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Assessing Changes in Freshwater and Marine Food Web Connections Following Restoration on the Penobscot River, Maine, Using Stable Isotope Analysis

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Assessing Changes in Freshwater and Marine Food Web Connections Following Restoration on the Penobscot River, Maine, Using Stable Isotope Analysis

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Abstract

Diadromous fish provide ecological subsidies to freshwater and marine food webs, connecting both ecosystems. A main goal of the Penobscot River Restoration Project was to increase connectivity between food webs by removing two mainstem dams, improving fish passage, and reintroducing river herring through stocking. Diadromous fish now reach historic spawning habitat that was not accessible for centuries. As a result, river herring runs in the Penobscot River increased from 2,336 fish in 2009 to over 3 million fish by 2018. To assess food web connectivity in the Penobscot watershed, I analyzed stable isotopes from samples collected before (2009-2010) and after (2020-2021) dam removals by sampling species ranging in trophic level from piscivorous fish to baseline primary consumers from three mainstem and three major upstream tributary sites. I targeted top fish predators that can consume adult river herring directly. Pre-restoration, I found little evidence of marine derived nutrient (MDN) assimilation in freshwater food webs, with the exception of a mainstem site below all dams. Post-restoration, MDN assimilation increased only below what is now the lowest dam on the river, likely due to migration delays aggregating more fish for a longer period of time than in free-flowing river sections. Where changes in MDN assimilation occurred, I saw evidence of bottom-up enrichment of the food web. This pattern of enrichment has been measured in smaller rivers with spawning runs dominated by river herring. These results may be one of the first in a river of this size (watershed area = 22,300 km^2) and restoration of this magnitude, suggesting that even in larger rivers with greater

"dilution effects," effects of river herring on transfer of nutrients from marine to freshwaters are detectable. In the Penobscot Watershed, river herring currently dominate the sea-run fish population but only comprise 20% of conservative estimates of historic run size based on spawning habitat available before dam construction. As sea-run species increase in abundance, I expect MDN to be detectable beyond points of aggregation.

List of Tables and Figures

Chapter 1: Introduction

Stable Isotope Analysis (SIA) has been used to better understand ecological subsidies, i.e., how energy or nutrients from one ecological system supports another ecological system, often with migratory organisms as vectors. In this short review I discuss ecological subsidies (with a specific review of river herring), how stable isotope analysis can address research questions related to food webs and trophic ecology, as well as how to address systematic challenges in using stable isotope data.

1.1 Ecological Subsidies

Many ecological studies use the framework of food webs to study communities and trophic relationships within and among those communities. Ecological subsidies make important contributions to many food webs and facilitate transfer both into and out of food webs via physical movement of individuals or nutrients (Polis et al., 1997). All ecological systems receive subsidies at some scale, and they vary in importance, role, and pathways in recipient food webs (Polis et al., 1997). For example, detritus inputs to forested streams drive primary and secondary productivity, and prey movements can subsidize resident consumer communities to grow larger than possible with prey derived solely from within the recipient food web (Polis et al., 1997). Ecological subsidies can enter food webs through organisms at many trophic levels, which can facilitate both bottom-up and top-down control. In some cases, these subsidies can drive trophic cascades within recipient food webs (Polis et al., 1997). Whereas subsidies are important natural

energy sources for food webs, spatial proximity, permeability, and physical connectivity determine the degree of transfer between food webs (Polis et al., 1997).

1.2 Sea-Run Fish as Marine Nutrient Vectors in Freshwater

Diadromous fish provide ecological subsidies to both freshwater and marine ecosystems due to their life cycle, which includes multiple migrations between systems. The large migrations of Pacific Salmon (*Oncorhynchus* spp.) are well documented in their ability to provide marine derived nutrient (MDN) subsidies to freshwater systems. The first studies using the stable isotopes ^{13}C, ^{15}N, and ^{34}S to trace MDN in freshwater systems studied Pacific Salmon nutrient transport and how those nutrients impact resident consumer growth rates (Hesslein et al., 1991; Kline et al., 1993). Many studies since then have used stable isotopes to trace MDN in freshwater systems on the Pacific coast and demonstrate the link between escapement rates of salmon and freshwater productivity (Bilby et al., 1996; Brock et al., 2007). Instances of North Atlantic diadromous fish providing the same kind of subsidies to both freshwater and marine systems are less studied, especially in large river systems.

North Atlantic diadromous fish include a diverse group of anadromous and catadromous species including Alewife (*Alosa pseudoharengus*), Blueback Herring (*A. aestivalis*), Atlantic Salmon (*Salmo salar*), American Shad (*A. sapidissima*), Sea Lamprey (*Petromyzon marinus*), Rainbow Smelt (*Osmerus mordax*), American Eel (*Anguilla rostrata*), Atlantic Tomcod (*Microgadus tomcod*), Striped Bass (*Morone*

saxatilis), Brook Trout (*Salvelinus fontinalis*), as well as Atlantic Sturgeon (*Acipenser oxyrhynchus oxyrhynchus*) and Shortnose Sturgeon (*Acipenser brevirostrum).* Whereas all these species contribute to ecological subsidies moved between systems, river herring (the collective term for the closely related Alewife and Blueback Herring) are historically highly abundant and important prey species in both freshwater and marine systems (Hall et al., 2012). Thus, they are potentially important vectors for ecological subsidies between systems through excretion, carcass loading, and direct consumption by predators.

Pathways of MDN incorporation depend on specific organisms and systems as sea-run species vary in rates of iteroparity and excretion in freshwater systems. MDN can be incorporated mainly through direct consumption, excretion, or carcass decomposition. Depending on the ratio of spawning adult river herring to out-migrating juveniles there may be an influx of additional nutrients to freshwater systems (Durbin et al., 1979; Barber et al., 2018). Moreover, freshwater predators of *Alosa spp.* derive a large portion of their nutrients from marine derived carbon during spawning runs of *Alosa* spp. through direct consumption of *Alosa* prey (Garman and Macko 1998; MacAvoy et al., 1998). Specifically, in the Penobscot Watershed, there is evidence of MDN incorporation in freshwater fishes in lakes following removal of lake outlet dams that restored Alewife access (Norris, 2012). Whereas river herring transport MDN to freshwater systems through direct consumption, their runs also contribute MDN through excretion and carcass loading.

Hanson et al. (2010) determined up to 35% of amphipod biomass could be MDN transported to freshwater systems through a river herring run. Other studies

provide estimates of Alewife excretion rates in freshwater and showed that excreted materials were rapidly incorporated into freshwater food webs (Post and Walters, 2009; Walters et al., 2009). River herring provide similar ecological subsidies to Pacific Salmon in freshwater, but in northern rivers only a subset of river herring die in freshwater, reducing their impact through carcass decomposition; however, carcass decomposition (or direct consumption) likely provides a greater magnitude of nutrient input per fish than through excretion alone.

In contrast to iteroparous river herring, Sea Lamprey are semelparous and die after spawning in freshwater streams and rivers, potentially contributing large quantities of marine derived nutrients to freshwater streams through carcass decomposition (Nislow and Kynard, 2009). Several studies have documented positive impacts of carcass loading by Sea Lamprey to freshwater stream productivity (Nislow and Kynard, 2009; Weaver et al., 2018). Similarly, carcass analogs of diadromous fish placed in freshwater streams in the Penobscot Watershed increased Atlantic Salmon young-of-year and parr growth and subsequent survival rates (Guyette et al., 2013). With this host of species and life histories, Atlantic coastal watersheds that support diadromous fish runs receive and incorporate MDN through multiple pathways, which result in higher productivity by the recipient freshwater food webs.

1.3 River Herring Life History

River herring are anadromous fishes born in freshwater lakes, ponds, rivers, and streams where they grow for a period as juvenile fish (0+ years) until

transitioning to the estuary (1-2+ year olds) and finally to the nearshore marine environment where they spend 3-5 years growing to maturity before running back up their natal rivers to spawn (Bigelow and Schroeder, 1953). River herring are also iteroparous, i.e., a proportion of individuals survives spawning in freshwater, returns to the ocean, and spawns again the following year (Bigelow and Schroeder, 1953).

This complex life history strategy allows fish to access the "best of both worlds," where spawning and juvenile fish experience relatively low rates of predation compared to the ocean, while adults can grow larger and faster in the ocean at the cost of higher relative levels of predation (Gross et al., 1988). Due to their life history that takes them across system boundaries with an annual migration, and their role as prey fish in both freshwater and marine systems, river herring can transfer ecological subsidies of materials and energy across system boundaries multiple times. Sea-run fish, and river herring specifically, are important connectors of marine and freshwater food webs that increase the productivity of both systems, but their life history can leave them susceptible to harmful anthropogenic activity.

1.4 Penobscot River Restoration Project

Damming and lack of effective fish passage create physical barriers to the migrations of sea-run fish to and from their spawning grounds. In comparison to early records from the 19th and 20th centuries, diadromous fish populations in the North Atlantic have declined by over 90% of their historic abundance for most

populations (Limburg and Waldman, 2009). Similarly, damming has significantly and negatively impacted diadromous species in Maine. Hall et al. (2011) suggests that 5% of lake access remained to diadromous fish in 1850 and approached a total loss of accessible habitat by 1860. The Penobscot River watershed was no exception.

The Penobscot River Restoration Project (PRRP) was born out of a goal to restore connectivity of the watershed and allow sea-run fish access to spawning habitat not seen in hundreds of years. An agreement between special interest groups, state, federal, and tribal governments, and private industry resulted in the removal of two mainstem dams along with fish passage improvements at another mainstem dam and a fish bypass channel of another major dam in the watershed (Day, 2009). The PRRP resulted in the opening of over 93% of historic habitat to American Shad and Blueback Herring, and over 53% increases in access to historic habitat for other sea-run species (Trinko Lake et al., 2012). However, the PRRP only opened 31% of historic habitat to Alewife due to the high numbers of small lake outlet dams in the watershed (Trinko Lake et al., 2012). The PRRP also changed the mainstem resident fish communities by physically changing habitat and increasing access between river reaches (Kiraly et al., 2014; Watson et al., 2018). Since these changes, the Penobscot River has seen increasing returns of diadromous fish, with around 3 million river herring, over 11,000 American Shad, over 1,500 Atlantic Salmon, and over 6,000 Sea Lamprey counted at fishways within the watershed in 2020 (Trap count statistics, 2020). As vectors of MDN's, these sea-run fish have the potential to transform freshwater systems in Maine and elsewhere, including

increasing freshwater system productivity. One technique used to trace MDN in freshwater systems is stable isotope analysis.

1.5 Stable Isotope Analysis

Stable isotope analysis (SIA) is a well-documented tool for assessing MDN incorporation, changes in trophic position, and feeding migrations of resident freshwater and marine fishes. Stable isotope data can be used to indicate food web connectivity and the degree to which river herring and other diadromous fishes transport energy and materials between systems (Garman and Macko, 1998). For example, ^{13}C isotopes are relatively depleted in freshwater compared to marine systems, which have elevated ^{13}C isotope ratios (Garman and Macko, 1998). Similarly, ^{34}S isotopes are more enriched in marine systems compared to freshwater systems (MacAvoy et al., 1998). When freshwater organisms incorporate MDN through diadromous fish predation, excretion, or remineralization, this elevated marine isotope signature is detectable in tissues. Similarly, if marine predators consume out-migrating juvenile anadromous fish, they incorporate this freshwater isotope signature, and it can be detected in samples of marine predators feeding on freshwater derived prey. ^{15}N isotopes are enriched at higher trophic levels because heavier isotopes of nitrogen are harder to excrete and bioaccumulate to greater amounts with each trophic level (Minagawa and Wada, 1984). $\delta^{15}N$ values corrected for baseline primary consumer isotope values allow researchers to calculate the trophic position of a given individual (Post, 2002).

When assessing connectivity between marine and freshwater food webs, stable isotope data from pre- and post-restoration efforts reveal a relative level of marine and freshwater derived nutrients transferred between systems. When using stable isotope data to calculate parameters such as trophic position, assumptions are made about trophic fractionation, natural isotope gradients, and body size. The next sections address some of these assumptions with examples drawn from previous literature.

1.5.1 Stable Isotope Fractionation and Trophic Position

Isotopic fractionation occurs when isotopes are discriminated against during mass dependent chemical and biological processes, resulting in unequal concentrations of isotopes within organisms and habitats (Vander Zanden and Rasmussen, 2001). Because many studies use $\delta^{15}N$ values to estimate trophic position, it is important to understand isotopic fractionation, how rates of fractionation change with species and size, and how differing rates of fractionation can bias those estimates of trophic position when using $\delta^{15}N$ isotopes (Vander Zanden and Rasmussen, 2001; Post, 2002). Here I discuss issues and common practices surrounding calculating trophic position including using primary consumers as baselines values, determining which fractionation rates should be used in trophic position calculations, the effect of body size on fractionation rates, and calculating error associated with trophic position estimates.

Trophic position is an important metric for evaluating food chain lengths, food web changes over time, and changes in species trophic niche (Post, 2002).

Trophic position is calculated using the following equation (Vander Zanden and Rasmussen, 2001):

$$\text{Trophic position}_{consumer} = [(\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/3.4] + 2$$

In this example equation, Vander Zanden and Rasmussen (2001) assume a trophic fractionation value of 3.4‰ for $\delta^{15}N$ isotope ratios, which is commonly used for the fractionation rate of $\delta^{15}N$ between trophic levels for fish. The first study to produce this value was Minagawa and Wada (1984), and this value has been widely used since then.

Estimates of trophic fractionation for fish have varied by species, type of study, and tissue types. In a literature review, Vander Zander and Rasmussen (2001) found that laboratory studies often reported lower fractionation values at an average of 2.9‰, whereas field studies showed higher fractionation values at 3.41‰ ((Table A-1). A more recent lab study of European Sea Bass (*Dicentrarchus labrax*) combined with literature review data to estimate a trophic fractionation value of 3-3.4‰ for fish muscle tissues but cautioned against using this value for whole fish, which they observed to have a lower fractionation value of 2.9‰ (Sweeting et al., 2007; Table A-1). Although there is variation in fractionation values across studies, using a fractionation value of 3.4‰ broadly captures the variability seen between studies (Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 2001; Post, 2002; Sweeting et al., 2007). In the following sections I discuss how these estimates of trophic fractionation can impact trophic position estimates, as

well as how to choose an appropriate baseline $\delta^{15}N$ value, which can be another significant source of error.

1.5.2 Determining Baseline Isotope Values

Previous studies have shown that trophic position estimates vary across sites (Vander Zanden and Rasmussen, 2001; Anderson and Cabana, 2007) and that using different baseline values or averaging values across different scales could change trophic position estimates by a factor of more than 1 level (Anderson and Cabana, 2007; SD = 0.67). One of those studies demonstrated that while taxonomic groups varied in $\delta^{15}N$ values among sites, differences remained consistent across trophic levels (Vander Zanden and Rasmussen 2001). In other words, whereas values differed among water bodies, they remained consistent among taxonomic groups. In a sensitivity analysis of trophic position estimates, one study found that calculations were most sensitive to a 1 SD change in baseline $\delta^{15}N$ values, suggesting that correct baselines are more important than small degrees of error in trophic fractionation values (Post, 2002). Therefore, identifying and setting a proper baseline $\delta^{15}N$ value for each sampling site is important when calculating trophic position.

Many studies state that temporal variability in primary producer isotope values was one of the largest obstacles to overcome when choosing baseline isotope taxa for trophic position calculations (Post, 2002; Anderson and Cabana, 2007; Jardine et al., 2014). Three papers used in this review investigated best practices for selecting and analyzing baseline $\delta^{15}N$ values including using different taxa and temporal sampling structure (Post, 2002; Jardine et al., 2014; Anderson and Cabana,

2007). Some studies used snails and mussels (benthic and pelagic primary consumers) as a way to account for these different pathways of primary production because mussels are long lived primary consumers that can mediate the temporal variability associated with primary producers (Post, 2002). As primary consumer tissues reflect long term trends, they can replace multiple samplings of primary producers throughout the year (Post, 2002; Anderson and Cabana, 2007). Using unionid mussels to correct $\delta^{15}N$ values was a common theme between all the papers except for one that studied riverine systems where these mussels were rare and sought alternative methods that compared to mussels (Jardine et al., 2014).

Alternatively, there seems to be a wide agreement that $\delta^{13}C$ values largely do not need to be adjusted for fractionation or that the fractionation adjustment is so close to 0‰ that it is negligible (Vander Zanden and Rasmussen, 2001; Post, 2002). Whereas fractionation is not a concern with carbon isotopes in this case, there are other systematic processes that can bias values and interpretation that must be addressed.

1.5.3 Watershed Scale Variation in Carbon Isotopes

Variation in nitrogen and carbon isotope ratios can be observed naturally at the watershed scale. Studies have documented inorganic $\delta^{13}C$ in rivers and streams on a predictable gradient from the headwaters to downstream habitats (Rasmussen et al., 2009). In headwaters, $\delta^{13}C$ isotope values closely align with soil CO_2 and weathering of substrate materials, which produces less enriched $\delta^{13}C$ isotope values (Rasmussen et al., 2009). Further downstream, $\delta^{13}C$ isotope values become more

aligned with atmospheric conditions and more enrichment of $\delta^{13}C$ is observed (Rasmussen et al., 2009). This gradient is also linked to the natural downstream increase in alkalinity and pH in river systems (Rasmussen et al., 2009). Since there is little fractionation of $\delta^{13}C$ as addressed above, invertebrate scrapers closely reflect autochthonous carbon sources. In contrast, shredders that feed heavily on allochthonous materials such as leaf litter do not reflect these local carbon sources and show a greater terrestrial signature (Rasmussen et al., 2009).

Studies have utilized this natural geochemical gradient in $\delta^{13}C$ isotope values to estimate inputs of allochthonous terrestrial leaf litter to aquatic food webs (Rasmussen, 2010). Terrestrial production does not differ systematically within a watershed and shows a "standard" isotope value across all sites (Rasmussen, 2010). One study used this observation to input terrestrial and aquatic primary producer isotope values into food web mixing models and to quantify the influence of terrestrial leaf litter in the aquatic food web (Rasmussen, 2010). Not surprisingly, shredders derived 85% of consumption from terrestrial leaf litter, with lesser values for collector/gatherers and filterers, and only 15% for herbivore/grazers (Rasmussen, 2010). This result adds more validity to use of invertebrate scrapers as proxies of autochthonous production between sites. However, the natural geochemical gradient of carbon isotopes is confounded in river systems that have MDN subsidies. Marine-sourced carbon is enriched in ^{13}C relative to freshwater and rapidly integrated into the food web, altering baseline values (Walters et al., 2009; Samways et al., 2018). The natural geochemical gradient can also be altered by the presence of dams, which change relatively shallow, free-flowing river reaches into

deep, thermally stratified impoundments, altering autochthonous production by favoring phytoplankton (Growns et al., 2014).

Because $\delta^{13}C$ becomes more enriched at lower river sites, it is sometimes difficult to distinguish between autochthonous production of the lower river and MDN transported by anadromous fish. For watersheds without significant marine-derived soils, sulfur isotopes offer another way to detect MDN. Sulfur stable isotopes are enriched in marine systems compared to freshwater systems (MacAvoy et al., 1998). Specifically, $\delta^{34}S$ can be used to determine marine origin when $\delta^{13}C$ values alone overlap with freshwater baseline values (MacAvoy et al., 1998). One study demonstrated that Pacific Salmon were enriched in $\delta^{34}S$ compared to freshwater fishes, and marine derived sulfur was incorporated into freshwater food webs (Kline et al., 2007). Using $\delta^{34}S$ isotope values can help to distinguish sources when $\delta^{13}C$ values alone could not do so (MacAvoy et al., 1998; Kline et al., 2007).

1.5.4 Site Specific Variation in Nitrogen Isotope Values

Whereas carbon isotope values exist along a predictable geochemical gradient within watersheds, $\delta^{15}N$ isotope variation by site is not as clear cut. Variation in $\delta^{15}N$ values is often correlated with anthropogenic influences on landscapes that change nitrate sources of watersheds (e.g., agriculture manure and chemical fertilizers; Chang et al., 2002; Mayer et al., 2002; Table A-2).

Mayer et al. (2002) used stable isotope ratios of $\delta^{15}N$ and $\delta^{18}O$ to determine sources of nitrate in 16 river systems in the northeastern United States. This study included more forested landscapes than other studies (Chang et al., 2002; Mayer et

al., 2002). Mayer et al. (2002) found $\delta^{15}N$ isotope values were positively correlated with urban and agricultural land use types, as percentage land use in the watershed draining to the sampling location (Table A-2). Forested areas had lower $\delta^{15}N$ values, suggesting nitrates derived from mineralization of soil organic matter (Mayer et al., 2002). A more recent study observed high levels of variation in $\delta^{15}N$ baseline values across watersheds and 68% of that variation was explained by anthropogenic activities (Anderson and Cabana, 2005). Anderson and Cabana (2005) found that $\delta^{15}N$ values vary greatly by river sites among many watersheds and that 88% of variation was explained by site effects (Table A-2). Anderson and Cabana observed minimal variation between trophic levels across lakes, suggesting that the differences in baseline values shifted the entire food web's isotopic values (Anderson and Cabana, 2005).

More recent studies have been influenced by the findings of Mayer et al. (2002) and Anderson and Cabana (2005) by understanding landscape contributiuins to food web isotope values (Cabana and Rasmussen, 1996; Anderson and Cabana, 2005; Bentoviglio et al., 2016; Hette-Tronquart et al., 2016, 2018; See table A-2 for summary). For example: Hette-Tronquart et al. (2016, 2018) demonstrated that stream food webs change along river gradients in $\delta^{13}C$ and $\delta^{15}N$ isotopes, and land use (and subsequent sources of nitrate). Bentoviglio et al. (2016) demonstrated that nitrate pollutants (wastewater, animal waste, fertilizer) can be traced and monitored using stable isotope values of freshwater aquatic food webs (Table A-2).

SIA is one way to better understand the role of ecological subsidies, especially that of sea-run fish in freshwater systems. Magnitude of subsidies, seasonal timing, and pathways of assimilations vary with species and specific watersheds. SIA is a powerful tool to address these questions when proper considerations are made for trophic fractionation, differences in baseline values, and other physiological processes that can influence isotope values. Using this type of analysis to assess marine derived nutrient subsidies provides a view into what has been lost by damming and blocking rivers and therefore mass spawning migration of diadromous fishes.

Chapter 2: Assessing Changes in Freshwater and Marine Food Web Connections Following Restoration on the Penobscot River, Maine, Using Stable Isotope Analysis

2.1 Introduction

Ecological subsidies are nutrients and energy provided to a recipient system by a donor system. Subsidies and their importance to productivity of recipient systems have been widely studied across ecological disciplines (Polis et al., 1997). A commonly used example is Pacific Salmon (*Oncorhynchus* spp.) life history where semelparous spawning adults migrate upstream into freshwater to spawn and die. Subsequently, their excretions, eggs, and carcasses provide high-quality marine derived nutrients (MDN) to relatively nutrient poor freshwater systems and riparian forests (Kline et al, 1993), showing the importance of subsidies to some ecosystem functions and productivity (Naiman et al., 2002). Examples from other systems include marine derived nutrient subsidies provided to terrestrial communities by seabirds, large herbivore migrations subsidizing freshwater rivers, and beach wrack providing energy and nutrients to coastal systems (Polis et al., 1997).

Similar to salmon on the Pacific Coast, sea-run fish on the Atlantic Coast of North America move energy and nutrients between fresh water and the ocean (Durbin et al., 1979; Garman and Macko, 1998). Studies first showed that anadromous *Alosa* species act as vectors of MDN subsidies to freshwater both by

direct consumption by freshwater predators and through excretion, egg deposition, and carcass loading (MacAvoy et al., 1998). Other Atlantic sea-run species provide MDN to freshwater systems and thus connect freshwater and marine food webs, including Rainbow Smelt (*Osmerus mordax*), Sea Lamprey (*Petromyzon marinus*), American Shad (*Alosa sapidissima*), Alewife (*A. pseudoharengus*), Blueback Herring (*A. aestivalis*), and Atlantic Salmon (*Salmo salar*), among others (see: Nislow and Kynard, 2009; MacAvoy et al., 2009; Guyette et al., 2013; Samways et al., 2015, 2018; Landsman et al., 2018; Weaver et al., 2018).

Large numbers (or biomass) of migratory species may subsidize recipient ecosystems in a variety of ways. At high abundances, sea-run fish act as keystone species that create an amplifying feedback loop within freshwater food webs; when spawning adults provide MDN subsidies that boost productivity of recipient systems their progeny use that productivity during freshwater rearing (Kline et al., 1993; Guyette et al., 2013). In freshwater systems where large quantities of low-quality inputs drive productivity, resident animals may select diadromous fish as a high-quality subsidy (Marcarelli et al., 2011), and these allochthonous subsidies might maintain resident predator populations at greater abundances than could be sustained without them (Marcarelli et al., 2011), both in freshwater and marine systems. On the East coast of North America, it has been hypothesized that restoration of sea-run fish may provide nearshore marine predators such as Atlantic Cod a greater diversity and abundance of forage fish, which may aid in restoring their depleted population levels (Ames, 2010; Hall et al., 2012). The potential importance of sea-run fish as subsidies for both freshwater and nearshore marine

systems has led to recent efforts to increase restoration of these fishes whose populations have undergone precipitous declines throughout North America (Limburg and Waldman, 2009).

Declines of sea-run fishes are attributed to dams blocking freshwater spawning habitat, pollution, overfishing, and loss of MDN subsidies to fresh water (Waldman and Quinn, 2022). The Penobscot River Watershed drains approximately 22,300 km^2 of Maine, USA (Trinko-Lake et al., 2012). Historic river herring (a collective term for Alewife and Blueback Herring) populations in the Penobscot River ranged from 14 to 18.91 million but declined to lows around 2,000 by 2010 (Laser, 2009). In 2013 the Penobscot River Restoration Project (PRRP) removed two lower mainstem dams (Veazie and Great Works Dams) and improved fish passage at two other dams (Milford and Howland Dams) (Day, 2009). These projects opened up at least 53% of historic freshwater spawning habitat to sea-run fish that was not accessible since head of tide dam construction in 1835 (Hall et al., 2011; Trinko-Lake et al., 2012). In addition to augmenting fish passage, the Maine Department of Marine Resources (MDMR) stocked river herring in watershed lakes (Laser, 2009). By 2020 trap counts indicate that sea-run fish populations have increased to 3 million river herring, 11,000 American Shad, over 1,500 Atlantic Salmon, and 6,000 Sea Lamprey (Trap count statistics, 2020). With increased connectivity resulting from greater access to historic habitat, and resulting higher numbers of fish, I anticipated greater influence of MDN on freshwater food webs.

One method to assess connectivity is through Stable Isotope Analysis (SIA), which utilizes naturally occurring gradients of isotope ratios between habitat types

to track animal migrations, trophic ecology, and food webs. In aquatic studies tracking MDN in freshwater, ^{13}C and ^{15}N are used to determine if resident freshwater fish and invertebrates assimilate MDN (Kline et al., 1993; Bilby et al., 1996; MacAvoy et al., 1998, 2001; Garman & Macko, 1998; Walters et al., 2009). Prey from marine systems are enriched in both ^{13}C and ^{15}N compared to prey from terrestrial or freshwater systems (Peterson and Fry, 1987). Within habitats, there is variation in $\delta^{13}C$ enrichment as a result of pelagic (more enriched) vs. benthic (less enriched) primary production pathways (Vander Zanden & Rasmussen, 2001). Over time, consumers' stable isotope values shift to reflect their prey (Fry, 2006).

To estimate the source contribution to consumer diets, stable isotope mixing models use isotope ratios of individual consumers and likely prey sources to quantify the relative contribution of different prey items to a consumer (Stock et al., 2018). Furthermore, models that use data from multiple time periods can indicate changes in trophic ecology over time. Other more advanced models can incorporate diet information and other covariates such as species, body size, and site to better understand mechanisms behind differences in consumer feeding habits (Stock et al., 2018). Stable isotope analysis and mixing models estimate feeding history over time, giving researchers valuable tools to detect and quantify MDN assimilation in freshwater systems that are not constrained by traditional diet study methods such as gut content analysis that only shows a snapshot of feeding history (MacAvoy et al., 2001).

As part of the PRRP, I assessed changes in MDN in freshwater food webs from 2009 (prior to mainstem dam removals) to 2021 (after removals). I had two main

objectives: (1) to understand if and where marine derived nutrients were assimilated into freshwater food webs since restoration took place, and (2) to analyze pathways by which marine derived nutrients enter freshwater food webs. I hypothesized that MDN would be most prevalent at sites below what is now the lowest dam on the river because of previous studies documenting the highest abundance of sea-run fish downstream of dams (Kiraly et al., 2014; Watson et al., 2018). I also hypothesized that MDN would enter the food web through direct consumption of the most abundant anadromous fish (river herring), rather than through the "remineralization" pathway or bottom-up enrichment of the freshwater food web. Previous studies showed bottom-up enrichment only in areas with a high density of sea-run fish of which populations in the Penobscot River are still relatively low compared to historic estimates (~20% for river herring). The Penobscot Watershed is also a much larger system than others investigated in previous studies (Laser, 2009; Post and Walters, 2009).

2.2 Methods

2.2.1 Study Design

This study utilized stable isotope analysis of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ from fish and invertebrate muscle tissue. Data were collected in two phases: pre-restoration (2009-2010) before the PRRP had begun dam removals and in the early period of river herring stocking in the watershed, and post-restoration (2020-2021) after mainstem dam removals, fish passage improvements, river herring stocking for > 10 years, and a substantial increase in river herring trap counts (2,336 to > 3 million

fish). Restoration in the Penobscot Watershed will continue past 2021 including further passage improvements, river herring stocking, and expected fish population level responses to increased access to spawning habitat (Laser, 2009).

Within each sampling phase, target species of fish and invertebrates were collected in both spring (May-June) and fall (September) to account for seasonal differences associated with timing of sea-run fish migration. A total of six sites within the Penobscot Watershed were sampled (described below); three sites in the lower mainstem of the river (hereafter referred to as Mainstem 1, 2, 3) and three sites in major upper tributaries (hereafter referred to as Tributary 1, 2, 3; Figure 1). Fish and invertebrates were collected in Penobscot Bay as a marine endmember (reference values that provide marine isotope values for comparison) for stable isotope analyses.

This sampling design allows for comparisons of stable isotope data in multiple ways: pre- and post-restoration, between seasons, and between mainstem and tributary sites. I also used SIA to check for ontogenetic diets shifts to understand the relationship between fish size and feeding history.

2.2.2 Sampling Locations

Mainstem sampling sites were located from below the Veazie Dam (head of tide, removed in 2015), upstream to the Great Works Dam (removed 2013) to the Milford Dam (now the lowest dam on the river; Figure 1). Sites in this reach experienced the most physical habitat change during this study. Other sites were located in major upstream tributaries of the Penobscot River above the Milford Dam

(Pushaw Stream with bankfull streamflow ~ 45 m^3/s [1,590 cfs]), the Piscatiquis River (bankfull streamflow ~ 308 m^3/s [10,900 cfs]), and the Passadumkeag River (bankfull streamflow ~ 78 m^3/s [2,770 cfs]) (Table 1; Figure 1; U.S. Geological Survey, 2022). Post dam removal, river herring spawning runs now reach each tributary after ascending a fish lift at the Milford Dam. More detailed information on sampling sites in the Penobscot Watershed can be found in Table 1.

Marine sampling in Penobscot Bay focused on five primary zones of the bay in relation to the river mouth (Figure 2). Samples were collected from at least one site in each zone at increasing distance to the river mouth. Fish samples were collected in open water areas (largely based on where fish could be caught with hook and line), and corresponding invertebrate samples were collected from the closest accessible intertidal area.

2.2.3 Field Sampling

In fresh water, fish were collected by boat electrofishing in collaboration with the Penobscot River Restoration Fish Survey at the University of Maine (see Kiraly et al., 2014; Watson et al., 2018). Sampling was conducted under University of Southern Maine IACUC #50509-01 (pre-restoration) and #071220-95 (post-restoration). Freshwater fish sampling prioritized top predators (generally Smallmouth Bass, *Micropterus dolomieu,* and Chain Pickerel, *Esox niger*) and common intermediate trophic level species (generally Redbreast Sunfish, *Lepomis auritus*). Freshwater primary consumers (freshwater mussels, snails) were collected by hand and used as site-specific baseline isotope values when calculating the

trophic position of secondary consumers and top predators. Freshwater mussels were Eastern elliptio (*Elliptio complanata*) and snails were either Family Planorbidae or Viviparidae depending upon availability (either hereafter referred to as "snails"). Fish were euthanized with an overdose of Tricaine mesylate and then placed on ice until they could be frozen at -20°C for processing at a later date.

In marine systems, fish were collected by hook and line. Marine fish sampling also targeted top predators and intermediate trophic level consumers (including Atlantic Cod, *Gadus morhura*, Atlantic Pollock, *Pollachius pollachius*, Atlantic Mackerel, *Scomber scombrus*). Marine primary consumers (periwinkles; Blue Mussel, *Mytilus edulis*; crabs) were collected by hand. Marine fish were treated in the same way as freshwater fish.

2.2.4 Lab Processing

Total length (± 1 mm) and weight (± 0.1 g; using a Sartorius GE812 microbalance) were measured for all fish samples. For snails and mussels, the entire foot muscle was extracted for stable isotope analysis. For fish, skinless dorsal muscle tissue fillets (> 1 g wet weight) or 5 mm biopsy plugs (non-lethal sampling in 2009/2010) were extracted for stable isotope analysis. Samples were placed in glass scintillation vials for drying in an IsoTemp oven at 55°C for at least 48 hours until the tissue was completely dry. Dried samples were immediately placed in a desiccator for a minimum of 30 min to cool and then ground to a fine powder using a mortar and pestle. No lipid extractions were performed on these samples prior to analysis.

Powdered samples of 1 ± 0.2 mg were packed into 3.5 × 5 mm or 4 × 6 mm tin capsules and indexed in 96 well microsampling plates for stable isotope analysis at the University of California Davis Stable Isotope Facility. Stable isotope analysis was performed for $\delta^{15}N$ and $\delta^{13}C$ (and pre-restoration $\delta^{34}S$ samples) using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) (UC Davis Stable Isotope Facility, 2020). Some Smallmouth Bass samples from lower river sites were also analyzed for $\delta^{34}S$ to confirm marine derived nutrient assimilation. $\delta^{34}S$ analysis was performed at the Marine Biological Laboratory at Woods Hole, Massachusetts using a Europa ANCA-SL elemental analyzer interfaced to a Europa 20-20 continuous flow isotope ratio mass spectrometer.

To investigate diets of top fish predators (from spring 2021 only), gut contents were collected from all top predators including Smallmouth Bass and Northern Pike (*Esox lucius*). Stomachs were dissected from each fish, and contents were sorted into taxonomic groups including macroinvertebrates, freshwater fish, river herring (adult), crayfish, and unidentifiable material. Proportions of total volume (using water displacement) for each taxonomic group in the stomach were recorded to the nearest 1 ml (Buckland et al., 2017).

2.2.5 Data Analysis

Comparisons of Mean $\delta^{13}C$ and $\delta^{15}N$

Mean $\delta^{13}C$ and $\delta^{15}N$ isotope values were analyzed by site using multiple one-sided Welch's t-tests because data from pre- and post-restoration often showed

heterogeneity in variance. Using a Levene's Test for equality of variances, 59 of 62 comparisons had significant unequal variances; thus, a test that assumes unknown and unequal variances was most appropriate. For every species for which I had data from both pre- and-post restoration, I compared differences of means that increased from pre- to post-restoration. Therefore "significant changes" are only identified for enrichment towards marine isotope values with an alpha level of 0.05. I corrected for multiple comparisons using the Benjamini-Hochberg correction (Benjamini & Hochberg, 1995).

Addressing Lipids

These samples did not undergo lipid extractions prior to SIA, and there were no corrections made from the raw isotope data. When comparing groups of species by site across pre- and post-restoration, 20 out of 62 groups had C:N ratios higher than 3.5, and only 1 group was greater than 5 (Table 2). Because lipids are depleted in ^{13}C, a sample with more lipids (evidenced by higher C:N) would be biased towards a freshwater isotope signal in this study (DeNiro & Epstein, 1977). Therefore, not correcting the minority of samples that warrant corrections constitutes a conservative approach to quantifying MDN input to freshwater systems.

Investigating Ontogenetic Diet Shifts

To investigate if Smallmouth Bass diet shifts related to size might confound my interpretation of MDN assimilation, I investigated the relationship between $\delta^{13}C$

and $\delta^{15}N$ values to individual fish total length using simple linear regression with the function geom_smooth (method = lm) in the ggplot2 R package (Wickham, 2016). For each simple linear regression, I reported the regression equation and R^2 values to assess whether there is a positive relationship, indicating ontogenetic diet shifts occurred. If a significant ontogenetic diet shift was identified, I reported more detailed total length data for each sample being compared to assess how length could influence the comparison. I used Analysis of Covariance (ANCOVA) to determine differences between linear regression line slopes pre- and post-restoration.

Confirming MDN using Sulfur Analysis

I compared sulfur values for Smallmouth Bass in the Mainstem 1 and 3 sites because these sites represent open access across all years (Mainstem 1) and changed access from blocked to open across years (Mainstem 3). For each comparison of Smallmouth Bass $\delta^{34}S$ values by site, I compared means using one sided Welch's t-tests to test for positive enrichment of $\delta^{34}S$ values in the fish tissue. Because there were only 2 comparisons for $\delta^{34}S$ data, I did not correct for multiple comparisons.

Estimating Trophic Position

Trophic positions for individual fish were calculated using baseline $\delta^{13}C$ and $\delta^{15}N$ values to weight the influence of benthic or pelagic resource use to $\delta^{15}N$ values for each fish (Post 2002). I used mean snail isotope values to represent benthic

production and mean mussel isotope values to represent pelagic production at each site (per Cabana & Rasmussen, 1996; Vander Zanden & Rasmussen, 2001; Post, 2002). I calculated trophic position for Smallmouth Bass and Redbreast Sunfish at the Mainstem 1 and Mainstem 3 sites where I had complete data sets of snail, mussel, and fish samples for pre- and post-restoration. These comparisons allowed me to distinguish between a site with unimpeded sea-run fish access before and after dam removal, and a site with levels of access that changed. Mean trophic position was then compared between pre- and post-restoration for both Smallmouth Bass and Redbreast Sunfish at both sites using one way Welch's t-tests.

Measuring Isotopic Niche Space using SIBER

To compare changes in isotopic niche areas between pre- and post-restoration, I used the R package Stable Isotope Bayesian Ellipses in R (SIBER) (Jackson et al., 2011). Understanding isotopic niche area is important to interpreting trophic ecology of consumer populations as it can inform levels of trophic specialization or generalization paired with raw isotope values. There is evidence from previous studies that expanding isotopic niche space indicates different resource use (Samways et al., 2018; Nolan et al., 2019). In some cases, isotopic niche can decrease when consumers are specializing on MDN in freshwater (Samways et al., 2018). Isotopic niche space estimates provide another layer of diet information that, in this case, can be used to piece together feeding habits with other metrics.

SIBER calculates multiple metrics that indicate isotopic niche (a proxy for trophic niche) for groups of individuals including the Total Area (TA), which is the

total convex hull area of points in the group, the Standard Ellipse Area (SEA), Standard Ellipse Area corrected for sample size (SEAc) and Standard Ellipse Area calculated in a Bayesian framework (SEAb) with associated credible interval estimates (Jackson et al., 2011). SEAc ellipses are slightly enlarged but retain the same ellipse shape for smaller sample sizes with the equation SEAc = –SEA(n–1) (n–2)–1 (Jackson et al., 2011). SEAb estimates the covariance matrix of the data set using Markov Chain Monte Carlo with uninformative or "vague" priors, 100,000 iterations and 10,000 burn in (Jackson et al., 2011).

In this analysis, all SIBER metrics were calculated for Smallmouth Bass and Redbreast Sunfish at four sites (M1, M2, M3, T3) and Redbreast Sunfish as well as Chain Pickerel at two other sites (T1, T2) where sample sizes for Smallmouth Bass were not sufficient. Because SEAb calculations are the only metrics that provide a credible interval, significant changes from pre- to post restoration are based on SEAb estimates falling outside the 95% credible interval of their comparable SEAb estimate.

Estimating MDN using Mixing Models

I used the R package MixSIAR to compare changes in the contribution of MDN to Smallmouth Bass and Redbreast Sunfish diets. MixSIAR is a Bayesian model that can incorporate informative priors. The mixing model assumed two sources, one representing freshwater production and one representing marine production (MDN). The freshwater nutrient signal was represented by freshwater mussels for two reasons: 1) isotope values were the most widely available for mussels both pre-

and post-restoration in our data set and 2) mussel stable isotope values remained highly consistent at each site from pre- to post restoration with only one significant shift in $\delta^{15}N$ detected (before corrections for multiple comparisons). Incorporating snails as another freshwater source value would confound results of the model as snails showed significant shifts in $\delta^{13}C$ at some sites (before corrections for multiple comparisons; Table 3). The marine nutrient signal was represented by adult Alewife captured on their upstream spawning run at Mainstem 1. These adult Alewife values were used throughout all sites with the assumption that spawning adult river herring do not remain in fresh water long enough to significantly change muscle isotope values (MacAvoy et al., 2001).

A separate model was run for fish at each sampling site with source inputs of freshwater mussels from that site for both pre- and post-restoration, and adult Alewife values representing the marine source. Consumers in each model were Smallmouth Bass (except for Tributary 1 and 2, which used Chain Pickerel due to low bass sample sizes) and Redbreast Sunfish from pre- and post-restoration. MixSIAR accounts for fractionation and variation in those values (Stock et al., 2018). Fractionation values and error were sourced from Post (2002) (3.4 ± 0.98 SD for $\delta^{15}N$ and 0.39 ± 1.39 SD for $\delta^{13}C$) and held constant for both freshwater and marine prey sources. Models were run with State (pre- or post-restoration) and Species as fixed effect categorical variables. Each model was run accounting for both process and residual error under MixSIAR's "multiplicative error" structure (Stock et al., 2018). These models were run using an "uninformative" or generalist prior, weighting each prey source as equally likely to be eaten by consumers. Models

utilized Markov Chain Monte Carlo with 100,000 iterations and 50,000 burn-in ("normal" model parameters recommended by the authors of the package; Stock et al., 2018). Model outputs included a mean diet proportion estimate with SD and associated credible intervals. Models were also run with length as a continuous effects covariate to estimate the relationship between %MDN and individual fish lengths.

2.3 Results

2.3.1 Testing for Mean Shifts Toward Marine Signal

For comparisons of pre- and post-restoration, most sites did not show consistent significant changes in mean isotope values across species with the exception of Mainstem 3 (below Milford Dam or first dam on the river in 2020/21; Figure 3). Sites in Tributaries 2 and 3 showed no significant shifts of $\delta^{13}C$ or $\delta^{15}N$ values for any species with comparisons from pre- and post-restoration (Tables 2, 3). No species at Mainstem 1 (below the former Veazie Dam site) showed significant shifts in either isotope. At the Mainstem 2 site (former Great Works Dam) I observed no significant shifts in $\delta^{13}C$ values and one significant shift in $\delta^{15}N$ (Golden Shiner +1.59‰; Table 4). At Mainstem 3, Smallmouth Bass, Redbreast Sunfish, and American Eel shifted significantly towards a more marine carbon signal (Table 3; Figure 3). At Tributary 1, Redbreast Sunfish shifted significantly towards a more marine nitrogen signal (Table 4).

When $\delta^{13}C$ values were averaged for many fish species across trophic guilds (Smallmouth Bass, Redbreast Sunfish, Chain Pickerel, Northern Pike, Pumpkinseed

Sunfish, American Eel, and White Sucker) the same trends were observed across sites. Only Mainstem 3 showed a consistent shift towards marine $\delta^{13}C$ values in both fish and invertebrate mean values (Fish: –27.67‰ ± 1.88 SD to –26.25‰ ± 2.5 SD; Invertebrate: –29.44‰ ± 1.38 to –27.52‰ ± 3.47 SD; Figure 2).

2.3.2 Magnitude of Ontogenetic Diet Shifts

Smallmouth Bass showed no significant relationship between $\delta^{13}C$ values and total length in spring, either pre- or post-restoration (ANCOVA p-value: 0.71; Figure 4), indicating no ontogenetic shifts in diet. In fall, $\delta^{13}C$ values were positively correlated with Smallmouth Bass total length, with a greater slope post-restoration than pre-restoration (ANCOVA p-value: 0.07; Figure 4). As expected, larger Smallmouth Bass were enriched in ^{15}N both pre- and post-restoration in both the spring (ANCOVA p-value: 0.40; Figure 5) and fall (ANCOVA p-value: 0.022; Figure 5). Relationships of both $\delta^{13}C$ and $\delta^{15}N$ to length had greater slopes in the fall seasons indicating some ontogenetic diet shifts being driven by marine-derived prey.

2.3.3 Confirming MDN Assimilation Using Sulfur Analysis

Pre-restoration, $\delta^{34}S$ mean values for Smallmouth Bass were 6.00‰ (± 2.23 SD) at Mainstem 1 and 2.02‰ (± 1.17 SD) at Mainstem 3 (Figure 6). Following restoration, values increased (+2.79‰) at Mainstem 3 and remained unchanged at Mainstem 1, reflecting similar trends in ^{13}C and ^{15}N (Table 3).

2.3.4 Estimating Trophic Position

While $\delta^{13}C$ and $\delta^{15}N$ values changed significantly for Smallmouth Bass at Mainstem 3, mean trophic position did not differ (+0.13; Table 5; Figure 7). At the Mainstem 1 site, mean trophic position did not change for Smallmouth Bass (–0.1), which aligned with no significant differences in mean $\delta^{13}C$ (0‰), and $\delta^{15}N$ (+0.01‰; Table 5; Figure 7). Mean trophic position values also did not differ for Redbreast Sunfish (Table 5; Figure 8).

2.3.5 Measuring Isotopic Niche Space

Of all Penobscot watershed sampling sites, only Mainstem 3 showed a consistent increase in estimates of isotopic niche based on SIBER analysis. For example, Mainstem 1 site Smallmouth Bass SEAb values decreased significantly (Table 6; Figure 9). In comparison, Mainstem 3 Smallmouth Bass SEAb values more than doubled (Table 6; Figure 9). All other sites except Mainstem 2 (which has largely overlapping credible intervals) showed decreases in all isotopic niche estimates for Smallmouth Bass or Chain Pickerel (Table 6; Figure 9).

Isotope niche estimates for Redbreast Sunfish showed similar trends as Smallmouth Bass and Chain Pickerel. Sites in Tributary 1, Tributary 3, and Mainstem 1 remained similar with largely overlapping credible intervals from pre- to post-restoration (Table 7). Redbreast Sunfish niche estimates at the Mainstem 2 site increased substantially (SEAb 4.96 to 14.51), but also had a large credibility interval (Table 7; Figure 10). The Mainstem 3 site showed a consistent increase similar to Smallmouth Bass where SEAb estimates more than doubled (Table 7; Figure 10).

Throughout the SIBER analyses, estimates of SEAc (corrected for sample size) remained similar to SEAb estimates (within 50% credible interval).

2.3.6 Estimating MDN using Mixing Models

Of five Penobscot Watershed sampling sites with sufficient data to accommodate analyses in MixSIAR, Smallmouth Bass, Chain Pickerel and Redbreast Sunfish showed similar changes in %MDN. Three sites decreased in %MDN for all three species (Figure 11). In contrast, %MDN increased in Smallmouth Bass at Mainstem 3 (+13.6%), Redbreast Sunfish at Mainstem 3 (+ 13.8%) and Tributary 2 (+5.7%), Chain Pickerel in Tributary 2 (+5%; Figure 11, Figure 12). Diet relationships to fish length as a covariate showed some evidence of a positive relationship for Smallmouth Bass at Mainstem 1 and 3 where %MDN increased as total length increased (Figure 13). At all other sites, there appeared to be no relationship between fish length and %MDN values of individual fish (Figure 13).

2.4 Discussion

Marine derived nutrient assimilation in Penobscot River food webs following the PRRP increased at Mainstem 3, which is below what is now the first dam on the river. Enrichment in $\delta^{13}C$ was measurable throughout the food web, suggesting MDN assimilation occurred through both bottom-up and direct consumption pathways. Multiple lines of evidence support this interpretation including $\delta^{34}S$ values, trophic position calculations, and modeling that accounts for variability in baseline and prey source values. Increases in isotopic niche space at Mainstem 3 could indicate MDN

use facilitated by multiple sea-run species. Evidence from other studies assessing the PRRP suggests that increases in MDN at the Mainstem 3 site are likely due to sea-run fish passage delays at Milford Dam, aggregating these fish for longer periods of time at this location (Izzo et al., 2016).

Estimates of isotopic niche space measured in this study are in line with previous published estimates from other systems. In a study of multiple rivers, each with a single prominent sea-run fish migration in Atlantic Canada, isotopic niche space decreased during the Alewife spawning season due to MDN specialization but increased significantly during spawning periods for all other sea-run species (Samways et al., 2018). This trend was observed in the Penobscot System as well; most sites showed decreases in isotopic niche space with the exception of Mainstem 3 (Table 6). Whereas river herring were the most abundant sea-run species, many other species were present in some numbers, which may explain the expansion of isotopic niche space. This result is consistent with the findings of Samways et al. (2018) that isotopic niche space did not always increase with MDN assimilation.

Similar mixing models showed the highest %MDN use in resident consumer fish was 35.9% in a river with a Rainbow Smelt run, and most spawning periods for each species showed %MDN use in the 20-29% range (Samways et al., 2018). These values are comparable to my estimates of %MDN in the diets of Smallmouth Bass and Redbreast Sunfish at Mainstem 3 post-restoration. %MDN estimates in another control river, with no sea-run fish providing MDN, ranged from 0.06% to 1.1% (Samways et al., 2018). Along the Penobscot watershed, tributary sites 1-3 ranged in

%MDN from 8.5-31.3%, suggesting some level of MDN assimilation pre-and post-restoration, but I saw no changes from pre to post restoration.

Why is Mainstem 3 showing more enrichment than other mainstem sites that have the same level of access and are located further downstream? I argue that this phenomenon is due to migration delays of sea-run fish at the Milford Dam, which is now the first mainstem dam that sea-run fish encounter when migrating upstream. Radio telemetry studies documented passage delays for sea-run Atlantic Salmon at the Milford Dam since restoration (new fish lift was operational in 2014; Izzo et al., 2016). This observation contrasts with other sites such as Mainstem 2 that have open access and unimpeded movement upstream (Izzo et al., 2016) where fish would likely not remain in the area long enough to significantly transfer MDN to food webs. In addition, fish monitoring studies on the Penobscot River reported that anadromous fish were most abundant below the lowest dam in the mainstem: the Veazie Dam before restoration (Mainstem 1) and below the Milford Dam (Mainstem 3) after restoration (Kiraly et al., 2014; Watson et al., 2018). I argue that most sites show no significant changes because sea-run fish migrations remain relatively small (~20% of historic estimates) and MDN do not change on a detectable level except where sea-run fish aggregate and migrations are delayed (Laser, 2009).
Another possible explanation for ^{13}C enrichment at Mainstem 3 is that physical habitat changes from an impoundment to a free-flowing river could change $\delta^{13}C$ values in food webs due to changes in the dominant autochthonous production pathway (pelagic vs. benthic) at the site (Freedman et al., 2014). However,

Mainstem 2 experienced similar habitat changes to Mainstem 3 and did not show the same patterns of change in isotope values pre- to post-restoration.

At sites where enrichment occurs, it appears throughout the food web. For all sites where Smallmouth Bass indicators of MDN assimilation shifted significantly, Redbreast Sunfish also showed significant shifts. Redbreast Sunfish are intermediary consumers that feed primarily on aquatic and terrestrial macroinvertebrates (Helms et al., 2018) and are too small to consume adult river herring directly. Redbreast Sunfish must receive MDN through different pathways than direct consumption of sea-run fish. They could receive enrichment by eating sea-run fish eggs (e.g., Samways et al., 2018), but the Mainstem 3 site (post restoration) is a fast-flowing section of river that would not be preferred spawning habitat for the sea-run species present. Modeled estimates of isotopic niche space and %MDN use in Redbreast Sunfish follow almost identical patterns to those of Smallmouth Bass. Whereas this relationship between changes in the two species is telling, analyses of trophic position and gut contents of Smallmouth Bass further support this idea.

Both Smallmouth Bass and Redbreast Sunfish at Mainstem 3 show no significant change in trophic position estimates because baseline values (snails) also shifted. Trophic position values are expected to increase with more fish prey being consumed (i.e., fish eat higher trophic-level prey; Post, 2002; Anderson and Cabana 2007). If Smallmouth Bass ate river herring directly and MDN were not incorporated into the bottom of the food web (mussels and snails), then Smallmouth Bass would appear to have even higher trophic levels based on enriched $\delta^{15}N$ in the

marine diet. Gut content analysis of Smallmouth Bass from post-restoration in Mainstem 3 in the spring shows a diet dominated by macroinvertebrates, with no detectable adult river herring (Table 8). Whereas stomach contents provide a snapshot of feeding, these results support findings from stable isotopes, which incorporate diet information on the scale of weeks to months (MacAvoy et al., 2001). Although sample size was small (n = 10 at Mainstem 1) for gut content analysis, these results agree with stable isotope analyses and suggest that direct consumption is likely a much rarer occurrence than previously thought.

Using length as a covariate in MixSIAR mixing models suggests a positive relationship between %MDN of Smallmouth Bass diet and fish size (Figure 13). However, this pattern is likely not due to direct consumption of river herring by large Smallmouth Bass. Although there are confirmed cases of Smallmouth Bass consuming Alewife directly (Figure 14), in general most bass collected in this study were too small to consume Alewife based on mouth gape estimates. Smallmouth Bass captured at Mainstem 3 averaged 290 mm total length (± 23.32 SD) post-restoration (Table 9). According to total length to gape width conversion equations for Smallmouth Bass (Schake et al., 2014), Mainstem 3 bass would have an average gape width of 36.65 mm (range 36.41 - 42.91 mm), well under the mean body height (from dorsal to ventral sides) of 63 mm (range of 49 - 82 mm) of Alewife samples collected in 2010 in the Penobscot River (Cronin-Fine et al., 2013). Some direct consumption of the smaller Blueback Herring may occur, although Blueback Herring currently represents only 24% of river herring in the Penobscot River (Wippelhauser et al., 2021). While direct consumption of river herring by very

large Smallmouth Bass may be physically possible strictly by gape limitations, other studies suggest that Smallmouth Bass often do not select for the largest prey items possible (Gaeta et al., 2018).

In contrast, Northern Pike, introduced in the time since pre-restoration sampling, were observed directly consuming river herring captured at the Tributary 1 site. Pike ranged in size from 400 to 670 mm and consumed river herring, as evidenced through gut content analysis where river herring made up almost all stomach mass by volume on average (Table 8). As Northern Pike continue to spread in the Penobscot watershed, their consumption of river herring likely will increase; Northern Pike select larger prey items compared to Smallmouth Bass (Gaeta et al., 2018), and Nolan et al. (2019) found that Northern Pike isotopic niche space increased in reaches below barriers (dams) with sea-run fish access compared to upstream reference sites.

The pattern of bottom-up enrichment has been detected in smaller rivers with spawning runs dominated by river herring. Biofilm (algae, bacteria, and fungi) standing stock increases and $\delta^{15}N$ enrichment occurs at some of the highest levels from Alewife runs compared to other sea-run species (Samways et al., 2015). Samways et al. (2018) also observed isotopic enrichment for all macroinvertebrate taxa studied except terrestrial leaf shredders in a river with an Alewife run. The annual spawning run of Alewife in Bride Brook, Connecticut, added a significant amount of nitrogen to the stream food web through excretion and mortality (Walters et al., 2009). Excretion by anadromous fishes can impact recipient food webs where fish are found at a high density (Post and Walters 2009). This

phenomenon may only take place at Mainstem 3 or other areas of high aggregations of sea-run fish for longer periods of time rather than free-flowing river sections that facilitate rapid migration to spawning areas. This study may be one of the first in a river of this size (watershed = 22,300 km^2), and where top fish predators assimilate MDN but not necessarily through direct consumption.

This study used a seasonal sampling design that should account for the entire period in which MDN transfer to freshwater organisms is possible. As noted in Samways et al. (2018) in their study of a river dominated by an Alewife run, %MDN in resident fish consumers peaked three weeks into the spawning period compared to two weeks for most other rivers with different sea-run species (Samways et al., 2018), suggesting that there may be a limited window in which %MDN in freshwater food webs would rise to detectable levels.

Maine DMR lists the peak fish run timing at the Milford Dam to fall between May 15 and June 7 for river herring (Bruchs et al., 2018). For my study, spring sampling occurred during the peak influx of MDN provided by river herring. My sampling in Spring 2021 was within the peak river herring run timing at the Milford Dam (May 15 – June 7; Bruchs et al., 2018); pre-restoration the spring sampling was late-May to mid-June. However, because muscle tissue has a slower turnover time than other tissues do (MacAvoy et al., 2001), fall sampling likely captured MDN consumed during the river herring spawning migration both upstream in May and June and as adults migrated downstream towards the ocean. Spring and fall sampling combined should account for all periods that sea-run fish were abundant at sampling sites when using dorsal muscle tissue. As expected I saw a higher

magnitude of change in fall as evidenced by the degree of ontogenetic diet shifts (Figure 4; Figure 5).

This study represents one of only a few known examples of using stable isotope analysis to assess food web changes following large scale river restoration for sea-run fish (see Tonra et al., 2015). Whereas MDN are detected at relatively few sites in great abundance at this point, the current sea-run fish populations in the watershed comprise only a fraction of conservative historic estimates (~20%; Laser, 2009). If sea-run fish populations continue to increase in the river, MDN assimilation in the Penobscot River food webs may increase to detectable limits at other sites and should be reassessed in the future. My results demonstrate that restoring and improving access to sea-run fish provides more marine derived nutrients to freshwater food webs, potentially increasing their productivity above pre-restoration levels. Increased productivity has positive implications for resident freshwater species, as well as for production of sea-run fishes and marine species supported by sea-run fish (Ames, 2010; Hall et al., 2012).

Aside from their ecological benefits, sea-run fish are also important components of local culture and economies through commercial harvest, recreation, and ecotourism (McClenachan et al., 2015). Sea-run fish are especially important to indigenous cultures and as a traditional sustenance source (USET Sovereignty Protection Fund, 2017). Managers should use the information provided here to justify improving access for sea-run fish to historic habitat and other efforts that work to increase sea-run fish populations to historic high levels. Moreover,

researchers could use this information to inform future studies on the effects of restorations in other watersheds.

Tables and Figures

Table 1. Sampling site descriptions with associated three letter codes, coordinates, and sea-run fish access by state of restoration.

Site Name	Latitude	Longitude	Pre Access	Post Access	Site Description
Mainstem 1	44.82403305	-68.6961323	Open	Open	Tidal freshwater free flowing mainstem Penobscot River. Located immediately below Veazie Dam until its removal in 2013.
Mainstem 2	44.84404089	-68.6970093	Blocked	Open	Pre-restoration: a mainstem impoundment behind Veazie Dam until its removal in 2013. Post-Restoration: a free-flowing section of mainstem Penobscot River.
Mainstem 3	44.92221637	-68.6347105	Blocked	Open	Pre-restoration: a mainstem impoundment behind Great Works Dam until its removal in 2012. Post-restoration: a tailwater of the Milford Dam on the mainstem Penobscot River.
Tributary 1	44.95231434	-68.7234121	Blocked	Partial	Pushaw Stream, a wetland-dominated free-flowing upper tributary to the Penobscot River.
Tributary 2	45.17605117	-68.5609659	Blocked	Partial	Passadumkeag River, a wetland-dominated free-flowing upper tributary to the Penobscot River.
Tributary 3	45.26142036	-68.869676	Blocked	Partial	Piscataquis River, a non-wetland-dominated free-flowing upper tributary to the Penobscot River. Increased sea-run fish access post-restoration with the bypass of Howland Dam at confluence with mainstem Penobscot River.

Table 2. Mean (± 1 SD) of C:N for all direct comparisons of fish and invertebrates with direct comparisons by site and state of restoration. Sample sizes are listed in Table 3.

Site and Species	Pre C:N Mean (SD)	Post C:N Mean (SD)
Mainstem 1		
Smallmouth Bass	3.23 (0.08)	3.26 (0.71)
Redbreast Sunfish	3.29 (0.09)	3.44 (0.16)
White Sucker	3.29 (0.12)	3.36 (0.08)
American Eel	4.03 (0.57)	4.99 (1.71)
Snails	3.97 (0.13)	3.83 (0.14)
Eastern Elliptio	3.78 (0.10)	3.93 (0.16)
Mainstem 2		
Smallmouth Bass	3.31 (0.13)	3.23 (0.04)
Redbreast Sunfish	3.44 (0.23)	3.43 (0.26)
White Sucker	3.39 (0.26)	3.38 (0.17)
American Eel	4.60 (1.55)	4.51 (1.80)
Snails	3.65 (0.13)	3.79 (0.09)
Golden Shiner	3.31 (0.05)	3.63 (0.12)
Mainstem 3		
Smallmouth Bass	3.31 (0.20)	3.23 (0.04)
Redbreast Sunfish	3.30 (0.12)	3.43 (0.30)
White Sucker	3.33 (0.11)	3.49 (0.36)
American Eel	5.61 (1.61)	3.73 (0.32)
Snails	3.99 (0.09)	3.80 (0.22)
Eastern Elliptio	3.91 (0.15)	3.84 (0.32)
Tributary 1		
Smallmouth Bass	3.20 (0.03)	3.16 (0.01)
Redbreast Sunfish	3.26 (0.08)	3.27 (0.02)
Chain Pickerel	3.17 (0.06)	3.19 (0.02)
White Sucker	3.25 (0.07)	3.26 (0.03)
Golden Shiner	3.23 (0.03)	3.33 (0.12)
Tributary 2		
Redbreast Sunfish	3.26 (0.15)	3.31 (0.05)
Chain Pickerel	3.18 (0.06)	3.22 (0.03)
White Sucker	3.46 (0.15)	3.29 (0.07)
Tributary 3		
Smallmouth Bass	3.35 (0.11)	3.38 (0.44)
Redbreast Sunfish	3.43 (0.16)	3.41 (0.16)
White Sucker	3.37 (0.21)	3.33 (0.16)
Eastern Elliptio	3.72 (0.11)	4.00 (0.17)
Common Shiner	3.38 (0.08)	3.85 (0.22)

Table 3. Mean (SD) $\delta^{13}C$ pre- and post-restoration by site and species. Degrees of freedom and p-value reported for one sided Welch's t-tests. Correction for multiple comparisons was made using the Benjamini-Hochberg method with an alpha level of 0.05 (significant p-values bolded).

Species	Pre $\delta^{13}C$ Mean	Post $\delta^{13}C$ Mean	df	p-value	Pre n	Post n
Mainstem 1						
Smallmouth Bass	-25.57 (1.22)	-25.57 (0.67)	29.44	0.91	27	11
Redbreast Sunfish	-25.35 (1.74)	-25.73 (0.96)	18.89	0.93	13	8
White Sucker	-25.56 (2.94)	-26.83 (2.51)	7.77	1.00	16	5
American Eel	-25.65 (1.85)	-28.71 (4.47)	2.09	0.98	22	3
Snails	-22.46 (1.15)	-21.35 (2.52)	4.50	0.59	2	6
Eastern Elliptio	-29.26 (2.39)	-29.45 (0.50)	8.90	0.91	9	7
Mainstem 2						
Smallmouth Bass	-26.02 (1.16)	-26.14 (1.70)	22.03	0.92	18	11
Redbreast Sunfish	-24.25 (2.78)	-24.59 (2.88)	9.99	0.94	6	6
White Sucker	-26.38 (4.08)	-26.57 (3.88)	7.98	0.91	5	5
American Eel	-27.28 (1.39)	-25.20 (1.69)	13.49	0.07	8	8
Snails	-25.07 (1.43)	-23.54 (1.49)	6.79	0.33	4	6
Golden Shiner	-26.18 (0.81)	-27.48 (1.11)	7.31	1.00	5	5
Mainstem 3						
Smallmouth Bass	-27.52 (0.90)	-25.41 (2.01)	15.01	**0.01**	19	11
Redbreast Sunfish	-27.23 (1.14)	-25.30 (1.41)	17.50	**0.02**	10	10
White Sucker	-27.44 (3.56)	-28.59 (3.97)	9.74	0.98	8	6
American Eel	-28.82 (2.43)	-25.07 (2.55)	11.51	**0.02**	11	8
Snails	-28.10 (1.29)	-24.49 (2.06)	6.29	0.08	3	6
Eastern Elliptio	-30.24 (0.78)	-30.55 (0.44)	7.26	0.99	2	6
Tributary 1						
Smallmouth Bass	-30.05 (1.34)	-28.28 (2.37)	1.17	0.63	5	2
Redbreast Sunfish	-29.31 (1.01)	-30.68 (0.85)	21.69	1.00	15	10
Chain Pickerel	-30.19 (0.72)	-31.02 (0.19)	6.68	1.00	15	2
White Sucker	-31.50 (1.73)	-31.51 (0.80)	16.06	0.90	12	10
Golden Shiner	-32.04 (0.71)	-30.47 (1.63)	11.31	0.08	15	10
Tributary 2						
Redbreast Sunfish	-29.05 (1.29)	-29.40 (0.36)	5.52	0.94	6	9
Chain Pickerel	-29.58 (0.83)	-29.09 (1.66)	10.50	0.35	8	6
White Sucker	-28.76 (0.97)	-27.62 (1.81)	6.12	0.44	5	5
Tributary 3						
Smallmouth Bass	-27.00 (0.74)	-28.08 (1.04)	16.48	1.00	11	12
Redbreast Sunfish	-25.25 (0.72)	-27.44 (0.86)	9.87	1.00	5	8
White Sucker	-27.80 (3.18)	-28.41 (0.83)	10.22	0.96	10	10
Eastern Elliptio	-30.32 (0.61)	-30.46 (0.45)	3.15	0.90	3	6
Common Shiner	-24.70 (0.72)	-28.01 (0.10)	4.35	1.00	5	2

Table 4 Mean (SD) $\delta^{15}N$ pre- and post-restoration by site and species. Degrees of freedom and p-value reported for one sided Welch's t-tests. Correction for multiple comparisons was made using the Benjamini-Hochberg method with an alpha level of 0.05 (significant p-values bolded).

Species	Pre $\delta^{15}N$ Mean	Post $\delta^{15}N$ Mean	df	p-value	Pre n	Post n
Mainstem 1						
Smallmouth Bass	10.48 (0.89)	10.49 (0.48)	32.75	0.89	27	11
Redbreast Sunfish	9.77 (0.67)	10.21 (1.08)	10.36	0.49	13	8
White Sucker	8.92 (1.26)	8.68 (0.75)	11.70	0.98	16	5
American Eel	8.81 (0.78)	9.01 (0.36)	5.25	0.57	22	3
Snails	5.44 (0.23)	4.92 (0.47)	4.04	1.00	2	6
Eastern Elliptio	3.92 (0.70)	4.82 (0.90)	11.08	0.13	9	7
Mainstem 2						
Smallmouth Bass	9.51 (1.06)	10.48 (0.95)	20.44	0.09	18	11
Redbreast Sunfish	8.80 (0.81)	9.78 (2.81)	5.82	0.57	6	6
White Sucker	8.37 (1.80)	8.52 (1.68)	7.96	0.87	5	5
American Eel	9.50 (0.55)	9.40 (1.22)	9.77	0.93	8	8
Snails	4.60 (0.09)	5.16 (0.44)	5.60	0.09	4	6
Golden Shiner	6.82 (0.42)	8.41 (0.38)	7.94	**0.01**	5	5
Mainstem 3						
Smallmouth Bass	9.63 (0.65)	10.64 (1.27)	11.75	0.08	19	11
Redbreast Sunfish	9.75 (0.66)	10.11 (1.47)	10.45	0.59	10	10
White Sucker	8.06 (0.63)	8.23 (0.64)	10.09	0.67	8	6
American Eel	9.16 (0.47)	10.15 (1.56)	8.01	0.29	11	8
Snails	4.21 (0.67)	4.89 (0.36)	2.61	0.41	3	6
Eastern Elliptio	4.10 (0.34)	4.31 (0.25)	6.02	0.53	2	6
Tributary 1						
Smallmouth Bass	11.24 (0.64)	11.10 (0.53)	1.54	0.96	5	2
Redbreast Sunfish	9.86 (0.34)	10.68 (0.57)	13.27	0.01	15	10
Chain Pickerel	10.01 (0.64)	10.73 (0.61)	1.31	0.49	15	2
White Sucker	8.95 (0.50)	9.26 (0.72)	15.69	0.42	12	10
Golden Shiner	9.75 (0.34)	9.77 (0.36)	18.60	0.88	15	10
Tributary 2						
Redbreast Sunfish	8.74 (0.28)	8.66 (0.26)	10.30	0.95	6	9
Chain Pickerel	8.14 (0.54)	8.48 (0.52)	11.79	0.40	8	6
White Sucker	8.11 (0.41)	8.32 (0.98)	5.37	0.73	5	5
Tributary 3						
Smallmouth Bass	10.74 (0.21)	10.52 (0.55)	19.85	1.00	11	12
Redbreast Sunfish	10.11 (0.26)	9.74 (0.36)	10.62	1.00	5	8
White Sucker	8.86 (1.08)	8.74 (0.50)	12.73	0.92	10	10
Eastern Elliptio	5.20 (0.70)	5.30 (0.40)	2.69	0.86	3	6
Common Shiner	8.91 (0.24)	8.37 (0.29)	1.60	1.00	5	2

Table 5. Pre- and post-restoration mean (SD) trophic position of Smallmouth Bass and Redbreast Sunfish by site. Degrees of freedom and p-value reported for one-sided Welch's t-tests.

Site and Species	Pre Mean	Post Mean	df	p-value
Mainstem 1				
Redbreast Sunfish	3.50 (0.32)	3.52 (0.27)	16.99	0.44
Smallmouth Bass	3.74 (0.26)	3.64 (0.20)	24.42	0.89
Mainstem 3				
Redbreast Sunfish	3.64 (0.21)	3.57 (0.42)	9.84	0.69
Smallmouth Bass	3.59 (0.32)	3.72 (0.34)	11.18	0.11

Table 6. Estimations of Smallmouth Bass and Chain Pickerel isotopic niche size generated from SIBER by site and state of restoration. Chain Pickerel was only used at Tributary 2. Column headings: TA = Total convex hull area; SEA = standard ellipse area; SEAc = standard ellipse area corrected for small sample size; SEAb = standard ellipse area Bayesian; 95% Lower and Upper associated with SEAb estimates.

Site and State	TA	SEA	SEAc	SEAb	95% Lower	95% Upper
Mainstem 1						
Pre-Restoration	10.26	2.97	3.10	2.85	1.98	4.38
Post-Restoration	1.75	0.85	0.95	0.85	0.47	1.66
Mainstem 2						
Pre-Restoration	8.90	3.19	3.39	3.13	1.92	5.14
Post-Restoration	6.92	3.70	4.11	3.56	1.80	6.73
Mainstem 3						
Pre-Restoration	8.40	2.11	2.18	2.10	1.44	2.99
Post-Restoration	8.33	4.65	5.17	4.70	2.38	8.83
Tributary 1						
Pre-Restoration	1.41	1.38	1.85	1.67	0.62	4.96
Tributary 2						
Pre-Restoration	1.50	0.93	1.08	0.97	0.47	2.16
Post-Restoration	0.66	0.55	0.69	0.49	0.19	1.29
Tributary 3						
Pre-Restoration	0.85	0.48	0.54	0.44	0.25	0.87
Post-Restoration	0.61	0.27	0.30	0.26	0.13	0.47

Table 7. Estimates of Redbreast Sunfish isotopic niche size generated from SIBER by site and state of restoration. Column headings: TA = Total convex hull area; SEA = standard ellipse area; SEAc = standard ellipse area corrected for small sample size; SEAb = standard ellipse area Bayesian; 95% Lower and Upper associated with SEAb estimates.

Site	TA	SEA	SEAc	SEAb	95% Lower	95% Upper
Mainstem 1						
Pre-Restoration	7.62	3.52	3.83	3.38	1.81	5.97
Post-Restoration	4.80	2.55	2.98	2.42	1.15	5.68
Mainstem 2						
Pre-Restoration	5.83	5.22	6.52	4.96	2.18	13.28
Post-Restoration	11.84	9.85	12.31	14.51	5.61	36.70
Mainstem 3						
Pre-Restoration	6.48	2.38	2.52	2.28	1.43	3.71
Post-Restoration	6.67	4.58	5.15	4.71	2.32	9.28
Tributary 1						
Pre-Restoration	2.31	0.99	1.07	0.97	0.53	1.63
Post-Restoration	2.67	1.35	1.52	1.31	1.04	1.61
Tributary 2						
Pre-Restoration	0.56	0.44	0.55	0.62	0.26	1.65
Post-Restoration	0.49	0.27	0.31	0.25	0.13	0.53
Tributary 3						
Pre-Restoration	0.37	0.33	0.44	0.37	0.14	1.14
Post-Restoration	0.77	0.48	0.56	0.60	0.21	1.29

Table 8. Stomach contents of Smallmouth Bass and Northern Pike by site. Values reported are mean proportion of diet by volume of prey items. Species codes: SMB = Smallmouth Bass, PIK = Northern Pike. Macro = Macroinvertebrates, FW = Freshwater, Un ID = Unidentifiable Material.

Site	Species	n	Mean Length (mm) and range	Weight (g)	Macro	FW Fish	River Herring	Crayfish	Un ID
MS1	SMB	13	296 (239-352)	351.1 (163.8-549.3)	0.43	0.16	0.00	0.13	0.28
MS2	SMB	9	310 (277-353)	396.7 (294.4-574.1)	0.16			0.22	0.62
MS3	SMB	10	271 (235-306)	253.8 (167.6-372.4)	0.66	0.09			0.25
TR1	PIK	7	473 (397-670)	NA		0.07	0.93		
TR3	SMB	6	286 (251-311)	341.6 (242.2-415.3)	0.69			0.31	

Table 9. Mean (range) of total length (mm) for all direct comparisons of fish mean isotope values by site and state of restoration. Sample sizes are consistent with Table 3.

Species	Pre Length	Post Length
Mainstem 1		
Smallmouth Bass	275.96 (165-407)	298.36 (239-344)
Redbreast Sunfish	183.33 (146-220)	112.88 (42-171)
White Sucker	303.81 (181-415)	261.2 (116-436)
American Eel	261.27 (73-450)	368.3 (203-592)
Mainstem 2		
Smallmouth Bass	293.85 (219-357)	310.56 (282-355)
Redbreast Sunfish		129 (53-160)
White Sucker	98.66 (12-200)	377 (325-465)
American Eel	441.66 (360-483)	282 (223-353)
Golden Shiner		5.8 (5.5-6.1)
Mainstem 3		
Smallmouth Bass	264.75 (71-436)	290.18 (272-322)
Redbreast Sunfish	163.26 (53-203)	140.2 (50-184)
White Sucker	250.88 (64-483)	343.66 (178-435)
American Eel	533.93 (186-720)	307.25 (235-372)
Tributary 1		
Smallmouth Bass	309.2 (288-372)	327.5 (320-335)
Redbreast Sunfish	160.53 (115-196)	114 (45-156)
Chain Pickerel	341.4 (211-437)	301.00
White Sucker	371.73 (230-455)	277 (130-435)
Golden Shiner	91.47 (80-116)	117.1 (56-165)
Tributary 2		
Redbreast Sunfish	144.5 (92-174)	118.44 (62-155)
Chain Pickerel	258.25 (206-305)	240.5 (190-360)
White Sucker	423.6 (386-460)	281.8 (257-316)
Tributary 3		
Smallmouth Bass	289.5 (246-321)	275.67 (251-311)
Redbreast Sunfish	104.2 (86-147)	144.88 (122-175)
White Sucker	330.9 (195-466)	290.1 (72-363)
Common Shiner	65.8 (54-71)	58.5 (54.63)

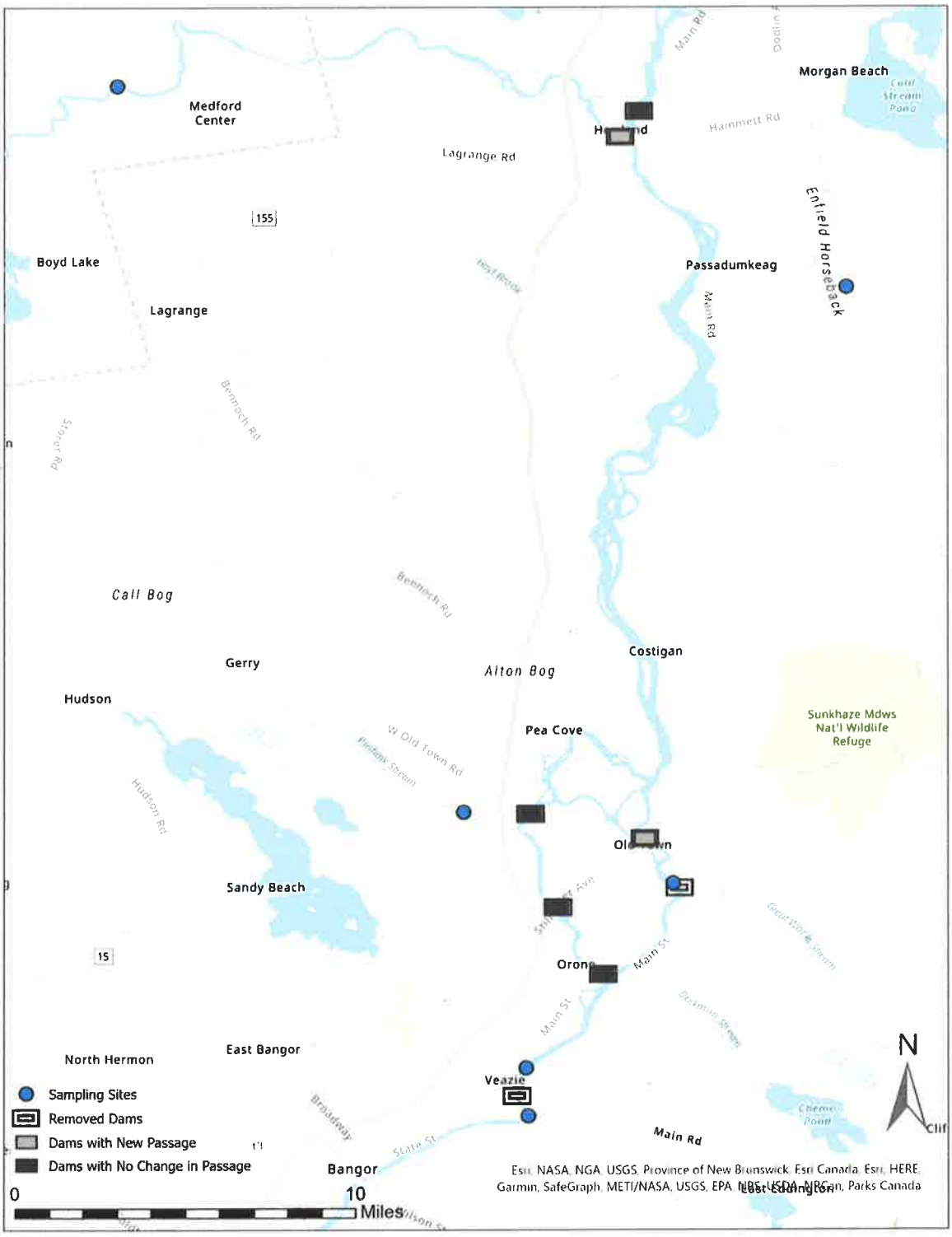

Figure 1. Sampling sites in the Penobscot River watershed. Sampling sites are marked by blue circles. Open rectangles are removed dams, gray rectangles are dams with passage improvements, and dark gray rectangles are dams with no new passage improvements. Note: lowest dam in dark gray sits just upstream of the mouth of the Stillwater River and does not block the mainstem Penobscot River.

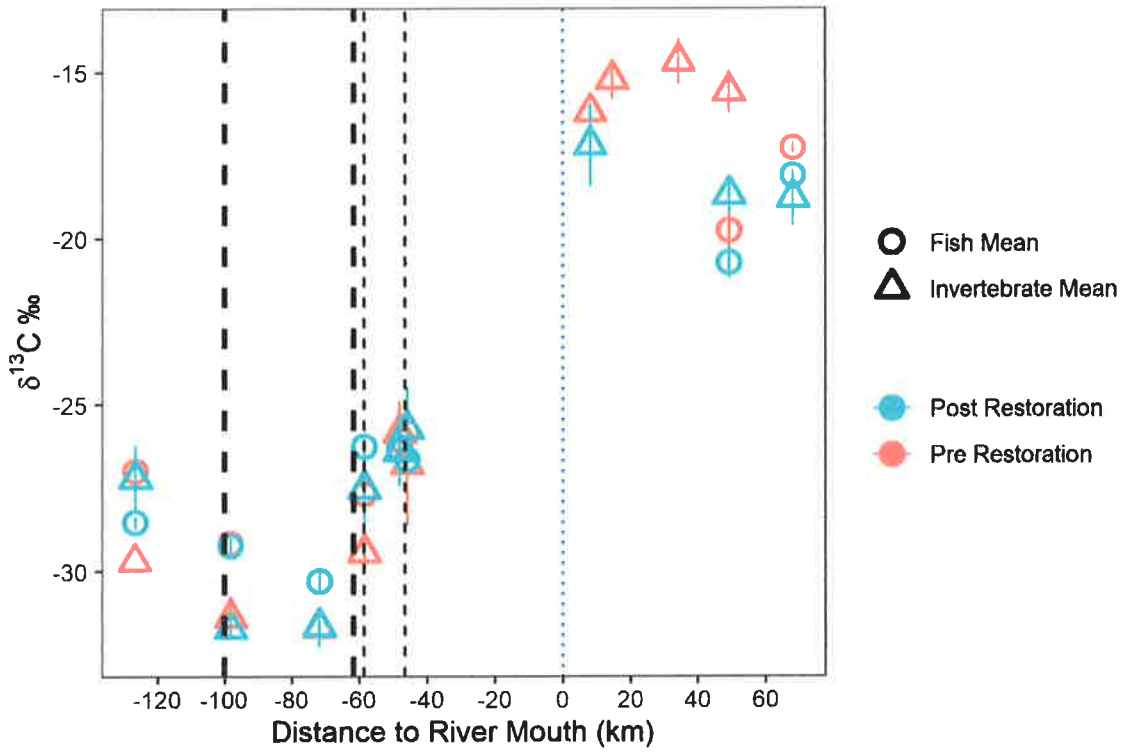

Figure 2. Mean fish and invertebrate $\delta^{13}C$ values pre- and post-restoration arranged by sampling sites in the Penobscot Watershed and Penobscot Bay. Error bars are ± 1 SE. Blue dashed line indicates the river mouth and the seaward extent of the estuary. Lightly dashed black lines indicate removed Veazie and Great Works Dams. Thick dashed black lines indicate dams with passage improvements (Milford and Howland Dam). Freshwater fish included in mean calculations: Smallmouth Bass, Redbreast Sunfish, Chain Pickerel, Northern Pike, Pumpkinseed Sunfish, American Eel, and White Sucker. Marine fish included in mean calculations: Atlantic Cod, Cunner, Atlantic Mackerel, Atlantic Pollock, and Acadian Redfish. Freshwater invertebrate mean values include mussels and snails. Marine invertebrate mean values include Blue Mussels, Periwinkles, Crabs, Urchins, and Seastars.

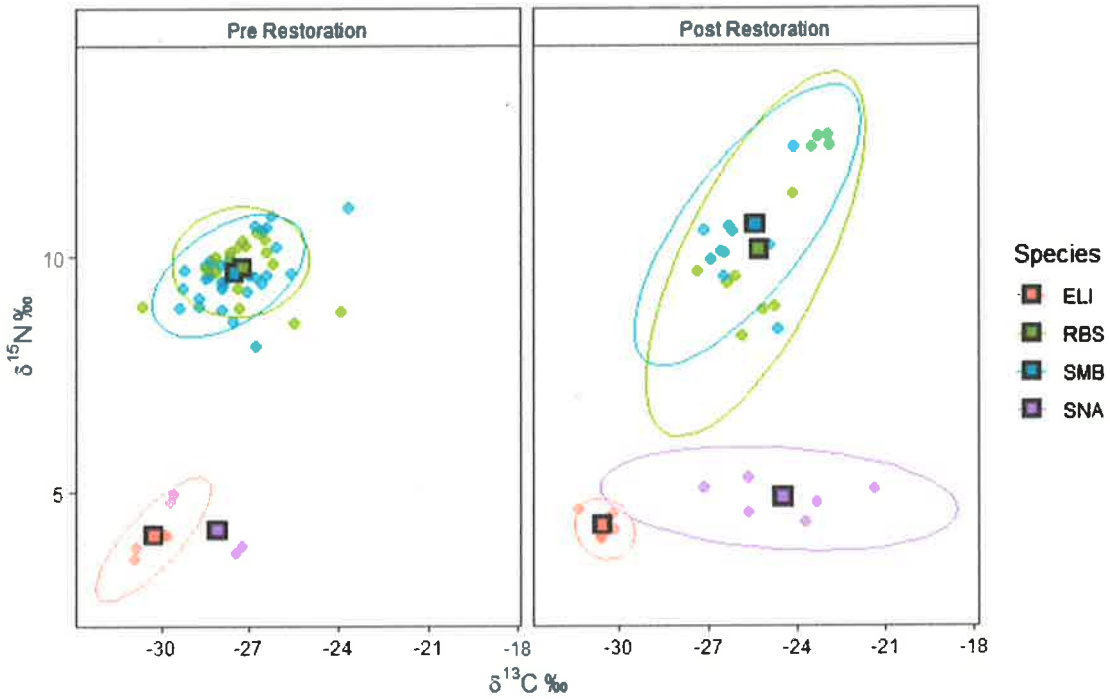

Figure 3. Isospace plot of $\delta^{13}C$ and $\delta^{15}N$ points at Mainstem 3. Species codes: ELI = Eastern Elliptio, RBS = Redbreast Sunfish, SMB = Smallmouth Bass, SNA = Snails. Points represent individuals. Squares represent mean value for the species. Ellipses are standard ellipses.

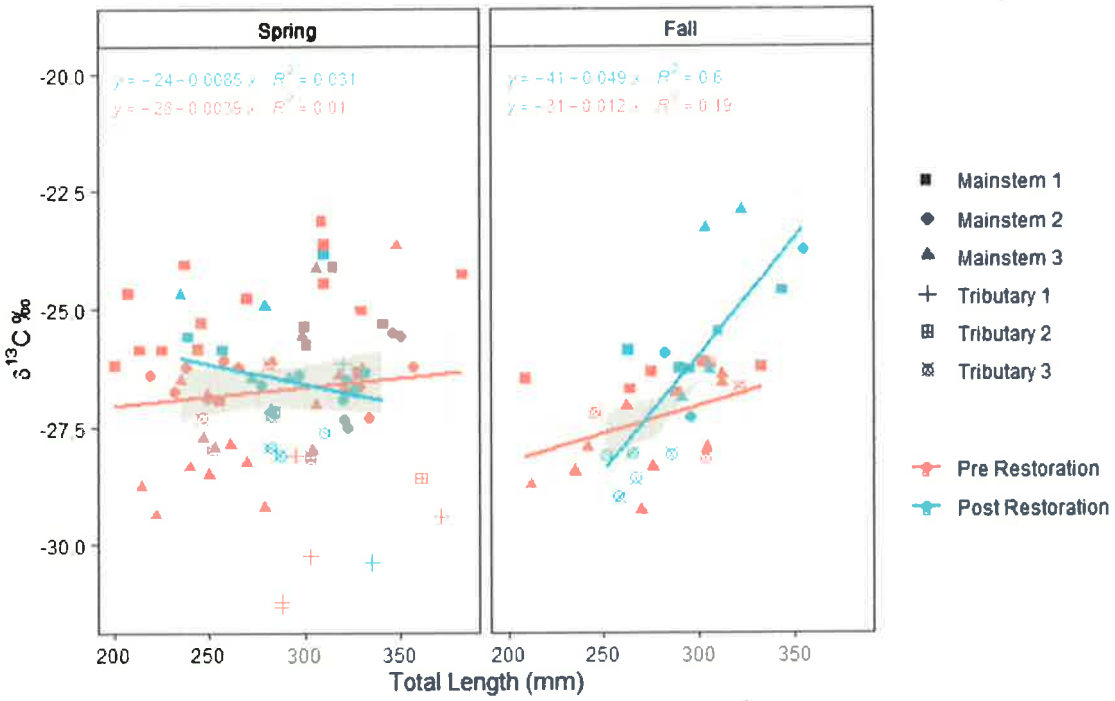

Figure 4. Relationship between individual Smallmouth Bass $\delta^{13}C$ values and total length pre- and post-restoration for spring and fall. Gray shading indicates SE. Only samples from post-restoration in fall suggest that ontogenetic (length-related) diet shifts occurred.

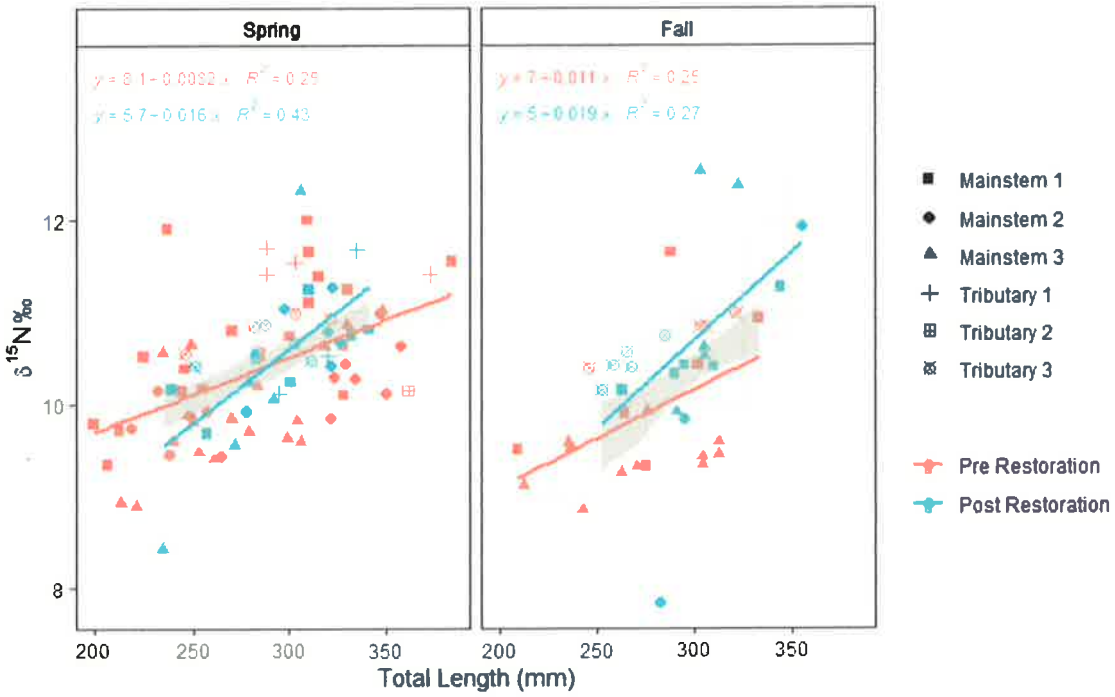

Figure 5. Relationship between individual Smallmouth Bass $\delta^{15}N$ values and total length pre- and post-restoration for spring and fall. Gray shading indicates SE.

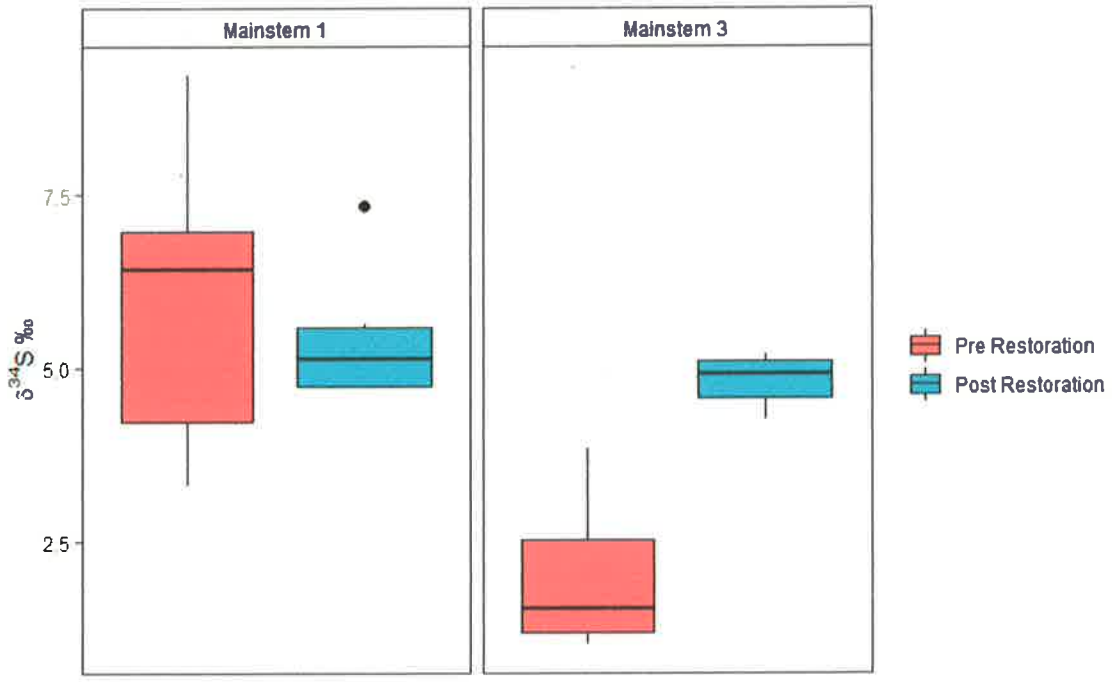

Figure 6. Box plots of mean $\delta^{34}S$ values for Smallmouth Bass pre- and post-restoration for two sampling sites. Vertical lines represent minimum and maximum values, boxes represent the range between first and third quartiles, horizontal bars represent medians, and points represent individuals outside of 1.5 interquartile range. n = 6 fish for each site at each sampling period.

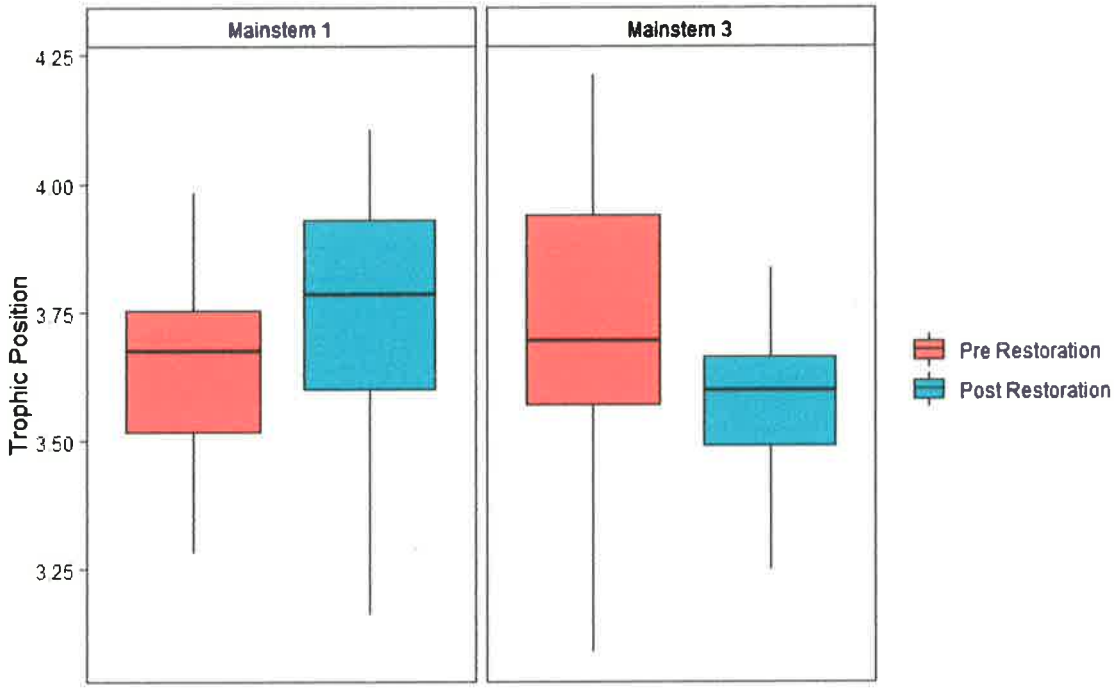

Figure 7. Box plots of mean trophic position estimates for Smallmouth Bass pre- and post-restoration for two sampling sites. Vertical lines represent minimum and maximum values, boxes represent the range between first and third quartiles, horizontal bars represent medians. Mainstem 1 sample sizes: pre-restoration: 27, post-restoration: 28. Mainstem 3 sample sizes: pre-restoration: 32, post-restoration: 20.

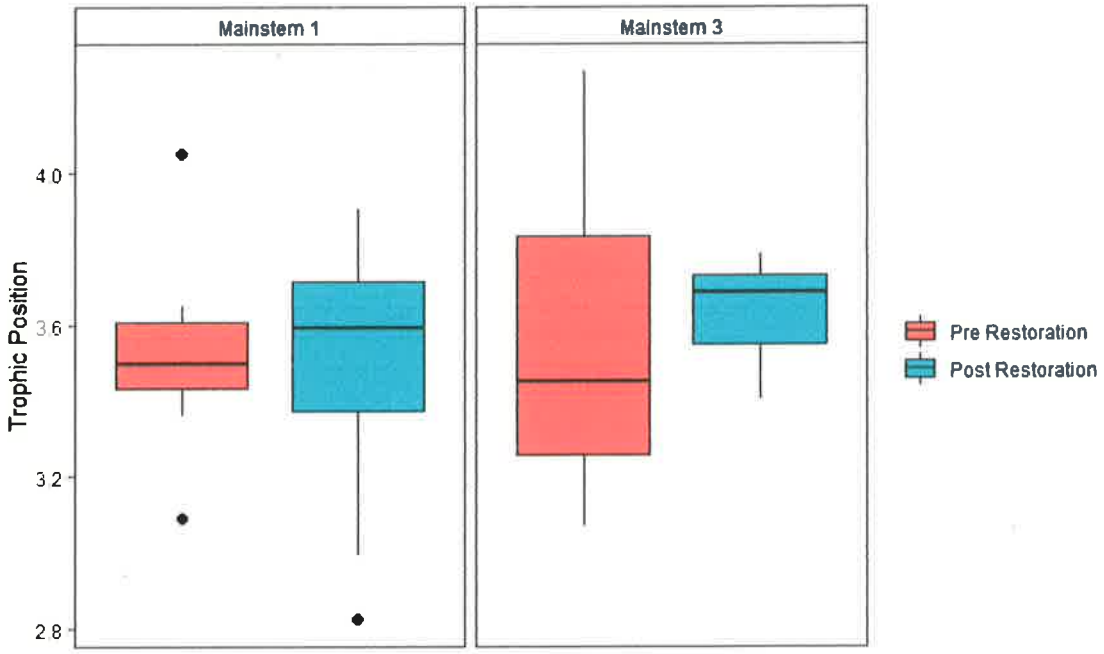

Figure 8. Box plots of mean trophic position estimates for Redbreast Sunfish pre- and post-restoration for two sampling sites. Vertical lines represent minimum and maximum values, boxes represent the range between first and third quartiles, horizontal bars represent medians, and points represent individuals outside of 1.5 interquartile range. Mainstem 1 sample sizes: pre-restoration: 13, post-restoration: 8. Mainstem 3 sample sizes: pre-restoration: 19, post-restoration: 10.

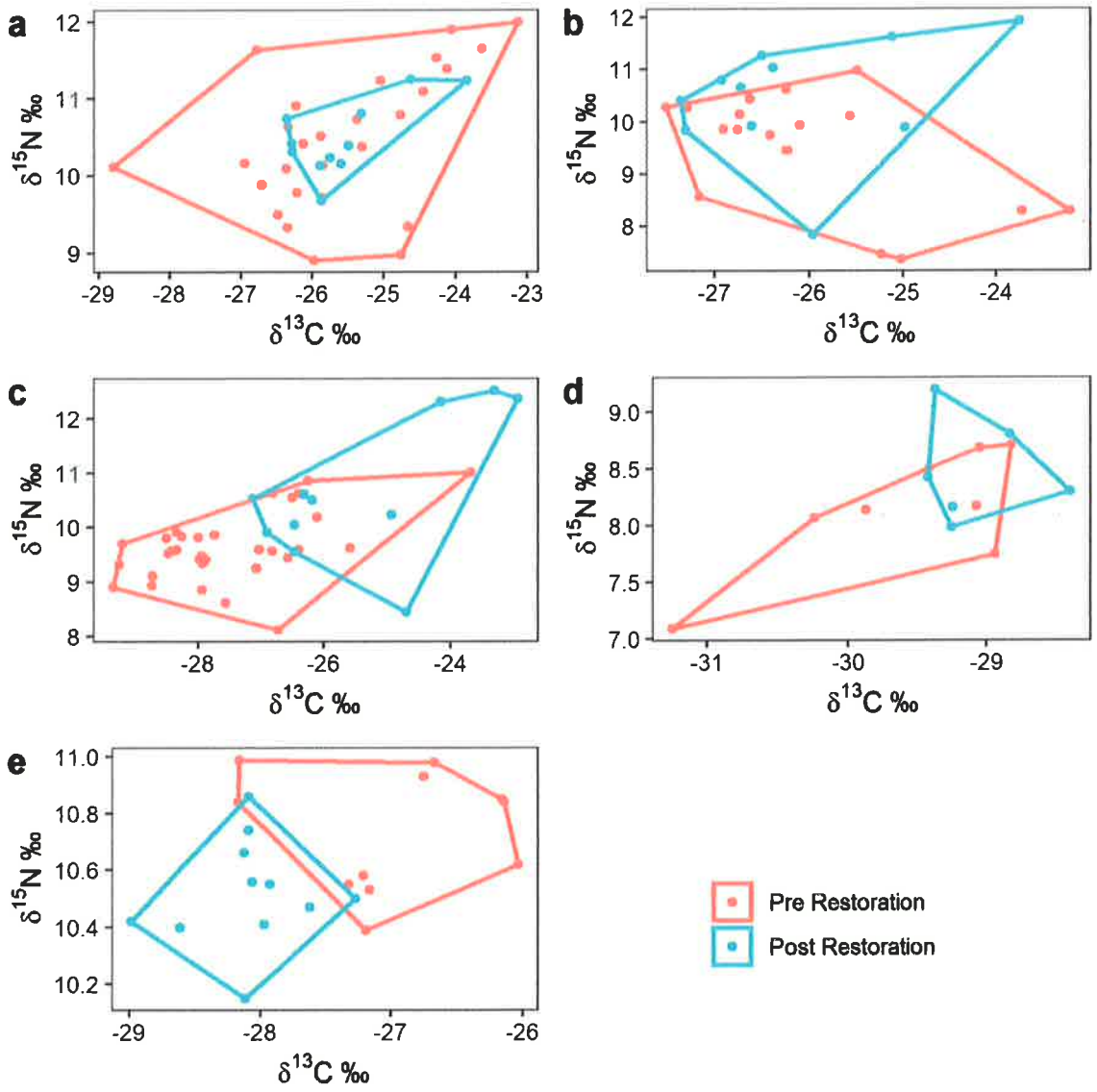

Figure 9. Total area calculations of isotopic space for Smallmouth Bass and Chain Pickerel by sampling site for pre-restoration (red) and post-restoration (blue). Points represent individual fish values. a) Mainstem 1; b) Mainstem 2; c) Mainstem 3; d) Tributary 2; e) Tributary 3.

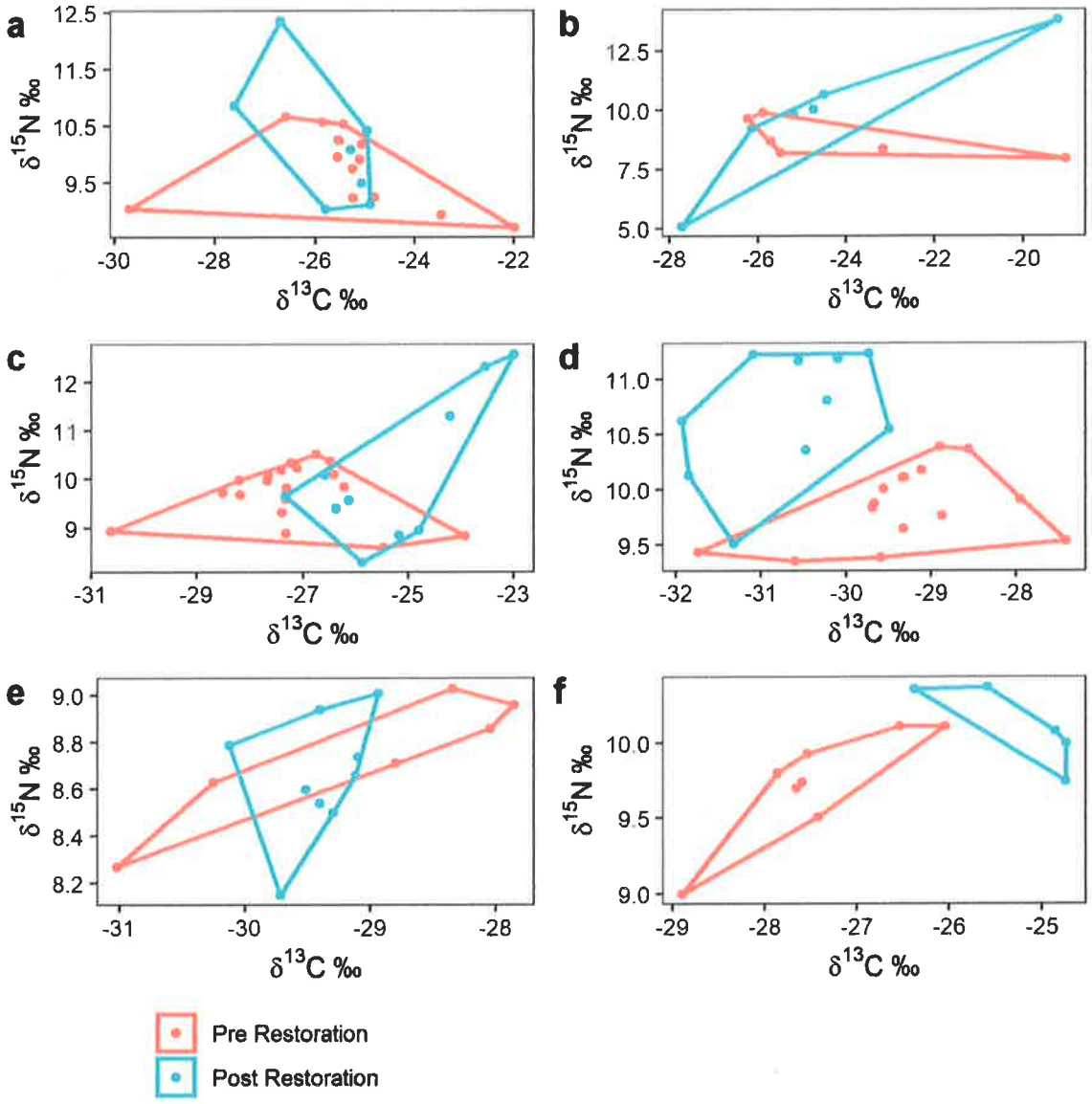

Figure 10. Total area calculations of isotopic niche space for Redbreast Sunfish by sampling site during pre-restoration (red) and post-restoration (blue). Points represent individual fish values a) Mainstem 1; b) Mainstem 2; c) Mainstem 3; d) Tributary 1; e) Tributary 2; f) Tributary 3.

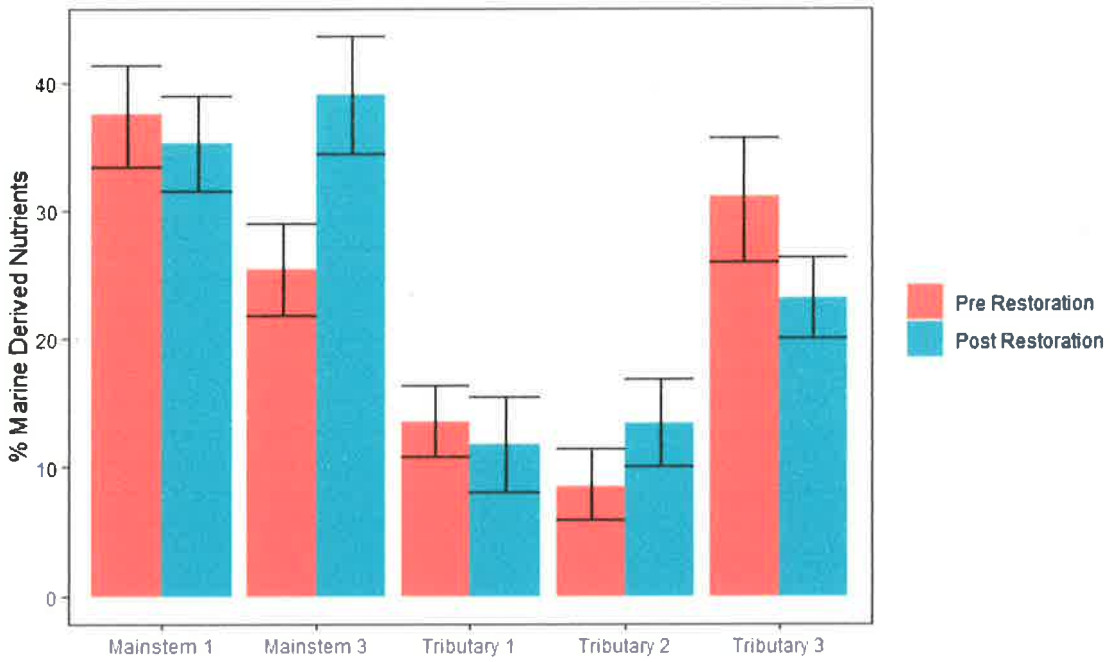

Figure 11. Mean MixSIAR estimates of marine derived nutrients for Smallmouth Bass and Chain Pickerel by site during pre-restoration (red) and post-restoration (blue)). Error bars display 95% credible intervals. Pre-restoration sample sizes (M1: 26, M3: 32, T1: 15, T2: 8, T3: 10. Post-restoration sample sizes: M1: 9, M3: 19, T1:15, T2: 6, T3: 5.

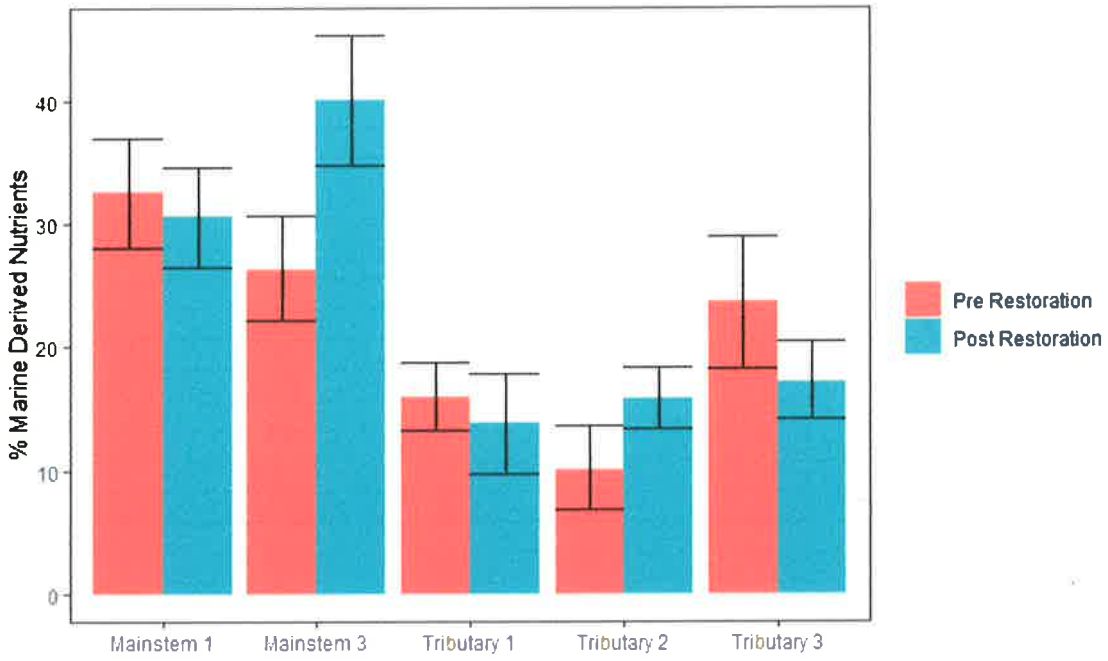

Figure 12. Mean MixSIAR estimates of marine derived nutrients for Redbreast Sunfish by site during pre-restoration (red) and post-restoration (blue). Error bars display 5-95% credible intervals. Pre-restoration sample sizes M1: 11, M3: 11, T1: 4, T2: 6, T3: 12. Post-restoration sample sizes: M1: 8, M3: 10, T1: 9, T2: 9, T3: 8.

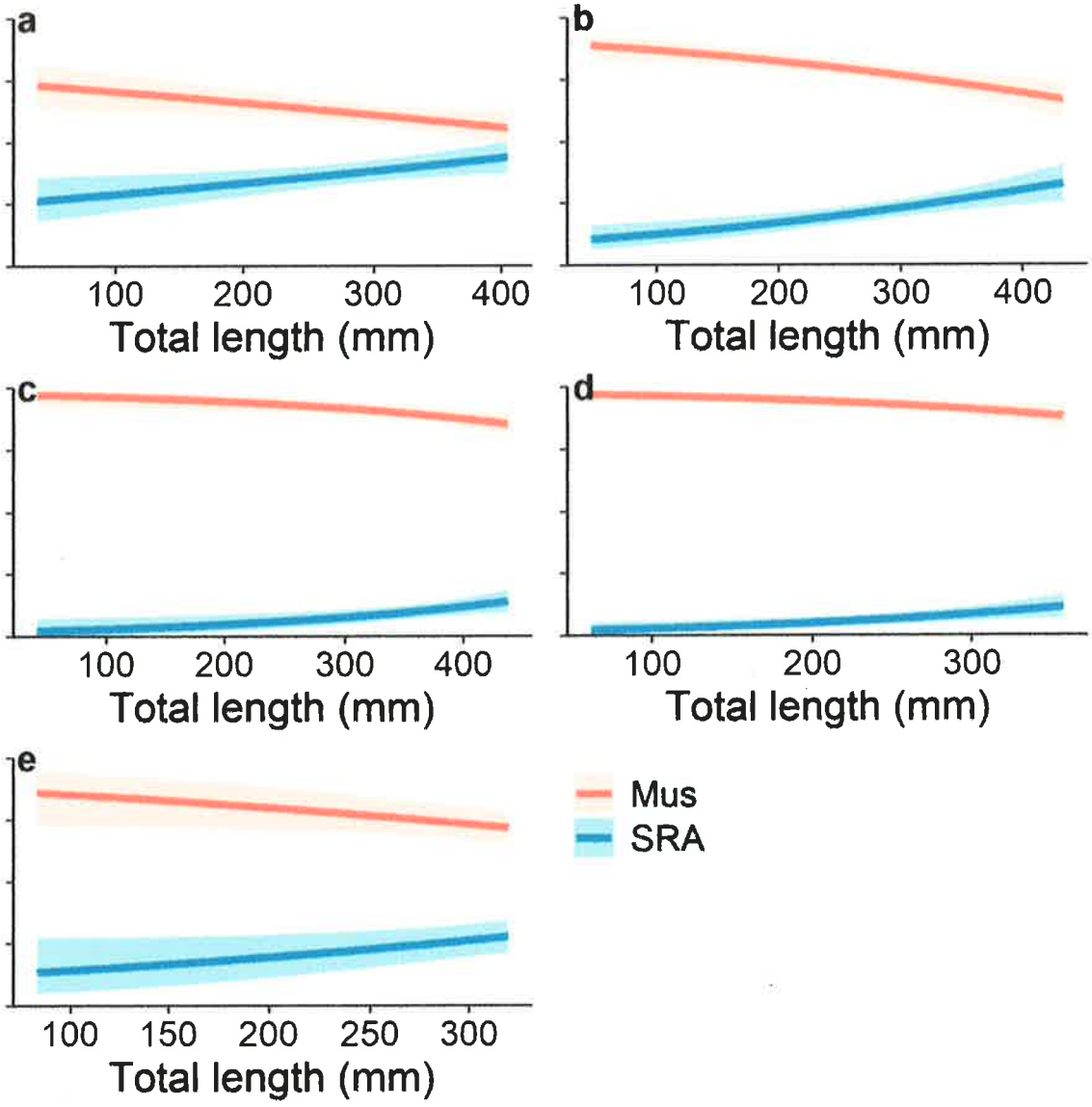

Figure 13. Total length to diet proportion relationships from MixSIAR mixing models of Smallmouth Bass and Chain Pickerel. Y-axis represents diet proportion; tick marks represent 25, 50, 75, and 100% of consumer diet. Red line shows %Freshwater diet proportions (represented by mussels (Mus)), blue line shows %MDN diet proportions (represented by adult Alewife (SRA)). Shaded regions indicate 95% credibility intervals. Chain Pickerel are in Tributaries 1 and 2 only. a) Mainstem 1; b) Mainstem 3; c) Tributary 1; d) Tributary2; e) Tributary 3.

Figure 14. Smallmouth Bass captured at the Milford Dam fishway with the tail of an adult river herring extending from its mouth. Photo credit: Spencer Campbell.

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Appendix A: Literature Review Summary Tables

Table A-1. Summary table of studies that determined their own trophic fractionation value for $\delta^{15}N$.

Paper	Field/ Lab Study	Study Species/ Taxa	$\delta^{15}N$ Fractionation Value
Vander Zanden and Rasmussen, 2001	Field Study	Lake Trout	3.49‰
Post, 2002	Combination	Many	3.4‰
Sweeting et al., 2007	Lab	Black Sea Bass	2.9 – 3.4‰

Table A-2. Summary table of studies that investigated differences in $\delta^{15}N$ across study systems.

Study	Study Systems	$\delta^{15}N$ Differences	Interpretation
Chang et al., 2002	Watersheds dominated by different land use types.	"Moderately distinct" values for each watershed by land use types.	Land use types can create watershed specific $\delta^{15}N$ that need to be accounted for the compare across systems.
Mayer et al., 2002	Watersheds with a variety of land use types (more forested).	Different nitrate values for watersheds could be identified by using $\delta^{15}N$ values.	Land use types can create watershed specific $\delta^{15}N$ with a greater difference between watersheds with a high degree of anthropogenic impact and those that remain mostly forested.
Cabana and Rasmussen, 1996	Lakes with varying levels of anthropogenic impact (human population density).	Increases in $\delta^{15}N$ with human population density within the lake watershed.	Isotope values vary along this predictable gradient meaning that baseline values need to be incorporated to accurately calculate values like trophic position to compare between lakes.
Anderson and Cabana, 2005	Watersheds with a range of land use types (focused on agriculture impacts).	Differences in $\delta^{15}N$ can mostly be attributed to site effects with little differences in feeding groups of primary consumers.	The differences in isotope values between feeding groups of primary consumers is negligible compared to the effect of site. Therefore, using site specific baseline values to correct $\delta^{15}N$ used in trophic position calculations, results can be compared across sites reliably.
Bentoviglio et al., 2016	Many sites with different pollution levels in one watershed.	$\delta^{15}N$ varied based on point-source pollution throughout the watershed.	Another example that demonstrates how δ15N vary by site and need to be corrected appropriately. This also demonstrates an application where this relationship is so strong it can identify point-source pollution within a watershed.

Appendix B: Penobscot Bay

Table B-1. Penobscot Bay mean $\delta^{13}C$ and $\delta^{15}N$ pre- and post-restoration by site and species.

Species	Pre $\delta^{13}C$	Post $\delta^{13}C$	Pre $\delta^{15}N$	Post $\delta^{15}N$	Pre n	Post n
Penobscot Bay A						
Crabs	-16.17		12.96		3	
Periwinkles		-14.44		8.51		3
Blue Mussels		-19.90		8.29		3
Penobscot Bay B						
Crabs	-15.81		12.89		4	
Periwinkles	-13.58		9.17		3	
Lobster	-16.23		13.74		2	
Blue Mussels	-18.26		6.99		4	
Sea Stars	-11.70		10.07		3	
Sea Urchins	-15.38		5.83		3	
Penobscot Bay C						
Crabs	-15.51		12.61		5	
Periwinkles	-14.28		8.00		4	
Lobster	-15.90		13.31		3	
Blue Mussels	-18.47		7.34		3	
Sea Stars	-11.13		8.45		6	
Sea Urchins	-17.03		5.95		3	
Penobscot Bay D						
Crabs	-16.00		12.11		10	
Periwinkles	-16.37		10.13		4	
Lobster	-16.24		13.68		4	
Blue Mussels	-17.76	-18.68	7.78	9.16	3	2
Sea Stars	-11.44		8.52		6	
Sea Urchins	-19.24		5.97		3	
Mackerel	-22.47	-20.72	13.25	10.70	3	5
Pollock	-17.02		12.63		3	
Sculpin	-16.70		13.34		1	
Penobscot Bay E						
Periwinkles		-15.69		7.90		3
Blue Mussels		-20.45		7.86		3
Pollock	-17.03	-18.59	12.81	12.45	7	4
Sculpin	-16.44		12.45		2	
Cod	-16.70	-17.79	12.93	14.47	8	7
Cunner		-18.23		12.82		4
Redfish	-18.01		11.94		8	
Sea Raven	-17.13		13.62		1	
Horse Mussel		-20.20		7.76		3

Appendix C: Kennebec River

Table C-1. Kennebec River mean $\delta^{13}C$ and $\delta^{15}N$ pre- and post-restoration by site and species.

Species	Pre $\delta^{13}C$	Post $\delta^{13}C$	Pre $\delta^{15}N$	Post $\delta^{15}N$	Pre n	Post n
Benton Falls						
Chain Pickerel	-26.45		13.01		1	
Smallmouth Bass	-24.04		13.28		1	
Eastern Elliptio	-32.81		8.55		3	
Snails	-24.13		9.49		1	
Burnham Dam						
Chain Pickerel	-27.12	-28.95	11.50	12.28	3	2
Smallmouth Bass	-29.12		10.34		3	
Eastern Elliptio	-33.67		8.09		7	
Snails	-31.60		8.29		1	
Lockwood Tailwater						
Smallmouth Bass	-24.86	-23.33	11.44	12.52	4	3
Eastern Elliptio	-29.53	-29.57	5.86	5.85	4	3
Snails	-23.47		6.81		1	
Juvenile Alewife		-26.87		9.15		5
Spottail Shiner		-25.26		9.38		3
Kennebec Sidney						
Smallmouth Bass	-22.67	-23.82	11.85	12.47	13	10
Eastern Elliptio	-28.86		7.12		5	
Snails	-22.85		7.49		3	
Striped Bass		-20.21		14.45		4
Lines Island						
Striped Bass	-16.73		14.06		2	

Appendix D: Penobscot River

Table D-1. Penobscot River mean $\delta^{13}C$ and $\delta^{15}N$ pre- and post-restoration by site and species. This data was not utilized in the main analysis due to lack of comparisons between pre- and post-restoration data sets.

Species	Pre $\delta^{13}C$	Post $\delta^{13}C$	Pre $\delta^{15}N$	Post $\delta^{15}N$	Pre n	Post n
Mainstem 1						
Sea Run Alewife	-19.13	-19.79	12.07	12.28	24	5
Juv. Blueback Herring	-25.00	-30.63	10.36	8.96	3	4
Atlantic Salmon	-20.25		12.55		4	
Chain Pickerel	-28.31		10.88		11	
Pumpkinseed Sunfish	-27.94		9.32		4	
Sea Lamprey	-17.82		12.13		2	
Blueback Herring		-19.89		12.31		5
Golden Shiner		-32.53		8.77		5
Fallfish		-26.91		9.03		5
Mainstem 2						
Brown Bullhead	-27.24		8.56		3	
Common Shiner		-27.38		8.44		5
Fallfish		-27.29		8.99		5
Eastern Elliptio		-29.80		4.32		5
Mainstem 3						
Brown Bullhead	-28.39		8.51		1	
Common Shiner	-27.28		8.03		5	
Golden Shiner	-25.70		8.36		5	
Fallfish		-26.81		9.40		5
Tributary 1						
Pumpkinseed Sunfish	-30.59		9.63		15	
Yellow Perch	-31.93		9.22		5	
Juv. Sea Run Alewife		-31.73		8.96		5
Blueback Herring		-19.45		11.92		5
American Eel		-32.36		9.69		2
Northern Pike		-28.11		11.51		7
Snails		-29.78		5.64		5
Sea Run Alewife		-19.07		12.07		5
Tributary 2						
Brown Bullhead	-29.22		7.99		3	
American Eel		-30.66		8.21		3
Fallfish		-28.29		7.47		5
Golden Shiner	-29.15		7.63		5	
Smallmouth Bass		-26.93		10.08		2
Tributary 3						

Juv. Sea Run Alewife	-30.69	7.02	5
Juv. Blueback Herring	-29.24	7.02	5
American Eel	-30.48	8.16	2
Fallfish	-27.91	8.97	9
Snails	-23.94	4.91	6