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# SHORT COMMUNICATION

# A voucher-based survey of arachnids from salmonid fish (*Oncorhynchus* spp.) gut contents

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**Abstract.** This investigation identifies arachnid prey of juvenile salmonid fishes (*Oncorhynchus* spp.) in a coastal stream in Marin County, California. Using morphological identification and DNA sequencing of the metazoan barcode gene (cytochrome *c* oxidase subunit I), arachnids recovered from salmon gut lavage samples were identified. The non-invasive specimen-specific sequencing approach allows for an association of the specimen with sequence data, something often lacking in metabarcoding or eDNA studies. To identify potential source habitats, the guilds of the spiders and mites studied were classified. No samples were contaminated, and genus-level identifications were achieved in all spiders, with species-level identifications in 38% of spiders. These findings provide insight into the arachnid prey within aquatic ecosystems, aiding our understanding of allochthonous contributions to freshwater environments and their fish predators. These contributions have implications for ecosystem dynamics, nutrient cycling, contaminant accumulation, and conservation broadly.

**Keywords:** Araneae, mites, COI, barcoding, terrestrial arthropod prey

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Freshwater streams of California are home to, and the breeding grounds of, numerous salmon species, including the Chinook [*Oncorhynchus tshawytscha* (Walbaum, 1792)], Coho [*O. kisutch* (Walbaum, 1792)], and Steelhead [*O. mykiss* (Walbaum, 1792)] (Katz et al. 2013). However, many salmon have displayed a decline in population in recent years due to climate change (Katz et al. 2013). Rising water temperatures in summer months are projected to result in a decline in the quality and quantity of freshwater environments suitable for salmon spawning and rearing (Mantua et al. 2010). Additionally, thermal stress in aquatic environments has been suggested as a contributing factor to increased mortality in adults returning to spawn (Mantua et al. 2010). Warming water temperatures in winter seasons, due to rain-on-snow events, have the potential to affect overwintering eggs of salmon species that spawn in autumn and hatch in spring (Hauer et al. 1997). More generally, freshwater habitats are under threat due to decreasing river levels as water is artificially stored and redirected for use in agriculture, industry, and domestic use (Grantham et al. 2010; Munsch et al. 2022). Combined, these factors have had negative impacts on salmon populations. For example, California's 2023 recreational fishing season was fully closed by the California Fish and Game Commission for the Klamath River Basin, Central Valley Rivers, and the Smith and Eel Rivers to allow for salmon populations to recover (CDFW News 2023). There is also a long legacy of the cultural importance of salmonids for the indigenous peoples of California dating well before European contact (Yoshiyama 1999; Yoshiyama & Fisher 2001; Madgic 2013). The continuance of this cultural practice is threatened by the decline of salmon due to the aforementioned anthropogenic habitat changes, as well as fishing practices that are less sustainable than the practices of indigenous peoples for time immemorial (Lufkin 1991). Furthermore, the economic impact of salmonids in California is massive. A recent fishery collapse and subsequent closures in 2008 and 2009 resulted in an estimated loss of $500 million–$2 billion to California's economy, and the loss of 5,000–23,000 jobs in the state (Schwarzenegger 2008, 2009; State of California Legislature 2010).

To conserve populations of salmonid fishes, we must understand their basic biology, including their feeding habits. Previous studies have shown that salmonids participate in a type of preferential feeding called drift foraging, wherein fish balance the energy costs of swimming in a current with the increased nutrient flow that faster currents provide (Rosenfield 2014). Ideal drift foraging sites are often low-flow, low-competition areas just downstream of high-flow rifles, so that fish can reap the benefits of high-flow whilst expending little energy on swimming (Rosenfeld et al. 2014). When engaging in this type of feeding, salmonids tend to select the largest, easiest prey, which is frequently terrestrial invertebrates with poor mobility in aquatic habitats (Nakano et al. 1999). Broadly, terrestrial prey constitute a large component of stream-dwelling fish diets. Hunt & Krokhin (1975) claimed that adult insects represent the most important terrestrial invertebrate food item for salmonids. Bridcut (2000) reported that terrestrial and aerial invertebrate prey comprise up to 86% of the salmonid diet.

Arachnids are regularly observed in studies on the composition of terrestrial invertebrates in salmonid diets. This is expected, as Naman et al. (2017) observed that spiders represent the third largest biomass component of terrestrial inputs to aquatic ecosystems. Hunt & Krokhin (1975) characterized predation on spiders by salmonids to be a "sporadic", yet not uncommon, event. Wipfli (1997) identified the percent biomass composition of spider prey to be similar between riparian old-growth and young-growth forests, findings which were corroborated by Allan et al. (2003). Krug et al. (2012) observed a 23% frequency of spiders found in salmon guts, regardless of time of year. Rundio & Lindley (2008) observed that arachnids represent 1.05–1.91% of all prey mass in salmonids, and that spiders contributed to wide standard errors in terrestrial biomass inputs due to their occasionally large size. Roon et al. (2018) quantified prey selectivity by salmon using Jacob's index of selectivity. In both the mite orders (defined as Acari) and other arachnids (Arachnida to the exclusion of Acari), negative values were recovered, indicating negative selection relative to the availability of such prey items in the environment. This selectivity index may speak more to whether these organisms are incidentally ingested. In all of these studies that have documented arachnids in salmonid guts, however, the taxonomic resolution is coarse, at the order-level. Never have arachnids in these works been identified to the family-level, let alone genus or species.

Jo et al. (2016) provided evidence for the efficacy of DNA barcoding in identifying soft-bodied prey items consumed by brown trout (*Salmo trutta* Linnaeus, 1758), stating that accurate identification of the fish's diet can be useful in monitoring an environment's status. Coddington

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et al. (2016) discuss thresholds for family-level and genus-level identification based on BLAST results and found that 91% matches and above capture a less than 5% misidentification rate at the family-level, and 95% matches and above capture a less than 5% misidentification rate at the genus level. These are the percent identity thresholds used for the purposes of this study. While species-level identification thresholds vary, Hebert et al. (2003) suggest a 2% divergence threshold between animal species (distinguishing 98% of animal species), and Barrett & Hebert (2005) found that a 3% divergence threshold worked for spiders they studied. This is complicated however, by the fact that not all species of spider submitted to genetic reference databases such as GenBank and BOLD are expertly identified, so sequences may match to multiple species either because of uniformity along the examined locus (in this case, *COI*) or because of misidentification. Accordingly, we sought to determine the types of arachnids that contribute to aquatic food systems to begin to understand their role and how they might enter such environments and discuss present limitations of identification using these methods. In this study, the gut contents of salmon were examined for mites and spiders, and these arachnids were then identified morphologically and by sequencing to the finest taxonomic scale.

## METHODS

**Gut lavage collection.**—Salmon of the genus *Oncorhynchus* [*O. kisutch*, Central California Coast evolutionarily significant unit; *O. mykiss* ssp. *irideus* (Gibbons, 1855), Central California Coast distinct population segment; and *O. tshawytscha*] were collected from a Marin County stream (Redwood Creek, from the lagoon to near the headwaters) in the Golden Gate National Recreation Area in summer 2022 using seining and electrofishing techniques. After anesthetizing with tricaine methanesulfonate (MS-222), data (age, site, species, mass, and fork length, measured as distance from the tip of the snout or jaw to the center of the fork in the tail) for each collected salmon were recorded. A non-lethal gut lavage technique was used to collect gut contents for later analyses, which were stored in at least 70% isopropyl alcohol (Meehan & Miller 1978; Pert 1993). For both *O. kisutch* and *O. mykiss irideus*, only individuals greater than or equal to 50 mm (fork length) were sampled for stomach contents. Gut contents of each specimen were examined, and any whole-bodied arachnids (spiders or mites) were recovered, noted, and placed in separate centrifuge tubes with 95% ethanol. This study was limited to whole-bodied spiders or mites to ensure morphological corroboration of DNA barcode results where possible. Four samples representing undetermined species of sculpin (Cottoidea) were also collected incidentally and examined in the same manner. Permit information for the collection of gut lavage samples from salmon and examining arachnids from these samples may be found in the Acknowledgements. Multiple Wilcoxon Rank-Sum tests (with an $\alpha$ of $P < 0.05$) were performed on the data in R to determine the relationship between fish age, mass, and fork length versus the presence of a consumed arachnid. For categorical data regarding fish age and species, a chi-squared test as well as Fisher's exact test (to examine whether any chi-squared values were due to lower values in contingency tables) were conducted to examine the relationship between fish age and presence of arachnid in gut.

**Morphological identification.**—Each spider specimen was visually identified through two rounds of keyed morphological identification using Ubick et al. (2017). Spiders were small enough to make identification by sight impossible, so a Leica MZ125 stereomicroscope (Wetzlar, Germany) was used. Spider specimens were placed into a shallow dish containing white or clear sand and enough 95% ethanol to completely submerge the specimen. Following DNA extraction, sequencing and identification using the National Institutes of Health website's BLAST (detailed subsequently), a second round of visual identification was performed to confirm or reject the original morphological identification and compare with barcode data. Morphological identification was largely not performed on the mites, as that requires clearing and slide mounting, which would have rendered them unsuitable for DNA extraction. In the case of mites for which DNA could not be recovered, a higher-level visual identification was made.

**DNA extraction, sequencing and identification.**—For spiders, two legs were removed from each specimen of good condition and placed into a new Eppendorf vial for DNA extraction, such that the specimen would have at least one leg in all four positions for use in future examination. No legs were removed from degraded or highly damaged specimens, and their DNA was extracted by a full body soak. All mite samples were subjected to full body soaks due to size. DNA extraction was performed according to directions set by QIAGEN QIAamp Micro DNA extraction kit (Hilden, Germany). For both spiders and mites, legs or bodies were placed directly into 180 µl of ATL buffer, with no tissue maceration being performed. The extraction procedure was modified by increasing the amount of proteinase K from 20 µl to 60 µl, and allowing the samples to incubate for roughly 16 hours in a VWR thermomixer (Radnor, PA, USA) at 350 rpm at 56°C. The optional step of adding 1 µl of 1 ng/µl carrier RNA was followed post-incubation and then with a single 50 µl elution with the kit's provided AE buffer to minimize DNA loss and dilution. DNA was quantified on a Qubit Fluorometer using a High Sensitivity Assay Kit (Thermo Fisher, Waltham, MA, USA). PCR was performed using the following reagents: PCR was performed using the following reagents: 2.50 µl Invitrogen 10× PCR Buffer, 1.00 µl 50 mM Invitrogen MgCl2, 1.00 µl 10 µM primer HCO, 1.00 µl 10 µM primer LCO, 0.50 µl 10mM dNTPs, 1.00 µl 10 mg/ml BSA, 0.25 µl 5U/µl Invitrogen *Taq* DNA Polymerase, and 15.75 µl deionized water. This was then multiplied by the number of samples needed to create a master mix. For each sample, 23 µl from the master mix was pipetted, followed by 2 µl of DNA or water in the case of a negative control for a total reaction volume of 25 µl. PCR tubes were placed into a Bio-Rad thermocycler (Hercules, CA, USA) with the following PCR cycle used: (1) 94°C for 2 minutes; (2) 95°C for 30 seconds; (3) 48°C for 30 seconds; (4) 72°C for 1 minute and 35 seconds; (5) repeat 2–4 for 40 cycles; (6) 71°C for 7 minutes; and a hold at 4°C. The quality of PCR products was examined using gel electrophoresis and then cleaned using ExoSAP-IT (Applied BioSystems) purification. The purified PCR product was prepared for outsourced sequencing by Elim Biopharmaceuticals, Inc. (Hayward, CA, USA). Sequenced DNA was assembled *de novo*, cleaned, and trimmed using the program Geneious version 2023 (Biomatters Ltd; online at https://www.geneious.com). Sequences were then searched on the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST; Altschul et al. 1990) to identify each arachnid specimen to the finest resolution possible. The percent identity threshold used for a family-level identification in this study was 91%, consistent with Coddington et al. (2016), and genus-level identifications were made at a 95% threshold. We use a 98% threshold for conclusive species-level identifications, stricter than what was necessary for Barrett & Hebert (2005), and consistent with Hebert et al. (2003), who found that a 2% sequence divergence generally distinguishes congeneric animal species. In the event there was a close secondary match above the 98% species threshold used (and within 0.25%), we have listed that other taxon as well.

**Classification of functional guilds and aquatic association.**—For spiders, the functional guild of each spider recorded in this study was classified according to Cardoso et al. (2011). Aquatic habitat associations in spiders were defined as any spiders regularly found along or in bodies of water, though this association may not be exclusive. This was determined through a brief literature review involving a combination of examining localities in taxonomic works and aquatic associations of any other species in the same genus. If a species was consistently recovered from aquatic-associated localities (i.e., beaches, streams, ponds) in taxonomic works, and there is precedent in that at least some members of the family are aquatic through the thorough review of aquatic spiders by Crews et al. (2020), a species was classified as aquatic habitat-associated. For mites, the aquatic or terrestrial designation was determined by investigation of the dominant genus or family-level ecology since species level-identifications were predicted to be infrequent.

## RESULTS

**Gut lavage collection.**—Of 179 total gut lavage samples examined, only eight had no identifiable prey. All remaining 171 gut content samples contained at least one arthropod, and most (approximately 137) of these contained putatively terrestrial arthropods. This number is approximate because in many cases the gut sample was too degraded or fragmented for an identification

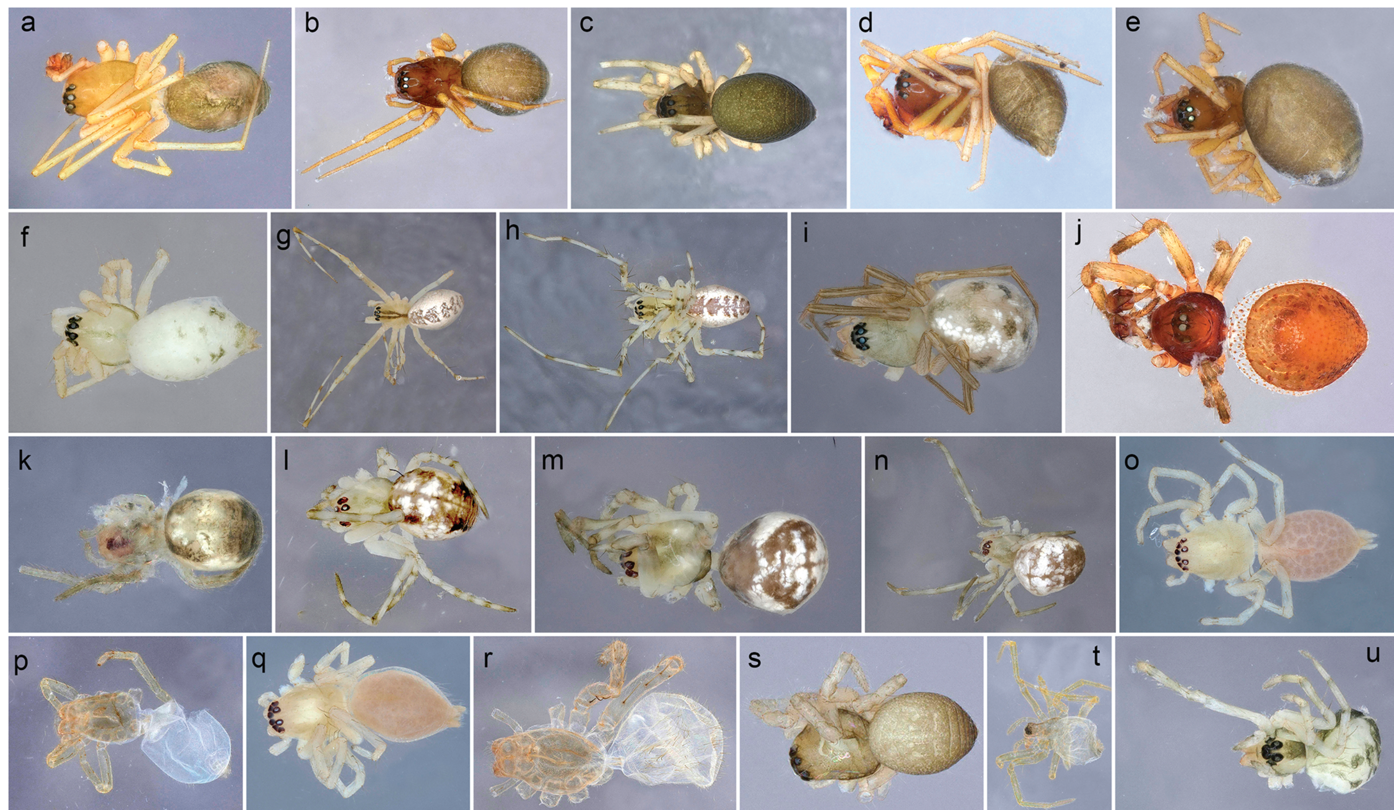


Figure 1a–u.—Images of spiders from salmon guts, showcasing the diversity of preservation quality and species diversity, with taxonomy relative to finest-scale BLAST result threshold. (a) 52.04.S1, Linyphiidae, *Agyneta protrudens*/cf. *darrelli*. (b) 148.09.S1, Linyphiidae, *Erigone aletris*/*dentosa*. (c) 175.01.S1, Linyphiidae, *Erigone aletris*/*dentosa*. (d) 268.03.S1, Linyphiidae, *Erigone aletris*/*dentosa*. (e) 586.05.S1, Linyphiidae, *Erigone aletris*/*dentosa*. (f) 148.10.S1, Linyphiidae, *Linyphantes Victoria*. (g) 586.07.S2, Linyphiidae, *Pityohyphantes subarcticus*/*alticeps*. (h) 586.07.S1, Linyphiidae, *Pityohyphantes subarcticus*/*alticeps*. (i) 729.06.S1, Linyphiidae, *Tenuiphantes tenuis*/sp. (j) 390.09.S1, Anapidae, *Gertschanapis* sp. (k) 390.02.S1, Tetragnathidae, *Metellina curtisi*. (l) 586.10.S1, Tetragnathidae, *Metellina curtisi*), (m) 614.11.51, Tetragnathidae, *Metellina curtisi*. (n) 614.15.S1, Tetragnathidae, *Metellina curtisi*. (o) 586.07.S3, Clubionidae, *Clubiona canadensis*/sp. (p) 2.22.S1, Clubionidae, *Clubiona pacifica*/*canadensis*. (q) 450.01.S1, Clubionidae, *Clubiona pacifica*/*canadensis*. (r) 53.08.S1. Anyphaenidae, *Anyphaena aperta*. (s) 2.01.S1, Linyphiidae, *Erigone aletris*/*dentosa*. (t) 614.04.S1, Linyphiidae, *Linyphantes orcinus*. (u) 450.03.S1, Linyphiidae, *Microlinyphia dana*. Images not to scale. Refer to Figures S1–S21 (Supplemental file 1) for photos with scale bars.

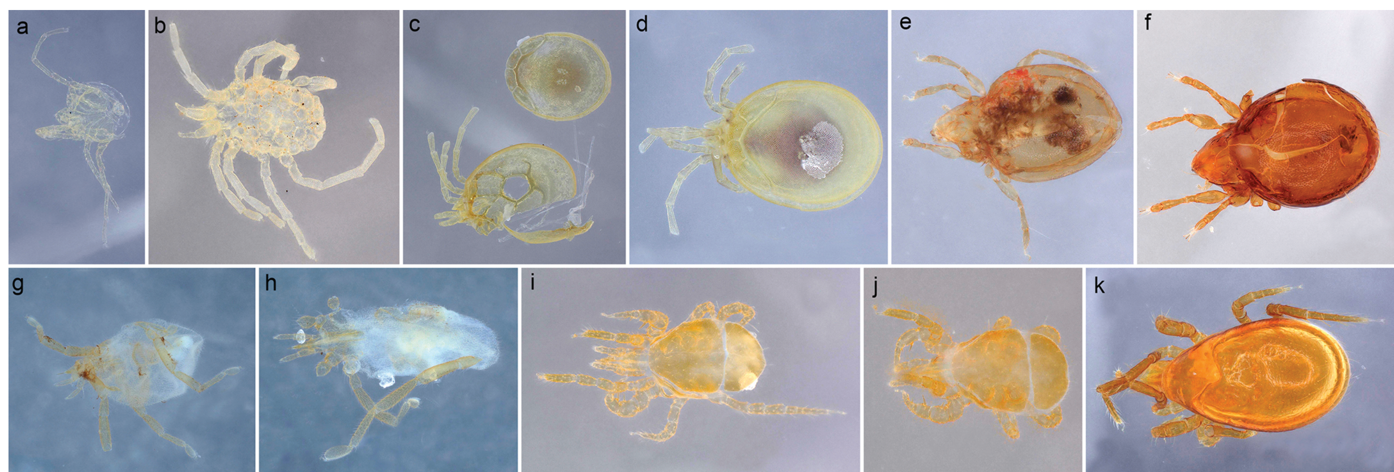


Figure 2a–k.—Images of mites from salmon guts, showcasing the diversity of preservation quality and broader diversity, with taxonomy relative to finest-scale BLAST result threshold, unless otherwise indicated. (a) 729.01.M1, Trombidiformes sp. (b) 729.06.M1, Trombidiformes sp. (c) 586.03.M1, *Torrenticola sierrensis*. (d) 586.09.M1, Trombidiformes sp. (e) 53.05.M1, Oribatida, *Hydrozetes* sp. - sight identification only by Dr. Zoë Lindo. (f) 53.08.M1, Oribatida, *Hydrozetes* sp. - sight identification only by Dr. Zoë Lindo. (g) 364.04.M1, *Balaustium* sp./Erythraeidae sp. (h) 364.08.M1, Erythraeidae. (i) 729.02.M1, Parasitidae sp. (j) 729.02.M2, Parasitidae sp. (k) 390.02.M1, Parasitiformes, *Pergamasus* sp. Images not to scale. Refer to Figures S22–S32 (Supplemental file 1) for photos with scale bars.

Table 1.—Results of molecular barcoding of arachnids from salmonid gut contents, with spiders sorted alphabetically by species, followed by mites. Fish species are members of the genus *Oncorhynchus* unless otherwise noted. BLAST result column represents at least 95% *COI* sequence identity for spiders and mites to the matched result. If there is a ? symbol in front of the family value, the sequence identity was greater than 85% but does not meet 91% similarity thresholds for family-level identity (Coddington et al. 2016). If there is a ? symbol in front of the genus under the BLAST result column, the sequence identity does not meet 95% similarity thresholds for genus-level identity (Coddington et al. 2016). If there is a ? symbol in front of the species name under the BLAST result column, the sequence identity does not meet 98% similarity thresholds for genus-level identity (Hebert et al. 2003, Barrett and Hebert 2005). Matches to multiple species indicate multiple species matches with >98% similarity thresholds. For spiders, * indicates a taxon not associated with aquatic habitats, † indicates a species associated with aquatic habitats. For mites, * indicates terrestrial species, ‡ indicates aquatic taxon. Lack of symbol indicates no clarity on whether the arachnid is aquatic or terrestrial-associated.

| Sample ID | Fish Species | BLAST Result | Family | Ecological Guild |
|---|---|---|---|---|
| 52.04.S1 | *O. tshawytscha* | *Agyneta protrudens*/cf. *darrelli* | Linyphiidae | Spider: Sheet web weavers* |
| 53.08.S1 | *O. kisutch* | *Anyphaena aperta* | Anyphaenidae | Spider: Other hunters* |
| 586.07.S3 | *O. kisutch* | *Clubiona canadensis*/sp. BIOUG22679-B03 | Clubionidae | Spider: Other hunters† |
| 2.22.S1 | *O. tshawytscha* | *Clubiona pacifica*/*canadensis* | Clubionidae | Spider: Other hunters† |
| 450.01.S1 | *O. mykiss irideus* | *Clubiona pacifica*/*canadensis* | Clubionidae | Spider: Other hunters† |
| 148.09.S1 | *O. kisutch* | *Erigone aletris*/*dentosa* | Linyphiidae | Spider: Other hunters† |
| 175.01.S1 | Cottoidea | *Erigone aletris*/*dentosa* | Linyphiidae | Spider: Other hunters† |
| 2.01.S1 | *O. mykiss irideus* | *Erigone aletris*/*dentosa* | Linyphiidae | Spider: Other hunters† |
| 268.03.S1 | *O. kisutch* | *Erigone aletris*/*dentosa* | Linyphiidae | Spider: Other hunters† |
| 586.05.S1 | *O. kisutch* | *Erigone aletris*/*dentosa* | Linyphiidae | Spider: Other hunters† |
| 390.09.S1 | *O. mykiss irideus* | *Gertschanapis ?shantzi* | Anapidae | Spider: Orb web weavers* |
| 614.04.S1 | *O. kisutch* | *Linyphantes orcinus* | Linyphiidae | Spider: Sheet web weavers* |
| 148.10.S1 | *O. kisutch* | *Linyphantes victoria* | Linyphiidae | Spider: Sheet web weavers* |
| 390.02.S1 | *O. kisutch* | *Metellina curtisi* | Tetragnathidae | Spider: Orb web weavers* |
| 586.10.S1 | *O. tshawytscha* | *Metellina curtisi* | Tetragnathidae | Spider: Orb web weavers* |
| 614.11.S1 | *O. mykiss irideus* | *Metellina curtisi* | Tetragnathidae | Spider: Orb web weavers* |
| 614.15.S1 | *O. tshawytscha* | *Metellina curtisi* | Tetragnathidae | Spider: Orb web weavers* |
| 450.03.S1 | *O. kisutch* | *Microlinyphia dana* | Linyphiidae | Spider: Sheet web weavers* |
| 586.07.S2 | *O. kisutch* | *Pityohyphantes ?subarcticus*/*?alticeps* | Linyphiidae | Spider: Sheet web weavers* |
| 586.07.S1 | *O. kisutch* | *Pityohyphantes ?subarcticus*/*?alticeps* | Linyphiidae | Spider: Sheet web weavers* |
| 729.06.S1 | *O. kisutch* | *Tenuiphantes tenuis*/*Tenuiphantes* sp. TTEN65 | Linyphiidae | Spider: Other hunters† |
| 729.01.M1 | *O. kisutch* | *Atractides* sp. BIOUG21865-G06 /aff. *nodipalpis* | ?Hygrobatidae | Trombidiformes (acariform mite)‡ |
| 364.04.M1 | *O. kisutch* | *Balaustium* sp. BOLD:ACE2704 /Erythraeidae sp. BIOUG16744-D10 | Erythraeidae | Trombidiformes (acariform mite)* |
| 364.08.M1 | *O. kisutch* | Erythraeidae sp. BIOUG16741-E05 /Erythraeidae sp. BIOUG20912-A02 | Erythraeidae | Trombidiformes (acariform mite)* |
| 53.05.M1 | *O. kisutch* | *Hydrozetes* sp. – sight identification | Hydrozetidae | Oribatida (sarcoptiform mite) |
| 53.08.M1 | *O. kisutch* | *Hydrozetes* sp. – sight identification | Hydrozetidae | Oribatida (sarcoptiform mite) |
| 729.02.M1 | *O. kisutch* | Parasitidae sp. BIOUG23517-H10 | Parasitidae | Mesostigmata (parasitiform mite)* |
| 729.02.M2 | *O. kisutch* | Parasitidae sp. BIOUG23517-H10 | Parasitidae | Mesostigmata (parasitiform mite)* |
| 390.02.M1 | *O. kisutch* | *Pergamasus* ? *crassipes* | Parasitidae | Mesostigmata (parasitiform mite)* |
| 729.06.M1 | *O. kisutch* | *Sperchon rostratus* | ?Sperchontidae | Trombidiformes (acariform mite)‡ |
| 586.03.M1 | *O. mykiss irideus* | *Torrenticola sierrensis* | Torrenticolidae | Trombidiformes (acariform mite)‡ |
| 364.02.M1 | *O. kisutch* | *Torrenticola* sp. BOLD:AAN9099/ BOLD:AAN9098 | ?Torrenticolidae | Trombidiformes (acariform mite)‡ |
| 586.09.M1 | *O. mykiss irideus* | *Torrenticola* sp. BOLD:AAN9099/ BOLD:AAN9098 | ?Torrenticolidae | Trombidiformes (acariform mite)‡ |

beyond the ordinal level. This number was calculated conservatively by excluding all insect orders, life stages, and other arthropod groups that contain both aquatic and terrestrial individuals. As the focus of this study was on the whole-bodied arachnids, which were the only organisms barcoded, no additional discussion of the other taxa is presented.

Of the 143 salmon collected with length and mass data, 126 were less than a year in age ("young-of-year"), 15 were more than one year in age, and two did not have life stage data recorded. Twenty-seven of those specimens had whole-bodied arachnids in their gut contents. Wilcoxon Rank-Sum tests ($\alpha$ of $P < 0.05$) on the salmon length, mass, and age versus the presence of an arachnid displayed no correlation between these factors, with smaller or younger fish being no more likely to have consumed an arachnid than a larger or older fish, as determined by both chi-squared and Fisher's exact tests (preferred for smaller sample sizes) ($P > 0.05$). Coho salmon (*O. kisutch*) ate spiders marginally more than the other salmon species examined ($P = 0.09$ for chi-squared test, $P = 0.07$ for Fisher's exact test), though this was not significant at $\alpha = 0.05$.

**Morphological and molecular identification.**—Identification was largely inconclusive beyond the family level, as several specimens were degraded or immature on consumption and thus were lacking in key anatomical traits used for morphological identification (Figs. 1, 2). Three specimens were too degraded to successfully identify to family using morphology (Fig. 1; see panels 16, 18, and 20). Ten samples were correctly identified to genus using morphology, and only one species, *Gertschanapis shantzi* (Gertsch, 1960), was morphologically identified as the same species from the genetic BLAST result (though the BLAST sequence identity was lower than the percent identity threshold of 98%). Only three samples were definitively adult males with full morphology of the pedipalps preserved (Fig. 1; see panels 1, 4, and 10).

Molecular barcoding was used for more accurate identification and was highly successful for both spider and mite specimens, with all samples except two mites generating a barcode corresponding to a spider or mite, respectively, with DNA concentrations for successfully sequenced extracts ranging from 0.18 to 23.6 ng/μL. Based on BLAST thresholds, the spiders were identified as belonging to five families, ten genera, and six definitive species, whereas the mites were placed into two families, two genera, and one definitive species (Table 1). In terms of ecological guilds for the spider samples, the most frequent guilds found in salmon guts were classified as

other hunters ($n = 10$), followed by sheet web weavers ($n = 6$), and orb web weavers ($n = 5$).

## DISCUSSION

Salmon in freshwater streams have displayed feeding preferences that target terrestrial invertebrates which have entered aquatic ecosystems (Nakano et al. 1999). Our work demonstrates that arachnids are a notable, albeit infrequent (15% of samples), prey item for salmonids. The consumed arachnids were found more so in coho salmon (*O. kisutch*), but this was only a moderate trend ($P < 0.1$, above $\alpha = 0.05$). There appeared to be no distinction between salmon under one year of age and at least one year regarding consumption of arachnids. While there were no trends between these age classes, perhaps investigations across broader age and size classes may provide additional insight. That these factors had no observable influence on preferential consumption of arachnids might suggest that arachnids consumed in aquatic ecosystems are indiscriminately foraged. In addition to ontogeny, there are seasonal factors relating to canopy cover, and stream-specific characters (width, depth, edge vegetation) that may have an impact on the incidence of arachnid ingestion (Cloe & Garman 1996; Nakano et al. 1999). While Jo et al. (2016) detected other terrestrial arthropods in samples of brown trout (*S. trutta*), no arachnids were recorded. However, spiders have previously been recorded from the guts of a variety of fishes in New Zealand (Hicks 1997).

We found that the identification of spiders depended upon molecular data gathered from each specimen, as accurate visual identification was highly inconsistent due to varying levels of specimen maturity and degradation. For most of the spiders, the sexual traits used to key specimens to genus or species were missing, which made genetic barcoding necessary for identification. A secondary morphological confirmation in the context of species barcode identification supported that the barcode in each case was not the result of contamination from other gut contents. One potential caveat to the use of molecular barcodes for species identification is the dependency on the quality and completeness of the reference database against which the barcodes are compared. While family- and genus-level identifications are generally reliable, species-level identifications should be treated with caution, as discussed in Coddington et al. (2016) because not all barcodes available in databases are expertly validated species identifications. Furthermore, some database barcodes are not identified and may be denoted by a "sp." or similar designation. This may be due to uncertainty on the end of the sequence submitter, but the "sp." notation can undermine confidence in a species-level identification. As mentioned before, an additional complexity could be lack of variation in this single barcode locus between closely related species. In cases where there were matches to multiple taxa at greater than the 98% threshold for species identification used in this study, we report both taxa in our table and figures. There are many efforts to develop regional barcode databases for certain taxa and these efforts will make species identifications increasingly reliable (see Blagoev et al. 2016; Astrin et al. 2016; Ashfaq et al. 2019).

An important consequence of this is that many metabarcoding or eDNA methods may result in specimen destruction. This study demonstrates the utility of non-destructive arachnid barcoding from animal gut contents, allowing for specimen vouchering from gut contents, for further identification if a match in sequence databases is not found, which is important, as Coddington et al. (2016) note that only 58% of spiders in BOLD are identified to species. Of the 21 identified spiders belonging to the orb-weaver, sheet web weaver, and other hunter guilds, a total of nine specimens were assigned an aquatic habitat association. The remaining 12 specimens were not considered aquatic habitat-associated according to our criteria. The lack of any trend toward aquatic habitat-associated spider species being found in salmon guts indicates that the species of spider that fall prey to salmon may be further-ranging than anticipated. However, the current understanding of the extent of aquatic habitat association in spider species is extremely limited, underlying the importance of natural history research with arthropods. It is important to note the small spatiotemporal scale of this study, where samples were sourced from a single stream in a single season. It is likely that the inputs of terrestrial organisms, in particular arachnids, varies by season and location and this would be a valuable direction for future research to address. This approach may aid ecologists interested in the identification of specific organisms from fish guts, particularly for studies of contaminants, nutrient cycling, or transitions of these from terrestrial to aquatic ecosystems, where understanding these processes often requires resolution at finer taxonomic levels (Chumchal et al. 2022; Hannappel et al. 2021; Todd et al. 2024).

### Data Availability

Data in this article are available in Supplemental Files 1-6. Sequence data are deposited on GenBank under accession numbers PV655121-PV655151. Accession numbers for each individual are available in Supplemental File 2. Results of BLAST search in a text file are available in Supplemental File 6.

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## SUPPLEMENTAL MATERIALS

Supplemental File 1.— Figures S1–S35, individual images of each arachnid with scale bars (S1-S32) and graphs for comparative analyses (S33-S35). Online at https://doi.org/10.1636/JoA-S-25-003.s1.

Supplemental File 2.—Data for arachnids in this study: sample code, fish species, collection locality, date, BLAST results, aquatic habitat association, and GenBank accession number. Online at https://doi.org/10.1636/JoA-S-25-003.s2.

Supplemental File 3.—Data for fish in this study, online at https://doi.org/10.1636/JoA-S-25-003.s3.

Supplemental File 4.—R script for analyses, online at https://doi.org/10.1636/JoA-S-25-003.s4.

Supplemental File 5.—Data for analyses in Supplemental File 4, online at https://doi.org/10.1636/JoA-S-25-003.s5.

Supplemental File 6.—Archive of BLAST search results (retrieved May 2025), online at https://doi.org/10.1636/JoA-S-25-003.s6.

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