

March 3, 2021

Via email: paula.wilson@deq.idaho.gov

Ms. Paula Wilson
Idaho Department of Environmental Quality
1410 North Hilton
Boise, ID 83706

Dear Ms. Wilson:

The Idaho Association of Commerce & Industry (IACI) is the leading trade association of Idaho businesses and represents hundreds of employer members of all sizes engaged in diverse commercial and industrial enterprises through the state. The arsenic water quality criteria values are used to set cleanup/remedial objectives, TMDLs, and requirements for wastewater discharge. Thus, these criteria will have direct impact on the IACI membership.

IACI submitted extensive comments to the Department of Environmental Quality (Department) in August 2020, reviewing the history of arsenic water quality criteria in Idaho, the regulatory framework for these standards, and a thorough evaluation of data on arsenic in Idaho waters and fish. To supplement the extensive August 2020 paper, attached is a paper by Arcadis that includes a literature review on the bioaccumulation of arsenic in the aquatic environment. Based on the findings of this literature review, this paper also includes further analysis of the Department's data on arsenic in surface waters and fish tissues. The totality of this work supports the direction that the Department has set regarding the human health water quality criteria for arsenic.

The Department has requested comments on Preliminary Rule Draft No. 1. This preliminary draft proposes the following human health water quality criteria for arsenic.

- For water and fish consumption/exposure, the water column concentration must meet the maximum contaminant level for inorganic arsenic in IDAPA 58.01.08 and the fish tissue concentration must not exceed 8 µg/kg.
- For fish only consumption/exposure, the fish tissue concentration must not exceed 8 µg/kg.

IACI supports these preliminary criteria. We believe that the fish concentration number satisfies the numeric criteria requirements for toxic pollutants in the CWA. Furthermore, these preliminary criteria are consistent with the technical findings from the extensive studies conducted by the Department and analysis of this data.

IACI appreciates the technical work that the Department has done in collecting data on arsenic in Idaho's waters and fish, and the use of this data in revising the arsenic human health water quality criteria. We thank you for the opportunity to comment on this work.

Sincerely,



Alex LaBeau
President

cc: Alan Prouty, IACI Environment Committee Chair

SUBJECT
IDEQ 2019 Preliminary Monitoring Findings

TO
Alan Prouty
J.R. Simplot Company

DATE
February 26, 2021

OUR REF

DEPARTMENT
Environment

PROJECT NUMBER
30039729

COPIES TO
None

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1. Executive Summary

Previous evaluations of paired fish tissue and surface water data collected by Idaho Department of Environmental Quality (IDEQ) in 2019 documented the absence of a relationship between inorganic arsenic (iAs, the form of arsenic in fish tissue that is assumed to be toxic) in fish tissue and surface water (Arcadis 2020, IACI 2020, IDEQ 2020a). In other words, bioaccumulation of iAs from water did not explain the concentrations of iAs observed in fish tissue.

Arcadis conducted a literature review of arsenic bioaccumulation and speciation in aquatic foodwebs to see if factors could be identified that might explain the iAs concentrations observed in fish collected by IDEQ in 2019. The literature review found that in freshwater aquatic ecosystems, the concentration of iAs in tissues of aquatic organisms decreases with increasing trophic level (i.e., biodilutes or biodiminishes) and, as a result, the vast majority of total arsenic (tAs) in upper trophic level fish species is present as non-toxic organic arsenic species.

Using the findings of the literature review, Arcadis evaluated the 2019 IDEQ data to identify the factors, if any, that affect iAs concentrations in tissue. The factors include those for which IDEQ had collected data, as well as others that could be determined based on life history of the collected fish species. The evaluation identified fish weight as the only factor with a statistically significant effect on iAs concentration in fish tissue.

Weight is likely related to trophic level position in the foodweb, with large individuals of most species being higher in the foodweb than smaller individuals. Inorganic As tissue concentrations tend to decrease with weight when all species collected by IDEQ in 2019 are grouped together, and more strongly when some species, such as trout, are evaluated separately. Assuming weight is a surrogate for trophic level, the decreasing iAs concentration with increasing weight is consistent with biodilution trends reported in the literature.

Other measures of the effect of trophic level were also investigated, including grouping species by feeding guild and by whether they are forage or non-forage species. Inorganic As tissue concentrations increased with increasing iAs water concentration in herbivorous and insectivorous species, both representing lower trophic levels, and decreased in piscivorous species, the latter representing higher trophic levels. Similar trends were observed between forage fish (assumed to represent lower trophic levels) and non-forage fish (assumed to

represent upper trophic levels). In addition, the ratio of inorganic to organic arsenic species decreases with increasing trophic level providing further evidence that metabolic transformation processes in aquatic foodwebs result in low and decreasing concentrations of iAs in upper trophic level organisms.

Arcadis also examined whether the relationship between iAs in surface water and fish tissue varied by Idaho river basin. While surface water iAs concentrations are relatively distinct among river basins, fish tissue iAs concentrations show substantial overlap between basins, supporting the previous analyses that show iAs concentrations in fish tissue are independent of iAs concentrations in surface water.

In conclusion, the 2019 Idaho data are consistent with findings reported in the literature that iAs is biotransformed by many aquatic species to non-toxic organic arsenic species and, thus, undergoes biodilution in aquatic foodwebs. As a result, iAs has limited potential to bioaccumulate in most species of fish and the vast majority of tAs in fish tissue is present as non-toxic organic arsenic species.

2. Introduction and Summary of Previous Analyses

Previous evaluations by Arcadis and others of the paired IDEQ 2019 surface water and fish tissue arsenic concentration data found that statistically significant relationships do not exist between the concentration of tAs in surface water and fish tissue (Arcadis 2020, IACI 2020), iAs in surface water and fish tissue (Arcadis 2020, IACI 2020, IDEQ 2020a) and iAs in fish tissue and tAs in surface water (Arcadis 2020, IACI 2020).

Previous evaluations also found no consistent trend across species, with tissue concentrations increasing with increasing surface water concentrations for some species and decreasing for other species (Arcadis 2020). Notably, in trout species, the concentration of iAs in tissue tended to decrease with increasing iAs or tAs concentration in surface water. The outcome of these evaluations indicates that using a bioaccumulation factor (BAF) to describe the relationship between arsenic in surface water and fish tissue is not applicable to fish in Idaho given that the concentration of arsenic in fish tissue is independent of the concentration of arsenic in surface water (Arcadis 2020, IACI 2020, IDEQ 2020b).

As part of the 2019 fish tissue collection program, IDEQ field teams recorded the length and weight of fish comprising each tissue sample. When the iAs concentration of each composite tissue sample was plotted versus the average weight of the fish comprising each composite tissue sample, a slight, not statistically significant, trend of decreasing iAs tissue concentration with increasing iAs surface water concentration was observed (Arcadis 2020). However, with the exception of bridgelip suckers collected from More's Creek, all fish weighing more than about 50 grams had an iAs concentration of about 1 microgram per kilogram ($\mu\text{g}/\text{kg}$) or lower (Arcadis 2020). The iAs concentration in fish weighing less than 50 grams ranged from less than 1 $\mu\text{g}/\text{kg}$ to about 10 $\mu\text{g}/\text{kg}$ (Arcadis 2020). Thus, the concentration of iAs in fish tissue does appear to be affected by fish weight, with larger fish having lower iAs concentrations than smaller fish, but the relationship appears to be represented better by a threshold than a gradual change (Arcadis 2020). For most species of fish, larger individuals likely occupy higher trophic levels in the foodweb than smaller individuals. Larger fish may also have a different diet composition than smaller fish.

To better understand whether changes in trophic level affect the concentration of arsenic species in aquatic foodwebs, a literature review was conducted to identify factors that might help explain the iAs concentrations observed in fish collected by IDEQ in 2019. The remainder of this technical memorandum presents the general findings of the literature review (short summaries of key papers are provided in Appendix I) followed by additional

analyses of the 2019 IDEQ fish tissue data to understand whether the arsenic accumulation trends observed in those data are unique to Idaho or are consistent with observations reported in the literature. The analyses include:

- a multivariate analysis to identify which, if any, parameters have a statistically significant effect on iAs concentrations in fish tissue;
- additional evaluation of the effect of weight on tissue iAs concentration;
- investigation of the effect of feeding guild on the relationship between iAs concentration in tissue and surface water;
- investigation of the effect of forage vs. non-forage fish on the relationship between iAs concentration in tissue and surface water; and
- evaluation of the effect of river basin on iAs surface water and tissue concentrations.

Brief conclusions are presented at the end of this Technical Memorandum.

3. Literature Review

Arsenic biomagnification and bioaccumulation literature was reviewed to identify general observations and findings. That information was then used to evaluate whether patterns observed in fish collected by IDEQ in 2019 are similar to those reported in the literature, as well as to identify additional areas of investigation.

Arsenic Biomagnification in Freshwater Aquatic Ecosystems

In freshwater systems, iAs concentrations decrease with increasing trophic level in the foodweb. This phenomenon is referred to as biodilution (or biodimution), in contrast to biomagnification in which the concentration of a substance increases with increasing trophic level (Chen and Folt 2000, Chen et al. 2008, Cheng et al. 2013, Chetalat et al. 2019; Dovick et al. 2015, Lopez et al. 2016, Maeda et al. 1990, Rahman et al. 2012, U.S. Environmental Protection Agency [USEPA] 2003). Inorganic arsenic does bioaccumulate at lower trophic levels, including aquatic plants (e.g., periphyton), phytoplankton, and zooplankton (Lopez et al. 2016), but those concentrations are not transferred to upper trophic level organisms. For example, in a three-level foodweb (phytoplankton, zooplankton, fish) in northeastern United States lakes, tAs was elevated in small zooplankton, but not larger zooplankton, and was negligible in fish tissue (Chen and Folt 2000). Laboratory studies using a three-step foodweb (algae, shrimp, fish) showed that the iAs species, AsV, accumulated from water into the lower trophic levels, but was not transferred to higher trophic levels (Maeda et al. 1990, Kuroiwa et al. 1994). This biodilution pattern is confirmed by studies that used stable isotope concentrations to more precisely define the trophic levels of organisms in the foodweb (e.g., Revenga et al. 2012, Otter et al. 2012).

Arsenic Bioaccumulation in Freshwater Aquatic Ecosystems

Biological factors (e.g., species, size, age) and ecological factors (e.g., diet composition, trophic position) may influence the accumulation of arsenic in aquatic organisms (e.g., Otter et al. 2012; Mason n.d.). For example, the accumulation of metals in invertebrate taxa depended on their place in the foodweb, their feeding behavior, and their specific habit (lenitophilic/rheophilic species; Culioli et al. 2009).

While foodweb studies in the field show that arsenic concentrations in upper trophic level organisms are much lower than in lower trophic level organisms, arsenic exposed fish in laboratory settings without a foodweb do bioaccumulate arsenic in their tissues. Ciardullo et al. (2008) found that tAs concentrations in gills, kidney, liver, muscle, and skin tissues of rainbow trout in aquaculture peaked at 14 months of age and then decreased in adults due to dilution with growth. Tilapia exposed to iAs through the diet showed a linear accumulation of arsenic in all tissues within the first 10 days of exposure, but remained stable in the subsequent 20 days of exposure (Pei et al. 2019).

While upper trophic level aquatic organisms, including fish, can bioaccumulate arsenic, internal bioregulation appears to convert the inorganic more toxic species of arsenic (e.g., AsIII and AsV) to non-toxic organic species (e.g., methylated forms of arsenic and arsenobetaine) (USEPA 2003). Several field and laboratory studies have confirmed this metabolic transformation. For example, organic arsenic species (arsenobetaine and dimethylarsinic acid) were the dominant arsenic species in muscle of fish studied in an arsenic contaminated lake in Patagonia; no iAs species were detected (Juncos et al. 2019). Arsenobetaine was the dominant arsenic species in a study of field-collected fish, representing 58% of the tAs concentration in carp, 89% of the tAs concentration in eel, and 95% of the tAs concentration in both mullet and chub (Ciardullo et al. 2010). Arsenic speciation analysis revealed that tAs in laboratory-exposed rainbow trout muscle tissue was almost exclusively present as the organic arsenic species, arsenobetaine (Ciardullo et al. 2008). A laboratory study of tilapia exposed to iAs species (AsIII and AsV) found that 90% of tAs concentrations in muscle, liver, and gill tissues were comprised of organic species, while only 30-80% of tAs in the gastrointestinal tract was comprised of organic species (Pei et al. 2019). Cui et al. (2021) found that after a 40-day exposure to AsIII and AsV in a laboratory setting, the fraction of organic arsenic (arsenobetaine) in carp tissue was 70-80%. Additionally, proportions of arsenobetaine increased in both the AsIII and AsV treatments in this study, with prolonged exposure, suggesting that fish had an adaptive response by increasing iAs biotransformation into the non-toxic arsenic species (Cui et al. 2021).

Overall, these findings highlight the low bioaccumulation potential of arsenic (and other trace elements) in upper trophic level fish and are consistent with the trend observed in the 2019 IDEQ data, where the average percent organic arsenic in fish tissue was 97% and no statistically significant relationships ($p=0.05$) were observed between tAs in surface water and fish tissue or between iAs in surface water and fish tissue.

4. Analyses

Based on the literature review, additional evaluations of the 2019 IDEQ surface water and fish tissue data were conducted to identify factors that might affect the concentrations of arsenic in fish tissue and the relationship between arsenic in surface water and fish tissue.

Multivariate analysis of the concentration of inorganic arsenic in fish tissue

A multivariate analysis was conducted to identify which parameters, if any, when evaluated holistically, have a statistically significant influence on the concentration of iAs in fish tissue. All parameters for which IDEQ had data and others based on review of life histories of collected species were included in the statistical model including: trophic level (forage or non-forage fish), feeding guild (herbivorous, insectivorous, piscivorous), river basin, species, average fish sample weight, concentration of iAs in water, concentration of tAs in water, and concentration of tAs in fish tissue.

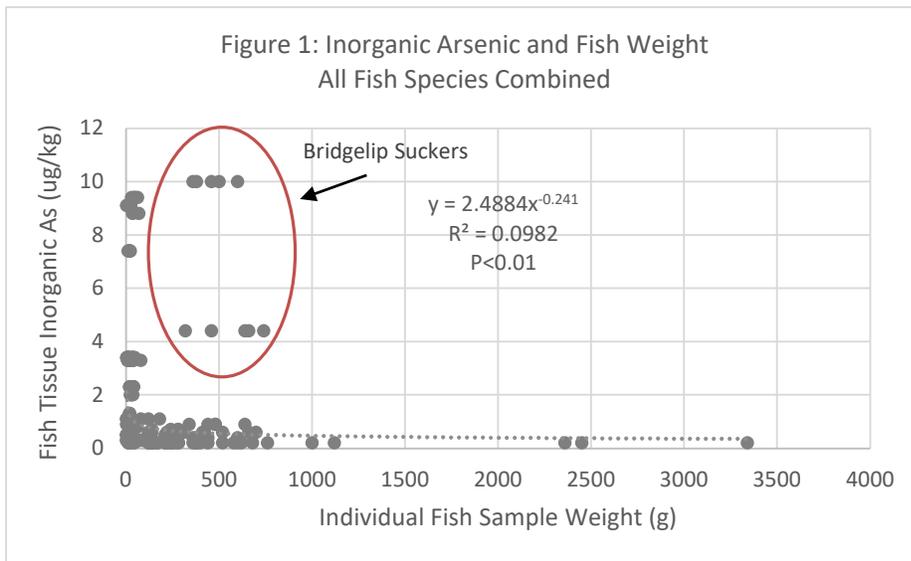
Fish species collected in 2019 were assigned to herbivorous, piscivorous, or insectivorous feeding guilds based on USEPA (1999). Bridgelip sucker was the only herbivorous fish. Insectivorous fish included catfish sp., cutthroat trout, dace sp., mountain whitefish, redbreast shiner, and sculpin sp. Piscivorous fish included brook trout, brown trout, largemouth bass, northern pikeminnow, rainbow trout, and smallmouth bass. Species collected were also assigned as forage fish (feeding on aquatic plants, zooplankton, and/or aquatic invertebrates) or non-forage fish (feeding primarily on aquatic invertebrates and/or smaller fish) based on diet data from USEPA (1999). Forage fish included sculpin sp., dace sp., bridgelip sucker, mountain whitefish, and redbreast shiner. Non-forage fish included brook trout, brown trout, catfish sp., cutthroat trout, largemouth bass, smallmouth bass, northern pikeminnow, and rainbow trout.

Weight was the only parameter with a statistically significant effect on the concentration of iAs in fish tissue (Table 1, see last column for p-values).

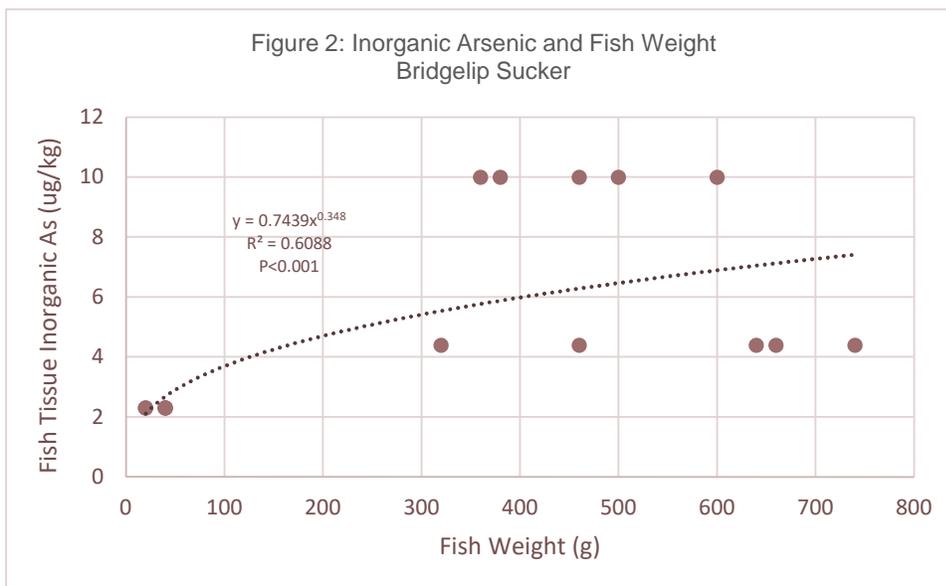
Table 1. Results of multivariate analysis of the effect of several parameters on the iAs fish tissue concentration

Parameter	Type III Sum of Squares	Degrees of Freedom	F-Ratio	P-value
Basin	0.64	5	0.71	0.63
Species	2.63	10	1.53	0.22
Trophic Level	0.01	1	0.04	0.85
Feeding Guild	0.00	2	0.00	0.99
Average Fish Sample Weight	1.45	1	7.95	0.01
Tissue Total As	0.14	1	0.78	0.39
Surface Water Total As	0.43	1	2.34	0.14
Surface Water Inorganic As	0.22	1	1.20	0.29
Error	4.38	24		

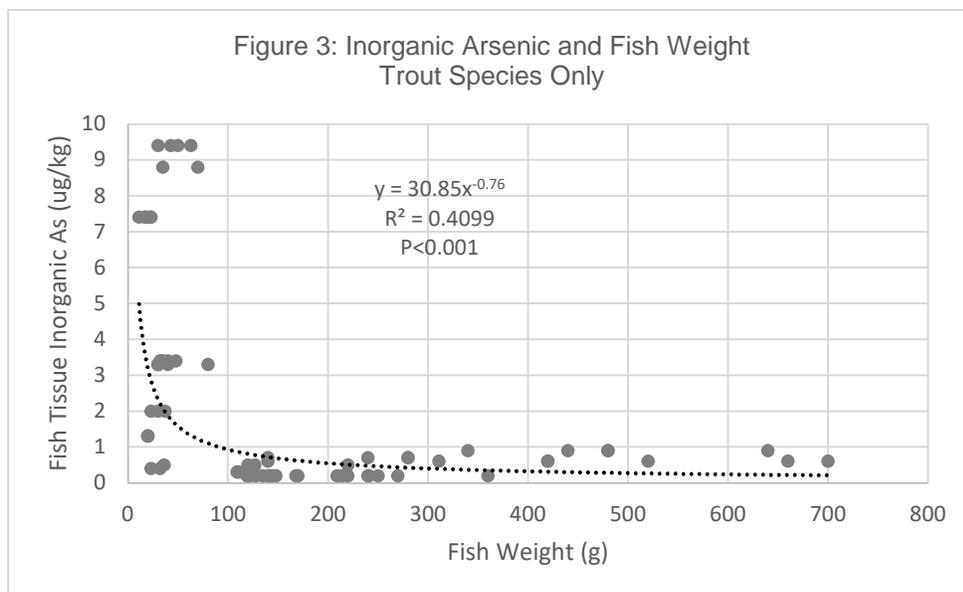
As described above in Section 2, when the iAs concentration of each composite tissue sample was plotted versus the average weight of the fish comprising each composite tissue sample, the slight trend of decreasing iAs tissue concentration with increasing iAs water concentration was not statistically significant. However, it did appear that a threshold existed in the relationship, where small fish (<50 grams) had a large range of iAs concentrations while virtually all larger fish (>50 grams) had an iAs concentration less than or equal to about 1 µg/kg (Arcadis 2020). If the weights of the individual fish comprising a composite sample vary, averaging the weights may dilute a potential effect on the concentration of iAs in fish tissue. When weights of individual fish (rather than the average weight of all fish that made up a composite tissue sample) are plotted versus iAs tissue concentration, the decreasing trend of iAs in fish tissue with increasing weight is statistically significant ($p < 0.01$), but still relatively weak in that the regression explains only about 10% of the variation ($R^2 = 0.098$, Figure 1). With the exception of bridgelip suckers, the above mentioned threshold also is apparent, with fish weighing less than about 100 grams having a large range of iAs tissue concentrations and fish weighing more than 100 grams having an iAs tissue concentration equal to or less than 1 µg/kg (Figure 1).



Bridgelip suckers samples appear to have a different pattern of weight to iAs tissue concentration than samples comprised of other species. In bridgelip suckers, the iAs concentration in tissue increases with increasing weight and the increase is statistically significant ($p < 0.001$, Figure 2). A review of the literature suggests that the bridgelip sucker diet, and therefore position in the foodweb, changes from being comprised of primarily aquatic insect larvae as small fish (<150 millimeters [mm] in length) to being comprised of primarily herbivorous (consuming aquatic plant periphyton) as suckers grow (Dauble 1980). The change in diet with increasing weight and transition from an upper trophic level to a lower trophic level likely explains the increase in iAs tissue concentration with increasing weight. This change in diet and trophic level, from insectivorous juveniles to primarily herbivorous, lower trophic level adults, is unusual for freshwater fish. Most species tend to transition from omnivorous or insectivorous juveniles to primarily insectivorous or piscivorous upper trophic level adults, such as most trout and bass species (Behnke 1992, Scott and Crossman 1973).



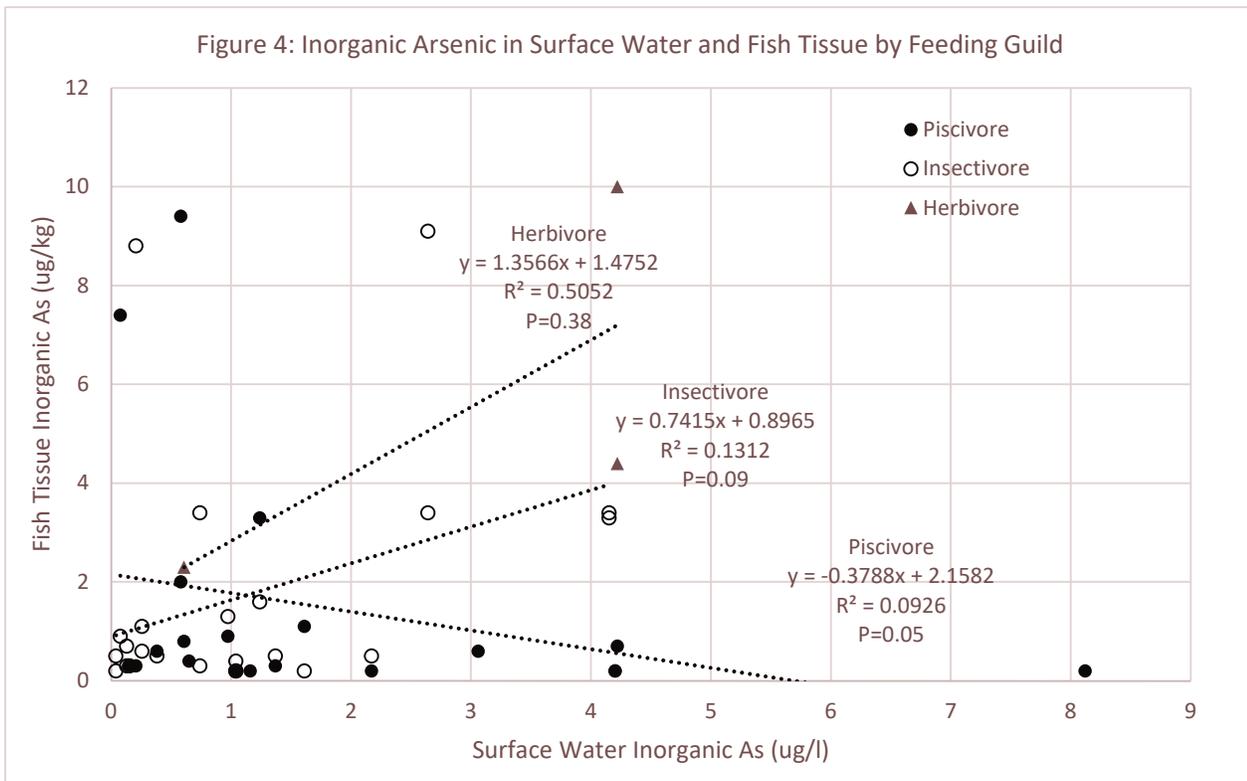
The effect of weight on iAs concentration in fish tissue was also examined in trout species (brown trout, rainbow trout, cutthroat trout, and brook trout). These trout species are primarily piscivorous, and therefore considered upper trophic level organisms. In trout, iAs tissue concentrations decrease with increasing weight and the trend is statistically significant ($p < 0.001$, $R^2 = 0.41$, Figure 3).



The apparent decrease in iAs concentration in fish tissues in larger fish is consistent with the literature that shows iAs concentrations decrease in fish that are larger and, therefore, are assumed to be upper trophic level species.

Effect of feeding guild on the relationship between inorganic arsenic in surface water and fish tissue

Fish species collected in Idaho waters in 2019 were assigned to herbivorous, insectivorous, or piscivorous feeding guilds based on USEPA (1999). The relationships between iAs in surface water and fish tissue for herbivorous fish (consisting entirely of bridgelip suckers) and for insectivorous fish were not statistically significant ($p = 0.38$ and 0.09 , respectively, Figure 4). For piscivorous fish, a statistically significant but weak negative relationship ($R^2 = 0.09$, $p = 0.05$) exists between iAs in water and tissue (Figure 4). The feeding guild assignments assume that the individual fish in the dataset were adults. Juveniles of some piscivorous species have diets that differ from than adults (e.g., the diet of juveniles may be primarily insects). In addition, common carp were excluded from this analysis because it is the only species that is primarily omnivorous and there was only one carp sample in the dataset.



Piscivorous and insectivorous fish samples had similar average weights, although piscivorous fish were slightly longer than insectivorous fish in this dataset (Table 2). Herbivorous fish (bridgelip sucker) were longer and heavier than the insectivorous and piscivorous fish. Concentrations of tAs were highest in piscivorous fish, followed by herbivorous fish, and lowest in insectivorous fish. Concentrations of iAs were highest in herbivorous fish, then insectivorous fish, and lowest in piscivorous fish.

Table 2: Summary statistics of length, weight, and arsenic tissue concentrations for feeding guilds

Guild	Mean Sample Weight (grams) (Min – Max)	Mean Sample Length (mm) (Min – Max)	Mean Inorganic Arsenic (µg/kg) (Min – Max)	Mean Total Arsenic (µg/kg) (Min – Max)
Herbivorous	352 (20 - 740)	295 (135 - 400)	5.57 (2.3 - 10)	75.3 (31 - 108)
Insectivorous	192 (2 - 2,450)	183 (60 - 540)	1.74 (0.2 - 9.1)	66.7 (11 - 278)
Piscivorous	204 (11 - 3,340)	252 (110 - 630)	1.47 (0.2 - 9.4)	91.9 (0.9 - 583)

These results are consistent with the findings of the literature review that suggest iAs bioaccumulation is higher in lower trophic levels, such as the herbivorous bridgelip sucker, and lower in upper trophic level piscivorous fish (Chen and Folt 2000, Maeda et al. 1990, Revenga et al. 2012). These results also support that general observation that iAs biodilutes in freshwater ecosystems.

Effect of trophic level on the relationship between inorganic arsenic in surface water and fish tissue

Fish collected in 2019 were classified as forage fish (feeding on aquatic plants, zooplankton, and/or aquatic invertebrates) or non-forage fish (feeding primarily on aquatic invertebrates and/or smaller fish). A slight, not statistically significant, trend of decreasing iAs concentration in fish tissue with increasing iAs in surface water was observed for non-forage fish (brook trout, brown trout, catfish sp., cutthroat trout, largemouth bass, smallmouth bass, northern pikeminnow, rainbow trout; Figure 5). However, forage fish (sculpin sp., dace sp., bridgelip sucker, mountain whitefish, reidside shiner), had a statistically significant positive relationship between iAs in surface water and in fish tissue ($p=0.005$, $R^2=0.49$, Figure 5). Forage fish in this dataset tended to be smaller than the non-forage fish, as expected. Compared to non-forage fish, forage fish also had the highest concentrations of iAs and the lowest concentrations of total As (Table 3).

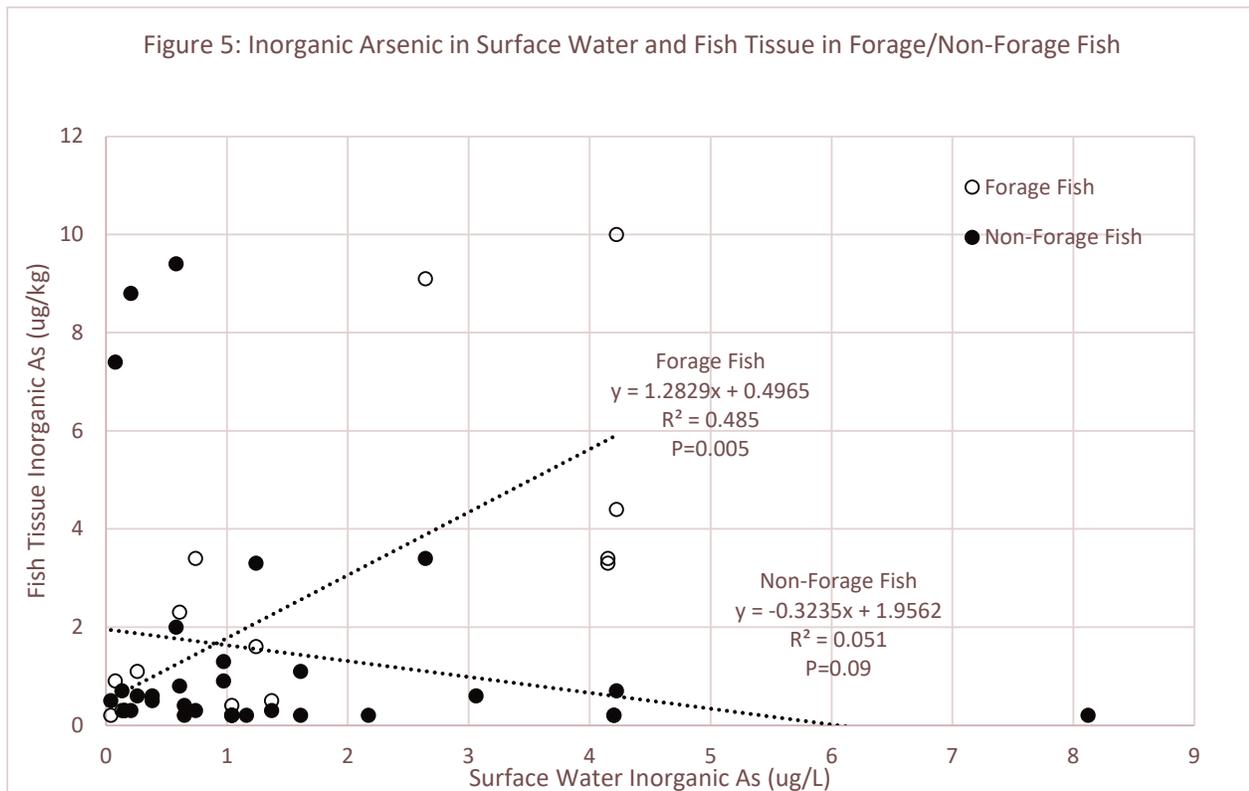


Table 3: Summary statistics of length, weight, and arsenic tissue concentrations for forage and non-forage fish

Trophic Level	Mean Sample Weight (grams) (Min – Max)	Mean Sample Length (mm) (Min – Max)	Mean Inorganic Arsenic (µg/kg) (Min – Max)	Mean Total Arsenic (µg/kg) (Min – Max)
Forage Fish	160 (2 - 740)	180 (60 – 400)	2.46 (0.2 – 10)	53.6 (11 – 145)
Non-Forage Fish	283 (11 - 3340)	252 (110 – 630)	1.48 (0.2 – 9.4)	89.0 (0.9 – 583)

Consistent with the feeding guild results above and the data in the literature, these results also support that iAs biodilutes in freshwater systems (e.g., Chen and Folt 2000, Chetalat et al. 2019; Dovick et al. 2015, Lopez et al. 2016, Maeda et al. 1990, Rahman et al. 2012, USEPA 2003). These results also match the bioaccumulation data in the literature, which found that lower trophic level species tend to have higher iAs concentrations, whereas upper trophic level species have much lower iAs concentrations (e.g., Chen and Folt 2000, Revenga et al. 2012).

Variation in ratios of organic arsenic to inorganic arsenic among species

The literature reviewed suggested that larger and, therefore, presumably upper trophic level fish are likely to have lower proportions of iAs to organic arsenic compared to lower trophic level organisms. The ratio of iAs to organic arsenic in forage fish tissue (species that are feeding lower in the foodweb), including bridgelip sucker (an herbivore), sculpins sp., redbelt shiner, mountain whitefish, and dace sp. did not appear to change substantially with increasing weight (Figure 6). The ratio of iAs to organic arsenic in brown trout, cutthroat trout, and rainbow trout, representing upper trophic level fish, appeared to decline with increasing body weight (Figure 7). The absence of a trend in the iAs to organic arsenic ratio versus size in forage species is consistent with the hypothesis that they represent a lower trophic level, while the decrease in the ratio with increasing size for non-forage (predator) species indicates they transition to higher trophic levels as they increase in size. Perhaps the most important observation about intra- and interspecies differences in the iAs to organic arsenic ratio is that it reaffirms that metabolic transformation processes in aquatic foodwebs result in low and decreasing concentrations of iAs in upper trophic level organisms.

Figure 6: Ratio of Inorganic to Organic Arsenic and Average Fish Weight Forage Fish Species

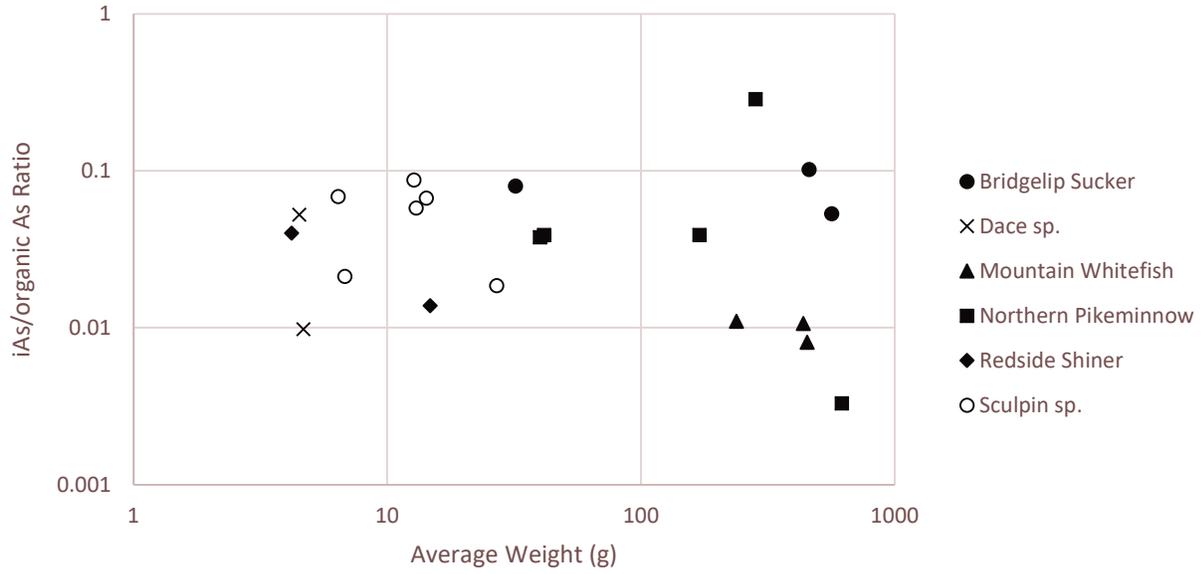
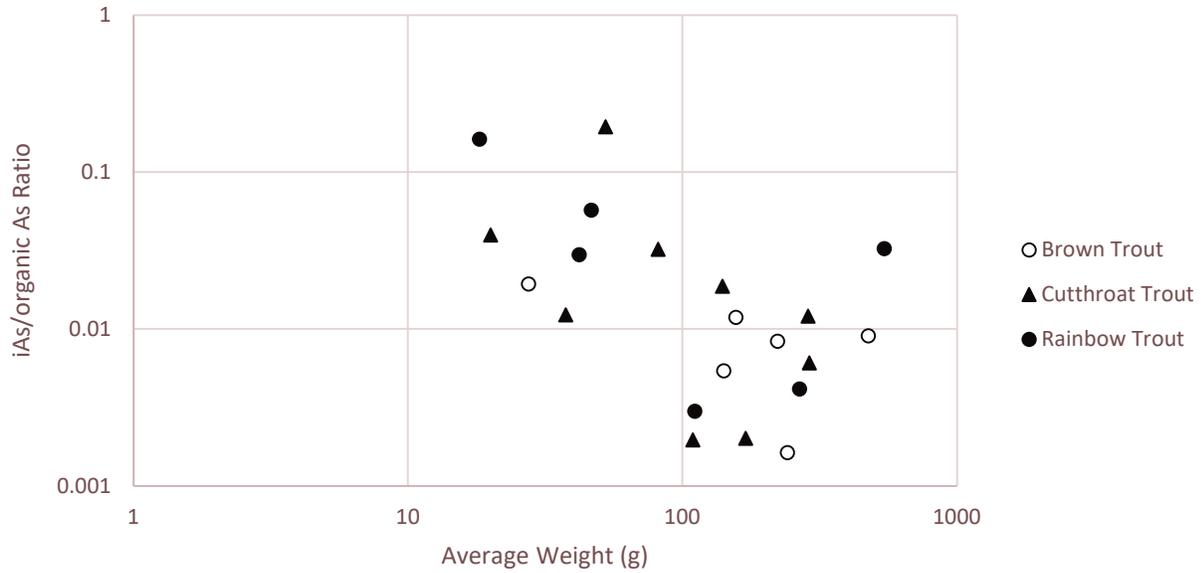


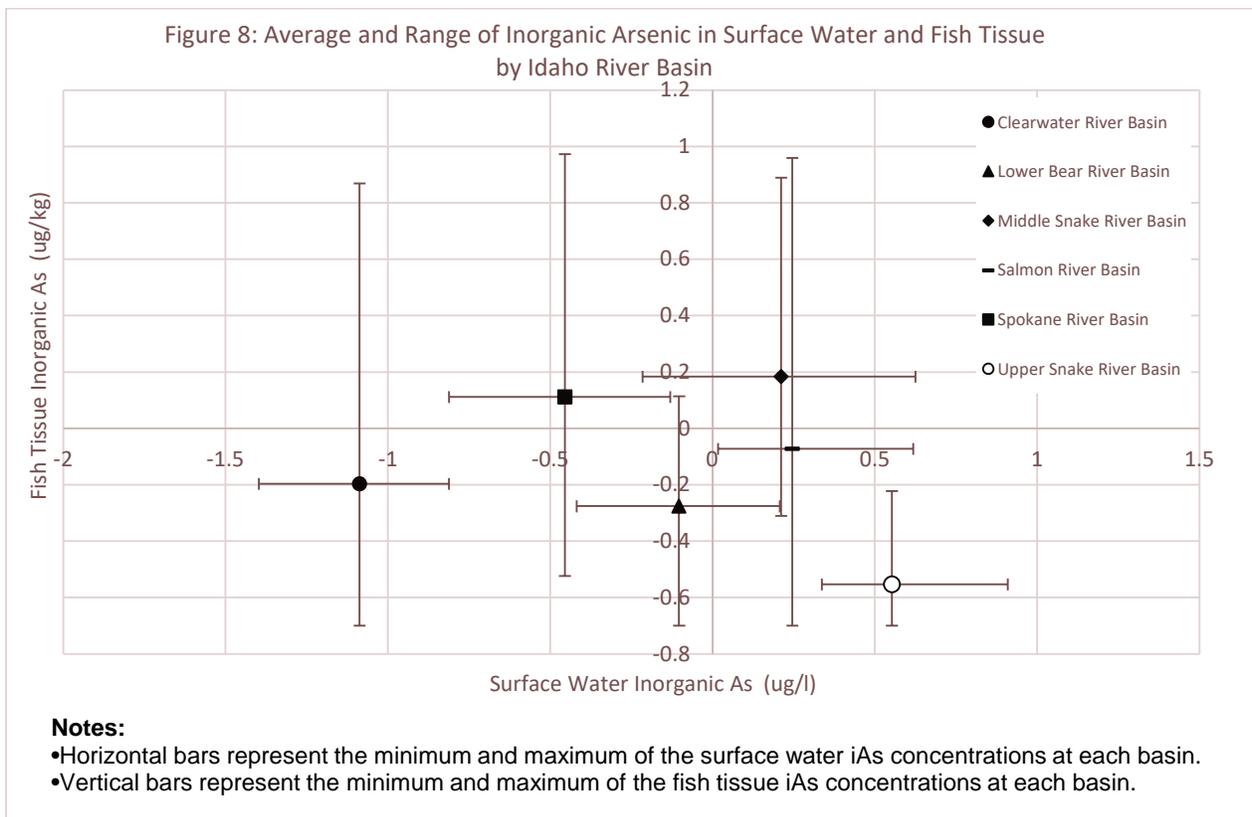
Figure 7: Ratio of Inorganic to Organic Arsenic and Average Fish Weight Non-Forage Fish Species



Effect of river basin on the relationship between inorganic arsenic in surface water and inorganic arsenic in fish tissues

A mixed linear regression model was used to examine whether the relationship between iAs in surface water and fish tissue was affected by Idaho river basin. In the model, river basin was the random effect, allowing the model to estimate the amount of variation that the fish in each river basin are contributing to the overall relationship between iAs in surface water and fish tissue. The 2019, IDEQ sample locations were assigned to six Idaho river basins using the U.S. Geological Survey Hydrologic Use Codes (HUC; see Appendix II for river basin assignments). No statistically significant effect of river basin on the relationship between iAs in surface water and fish tissue was identified (df=42, F=1.775, p=0.19).

Surface water iAs concentrations (horizontal bars in Figure 8) are relatively distinct among river basins. However, fish tissue iAs concentrations (vertical bars in Figure 8) show substantial overlap. The comparison among basins adds additional support to previous analyses of the 2019 IDEQ data that show iAs concentrations in fish tissue are independent of iAs concentrations in surface water.



Conclusions

The results of the analyses of the 2019 Idaho paired surface water and fish tissue arsenic data presented above are consistent with biomitigation trends observed in both field and laboratory studies in the literature across a wide variety of study locations and freshwater aquatic foodwebs. As established previously for the 2019 Idaho

data, the concentrations of neither iAs nor tAs in fish tissue can be predicted based on the concentrations of arsenic in surface water. The finding that most of the tAs in fish tissue is present as inorganic species is consistent with arsenic speciation reported in the literature. Further, the finding that iAs in fish tissue is related to fish weight (a proxy measure for trophic level and/or feeding guild), feeding guild, and trophic level also follow patterns reported in the literature. The decrease in the concentration of iAs in fish tissue with increasing size and trophic level are likely the result of metabolic transformation of accumulated species of inorganic species to non-toxic organic arsenic species by organisms in each level of the aquatic foodweb.

The results of the analyses presented in this technical memorandum not only continue to support previous analyses that found no relationship between iAs concentrations in fish tissue and surface water, but identify factors that do affect, and can help explain, the concentrations observed in fish collected from Idaho surface waters. Those factors include the size and trophic level of collected fish, and the biotransformation of inorganic to organic species of arsenic by organisms in the aquatic food web leading to biodilution of iAs concentrations. The trophic level differences and metabolic biotransformation explain the low concentration of iAs in larger fish (>100 grams) collected by IDEQ in 2019, regardless the concentration of iAs in the surface water from which the fish were collected.

5. References

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- Maeda, S., A. Ohki, T. Tokuda and M. Ohmine. 1990. Transformation of arsenic compounds in a freshwater food chain. *Appl. Organomet. Chem.* 4:251-254.
- Mason, R.P. n.d. An Investigation of the Influence of Water Quality on the Mercury, Methylmercury, Arsenic, Selenium and Cadmium Concentrations in Fish of Representative Maryland Streams. Maryland Department of Natural Resources. CBWP-MANTA- AD-02-1.
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- Pei, J. J. Zuo, X. Wang, J. Yu, L. Liu, W. Fan. 2019. The Bioaccumulation and Tissue Distribution of Arsenic Species in Tilapia. *International Journal of Environmental Research and Public Health* 16: 757-768.
- Rahman, M.A., H. Hasegawa, R. Peter Lim. 2012. Bioaccumulation, biotransformation and trophic transfer of arsenic in the aquatic food chain. *Environmental Research* 116:118–35.
- Revenga, J.E., L.M. Campbell, M.A. Arribere, S.R. Guevara. 2012. Arsenic, cobalt and chromium food web biodilution in a Patagonia mountain lake. *Ecotoxicology and Environmental Safety* 81: 1-10.
- Robinson, B.H., R.R. Brooks, H.A. Outred, J.H. Kirkman. 1995. Mercury and arsenic in trout from the Taupo Volcanic Zone and Waikato River, North Island, New Zealand. *Chemical Speciation and Bioavailability* 7: 27-35.
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APPENDIX I - Literature Summaries

- Chen, C.Y., and C.L. Folt. 2000. Bioaccumulation and diminution of arsenic and lead in a freshwater food web. *Environ. Sci. Technol.* 34:3878-3884.

(Field Study) Bioaccumulation and biomagnification of As in water and four food web components (particulates, two size fractions of zooplankton, and six species of fish) were measured in Upper Mystic Lake (Massachusetts, USA). Total As levels in small and large plankton and fish diminished with increasing trophic level. Arsenic was elevated in lower trophic levels relative to food webs in uncontaminated lakes. The highest As concentrations were found in planktivorous species that feed directly on metal-enriched zooplankton. Despite elevated As in zooplankton, pronounced diminution between zooplankton and fish in Upper Mystic Lake appears to result in concentrations of As in fish that do not differ from uncontaminated systems.

- Chen CY, Pickhardt PC, Xu MQ, Folt CL. 2008. Mercury and arsenic bioaccumulation and eutrophication in Baiyangdian Lake, China. *Water, Air, and Soil Pollution* 190:115–27

(Field Study) Arsenic and other metals concentrations were measured in water, two size fractions of zooplankton, and fish from 20 lakes in contaminated to pristine watersheds in the northeastern United States (New England and New York). Bioaccumulation of As diminished with increasing trophic level. Aqueous and zooplankton metal levels were not significant predictors of As levels in fish.

- Cheng, Z., K-C. Chen, K-B, Li, X-P., Nie, S.C. Wu, C. K-C. Wong, M-H. Wong. 2013. Arsenic contamination in the freshwater fish ponds of Pearl River Delta: bioaccumulation and health risk assessment. *Environmental Science and Pollution Research* 20: 4484-4495.

(Field Study) This study examined the extent of As contamination in fish pond sediment and five common species of freshwater fish (northern snakehead [*Channa argus*], mandarin fish [*Siniperca chuatsi*], largemouth bass [*Lepomis macrochirus*], bighead carp [*Aristichthys nobilis*] and grass carp [*Ctenopharyngodon idellus*]) collected from 18 freshwater fish ponds around the Pearl River Delta (China). The biota sediment accumulation factor showed that omnivorous fish and zooplankton accumulated higher concentrations of heavy metals from sediment than carnivorous fish. No biomagnification or bio-diminution of As from lower trophic levels (zooplankton) to fish in the aquaculture ponds was found.

- Chetalat, J., P.A. Cott, M. Rosabal, A. Houben, C. McClelland, E.B. Rose, M. Amyot. 2019. Arsenic bioaccumulation in subarctic fishes of a mine-impacted bay on Great Slave Lake, Northwest Territories, Canada. *PLoS ONE* 14(8): e0221361.

(Field Study) Total concentrations of As and other metals were measured in 13 species of fish, as well as primary producers and consumers in mine-impacted Yellowknife Bay (Great Slave Lake, Northwest Territories, Canada). Arsenic did not bioaccumulate in long-lived, slow-growing subarctic fishes. Foodweb biodilution of tAs occurred between primary producers, aquatic invertebrates, and fish. Pelagic-feeding species had higher tAs concentrations compared to littoral fishes.

- Ciardullo, S. F. Aureli, A. Raggi, F. Cubadda. 2010. Arsenic speciation in freshwater fish: Focus on extraction and mass balance. *Talanta* 81: 213-221.

(Field Study) Arsenic speciation in muscle tissues of freshwater fish was investigated in a study of eel (*Anguilla anguilla* L.), flathead grey mullet (*Mugil cephalus* L.), chub (*Leuciscus cephalus* L.), and carp (*Cyprinus carpio* L.) collected from the Tiber river (Italy). The inorganic arsenical arsenobetaine was the dominant As compound,

representing 58% of the tAs concentration in carp, 89% of the tAs concentration in eel, and 95% of the tAs concentration in both mottlet and chub.

- Ciardullo, S. F. Aureli, E. Coni, E. Guandalini, F. Iosi, A. Raggi, G. Rufo, F. Cubadda. 2008. Bioaccumulation potential of dietary arsenic, cadmium, lead, mercury, and selenium in organs and tissues of rainbow trout (*Oncorhynchus mykiss*) as a function of fish growth. *Journal of Agriculture and Food Chemistry* 56: 2442-2451.

(Lab Study) Rainbow trout were fed dietary As (the study does not identify the species of arsenic used in the feeding study) for three years in a laboratory study. Concentrations of As in gills, kidney, liver, muscle, and skin tissues did not increase over time due to dilution with growth. The concentration of As in muscle tissue peaked at 14 months and then decreased in adult specimens. Speciation analysis revealed that the As species in muscle tissue was almost exclusively present in the form of the non-toxic organic As, arsenobetaine. These findings highlight the low bioaccumulation potential of toxic trace elements such as As in rainbow trout following dietary exposure.

- Cui, D., P. Zhang, H. Liu, Z. Zhang, Y. Song, Z. Yang. 2021. The dynamic changes of arsenic biotransformation and bioaccumulation in muscle of freshwater food fish crucian carp during chronic diet-borne exposure. *Journal of Environmental Sciences* 100: 74-81.

(Lab Study) This was a 40-day laboratory study on dietary exposure of iAs (AsIII and AsV) exposure in the benthic freshwater fish, the crucian carp (*Carassius auratus*). After 40 days of exposure, arsenobetaine comprised 87.9% and 70.4% for the AsIII and AsV treatment. Proportions of arsenobetaine increased in both the iAs treatments with prolonged exposure, suggesting that fish had an adaptive response by increasing iAs biotransformation into the non-toxic species, arsenobetaine.

- Culioli, J-L., A. Fougouire, S. Calendini, C. Mori, A. Orsini. 2009. Trophic transfer of arsenic and antimony in a freshwater ecosystem: A field study. *Aquatic Toxicology* 94: 286-293.

(Field Study) Arsenic concentrations in water and biota (bryophytes, benthic macroinvertebrates and fish) of the Bravona River (Corsica, France) were measured. The pattern of accumulation of As in the food chain decreased as follows: macroinvertebrates > bryophytes > water > fish tissues. The accumulation of metals in invertebrate taxa depended on their place in the food chain, their feeding behavior, and their specific habit (lenticophilic/rheophilic species). Concentrations of As decreased with increasing trophic level.

- Dovick, M.A., T.R. Kulp, R.S. Arkle, D.S. Pilliod. 2015. Bioaccumulation trends of arsenic and antimony in a freshwater ecosystem affected by mine drainage. *Environmental Chemistry* 13: 149-159.

(Field Study) Arsenic concentrations in water and substrate samples, and in tissues of organisms representing several trophic levels were measured at the Stibnite Mine in Idaho. Bioaccumulation of As was observed in stream organisms with the following trend of bio-diminution with increasing trophic level: primary producers > tadpoles > macroinvertebrates > trout. This study adds to a number of recent investigations reporting bioaccumulation, but not biomagnification, of As in food webs. Results suggest that tadpoles, in particular, may be more resistant to metalloid contamination than previously assumed.

- Juncos, R. M. Arcagni, S. Squadrone, A. Rizzo, M. Arribere, J. P. Barriga, M.A. Battini, L.M. Campbell, P. Brizio, M.C. Abete, S.R. Guevera. 2019. Interspecific differences in the bioaccumulation of arsenic of three Patagonian top predator fish: Organ distribution and arsenic speciation. *Ecotoxicology and Environmental Safety* 168: 431-442.

(Field Study) Interspecific differences in arsenic bioaccumulation and organ distribution (muscle, liver, kidney and gills) in three predator fish (creole perch, rainbow trout and brown trout) from a Patagonian lake impacted by volcanic eruptions were studied. Organic arsenic species (arsenobetaine and dimethylarsinic acid) were the dominant As species in muscle of these fish. No iAs species were detected.

- Kuroiwa, T., K. Mawatari, A. Ohki, K. Naka, S. Maeda. 1994. Biomethylation and biotransformation of arsenic in a freshwater food chain: Green alga (*Chlorella vulgaris*) → shrimp (*Neocaridina denticulata*) → killifish (*Oryzias latipes*). Appl. Organomet. Chem. 8: 325–333.

(Lab Study) Total As accumulation was examined in food chain (green algae [*Chlorella vulgaris*] → shrimp [*N. denticulata*] → killifish [*O. latipes*]), collected from the natural environment. Total As concentrations decreased by one order of magnitude or more with increasing trophic level. The concentration of methylated As relative to tAs increased successively with increases in the trophic level.

- Liao, C-M., B-C. Chen, S. Singh, M-C. Line, C-W. Liu. 2003. Acute Toxicity and Bioaccumulation of Arsenic in Tilapia (*Oreochromis mossambicus*) from a Blackfoot Disease Area in Taiwan

(Field Study) Bioaccumulation and toxicity of As in tilapia from fish farm ponds in Taiwan were examined. Arsenic concentrations in all tissues were allometric, but negatively correlated with fish body weight.

- Liu, C-W., C-P. Liang, K-H. Lin, C-S. Jang, S-W. Wang, Y-K. Huang, Y-M. Hseuh. 2007. Bioaccumulation of arsenic compounds in aquacultural clams (*Meretrix lusoria*) and assessment of potential carcinogenic risks to human health by ingestion. Chemosphere 69: 128-134.

(Field Study) This study surveyed the tAs and As species content in clams (*Meretrix lusoria*) farmed in southwestern Taiwan as well as pond water and sediment. The average ratios of iAs content to tAs content in clams ranged from 12% to 14%.

- Lopez, A.R., D.R. Hesterberg, D.H. Funk, D.B. Buchwalter. 2016. Bioaccumulation Dynamics of Arsenate at the Base of Aquatic Food Webs. Environmental Science and Technology 50: 6556-6564.

(Lab Study) This study examined the As bioaccumulation dynamics in lotic food webs: accumulation in periphyton and subsequent trophic transfer to benthic grazers. Periphyton bioconcentrated As between 3,200–9,700-fold (dry weight) over 8 days without reaching steady state, suggesting that periphyton is a major sink for arsenate. However, As-enriched periphyton as a food source for the mayfly *Neocloeon triangulifer* resulted in negligible As accumulation after exposure for a full life cycle. Estimated dietary assimilation efficiency in several primary consumers ranged from 22% in the mayfly *N. triangulifer* to 75% in the mayfly *Isonychia* sp.

- Maeda, S., A. Ohki, T. Tokuda and M. Ohmine. 1990. Transformation of arsenic compounds in a freshwater food chain. Appl. Organomet. Chem. 4:251-254.

(Lab Study) The transport and transformation of arsenic were investigated in the food chain *Chlorella* and *Phormidium* sp. (autotrophic freshwater algae)–*Moina* sp. (zooplanktonic grazer)–*Poecilia* sp. (carnivorous guppy). Algae were grown in the presence of As (as Na₂HAsO₄) and then fed to *Moina*, which were in turn fed to the guppy. The guppy had the lowest As concentrations in the food chain. Arsenic in algae was almost all in inorganic species while 85% of the As in the guppy was in di- and tri-methylated species.

- Maeda, S., K. Mawatari, A. Ohki, K. Naka. 1994. Arsenic metabolism in a freshwater food chain: Blue–green alga (*Nostoc* sp.) → shrimp (*Neocaridina denticulata*) → carp (*Cyprinus carpio*). Appl. Organomet. Chem. 7: 467–476.

(Lab Study) Bioaccumulation and biomethylation of iAs were investigated in a three-step fresh-water food chain consisting of an autotroph (blue- green alga: *Nostoc* sp.), a herbivore (shrimp: *Neocaridina denticulata*) and a carnivore (carp: *Cyprinus carpio*). Arsenic(V) was accumulated from the water phase and underwent methylation by all three components of the food chain. Arsenic was mostly accumulated in the gut of the carp and the predominant arsenical was the monomethylarsenic species. Arsenic accumulation via food decreased by one order of magnitude and the relative concentration of methylated arsenic to tAs accumulated increased successively with increased trophic level.

- Mason, R.P. n.d. An Investigation of the Influence of Water Quality on the Mercury, Methylmercury, Arsenic, Selenium and Cadmium Concentrations in Fish of Representative Maryland Streams. Maryland Department of Natural Resources. CBWP-MANTA- AD-02-1.

(Field Study) Fish (sunfish, pickerel, minnows, bass, and catfish) and water were sampled from several stream locations in Maryland. No relationship was found between fish weight and As concentration and it appears that the larger fish have lower As concentrations.

- Otter, R.R., F.C. Bailey, A.M Fortner, S.M. Adams. 2012. Trophic status and metal bioaccumulation differences in multiple fish species exposed to coal ash-associated metals. *Ecotoxicology and Environmental Safety* 85: 30-36.

(Field Study) Arsenic in fish tissues from rivers contaminated by the Tennessee Valley Authority Kingston Fossil Plant coal ash spill were analyzed. Stable isotope analysis confirmed differences in trophic levels among the studied fish species: largemouth bass, white crappie, bluegill, and redear sunfish. Redear sunfish, a lower trophic level fish, had the highest As concentrations of the species examined.

- Pei, J. J. Zuo, X. Wang, J. Yu, L. Liu, W. Fan. 2019. The Bioaccumulation and Tissue Distribution of Arsenic Species in Tilapia. *International Journal of Environmental Research and Public Health* 16: 757-768.

(Lab Study) Tilapia were exposed to the iAs species AsIII and AsV for 32 days. The accumulation of As in all tissues increased linearly in the first 10 days of exposure, while the As levels remained stable in the following 20 days of exposure. More than 90% of As was converted into organic species in liver, gill, and muscle, while organic As species comprised between 30–80% of tAs in the GI tract. The percentage of organic As in muscle was the highest, followed by gill, liver, and intestine, and arsenobetaine was the main form of organic As.

- Rahman, M.A., H. Hasegawa, R. Peter Lim. 2012. Bioaccumulation, biotransformation and trophic transfer of arsenic in the aquatic food chain. *Environmental Research* 116:118–35.

(Review Paper) Review paper examining bioaccumulation and biomagnification of arsenic in aquatic ecosystems. Although iAs species dominate both marine waters and freshwaters, it is biotransformed to methyl and organoarsenic species by aquatic organisms. Phytoplankton is considered a major energy source for aquatic foodwebs, and this autotrophic organism plays an important role in the biotransformation and distribution of As species in the aquatic environment.

- Revenga, J.E., L.M. Campbell, M.A. Arribere, S.R. Guevara. 2012. Arsenic, cobalt and chromium food web biodilution in a Patagonia mountain lake. *Ecotoxicology and Environmental Safety* 81: 1-10.

(Field Study) Stable isotopes of nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) were used to characterize metals trophodynamics in the Lake Moreno (Argentina) food web. Arsenic was found to biodilute, confirmed by elevated As concentrations in phytoplankton and zooplankton, while lower concentrations were found in sport fish such as rainbow trout.

- Robinson, B.H., R.R. Brooks, H.A. Outred, J.H. Kirkman. 1995. Mercury and arsenic in trout from the Taupo Volcanic Zone and Waikato River, North Island, New Zealand. *Chemical Speciation and Bioavailability* 7: 27- .

(Field Study) Arsenic concentrations in brown and rainbow trout tissue from lakes and rivers in New Zealand were of the same order of magnitude as the surface water from which they were taken. There was no relationship between body weight and As concentrations in trout.

- USEPA. 2003. Technical summary of information available on the bioaccumulation of arsenic in aquatic organisms. EPA-822-R-03-032. September 2003.

Review and summary of literature As bioaccumulation factors and biomagnification in freshwater and marine aquatic ecosystems.

- Williams, L. R.A. Schoof, J.W. Yager, J.W. Goodrich-Mahoney. 2006. Arsenic bioaccumulation in freshwater fishes. *Human and Ecological Risk Assessment* 12: 904-923.

(Review paper) Review paper of laboratory and field arsenic bioaccumulation studies in freshwater ecosystems. Field studies found that there was no significant relationship between As concentrations in surface water and fish tissues. Arsenic data assumed to be tAs.

APPENDIX II – River Basin Assignments

Table X: Idaho 2019 river basin assignments

River Basin	Waterbody Name	Sample ID
Lower Bear River Basin	Bear River	ASP004A
Lower Bear River Basin	Bear River	ASP004B
Lower Bear River Basin	Bear River	ASP005A
Lower Bear River Basin	Bear River	ASP005B
Lower Bear River Basin	Whiskey Creek	ASP007A
Lower Bear River Basin	Whiskey Creek	ASP007B
Lower Bear River Basin	Maple Creek	ASP008A
Lower Bear River Basin	Maple Creek	ASP008B
Clearwater River Basin	Warm Springs Creek	ASP026A
Clearwater River Basin	Warm Springs Creek	ASP026B
Clearwater River Basin	Red River	ASP027A
Clearwater River Basin	Red River	ASP027B
Clearwater River Basin	Cranberry Creek	ASP031A
Clearwater River Basin	Cranberry Creek	ASP031B
Clearwater River Basin	Potlatch River	ASP035A
Spokane River Basin	Potlatch River	ASP035B
Spokane River Basin	Saint Joe River	ASP051A
Spokane River Basin	Saint Joe River	ASP051B
Spokane River Basin	Hayden Creek	ASP052A
Spokane River Basin	Hayden Creek	ASP052B
Spokane River Basin	North Fork Coeur d'Alene River	ASP056A
Spokane River Basin	North Fork Coeur d'Alene River	ASP056B
Spokane River Basin	Rock Creek	ASP062A
Spokane River Basin	Rock Creek	ASP062B
Salmon River Basin	Salmon River	ASP076A
Salmon River Basin	South Fork Salmon River	ASP088A
Salmon River Basin	South Fork Salmon River	ASP088B
Salmon River Basin	Salmon River	ASP090A
Salmon River Basin	Salmon River	ASP090AD
Salmon River Basin	Salmon River	ASP090B
Salmon River Basin	Salmon River	ASP090c
Salmon River Basin	Seafoam Creek	ASP091A
Salmon River Basin	Seafoam Creek	ASP091B
Middle Snake River Basin	Granite Creek	ASP100A
Middle Snake River Basin	Granite Creek	ASP100B
Middle Snake River Basin	Marys Creek	ASP102A
Middle Snake River Basin	Marys Creek	ASP102B

Middle Snake River Basin	Mores Creek	ASP104A
Middle Snake River Basin	Mores Creek	ASP104B
Middle Snake River Basin	Mores Creek	ASP104BD
Middle Snake River Basin	Weiser River	ASP105A
Middle Snake River Basin	Weiser River	ASP105B
Upper Snake River Basin	Snake River	ASP122A
Upper Snake River Basin	Snake River	ASP122B
Upper Snake River Basin	Rock Creek	ASP123A
Upper Snake River Basin	Henrys Fork	ASP126A
Upper Snake River Basin	Henrys Fork	ASP126AD
Upper Snake River Basin	Salmon Falls Creek	ASP127A

Enc. [Enclosures]