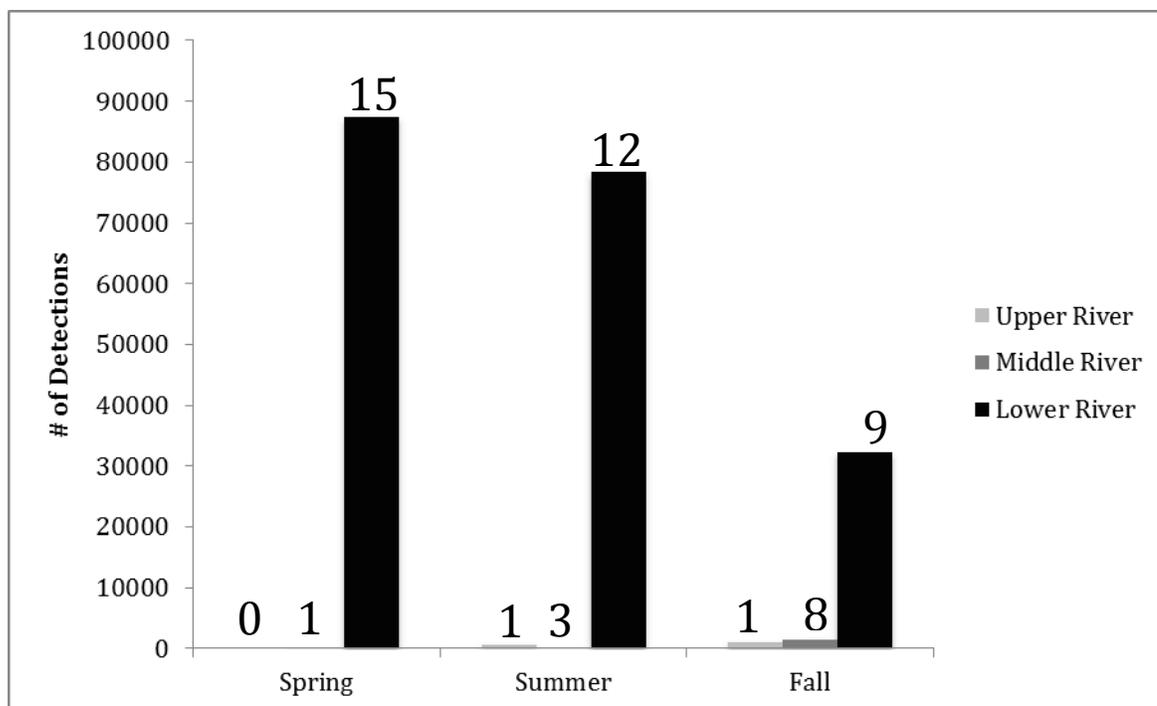


Figure 3. The total number of Atlantic sturgeon detections decoded by receivers in the upper river, middle river and lower river reaches of the Penobscot River in each season sampled. The total number of tags recorded is shown at the top of each column.

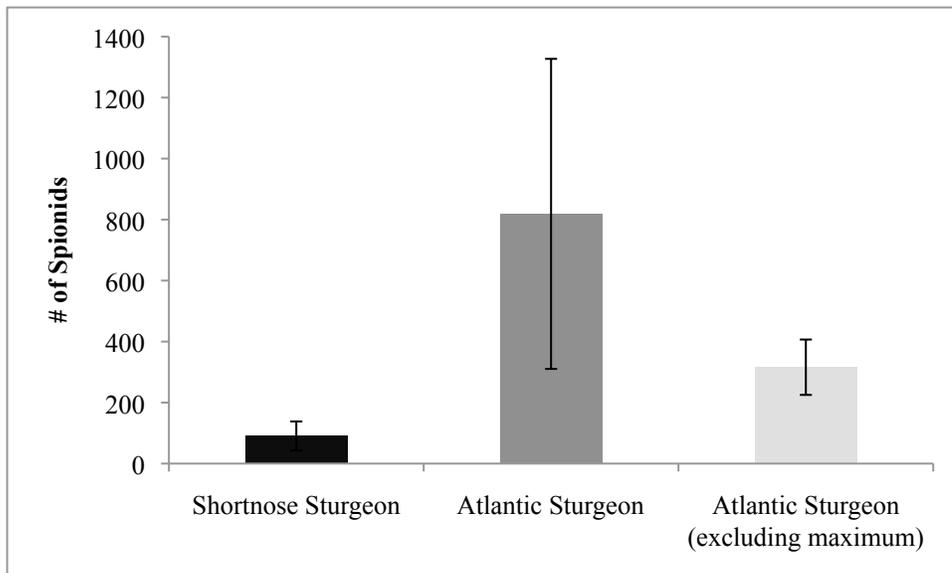


Figure 4. Mean number of spionid polychaetes collected per sturgeon stomach content sampled in the Penobscot River. The error bars indicate the standard error of each sample.

Tables

Table 1. The total number of ponar grabs taken and the total number shortnose and Atlantic sturgeon gastric lavage performed by season and according to river position in the Penobscot River. No sturgeon were caught in the middle river. The upper river is river km 34-43, the middle river is river km 26-31, and the lower river is river km 21-24.

Season	Ponar sample (successful)	Shortnose sturgeon lavage (empty stomachs)	Atlantic sturgeon lavage (empty stomachs)
Spring (May, June)	Upper river: n=15 (0) Middle river: n=12 (9) Lower river: n=13 (11)	Upper river: n=0 Lower river: n=2	Upper river: n=0 Lower river: n=4
Summer (July, August)	Up river: n=43 (0) Middle river: n=0 Lower river: n=18 (17)	Upper river: n=3 Lower river: n=6	Upper river: n=0 Lower river: n=3 (1)
Fall (September, October)	Upper river: n=11 (0) Middle river: n=3 (3) Lower river: n=11 (6)	Upper river: n=7 (7) Lower river: n=0	Upper river: n=1 (1) Lower river: n=0

Table 2. Total number and percent composition of taxa identified in the middle river in the spring and the lower river of the Penobscot River in the spring and summer.

		Total Number			Percent Composition		
		Middle River	Lower River		Middle River	Lower River	
		Spring	Spring	Summer	Spring	Spring	Summer
Polychaete	Ampharetidae	0	10	55	0.0	2.1	6.9
	Capitellidae	0	7	3	0.0	1.4	0.4
	Flabilligeridae	0	2	0	0.0	0.4	0.0
	Nereididae	0	0	3	0.0	0.0	0.4
	Spionidae	4	316	698	4.5	64.9	87.7
Oligochaete	Unk Fam.	60	0	0	67.1	0.0	0.0
	Naididae	4	0	0	4.5	0.0	0.0
Mollusca	Bivalvia	9	3	3	10.1	0.6	0.4
	Gastropod	5	90	4	5.6	18.5	0.5
Crustacean	Anthuridae	0	55	15	0.0	11.3	1.9
	Corophium	0	2	0	0.0	0.4	0.0
	Cumacean	0	0	2	0.0	0.0	0.3
	Gammarus	0	0	7	0.0	0.0	0.9
	Mysidae	0	0	2	0.0	0.0	0.3
Insecta	Chironomidae Larvae	8	2	3	9.0	0.4	0.4
	Clupeidae Larvae	3	0	1	3.4	0.0	0.1

Table 3. Percent occurrence (%O) and average abundance of spionids in mud, sand and gravel collected in the Penobscot River. SD represents \pm one standard deviation from the mean.

Substrate (n)	%O	Mean Abundance (Individual/m²) (SD)
Mud (8)	30.4	1725 (1025)
Sand (14)	60.9	4175 (4815)
Gravel (2)	8.7	3445 (2181)

Table 4. Simpson's diversity index of the spring and summer benthic communities. The percent similarity and Morisita's Index of Similarity of the spring and summer communities in the lower reach of the Penobscot River.

	Season: Spring	Summer
Simpson's Diversity Index	0.468	0.774
Percent Similarity	70.5	
Morisita's Index of Similarity	0.924	

Table 5. Simpson's diversity index of the middle river and lower river spring benthic communities. The percent similarity and Morisita's Index of Similarity of the middle and lower river benthic communities in the Penobscot River in the spring.

	Middle River	Lower River
Simpson's Diversity Index	0.363	0.468
Percent Similarity	11.1	
Morisita's Index of Similarity	0.0967	

Table 6. Mean number (N), standard deviation (sd), mean percent number (%N), and percent occurrence (%O) of prey categories found in the stomach contents of shortnose sturgeon and Atlantic sturgeon caught in the lower reach of the Penobscot River in the spring and summer.

	Shortnose Sturgeon						Atlantic Sturgeon					
	Spring (n=2)			Summer (n=6)			Spring (n=4)			Summer (n=2)		
	N (SD)	%N	%O	N (SD)	%N	%O	N (SD)	%N	%O	N (SD)	%N	%O
Anthuridae	0	0	0	1.17 (2.1)	16.9	33.3	0	0	0	1(1.41)	0.18	50
Mysidae	0	0	0	0	0	0	0	0	0	1(1.41)	0.18	50
Nereididae	0	0	0	0.833 (2.0)	7.6	16.7	0	0	0	0	0	0
Spionidae	119 (167)	100	100	66.17 (118)	75.5	83.3	1033 (1544)	100	100	391 (225)	99.4	100

Table 7. Ivlev's electivity index (E_i) between percent number of organisms in the benthic community (%Ben) and percent number of organisms in the stomach contents of shortnose sturgeon (SNS) and Atlantic sturgeon (AST) caught in the lower reach of the Penobscot River in the spring and summer.

Organism	Spring			Summer		
Family	%Ben	E_i AST	E_i SNS	%Ben	E_i AST	E_i SNS
Anthuridae	11.3	0	0	1.9	-0.831	0.794
Mysidae	0.0	0	0	0.3	-0.181	0.000
Nereididae	0.0	0	0	0.4	0.000	0.902
Spionidae	64.9	0.213	0.213	87.7	0.065	-0.072

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Author's Biography

Matthew P. Dzaugis was born and raised in Holden, MA and graduated from Wachusett Regional High School in 2009. He has been interested in the sciences for his entire life and as a child enjoyed wondering off to salt water marshes and tidal pools looking for crabs, fish, clams and mussels. Matthew has extensive experience in the field of paleontology studying the enigmatic Ediacaran fauna in Australia, which he considers ancient marine biology. Early in high school he became SCUBA certified and hasn't looked at land since. He enjoys all outdoor activities especially SCUBA diving, skiing, soccer and lacrosse. Matthew majored in marine science with a concentration in biology at the University of Maine. In the fall of his freshman year he became involve in a research lab and had the opportunity to work with sturgeon for the four years he attended the University of Maine. Upon graduation in 2013, Matthew plans to spend the summer hiking the 750 miles of the New England portion of the Appalachian Trail before attending the University of Texas at Austin for graduate school.