**Table S1.** Summary of number, size ranges and references on relationships between carbon content (C, in µg), prosome or total length (L, in µm), volume (V, in µL), ash-free dry weight (ADW in µg) and dry weight (DW, in µg) for prey found in larval rainbow smelt gut contents.

| Prey item | n | Size range | Measure | Equation | Reference |
|---|---|---|---|---|---|
| **Copepoda** | | | | | |
| **Eggs** | 6950 | 40–122 | V | $4/3\ ((L/1000)/2)^3$ | |
| | | | C | 140V | Kiørboe *et al.*, 1985 |
| **Nauplii** | 18 | 132–362 | DW | $3.009\ (L/1000)^{1.706}$ | Culver *et al.*, 1985 |
| | | | C | $10^{(\log DW - 0.499)/0.991}$ | Wiebe, 1988 |
| ***Eurytemora* spp. (N1–N6)** | 232 | 112–404 | DW | $3.009\ (L/1000)^{1.706}$ | Culver *et al.*, 1985 |
| | | | C | $10^{(\log DW - 0.499)/0.991}$ | Wiebe, 1988 |
| **Copepodites** | 29 | 275–705 | DW | $7.047\ (L/1000)^{2.399}$ | Bottrell *et al.*, 1976 |
| | | | C | 44.7% DW | Mauchline, 1998 |
| ***Eurytemora* spp. (C1–C6)** | 1970 | 289–1028 | DW | $10^{2.088\ (L/1000) - 0.859}$ | Burkill & Kendall, 1982 |
| | | | C | 44.7% DW | Mauchline, 1998 |
| ***Halicyclops* spp. (C1–C6)** | 23 | 318–401 | DW | $7.047\ (L/1000)^{2.399}$ | Copepod conversion; Bottrell *et al.*, 1976 |
| | | | C | 44.7% DW | Mauchline, 1998 |
| **Cyclopoida** | 7 | 123–793 | DW | $7.047\ (L/1000)^{2.399}$ | Copepod conversion; Bottrell *et al.*, 1976 |
| | | | C | 44.7% DW | Mauchline, 1998 |
| ***Acanthocyclops robustus*** | 1 | 792 | DW | $7.047\ (L/1000)^{2.399}$ | Copepod conversion; Bottrell *et al.*, 1976 |
| | | | C | 44.7% DW | Mauchline, 1998 |
| **Diplostraca** | 1 | 180 | C | $10^{4.15 \log L - 11.15}$ | Uye, 1982 |
| ***Bosmina* spp.** | 285 | 105–630 | DW | $(10^{4.849 \log(L/1000) - 3.857} \times 10^5)/1000$ | Rosen, 1981 |
| | | | C | $10^{(\log DW - 0.499)/0.991}$ | Wiebe, 1988 |
| **Mysidacea** | 6 | 476 –3053 | DW | $6.605\ (L/1000)^{2.57}$ | Chigbu & Sibley, 1996 |
| | | | C | $10^{(\log DW - 0.499)/0.991}$ | Wiebe 1988 |
| ***Neomysis americana*** | 19 | 1917–11121 | DW | $6.605\ (L/1000)^{2.57}$ | Chigbu & Sibley, 1996 |
| | | | C | $10^{(\log DW - 0.499)/0.991}$ | Wiebe, 1988 |
| ***Gammarus* spp.** | 15 | 778–2380 | DW | $9.616\ (L/1000)^{2.604}$ | Pöckl, 1992 |
| | | | C | $10^{(\log DW - 0.499)/0.991}$ | Wiebe, 1988 |
| ***Gammarus tigrinus*** | 1 | 1609 | DW | $9.616\ (L/1000)^{2.604}$ | Pöckl, 1992 |
| | | | C | $10^{(\log DW - 0.499)/0.991}$ | Wiebe, 1988 |
| **Ostracoda** | 2 | 512–629 | AFDW | $0.0228\ L^{2.3698}$ | Mumm, 1991 |
| | | | C | $10^{(\log AFDW - 0.410)/0.963}$ | Wiebe, 1988 |
| ***Crangon septemspinosa*** | 1 | 2500 | DW | $10^{0.039(L/100) + 0.51}$ | Wilcox & Jeffries, 1973 |
| | | | C | $10^{(\log DW - 0.499)/0.991}$ | Wiebe, 1988 |
| **Gastropoda** | 1 | 306 | DW | $6.07\ (L \times 10^{-6})^{2.59} \times 10^8$ | Legendre & Michaud, 1998 |
| | | | C | 22.1% DW | Omori, 1969 |
| **Unidentified material** | | | DW | $109.08\ V^{0.9591}$ | Sirois & Dodson, 2000a |
| | | | C | $10^{(\log DW - 0.499)/0.991}$ | Wiebe, 1988 |

**Table S2.** Results of permutational analyses of variance (PERMANOVA) performed on carbon weight data from visual analysis of larval gut contents and data from molecular *Eurytemora* spp. identification in larval gut contents via qPCR. PERMANOVA analyses were based on Bray-Curtis dissimilarities (Bray & Curtis 1957) and were performed using 9999 permutations. The homogeneity of dispersion was verified prior to each PERMANOVA. For visual data, individual larvae were tested using the carbon weight (µgC) of each prey consumed divided by the standard length (mm) of the larva. Preliminary tests revealed no statistical influence of larval lineage in diet differences ($p > 0.05$), so this factor was removed from the analysis and two-way PERMANOVA were employed for diet composition comparison. For molecular data, diet composition in terms of the complex *E. affinis* NAC/*E. carolleeae* based on percentage of qPCR results per station was determined. Stations within salinity zones were considered as replicates.

| **Factor** | **df** | **Visual** | | **Molecular** | |
|---|---|---|---|---|---|
| | | **Pseudo-F** | **p-value** | **Pseudo-F** | **p-value** |
| Salinity habitat | 2 | 4.331 | **0.001** | 6.258 | **0.013** |
| Survey | 3 | 2.163 | **0.003** | 1.411 | 0.261 |
| Salinity habitat×survey | 6 | 3.262 | **0.001** | 0.928 | 0.507 |

**Table S3.** Diet composition expressed as percentage of prey-specific index of relative importance (%PSIRI) for the prey categories identified in rainbow smelt larval diet in each salinity zone sampled throughout summer 2021 in the MTZ of the St. Lawrence Estuary.

| | | | | | **Limnetic** | | | | **Oligohaline** | | | | **Mesohaline** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Class** | **Order** | **Family** | **Item** | **Stage** | **Mid-June** | **Late June** | **Mid-July** | **Early Aug.** | **Mid-June** | **Late June** | **Mid-July** | **Ear. Aug.** | **Mid-June** | **Late June** | **Mid-July** | **Early Aug.** |
| Branchiopoda | Diplostraca | Bosminidae | *Bosmina sp.* | | 5.32 | 4.39 | 5.52 | 2.68 | 8.51 | 0.29 | 0.06 | | | | | |
| | | | Unidentified Cladocera | | | | | | 0.16 | | | | | | | |
| Malacostraca | Amphipoda | Gammaridae | *Gammarus tigrinus.* | | | | | 2.41 | | | | | | | | |
| | | | *Gammarus sp.* | | 4.12 | | 0.16 | 6.61 | | 2.68 | | | | | | |
| | Decapoda | Crangonidae | *Crangon septemspinosa* | Zoea | | | | | | | | | | 2.32 | | |
| | Mysida | Mysidae | *Neomysis americana* | | | | | | | | 5.15 | 33.25 | | 21.50 | | |
| | | | Unidentified Mysida | | | | | | | 6.25 | | 1.13 | | 3.30 | | |
| Maxillopoda (Copepoda) | Calanoida | Temoridae | *Eurytemora sp.* | N1 | | 0.30 | | | 0.23 | | | | | | | |
| | | | | N2 | | 1.28 | | | 0.92 | | | | | | | |
| | | | | N3 | | 0.74 | | | 5.97 | | | | | | 0.35 | |
| | | | | N4 | | 1.60 | | | 6.92 | | | | | | 0.36 | |
| | | | | N5 | | 2.52 | | | 6.34 | | | | | | 2.03 | |
| | | | | N6 | | 1.92 | | | 6.80 | | | | | | 0.43 | |
| | | | | C1 | | 1.09 | | | 2.49 | | | | 3.27 | | 2.84 | |
| | | | | C2 | | 1.97 | | | 0.58 | | | 0.13 | | | 3.95 | 1.82 |
| | | | | C3 | | | | | | | | 0.18 | 4.38 | | 0.60 | 2.77 |
| | | | | C4 | | 0.33 | | 0.07 | | | | 3.85 | 3.54 | 0.96 | 6.82 | 10.40 |
| | | | | C5 | 1.59 | 1.73 | 1.17 | 0.78 | 6.95 | 0.26 | 3.33 | 8.44 | | 20.24 | 13.40 | 41.55 |
| | | | | C6 (fem.) | 27.17 | 13.09 | 26.15 | 38.93 | 21.31 | 16.24 | 21.06 | 7.59 | 12.51 | 11.49 | 17.46 | 5.31 |
| | | | | C6 (male) | 15.27 | 56.91 | 22.33 | 18.96 | 4.89 | 44.19 | 38.25 | 24.66 | 36.40 | 16.09 | 31.55 | 19.15 |
| | | | | Eggs | 23.63 | 8.93 | 32.91 | 28.40 | 7.63 | 19.13 | 32.14 | 18.34 | | 5.95 | 9.02 | 7.92 |
| | Cyclopoida | Cyclopidae | *Acanthocyclops robustus* | C1–C6 | 4.57 | | | | | | | | | | | |
| | | | *Halicyclops sp.* | C1–C6 | | 1.92 | | 0.27 | | | | | | | | |
| | | | | Eggs | | | | 0.73 | | | | | | | | |
| | | | Unidentified Cyclopidae | C1–C6 | | | 0.01 | | 0.16 | | | 0.26 | | | 0.51 | |

**Table S3**. *Continued.*

| | | | | | **Limnetic** | | | | **Oligohaline** | | | | **Mesohaline** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Class** | **Order** | **Family** | **Item** | **Stage** | **Mid-June** | **Late June** | **Mid-July** | **Early Aug.** | **Mid-June** | **Late June** | **Mid-July** | **Early Aug.** | **Mid-June** | **Late June** | **Mid-July** | **Early Aug.** |
| | | | Unidentified Copepoda | C1–C6 | 1.07 | 1.28 | | | 19.75 | | | | 0.84 | | | |
| | | | | N1–N6 | | | | 0.16 | 0.25 | 7.37 | | 0.13 | | 2.62 | 0.84 | 11.08 |
| | | | | Eggs | | | | | 0.13 | | | | 3.84 | | | |
| Ostracoda | | | Unidentified Ostracoda | | | | | | | 0.05 | | | | | 0.76 | |
| Gastropoda | | | Unidentified Gastropoda | | | | | | | | | | | 1.39 | | |
| Unidentified material | | | | | 17.27 | | 11.74 | | | 3.53 | | 2.03 | 35.24 | 14.14 | 9.09 | |

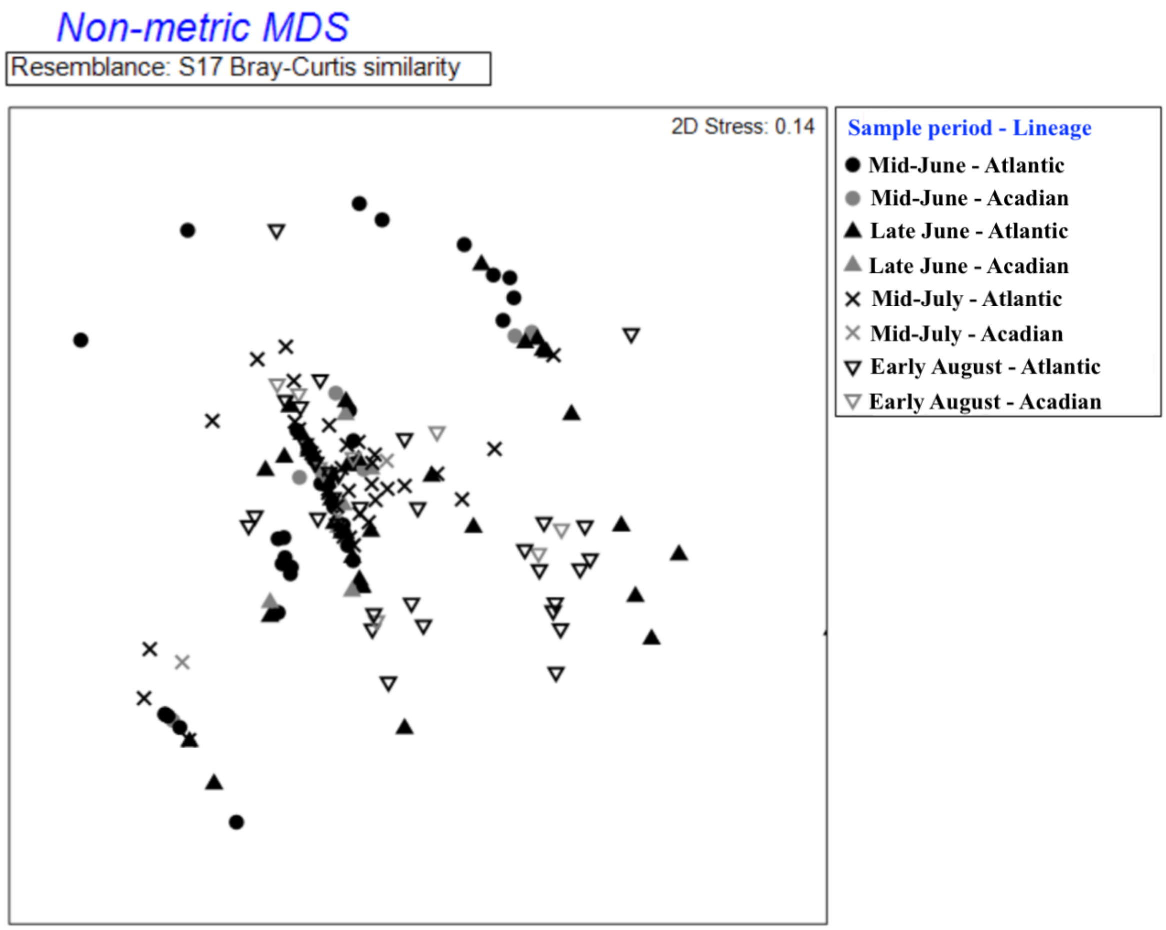


**Figure S1.** Non-metric multidimensional scaling (nMDS) analysis plots of rainbow smelt diet in the four sampling periods separated by lineage (Atlantic or Acadian).

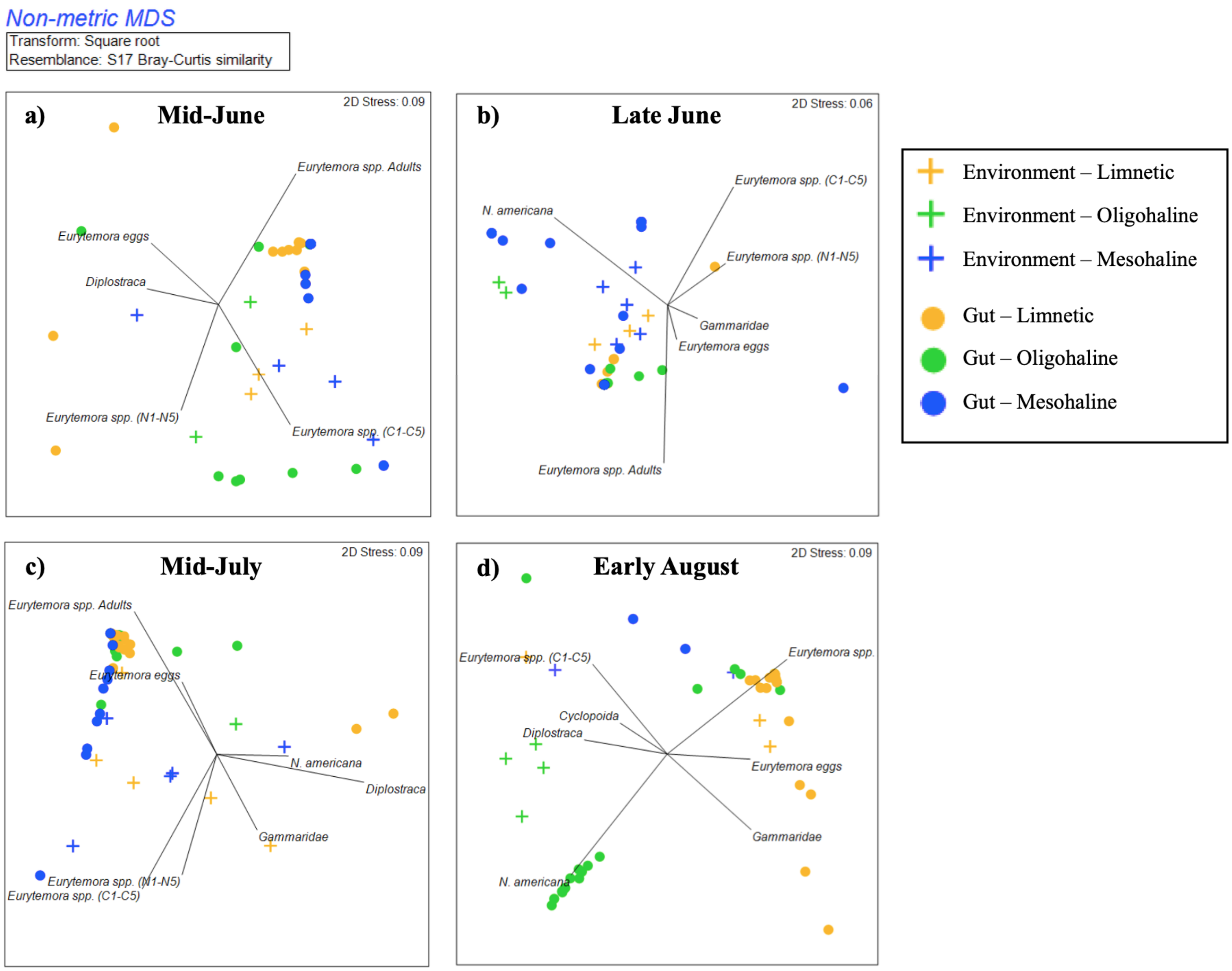


**Figure S2.** Non-metric multidimensional scaling (nMDS) analysis plots of rainbow smelt diet and prey field composition across the salinity habitats of the MTZ (limnetic, oligohaline and mesohaline) for mid-June, late June, mid-July and early August.

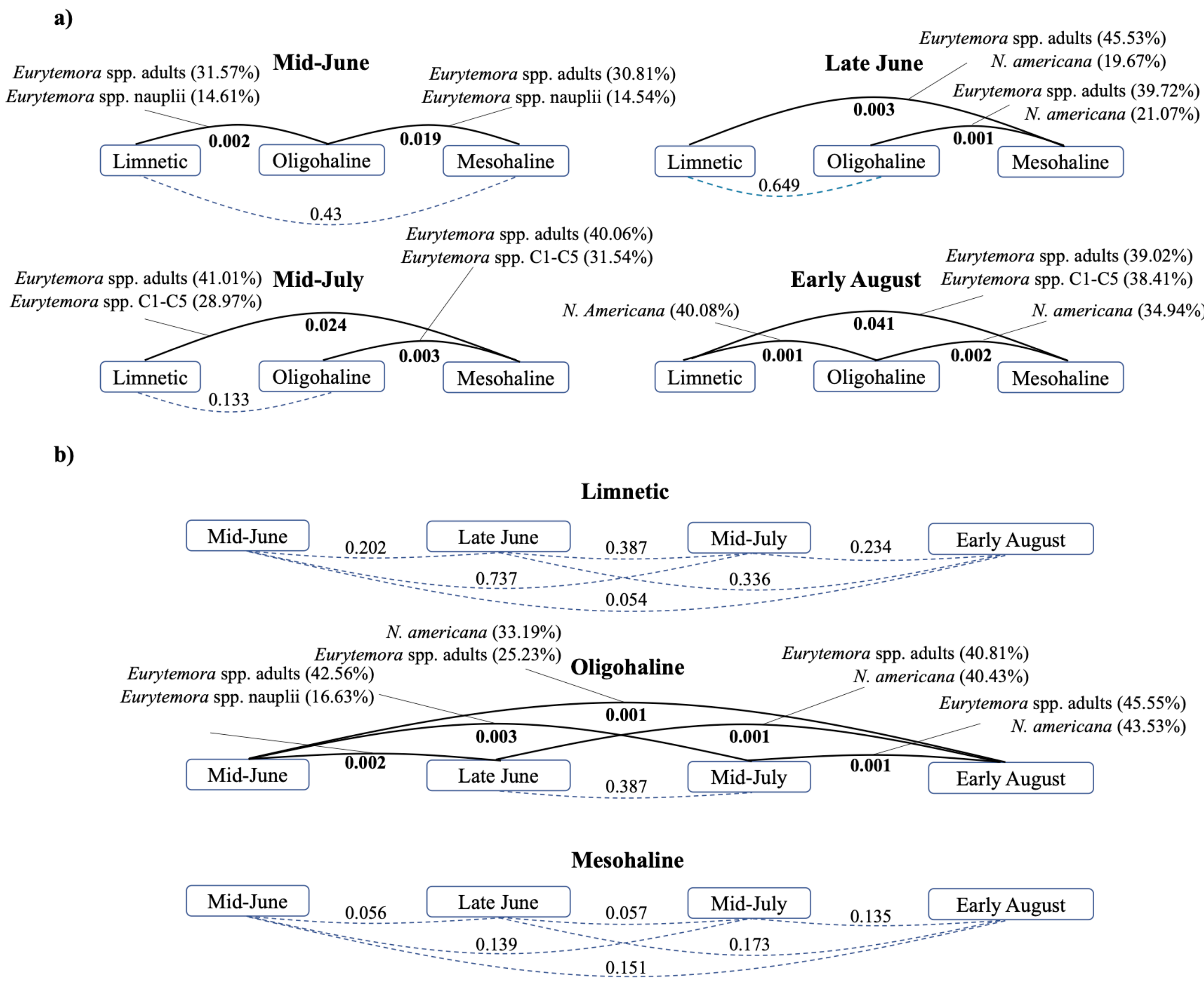


**Figure S3.** Spatial-temporal comparisons of larval diet through salinity habitats and time, based on visual gut contents. Summary of pair-wise comparisons from the permutational analysis of variance (PERMANOVA). Spatial comparisons between salinity habitats in each survey (a) and temporal comparisons of each salinity habitat through the sampling period (b) are shown with significant interactions and p–values in bold. Prey with highest percent contribution to the dissimilarity (SIMPER) are shown for interactions with significant p-values.

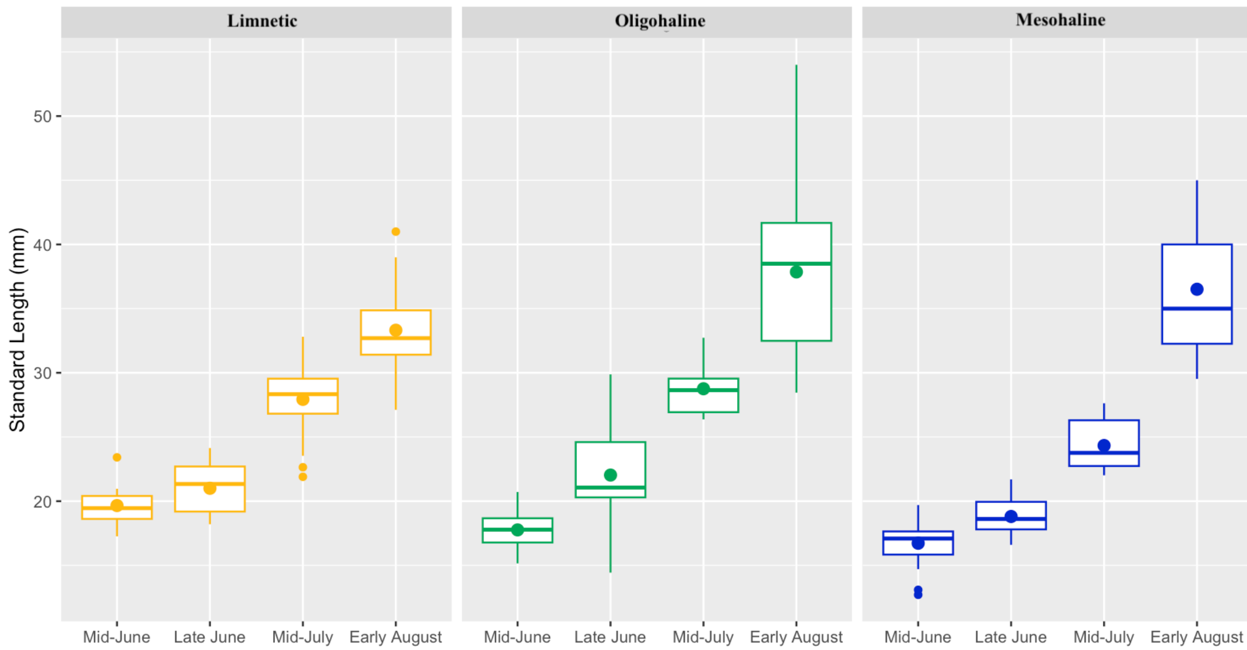


**Figure S4.** Mean standard length (mm) of rainbow smelt larvae by salinity habitat in summer 2021. Boxplots show the median (horizontal line), interquartile range (IQR, box), and whiskers extending to 1.5 times the IQR. Outliers are indicated by small dots beyond the whiskers, while larger filled circles represent the mean values for each group.

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