FEDERAL ASSISTANCE
FINAL PERFORMANCE REPORT

ALASKA DEPARTMENT OF FISH AND GAME
SPORT FISH DIVISION
PO Box 115526
Juneau, AK 99811-5526

STATE WILDLIFE GRANT (SWG)

STATE: Alaska

GRANT: T-37-1
PROJECT: P-01

WORK LOCATION: Homer
PERIOD DURATION: July 1, 2017–June 30, 2018

PROJECT REPORTING PERIOD: July 1, 2017–June 30, 2018

PROJECT TITLE: Headwater stream nutrient export and downstream community response.

PROJECT AUTHORS: Coowe Moss Walker and Ryan King

Project Objectives:
This project investigates the downstream nutrient export from headwater streams to lower river reaches of the four major river drainages of the Kenai Lowlands, Alaska. This information can be used to prioritize conservation and management of these watersheds for the support and continued resilience of salmonids.

Objectives:

1. Estimate the quantity, quality and species composition of basal food resources, particularly periphyton (attached algae and microbes);

2. Estimate density, biomass and species composition of benthic macroinvertebrates, which are the primary food source of juvenile salmonids;

3. Estimate whole stream metabolism; and

4. Continue to collect and analyze water samples to relate differences in chemistry to the biological response variables.

Summary of Project Accomplishments:
The Kenai Lowlands region of the southern Kenai Peninsula, Alaska includes four major watersheds widely recognized for supporting Coho salmon (*Onchorhynchus kisutch*), Chinook salmon (*O. tshawytscha*) and Dolly Varden (*Salvelinus malma*) populations that are valued as sport and commercial fisheries, and are an important part of local culture (ADFG 2015). Over the past decade, research led by the Kachemak Bay National Estuarine Research Reserve (KBNERR) has demonstrated that virtually all headwater streams in the Kenai Lowlands support at least one life-history stage of salmonid (King et al. 2012). Our studies have revealed that productivity in these headwaters is driven by nitrogen, fixed by alders in surrounding watersheds, supports streams and riparian wetlands (Walker et al. 2012, Hiatt et al. 2017, Callahan et al. 2017, Whigham et al. 2017), subsidizing instream invertebrate production (Shaftel et al. 2011, Shaftel et al. 2012) and juvenile salmonid diets and productivity (Dekar et al. 2012, King et al. 2012). We have also shown that peatlands, which are common in Kenai Lowland headwater stream landscapes, contribute substantial amounts of dissolved organic carbon (DOC) additions to headwater streams (Walker et al. 2012).

This carbon can be important energetically for salmon rearing headwater streams, especially when there is alder derived nitrogen already present (King et al. 2015, Robbins et al. *submitted*).

Building on these studies, we hypothesized that there may be ecological “hotspots” where wetlands and alder cover in headwater streams supply dissolved organic carbon and dissolved organic nitrogen that synergistically enhance stream productivity, and that this productivity transfers downstream. We conducted initial investigations to determine the spatial and temporal patterns of downstream nutrient export. Results from this preliminary investigation indicated that nutrient sources (alder and peatlands) matched up with the patterns in water chemistry downstream. Where there is high alder cover in headwaters, there is more nitrogen in lower stream reaches, and where there is high peatland cover in headwaters, there is more carbon in lower stream reaches, supporting our hypothesis that export of headwater stream productivity is occurring (Walker and King 2017).

The study documented in this report investigated the effects of the nutrients exported from headwaters on downstream food webs, particularly primary productivity and biota which constitute key food resources for juvenile Chinook and Coho Salmon. We sampled and analyzed stream chemistry, discharge, basal food resources, and stream macroinvertebrates along a river continuum from headwaters to lower reaches in the major rivers of the Kenai Lowlands. Results confirmed the downstream export of nutrients, and showed that these nutrients are driving the quality of juvenile salmonid food resources in lower river reaches through enriched omega3:omega6 fatty acid ratios.

Study Sites: We selected sites in the lower reaches of each Kenai Lowland river, a trio of sites in two different headwater settings, and four mid river locations m for a total of 16 sites (Figure 1). Each trio included 1) a 1st order stream draining upper watersheds with high alder cover; 2) a 1st order stream draining an upper watershed with high peatland cover; and 3) the stream below the confluence of the two 1st order streams. Alder cover was assessed from previously mapped alder completed by KBNERR (Walker et al. 2013). For peatland cover, we used the Kenai Lowlands wetlands map (Gracz et al. 2008). Specific locations for sampling were chosen based on previous knowledge of streams, access and property ownership. We delineated catchment boundaries with 1-m resolution LiDAR.

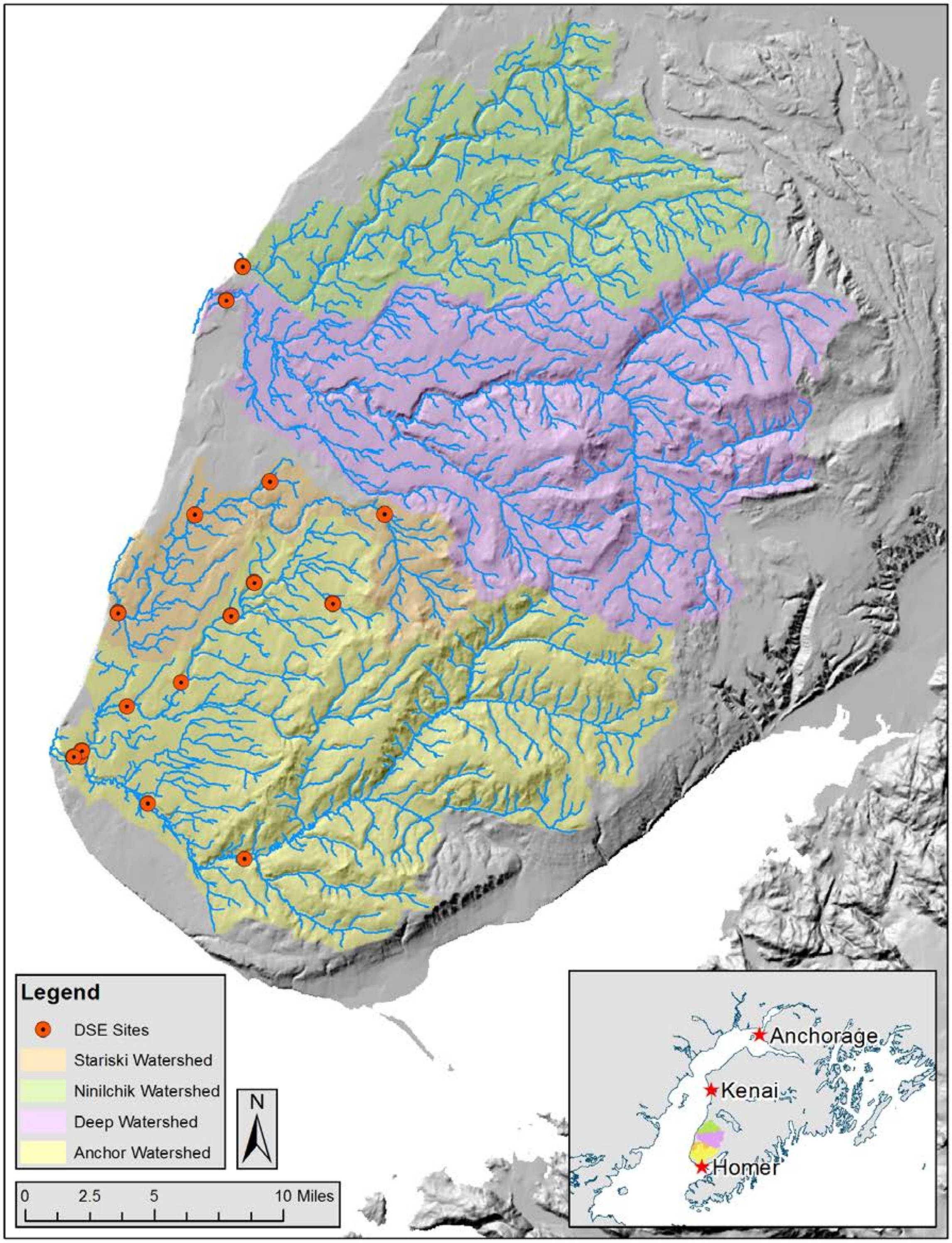


Figure 1. Map showing sample collection locations.

Methods

Discharge and water chemistry

Data used in this study includes water and discharge samples, collected in October of 2016, early May through October 2017, and late April through June 2018. Earlier spring sampling during high water conditions in 2018 was made possible due to the use of an acoustic doppler profiler (Sontek®), loaned by the Kenai Watershed Forum (Figure 2).

Figure 2. The Acoustic Doppler Profiler (SonTek) allowed us to measure stream discharge during high flow events. Prior to the use of the SonTek, sampling discharge high water events required ropes and taller staff members (above right). A graphical output from the profiler illustrates a rough outline of the river bottom and colored cells representing water speed in feet per second (below).

Water samples were collected in 50ml duplicates in the field and analyzed for total N (TN), NH4, NO3+NO2 (NOx-N), total P (TP), orthophosphate (PO4-P), and dissolved organic C (DOC). Samples for dissolved nutrients were filtered in the field with a 0.45 μm filter (Whatman, Ltd., Maidstone, Kent, UK) and syringe. All samples were kept cool in the field and then frozen upon returning to the lab until delivery to Baylor University. At Baylor's Center for Reservoir and Aquatic Systems Research lab they were analyzed on a Lachat QuikChem 8500 series 2 continuous flow injection analyzer (Lachat Instruments, Loveland, Colorado, USA). DOC and TDC were analyzed using a Shimadzu TOC-Vcsh (Shimadzu Corporation, Kyoto, Japan). Dissolved inorganic carbon was calculated as TDC – DOC = DIC. All analyses followed standard methods (APHA 1998). Stream velocities and discharge measurements were made downstream of the chemistry samples using either a Marsh-McBirney Flo-Mate™flow meter and a top-setting wading rod (Marsh-McBirney, Frederick, MD), following Lazorchak et al. (1998), or the Sontek. Results for each analyte over the time series were plotted to look for patterns in nutrients across sites and time. We included results from our previous years study to determine if inter-annual trends were evident.

Periphyton, benthic organic matter, invertebrates

We collected epilithic periphyton by grabbing five large gravel or cobbles spread evenly across each of three transects in riffles at each site (N=15 rocks). We chose rocks that did not show evidence of being recently tumbled or scoured. Rocks were then scraped and slurries were placed in a dark bottle on ice. Rock surface area was determined by tearing foil along the area of the top of the rock and using a foil mass-area relationship.

We collected benthic organic matter (BOM) by inserting an open-bottomed bucket (530 cm^2) ~5 cm into substrates in areas of the stream <~40 cm deep to discourage the continuous input of sestonic OM (OM transported in the stream water column) overtopping the bucket. Because we had collected periphyton already, we removed cobbles and large gravels from the bucket enclosure so that BOM collection was focused on the detrital portion. We vigorously disturbed and swirled the substrate in the open bucket to entrain OM in the water column. Then, we used a bottle to scoop samples out of the enclosure and poured the contents through nested sieves to collect coarse (1 mm mesh), fine (250 mm) and very fine particulate OM fractions (73 µm). We did this at at least three locations in each stream. We sampled dominantly in shallow habitat, but sampled across the representative substrate at each site. BOM samples were kept in whirl-pak bags and placed on ice. We collected sestonic OM from the thalweg in dark bottles and placed on ice. Where present, we additionally collected filamentous algae, bryophytes and conditioned *Calamagrostis canadensis* (blue-joint grass) litter and placed them on ice in the dark.

Benthic invertebrates were collected qualitatively by using a D-net to collect kicked sediment or undercut banks. We also used forceps to pick invertebrates off of rocks and placed the invertebrates directly into plastic vials. Samples were kept on ice, and D-net samples were picked and sorted in the lab. Abundant and large-bodied taxa (i.e., enough mass for stable isotope analysis) were placed in microcentrifuge tubes and placed back on ice until freezing in liquid nitrogen.

Lab processing occurred no more than eight hours post-collection. We homogenized periphyton slurry on a stir plate and filtered aliquots (5-10 mL) onto glass fiber filters (0.7 um pore size) for ash-free dry mass (AFDM; pre-combusted, pre-weighed filters; N=2), chlorophyll-a (N=2) and fatty acid analysis (N=3). Seston was also filtered onto glass fiber filters for AFDM (N=2), chlorophyll-a (N=2), stable isotopes (N=2) and fatty acid analysis (N=3). The remaining periphyton slurry was frozen for stable isotope analysis. Any filters for AFDM, chlorophyll-a, or stable isotope analysis were frozen in foil packets. BOM, filters (seston and periphyton), filamentous algae, bryophytes and invertebrates for fatty acid analysis and invertebrates for stable isotope analysis were frozen in liquid N_2 and shipped to Baylor University overnight on dry ice and frozen at -80 °C.

At Baylor University, all samples for fatty acid analysis (and whole samples for stable isotope analysis) were freeze-dried. Additionally, any samples destined for fatty acid analysis were always stored under N_2 atmosphere at -80 °C for any future storage. Periphyton slurry and filters for AFDM determination or stable isotope composition were dried at 60 °C for 48 hours. Invertebrates, bryophytes and filamentous algae were cleaned of any foreign debris (e.g., detritus, sand) prior to stable isotope or fatty acid analysis. Chlorophyll-a and AFDM in seston and periphtyon were determined following methods in Biggs and Kilroy (2000). For stable isotope analysis, invertebrates were ground into a fine powder using a weighing spatula and placed in weighed silver capsules. BOM was ground to a fine powder in a Biospec Mini-BeadBeater (Bartlesville, OK). Aliquots of thawed periphyton slurries were dried at 60 °C for 48 hours in tin weigh pans. Dried slurry was scraped into weighed silver capsules. All stable isotope samples were fumigated with HCl to remove any inorganic carbon. Silver capsules were then wrapped in tin capsules and analyzed for δ^{13}C and δ^{15}N on a Thermo-Electron Delta V Advantage IRMS at Baylor University.

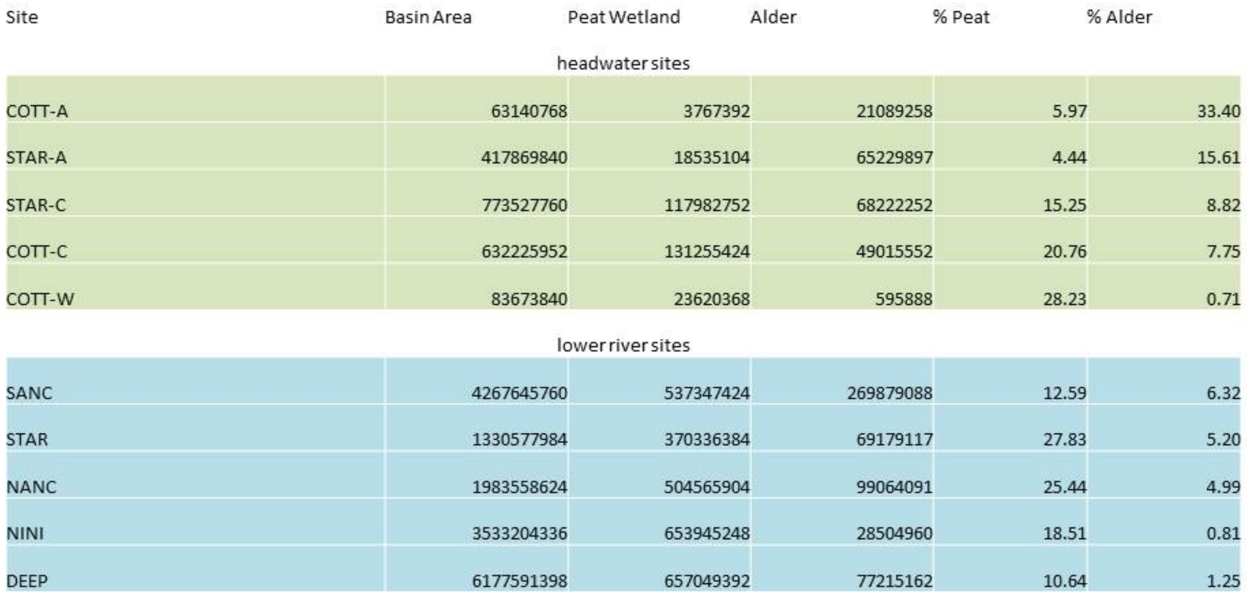
Invertebrates analyzed for fatty acids were left whole and 5-10 mg were shipped on dry ice overnight to Microbial ID, Inc. (Newark, Delaware) for lipid extraction and quantification of phospholipid fatty acid (PLFA) composition. PLFAs are presented as percent composition, where known PLFA gas chromatograph peaks were quantified relative to the entire area of PLFA peaks present.

Figure 3. Field and laboratory efforts provided opportunities NOAA Hollings Scholar student Anna Lowlein (above left), graduate students from Baylor University, Sunshyne Hendrix and Caleb Robbins (upper right) and UAA Kenai Peninsula College Semester by the Bay student Sara Coble (lower left and right). Also shown is Ryan King collecting macroinvertebrates at a headwater stream.

Results

In Table 1, sites are arranged first by headwater or lower river, and second by percent alder. Headwater stream sites with high percentages of alder in their watersheds (COTT-A and STAR-A) had higher nitrogen levels (N03 and NH4), and headwater stream sites with higher percent peat wetlands in their watersheds (COTT-C and STAR-C) had higher carbon levels. The confluence below where these two types of streams joined exhibited intermediate N and C levels, showing that both nitrogen and carbon were being exported. Of the lower river sites, Anchor River and Stariski sites (SANC, NANC, STAR) had the highest percent alder in their watersheds, with Ninilchik and Deep Creek having significantly less alder (Table 1).

Table 1. Watershed area, alder and peatland cover for each of the study site locations. Sites are arranged with headwater sites (in green) above, and lower river sites (in blue) below.



Site	Basin Area	Peat Wetland	Alder	% Peat	% Alder
headwater sites					
COTT-A	63140768	3767392	21089258	5.97	33.40
STAR-A	417869840	18535104	65229897	4.44	15.61
STAR-C	773527760	117982752	68222252	15.25	8.82
COTT-C	632225952	131255424	49015552	20.76	7.75
COTT-W	83673840	23620368	595888	28.23	0.71
lower river sites					
SANC	4267645760	537347424	269879088	12.59	6.32
STAR	1330577984	370336384	69179117	27.83	5.20
NANC	1983558624	504565904	99064091	25.44	4.99
NINI	3533204336	653945248	28504960	18.51	0.81
DEEP	6177591398	657049392	77215162	10.64	1.25

Discharge patterns were similar across years of sampling and across basins (Figure 4). For the headwater sites, Cottonwood and Stariski (right panels), the confluence streams had higher discharge, which again would be expected as they represent the joining of two smaller streams. In all systems, discharge peaks were in late April, corresponding to snow melt, and in fall, corresponding to heavy rain events.

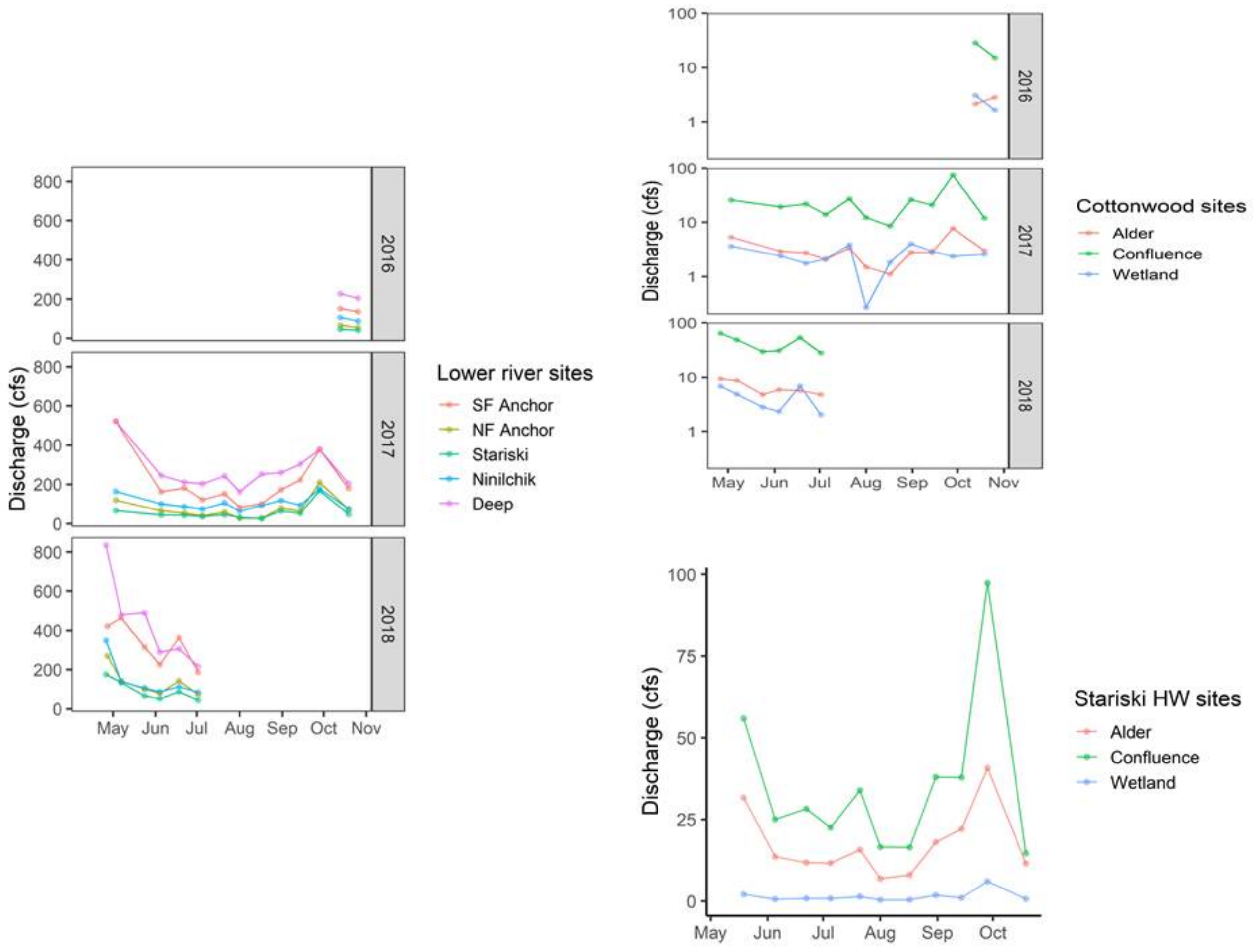


Figure 4. Discharge patterns over time in lower river reaches (left) and headwater sites (right).

Patterns in nitrite-nitrate N show that headwaters with higher percentages of alder have higher levels of N03 (Figure 5). Streams below the confluence of an alder–rich stream show high N as well, and lower river sites in basins with higher alder cover in headwater streams settings have higher N03 levels than those with lower percent alder. Ammonium (NH4) patterns are similar to NO3, showing that headwaters with alder have higher levels as would be expected, and that N is conveyed downstream, influencing the confluence of larger streams and on down to the lower river reaches (Figure 6).

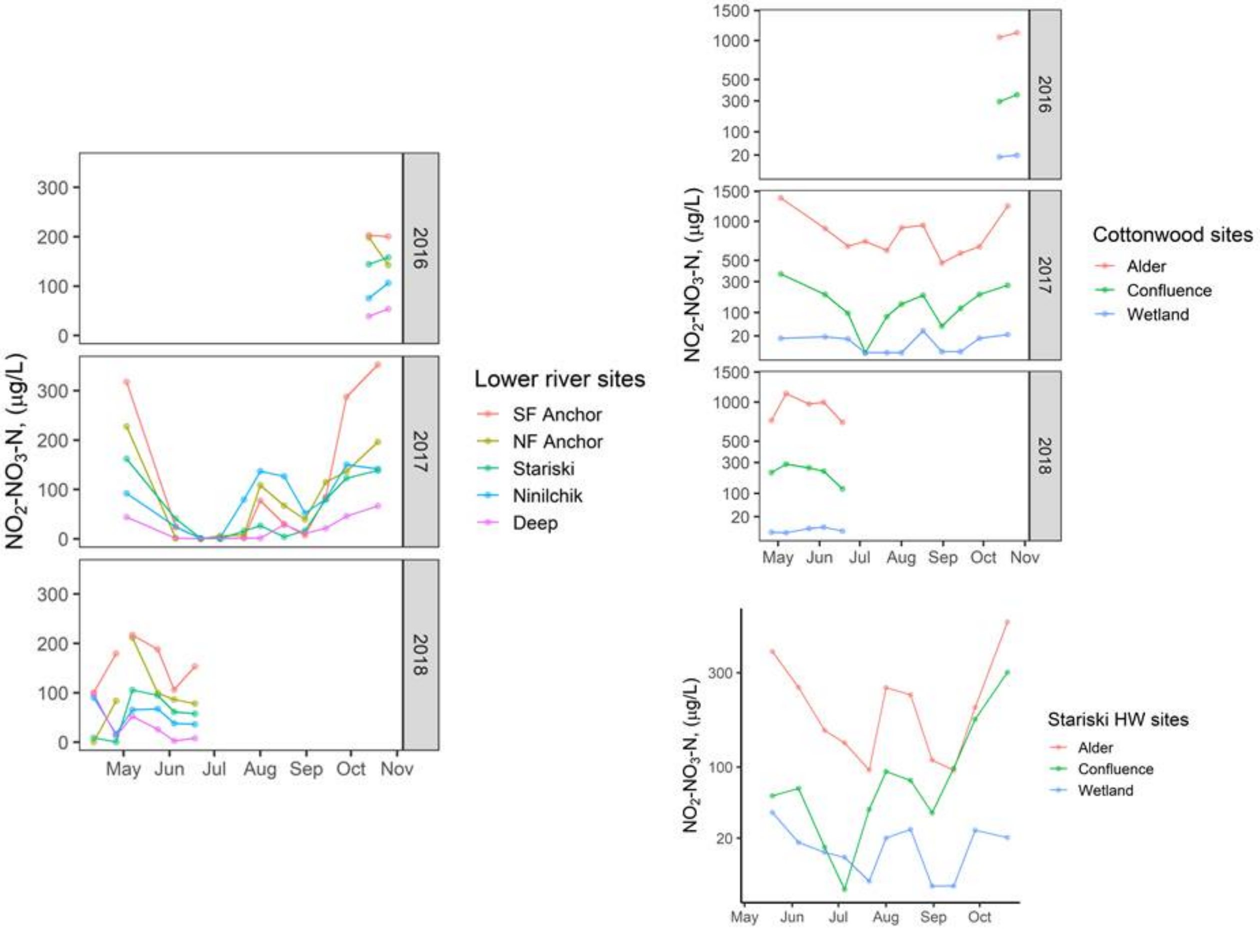


Figure 5. Nitrite (N02) and nitrate (N03) patterns over time in lower river sites (left) and headwater sites (right).

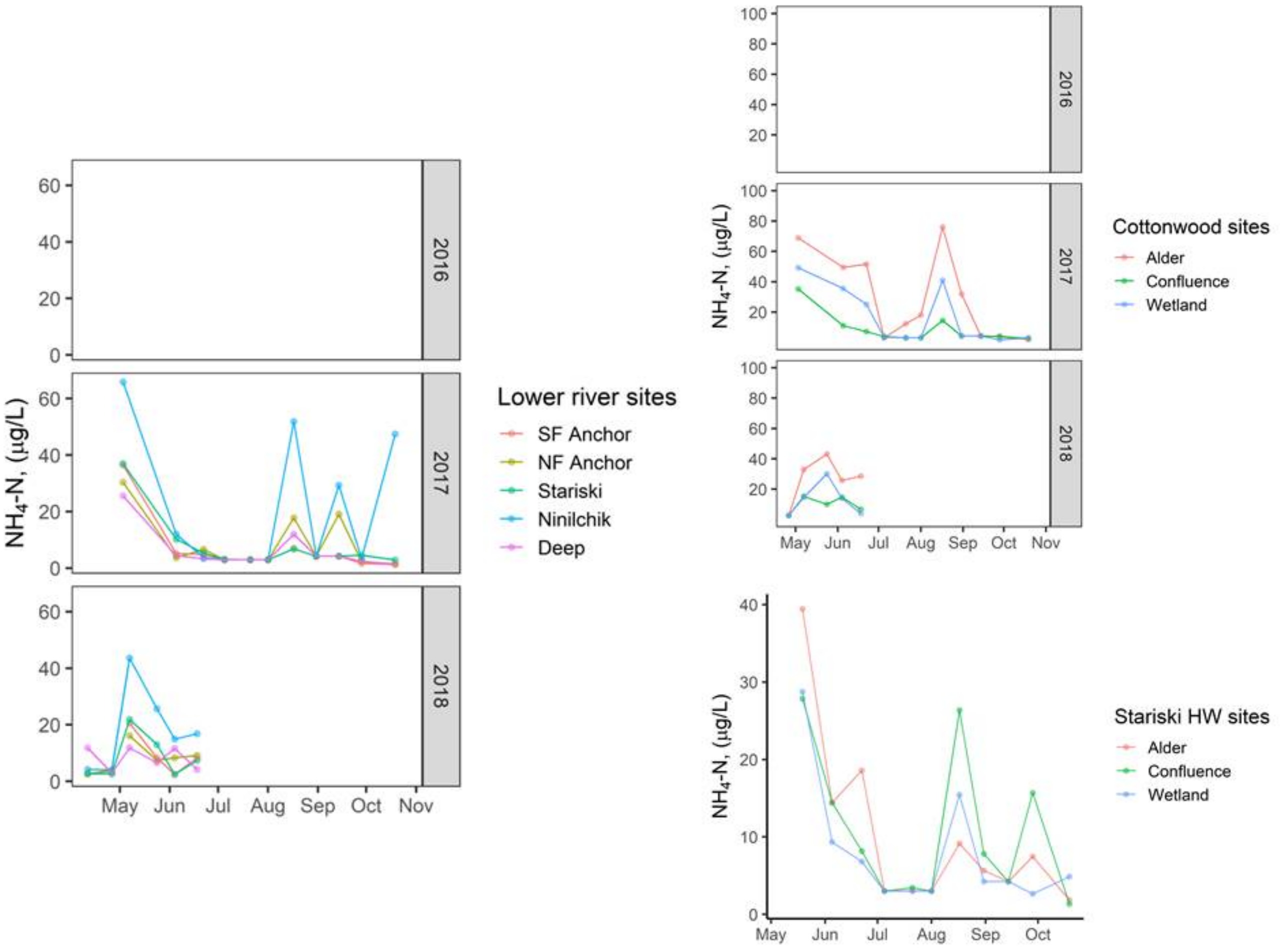


Figure 6. Ammonium N (NH4) patterns over time in lower river sites (left) and headwater sites (right).

Patterns in carbon show that headwaters with more peat wetlands have higher dissolved organic carbon levels (DOC), and that this carbon is exported downstream. Lower river reaches also reflect overall peat wetland cover in their basins, with Ninilchik (NINI), Stariski (STAR) and North Fork of the Anchor (NANC) showing the highest DOC levels, followed by South Fork of the Anchor (SANC) and Deep Creek (DEEP) (Figure 7).

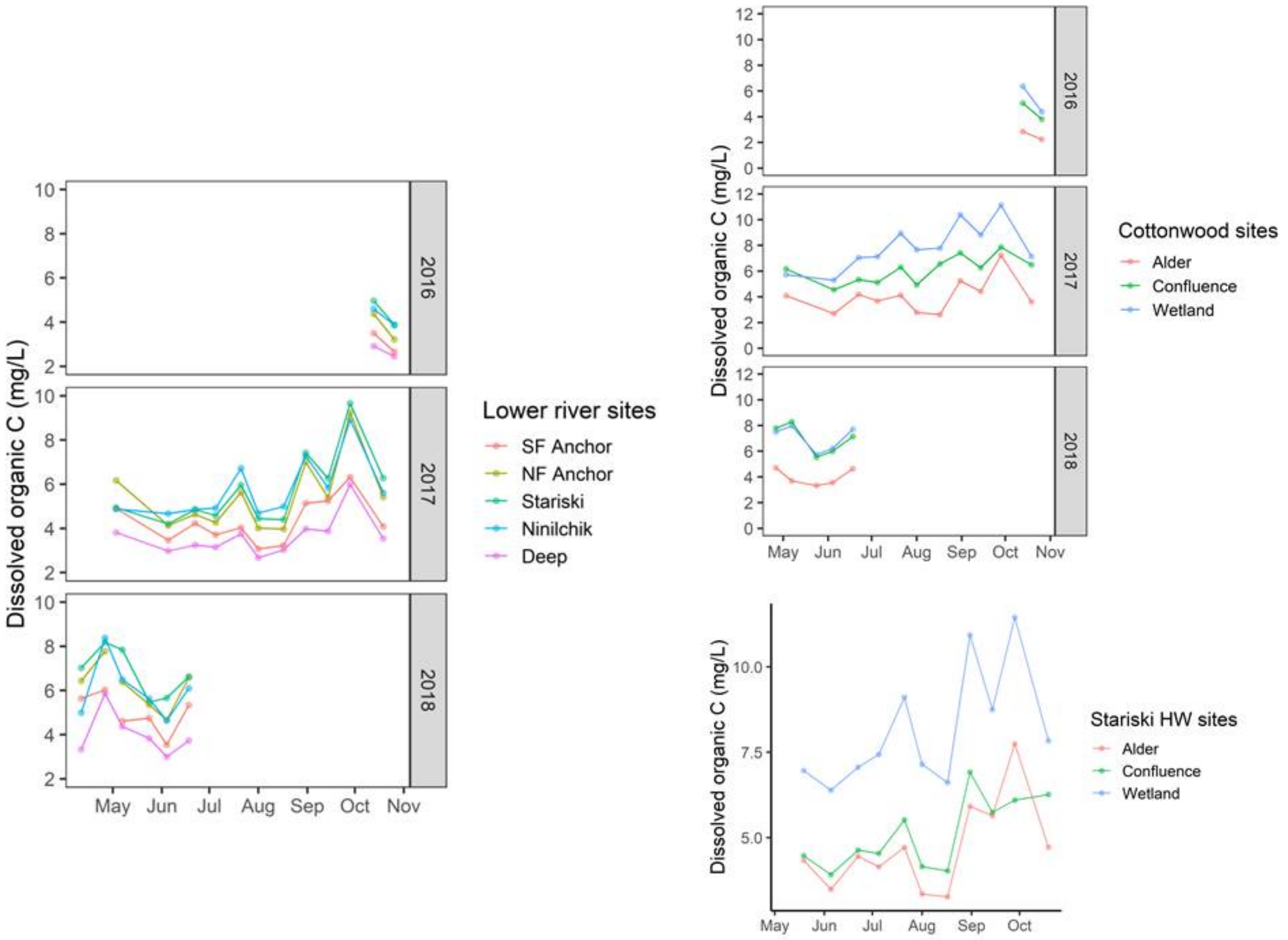


Figure 7. Patterns in dissolved organic carbon (DOC) over time in lower river reaches (left) and headwater sites (right).

Polyunsaturated fatty acids (PUFAs) in streams are derived from algae and are transferred up trophic levels, with omega 3 PUFAs preferentially retained in stream food webs when available (Guo et al. 2017). In our study, we used the raio of omega3:omega6 fatty acids in stream taxon to explore the effects of headwater stream nutrients on food webs (Figure 8). Higher ratios of omega3:omega6 are associated with alder-derived N, presumably because N enrichment enhances the growth of algae that produce omega 3 PUFAs. Results showed that seston, (fine particulate organic matter), and invertebrate species that feed on fine detritus, such winter stoneflies (*Capnidae Nemourida*, *Gammarus,* and *Ryhcophila),* tend to have low omega3:omega6 fatty acid ratios, whereas species such as *Drunella* and *Glossosoma* that feed on algae have higher ratios (Figure 8). Periphyton exhibit mid range omega3:omega6 fatty acid ratios, reflecting the complex mixture of algae, cyanobacteria, heterotrophic microbes and detritus that periphyton is composed of.

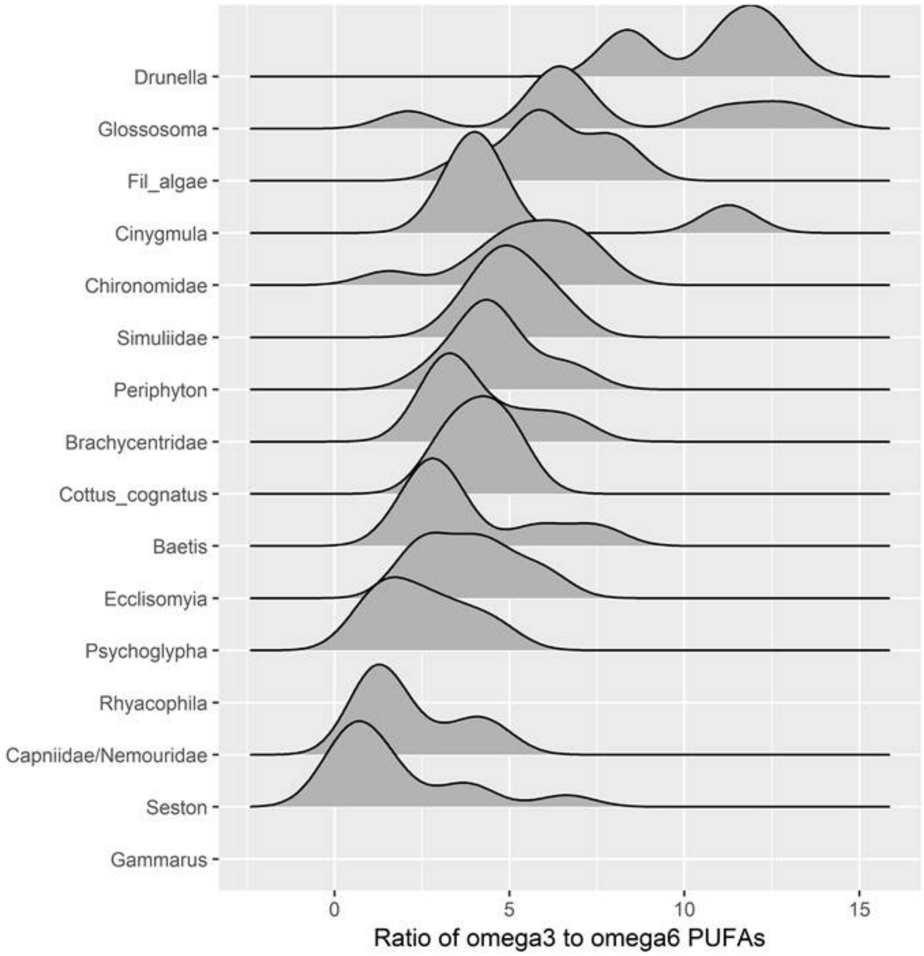


Figure 8. Stream taxon ordered by ratio of omega3:omega 6 fatty acids.

Plotting omega3:omega6 fatty acid ratios in taxa along a gradient of peatland influence shows that wetland sites are more dependent on allothonous carbon sources (from the peat wetlands), whereas higher gradient alder sites are more dependent upon autochthonous sources (Figure 9). Further, there is a balance between the two as we move downstream to the lower river sites, which appear to depend significantly on allochthonous material from peat wetlands, but also show patterns consistent with periphyton and algae sources that reflect the importance of alder-derived N. A strong autochthonous signal is evident coming from the higher gradient, alder-rich, but peatland poor locations, even within the same taxon. *Drunella* for example, has high omega3:omega6 ratios in alder rich locations, and lower ratios in alder-poor/peatland rich locations. The floor for the ratios is similar across all sites, indicatomg that the peatland signature is consistent across all locations. However, peatlands have less influence on the food web in locations where they contribute less organic matter, and alder is contributing more nitrogen for periphyton and algae growth.

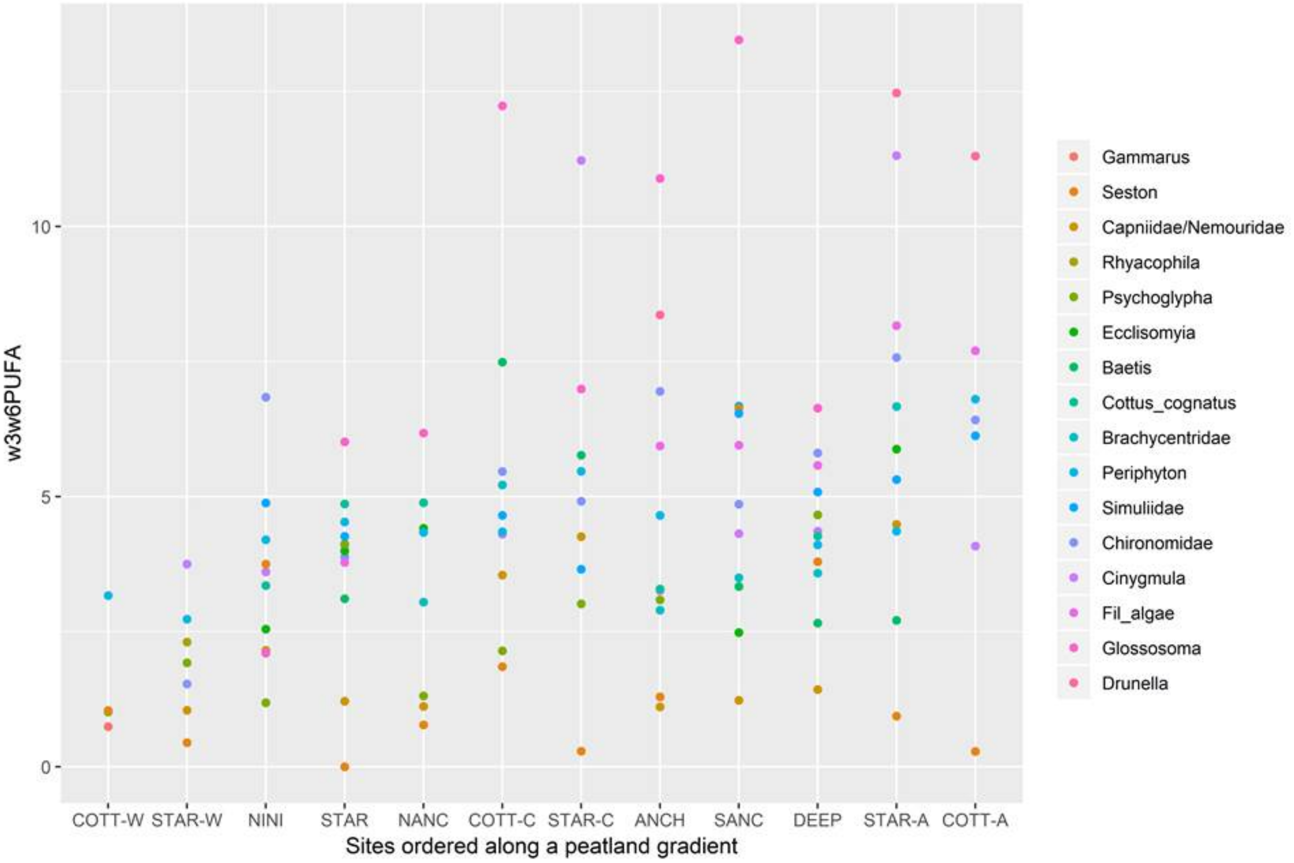


Figure 9. Ratios of omega3:omega6 fatty acids in stream taxon ordered by site along a peatland influcnce gradient.

Another way to view this data is by arranging the omega3:omega6 fatty acid ratios along a site gradient ordered by peatland contribution (Figure 10). Here, it is evident that higher ratios are associated with sites that have higher alder cover, and lower ratios are associated with sites that have more peatland cover. Confluence sites and lower river reaches are in the mid-range, reflecting the contributions of both alder and peatlands to stream food webs.

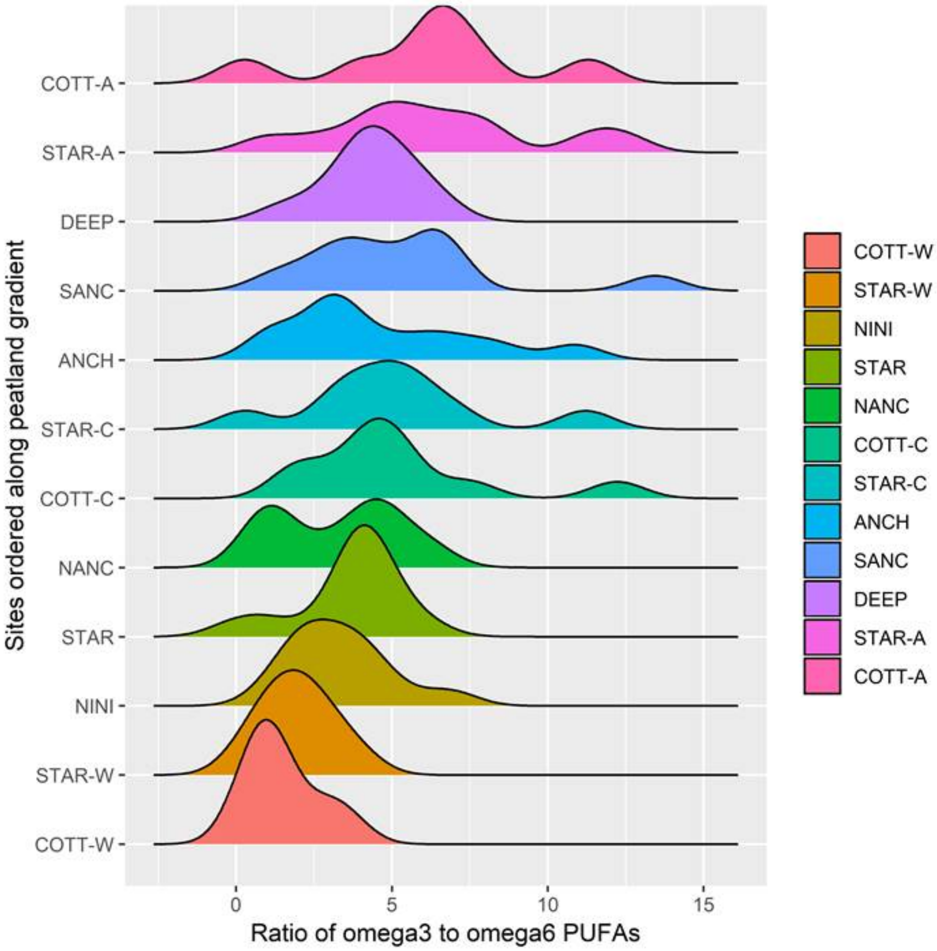


Figure 10. Plot of omega3:omega6 fatty acid ratios ordered by site along a peatland gradient.

Discussion

Juvenile Chinook and Coho salmon, as well as Dolly Varden prosper in the freshwater habitats of the Kenai Lowlands. Understanding how terrestrial habitats influence stream productivity and thus juvenile salmonid growth is important, as it is a primary determinant of survivorship in the transition to saltwater, and ultimately of recruitment of returning adults (see Groot & Margolis 1991). The data from this study shows that the food base for these fish, whether they are present in the headwaters or in lower river reaches is being driven by nutrients coming from headwater stream watersheds.

Nutrient patterns matched up to the sources in each basin, especially in the headwater sites. Where there is high alder cover, there is more nitrogen in the streams, and where there is high peatland cover, there is more carbon in the streams. The data clearly shows that the nitrogen and carbon derived from headwater stream sites is being conveyed downstream, enhancing the quality of basal resources and invertebrates along the way, and thus confirming our hypothesis that export of headwater stream productivity is supporting downstream productivity. A spring 'flush' of nutrients is noticeable across all the sites in late April, especially for DOC in the North Fork Anchor and Stariski Creek watersheds, which have high amounts of peatlands, and for inorganic N in the South Fork Anchor, Stariski and North Fork Anchor, which have high alder cover. The concurrent timing of maximum stream nitrogen derived from alder in the spring corresponds to salmon fry emergence, further indicating the potential importance of this subsidy to headwater stream ecosystems (Shaftel et al. 2011). These results emphasize that alder derived N is a very important source to the lower river reaches, particularly the Anchor River (both North and South Forks), and Stariski Creek, where there are have large amounts of alder associated with river headwaters.

Fatty acid ratios in stream food webs reflect the large amounts of fine benthic organic matter exported downstream from the expansive peatlands of the region, and the influence of alder derived N. The river continuum concept (RCC) suggests that allochthonous detritus is the major energy source in shaded headwaters, whereas the importance of autochthonous foods and fine particulate organic matter increases in wider downstream sites along the river continuum (Vannote et al. 1980). In the Kenai Lowlands region, headwater streams are typically not well shaded as they drain through un-forested settings. This provides for increased autothonous production, especially where these streams are enhanced with alder-derived N. Alder in the surrounding watersheds of headwaters also enhances riparian vegetation, especially the ubiquitous *Calamagrostis* grasses which line streams. These grasses fall into the streams, becoming N enriched substrates for bacteria and invertebrates, which are subsequently food for salmonids. Thus, alder enhances autothonous and allothonous energy sources in Kenai Lowland streams (Dekar et al. 2012). Where alder is not prevalent, peatland carbon is the dominant source of allothonous inputs, providing a baseline of dissolved organic carbon that fuels food webs. When alder is present, the added nitrogen clearly enhances omega3:omega6 ratios, and presumably, productivity. This underscores the value of aiming conservation measures in watersheds with both alder and peatlands, and especially in high alder cover locations.

As the Kenai Lowland region faces habitat changes due to a warming climate and human developments, it is critical that the watersheds be treated as connected systems, from headwaters to estuary in order to maintain juvenile salmon productivity, and ultimately recruitment to adults. The project represented in this report has contributed science that can be used for decision-making directed at maintaining integrate functioning landscapes and watersheds in order to maintain regional salmonid populations. Project results were presented to the Kenai Peninsula Fish Habitat Partnership (April 2018), the, the American Fisheries Society meeting (May 2018), Kachemak Bay NERR Community Council (June 2018), and were featured in the Kachemak Bay NERR Quarterly Report. The project provided opportunities for several student interns (undergraduate and graduate), as well as volunteers to assist with data collection and laboratory analyses, enhancing local, regional and national workforce development in fisheries science.

Other useful results from the project stemmed from conversations with local Alaska Department of Fish and Game, Sport Fish Division, who identified discharge rating curves as a valuable product that could be derived from the project (Figure 11). Raw data and rating curves have been provided to ADFG staff, who will use them to assist with predicting instream fish counts and management (*M. Booze pers. comm.*).

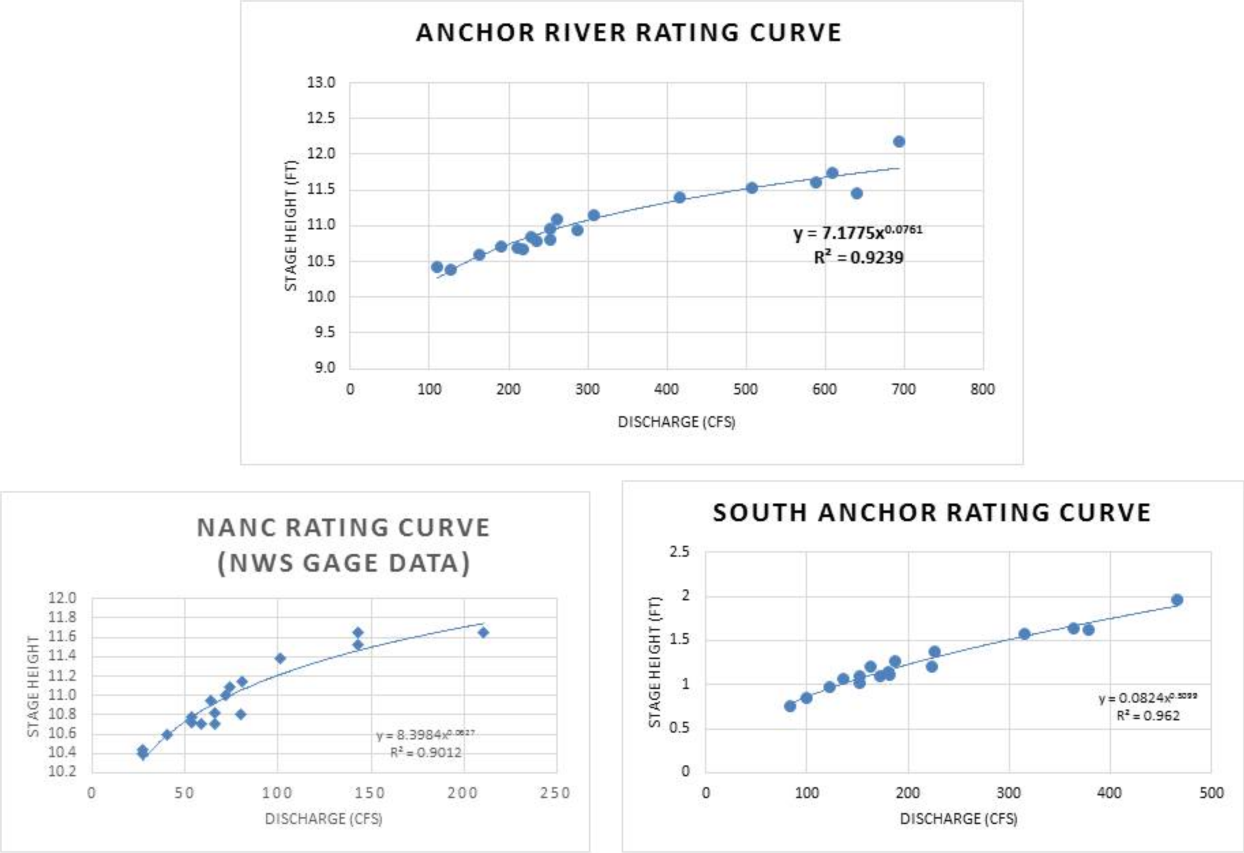


Figure 11. Discharge rating curves based on stage height for both branches and the main stem of the Anchor River.

In conclusion, the findings of this study support our hypothesis that alder and peatlands in the Kenai Lowlands are very important to stream productivity and riparian zones in headwater regions. Results clearly show that headwaters influenced by high contributions of alder and wetlands should be viewed as vital and complementary components that support downstream productivity, and that these headwaters should be considered in conservation efforts to protect and promote the productivity salmon-bearing streams downstream.

Acknowledgments

We thank the Kenai Watershed Forum for loaning the use of the SonTek acoustic Doppler profiler in order to obtain high flow discharge measurements. NOAA Hollings Scholar Anna Lowlein did the data analysis for the discharge rating curves, and helped with fieldwork along with fellow Hollings Scholars Megan Hazlett and Ashley Bangs in 2018. UAA Semester by the Bay student intern Sara Coble assisted with field work in 2017. KBNERR staff Chris Guo, Syverine Bentz, Steve Baird, Jasmine Maurer, Dana Nelson and Alice Rademacher assisted with field and laboratory work in 2017 and 2018. Baylor University water chemistry analyses were led by Jeff Back, and Baylor student Sunshyne Hendrix assisted with field work in 2018. Baylor PhD student, Caleb Robbins, and KBNERR technician Jacob Argueta deserve special recognition for their outstanding dedication to

the project. Additional project support (funding, facilities and and analyses) were provided by Baylor University and KBNERR.

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Final Report Status: This performance report is the final report for this project during the reporting period (July 1, 2017–June 30, 2018).

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