



Seasonal and longitudinal variability of zooplankton assemblages along a river-dominated estuarine gradient

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ABSTRACT

The Columbia River Estuary (CRE) on the Pacific coast of North America is a highly modified river-dominated estuary. Land use, human development and tributary input vary along the length of the estuary, which may impact the distribution of zooplankton; however the longitudinal and temporal variability of zooplankton have not been evaluated along the tidally-influenced length of the CRE, which extends 234 river kilometers (rkm) inland. To evaluate zooplankton dynamics along the estuarine gradient, zooplankton and environmental data were collected monthly from five sites distributed along 193 rkm (82% of the length) of the estuary over a 2-year period (2016–2018). We found that zooplankton abundance and assemblage structure exhibited strong seasonal dynamics and that abundance was higher in the year with lower river discharge. At the only saline site sampled near the river mouth, assemblages were comprised of greater proportions of estuarine and marine taxa relative to tidal freshwater sites. Zooplankton assemblages at the river mouth were best explained by salinity, discharge and temperature, while assemblages at tidal freshwater sites were best explained by temperature and season. Non-native taxa, notably the calanoid copepod *Pseudodiaptomus forbesi*, comprised over 50% of zooplankton abundance at tidal freshwater sites during late summer - early autumn, but were largely absent at the river mouth. Assemblages were highly similar among tidal freshwater sites despite differences in tributary input, land use and development along the estuarine gradient. Our findings suggest that low residence times in river-dominated estuaries, such as the CRE, may contribute to homogenization of zooplankton assemblages, particularly during the high flow period and within the tidal freshwater reach.

1. Introduction

Estuaries are productive habitats at the interface of riverine and marine systems, and are critical habitat for many resident and migratory species, including invertebrates, fish, birds and marine mammals (Day et al., 2012). Zooplankton are an integral component of estuarine food webs, and serve as a link between phytoplankton and higher trophic levels (Day et al., 2012). Zooplankton abundance and assemblage composition can vary temporally and spatially in estuaries in response to natural variability in environmental conditions or anthropogenic stressors, with implications for food web processes and ecosystem functioning (Winder and Jassby, 2011; Almeida et al., 2012; Hébert et al., 2017).

Zooplankton assemblages in estuaries exhibit spatial and temporal variability in response to dynamic environmental and hydrological conditions that vary according to position within the estuarine gradient

(Miller, 1983; Soetaert and Van Rijswijk, 1993; Roman et al., 2005). In near-coastal estuarine regions, an upstream-downstream salinity gradient exists and its extent is dependent on river discharge and the tidal cycle (Cloern et al., 2017). Zooplankton are distributed along this salinity gradient in accordance with taxon-specific physiological tolerances (Jeffries, 1962; Telesh and Khlebovich, 2010; Bollens et al., 2011). Zooplankton assemblages are further regulated by temperature (Fulton, 1983; Bollens et al., 2011; Dexter et al., 2015) and water residence time, with higher zooplankton abundance and biomass generally associated with longer residence times and lower rates of advective transport (Pace et al., 1992; Bum and Pick, 1996; Doubek et al., 2019; Breckenridge et al., 2020). Zooplankton assemblages are additionally structured spatially and temporally by biotic variables (Fulton, 1983; Jack and Thorp, 2002; Kayfetz and Kimmerer, 2017), with the distribution of zooplankton predators and prey varying temporally and spatially in estuaries in relation to physicochemical conditions (Morgan, 1990;

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Burić et al., 2007).

Estuarine zooplankton are also commonly affected by anthropogenic stressors and human modifications to riverine systems (e.g. Cloern and Jassby, 2012). Urban development and land use can increase nutrient loading, leading to eutrophication and adverse impacts to water quality (Sliva and Williams, 2001; Ahearn et al., 2005; Chen and Lu, 2014; Xia et al., 2016), with implications for zooplankton populations (Capriulo et al., 2002; Albaina et al., 2009; Zervoudaki et al., 2009). Studies evaluating relationships between land use and zooplankton assemblage structure are scarce, and have focused on lentic systems (e.g., Dodson and Lillie, 2001; Dodson et al., 2005) and riverine systems (e.g., Claps et al., 2009; Slugocki et al., 2019), whereas the effects of land use on estuarine zooplankton assemblages are, to our knowledge, unknown.

In large modified river systems, dams and hydropower operations alter flow and thermal regimes (Olden and Naiman, 2010) affecting zooplankton assemblages (Wang et al., 2016), and the warmer, slower waters of reservoirs incubate plankton compatible with these conditions, which are then advected downriver (Sherwood et al., 1990; Havel et al., 2009). Many estuaries of large river systems also have major shipping ports, which have been implicated in the spread of non-native zooplankton species via ballast water releases, resulting in dramatic assemblage shifts with potential food web impacts (Bollens et al., 2002; Winder and Jassby, 2011; Dexter and Bollens, 2020). Specifically, invasive zooplankton may prey upon, displace or compete with native taxa that support higher trophic levels (Telesh and Ojaveer, 2002; Hooff and Bollens, 2004; Chícharo et al., 2009; Bollens et al., 2012). One of the most infamous examples of a zooplankton invader is the ctenophore *Mnemiopsis leidyi*, whose invasion of the Black Sea resulted in dramatic predation-related declines in the abundance and diversity of meso-zooplankton and ichthyoplankton, and is partly credited with subsequent collapses of pelagic fisheries (Shiganova, 1998).

The Columbia River, that runs through the U.S. Pacific Northwest, is a prime example of a large, highly modified river, with 14 hydroelectric dams on its mainstem, extensive diking and dredging, and 5 major deep-water shipping ports within its estuary. Urban development also varies along the length of the Columbia River Estuary (CRE). The CRE extends 234 river kilometers (rkm) inland, of which the upper ~184 rkm is tidally-influenced freshwater (Fig. 1). Past studies of CRE zooplankton have revealed the presence of six non-native zooplankton taxa: three species of calanoid copepods (*Pseudodiaptomus inopinus*,

Pseudodiaptomus forbesi, and *Sinocalanus doerri*) and one cyclopoid copepod (*Limnithona tetraspina*) native to Asia, one cladoceran (*Bosmina coregoni*) of Eurasian origin, and the planktonic veliger larvae of the Asian clam (*Corbicula fluminea*) (Cordell et al., 2008; Bollens et al., 2012; Smits et al., 2013; Breckenridge et al., 2015; Dexter et al., 2015, 2020a, 2020b).

Non-native copepods are presumed to have been introduced to the CRE via ship ballast water releases (Cordell et al., 2008; Dexter et al., 2018a). *P. inopinus* was first documented in the CRE in 1990 and was present at high densities in the estuarine turbidity maxima region (Cordell et al., 1992). *P. forbesi* was first detected in the CRE in 1999, and by 2002 *P. inopinus* was rare and *P. forbesi* was abundant (Sytsma et al., 2004; Cordell et al., 2008). *P. forbesi* has since become the numerically dominant zooplankton taxon from late summer to early autumn throughout the CRE (Cordell et al., 2008; Bollens et al., 2012; Breckenridge et al., 2015; Dexter et al., 2015, 2020b) and has spread beyond the tidally-influenced reach to freshwater reservoirs over 600 rkm upstream (Cordell, 2012; Emerson et al., 2015). *B. coregoni* was first detected in the CRE in 2008 and its invasion mechanism(s) is unknown, but may include spread by migratory birds, recreational boating and direct hydrological connections (Smits et al., 2013). *C. fluminea* has been established in the Columbia River since the 1930's and is most commonly spread by humans via accidental or intentional introductions (Counts, 1986), but its larvae can also be spread passively by water currents (Isom, 1986).

A number of zooplankton sampling programs were carried out in the CRE from 1963 through 2006 (Haertel and Osterberg, 1967; Misitano, 1974; Jones et al., 1990; Morgan et al., 1997; Bollens et al., 2012; Breckenridge et al., 2015; Rollwagen-Bollens et al., 2020) that were primarily focused in the lower estuary, defined here as the region between the river mouth and the upstream extent of saltwater intrusion (~50 rkm). These past studies have identified the calanoid copepod *Eurytemora affinis*, and in recent years, *P. forbesi*, as the most abundant crustacean taxa in the lower estuary (Bollens et al., 2012; Breckenridge et al., 2015). Further upstream, a tidal freshwater site at Vancouver, WA (rkm 171) has been extensively sampled from 2005 to the present and is dominated by native freshwater taxa such as *Bosmina longirostris* (cladoceran), *Diacyclops thomasi* (cyclopoid copepod) and *Asplanchna* sp. (rotifer), with the exception of late summer to early autumn, when the non-native taxa *P. forbesi* and *C. fluminea* veligers have represented over 50% of total zooplankton abundance (Dexter et al., 2015, 2020b). These past studies are informative, but the lower estuary and tidal freshwater reach have not been concurrently sampled across an annual cycle. Some studies have examined zooplankton along the full length of the CRE estuarine gradient (Prahl et al., 1998; Cordell et al., 2008), however none exceeded two sampling events in a year. Sampling the full estuarine gradient across an annual cycle allows for improved understanding of how seasonally variable factors (e.g. freshwater flow, temperature, salinity) affect the spatial heterogeneity of zooplankton abundance and assemblage composition within the CRE.

In this study we investigate the longitudinal and temporal patterns of zooplankton assemblages and underlying environmental and landscape drivers along nearly 200 rkm of the upstream-downstream gradient of the CRE. We address two primary research objectives: (1) Evaluate seasonal and interannual variability of abundance and distribution of zooplankton taxa along the length of the estuary, here defined as the zone of tidal influence (sampled from rkm 4–197); and (2) Establish relationships between environmental variables (e.g. water temperature, salinity, river discharge, watershed land cover and human development) and zooplankton assemblages. This study is the first in this system to examine zooplankton in high (sub-seasonal) temporal resolution along the full length of the estuarine gradient. Furthermore, no previous study in the CRE has attempted to link zooplankton to local land use and development.

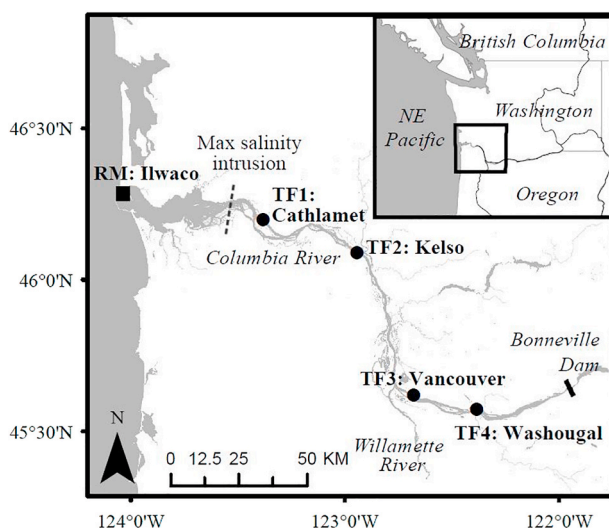


Fig. 1. Map of the Columbia River Estuary with locations of sampling sites. The river mouth site (RM) at Ilwaco is indicated by the filled black square and tidal freshwater sites (TF) are indicated by filled black circles. The dotted line represents the approximate maximum extent of salinity intrusion. Tidal influence extends to the base of Bonneville Dam.

2. Materials and methods

2.1. Study system

The Columbia River is the largest river by discharge on the Pacific coast of North America, with an average outflow of $\sim 7000 \text{ m}^3 \text{ s}^{-1}$ at its mouth near Astoria, Oregon (Sherwood et al., 1990). It flows 1930 rkm from southeastern British Columbia, Canada, to the Pacific Ocean and drains an area of 671,000 km^2 (Ebel et al., 1989). Flow rates typically range from 2000–16,000 $\text{m}^3 \text{ s}^{-1}$, with high flows from April–June driven by snowmelt, and low flows from July–October during the dry season (Chawla et al., 2008; Jay et al., 2015). Flows from November–March are variable and driven by coastal sub-basin precipitation, and enter the CRE through its major tributaries: the Willamette, Cowlitz and Lewis rivers (Simenstad et al., 1990).

The CRE extends 234 rkm inland from the Pacific Ocean to Bonneville Dam, the lowermost impoundment on the mainstem Columbia River. The CRE is an upwelling margin mesotidal estuary and is considered river-dominated due to its strong freshwater flows (Evans et al., 2013). Salinity intrusion extends 18–50 rkm upstream from the mouth of the estuary, dependent on tides and river flow conditions (Giese and Jay, 1989; Jay et al., 2015), while the remainder of the upstream estuary is tidally-influenced freshwater. Water residence time for the full tidally-influenced length of the CRE is approximately 3–23 days, estimated as the estuary volume divided by the inflow rate ($\text{m}^3 \text{ d}^{-1}$), and using a constant volume of 4.0 km^3 (surface area of 550 km^2 and mean depth of 7.3 m) (Hickey and Banas, 2003), and discharge of 2000–16,000 $\text{m}^3 \text{ s}^{-1}$. Residence time is highest during the low-flow period and lowest during the high-flow period. Based on circulation modeling, residence time for the lower estuary region ranges from 20 to 70 h for channels, but can exceed 120 h in lateral bays (Kärnä and Baptista, 2016). Land cover and development vary greatly along the longitudinal gradient of the estuary. Farthest downstream in the lower estuary, sites are predominately rural, while the region's major urban centers (Portland, OR and Vancouver, WA), are located near the confluence of the Willamette River with the Columbia River at rkm 162.

2.2. Sampling sites

We sampled five sites at public docks spanning 193 rkm of the upstream-downstream gradient of the CRE for zooplankton and environmental data during 2016–2018 (Fig. 1). Sites included Ilwaco, WA (rkm 4), Cathlamet, WA (rkm 64), Kelso, WA (rkm 108), Vancouver, WA (rkm 171) and Washougal, WA (rkm 197). The Ilwaco site, near the river mouth, is just inside Baker Bay and within the estuarine mixing zone, while the other four sites are above the extent of salinity intrusion and within the tidal fluvial zone (Simenstad et al., 1990). In January 2017, the Washougal dock suffered major damage in a winter storm and the site was permanently moved 2 rkm downstream to a comparable dock (rkm 195). Site geographic locations and characteristics are summarized

in Table 1.

2.3. Zooplankton sampling and data collection

Zooplankton samples and environmental data were collected as part of the Columbia River Estuary Science Education and Outreach (CRE-SCENDO) project, a two-year research collaboration that partnered Washington State University (WSU) Vancouver researchers with science teachers and students from five southwest Washington high schools (Rollwagen-Bollens et al., 2019). Field sampling was conducted by teachers and students during the academic year, and by WSU Vancouver researchers during academic breaks. Schools used identical sampling equipment and protocols, and teachers attended training workshops and supervised all student participants.

Samples were collected monthly at each site from October 2016–September 2018, with the exceptions that Kelso was not sampled during Year 1, and Ilwaco was not sampled in October 2016 nor in December 2017. This period encompassed two complete water years (Year 1: October 2016–September 2017; Year 2: October 2017–September 2018). At each site, three replicate zooplankton samples were collected during daylight hours by vertical hauls from 1 m above the bottom to the surface, using a 73- μm mesh, 0.5-m diameter ring net with attached flowmeter (Sea-Gear Corp.). Zooplankton samples were preserved in 70% ethanol and transferred to WSU Vancouver for detailed taxonomic analysis by trained staff members of the WSU Vancouver Aquatic Ecology Laboratory. High school students did not conduct zooplankton identifications. Water column total depth and water transparency (Secchi depth) were recorded, and temperature and salinity profiles were taken at 1 m intervals to a depth of 4 m using a YSI Pro 30 multisonde (YSI Inc.).

External data sources provided supplementary information on river discharge and land cover. Mean monthly river discharge was calculated by averaging daily data from the National Water Information System (NWIS) (www.waterdata.usgs.gov/nwis). Discharge at Vancouver (Site 14144700, rkm 171) was used for sites above the Willamette confluence (Vancouver and Washougal), and Beaver Army Terminal (Site 14246900, rkm 87) was used for sites below the confluence (Kelso, Cathlamet and Ilwaco). Land cover data from the National Land Cover Database 2011 were acquired from the Multi-Resolution Land Characteristics Consortium (MRLC) (www.mrlc.gov/data), and watershed boundaries from the USGS Watershed Boundary Dataset (WBD) (www.usgs.gov/core-science-systems/ngp/national-hydrography). Percent cover for 16 land cover classes were calculated in ArcMap (v. 10.5.1) for each watershed (WBD HUC10 scale) that contained a sampling site (Fig. A1). Percent cover was calculated by dividing the area classified to each land use type by the total area of the watershed.

2.4. Laboratory methods

Two replicate zooplankton samples were processed for each date and

Table 1

Site and sampling information. The Washougal site was moved in January 2017 after the first dock was damaged. Values for site depth, temperature and salinity are mean (range). Temperature and salinity are the average of the top 4 m of the water column.

Site Name	Ilwaco	Cathlamet	Kelso	Vancouver	Washougal-1	Washougal-2
Sampling Months	November 2016–September 2018 ^a	October 2016–September 2018	October 2017–September 2018	October 2016–September 2018	October 2016–December 2016	January 2017–September 2018
Latitude (°N)	46.285	46.201	46.094	45.622	45.575	45.577
Longitude (°W)	124.051	123.387	122.943	122.678	122.355	122.379
River KM (rkm)	4	64	108	171	197	195
Estuarine Zone	River mouth	Tidal freshwater	Tidal freshwater	Tidal freshwater	Tidal freshwater	Tidal freshwater
Depth (m)	3.6 (2–4.85)	5.6 (3.5–7.6)	9.9 (8.5–11.5)	10.4 (8–15.5)	4.3 (3–6)	6.1 (4–12)
Temperature (°C)	12.3 (4.3–19.7)	12.7 (2.8–22.4)	12.7 (6.3–22.4)	12.6 (2.4–22.5)	11.1 (5.0–15.1)	12.7 (3.8–23.4)
Salinity	7.4 (0.7–19.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

^a Not sampled October 2016 or December 2017.

site, except Cathlamet in February 2018 and Washougal in May 2018, for which only one replicate was available. Homogenized samples were subsampled using a Hensen-Stempel pipette and a minimum of 300 non-naupliar zooplankton ($>200\ \mu\text{m}$) were identified, staged and enumerated. Specimens were identified to the lowest practical taxonomic level using a stereomicroscope (Olympus SZ60) and taxonomic references (Cordell, 2012; Gardner and Szabo, 1982; Haney et al., 2013; Thorp and Covich, 2010). Cladocerans and copepods were identified to the genus or species level, with the exception that harpacticoid copepods were identified to order. Rotifers were identified to the family or genus level.

2.5. Statistical analyses

Zooplankton sample total volume was calculated by multiplying the flowmeter distance (m) by the area of the net mouth. To verify the data quality of student-collected zooplankton samples, we compared mean sample volumes from monthly student sampling at Vancouver to volumes of samples collected by WSU Vancouver Aquatic Ecology Lab researchers, who sampled zooplankton within a week's time of student sampling at the same dock in Vancouver throughout the duration of this study. We compared sample volumes for all months that students conducted zooplankton field sampling ($n = 18$ of 24 monthly collections). Student-collected sample volumes did not significantly differ from those collected by researchers (Paired t -test: $t = 1.14$, $df = 17$, $p = 0.17$).

Counts of zooplankton taxa were converted to abundance (individuals m^{-3}) using the subsample ratio and the total volume of the sample. Nauplii were excluded from all statistical analyses, and for abundance and assemblage analyses, copepodites I–V were aggregated but kept distinct from adults to reflect differences in their ecology. Taxa richness and the Shannon-Wiener diversity index (H) were calculated for each sample. For calculating the diversity indices only: organisms were aggregated to the lowest consistent taxonomic level (cladocerans and copepods to the genus level, rotifers to family), juvenile stages were combined with adults, and juveniles that could not be identified to the appropriate specificity were excluded.

Seasons were defined by dividing water years into equal intervals of 3 months (Autumn: October–December, Winter: January–March, Spring: April–June, Summer: July–September). Salinity and temperature were averaged over the 4-m depth profile, and for Ilwaco, temperature and salinity stratification were calculated as the difference between bottom and surface readings.

2.5.1. Variability of zooplankton abundance and diversity, and environmental conditions

Interannual, seasonal and spatial variability of zooplankton abundance (log transformed to achieve normality), alpha diversity (taxa richness and Shannon-Wiener diversity) and environmental conditions were assessed graphically and using three-way ANOVA (water year \times season \times site) (Zar, 2010). Only data from sites sampled during both years were included in the three-way ANOVAs, while subsequent two-way ANOVAs were run for Year 2 (season \times site) to include Kelso. When data did not meet the assumptions of normality of residuals or homogeneity of variance, non-parametric Aligned Rank Transform ANOVA (Wobbrock et al., 2011) or Welch's ANOVA (Zar, 2010) were used respectively. Aligned rank ANOVA was implemented in R using the *ARTool* package, and was used instead of the more commonly applied Kruskal-Wallis ANOVA as it allows for testing of factorial designs (Wobbrock, 2019).

2.5.2. Variability of zooplankton assemblage structure

Zooplankton assemblage structure and associations with explanatory variables were assessed using multivariate statistical techniques. For all multivariate analyses, rare taxa (present in $<5\%$ of samples at each site) were removed or aggregated into higher taxonomic groupings. Replicates were averaged to create one monthly sample per site. Zooplankton taxa abundance data were log ($x+1$) transformed to down-weight

dominant taxa, and Bray-Curtis dissimilarity was used to quantify differences between samples.

We tested for significant differences in zooplankton assemblage structure between water years, seasons and sites using permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001). We first tested a three-way model of year, season and site using only sites sampled both years. We then tested for multivariate homogeneity of group dispersions (a comparison between groups of the spread (variance) of the data points in multivariate space, which if violated can confound results), using the 'betadisper' function in R. Significantly different dispersions were found by season and site, so we subsequently ran season-specific PERMANOVAs, which had homogenous group dispersions. PERMANOVAs were also run on Year 2 independently in order to include Kelso. When effects were significant, we ran pairwise post hoc tests using the function 'pairwise.perm.manova' in the package *RVAideMemoire* (Herve, 2019). For the Year 2 season post hoc tests, we used Monte Carlo p -values instead of permutation based estimates due to small sample sizes (Anderson et al., 2008).

We next grouped samples based on similarity of assemblage composition using hierarchical agglomerative clustering analysis. We used the flexible beta linkage ($\beta = -0.25$) (McCune and Grace, 2002) and conducted the clustering analysis using the R package *cluster* (Maechler et al., 2018). The final number of clusters was selected to balance cluster stability (indicated by long stems on the dendrogram) and preservation of ecologically relevant groups. Indicator species analysis (ISA) (Dufrene and Legendre, 1997) was then used to identify taxa characteristic of each cluster. For each cluster, ISA produces an indicator value index for each taxon that takes into account the taxon's abundance within the cluster compared to its abundance across clusters (specificity), as well as the proportion of samples within the cluster that contain the taxon (fidelity). High indicator values occur when the taxon has both high specificity and high fidelity to the target cluster. We tested the statistical significance of indicator values with 1000 Monte Carlo randomizations using the R package *labdsv* (Roberts, 2016).

2.5.3. Associations with explanatory variables and visualization of assemblages

The full explanatory dataset included spatial (site, rkm, estuarine zone), temporal (water year, season, day of year), environmental (salinity, temperature, Secchi depth, river discharge, temperature stratification, salinity stratification) and watershed land cover (percent cover for 16 land cover classes) variables. However, not all variables were available for all sites, therefore three distinct explanatory data sets were created: one consistent across all sites, one for tidal freshwater sites and one for Ilwaco. To reduce data dimensionality and redundancy, pairwise correlations between numeric explanatory variables were examined for each data set. Variables were standardized, correlations calculated, and when pairs were highly correlated (Pearson's $r > 0.7$), only one variable was retained. In subsequent analyses, each retained variable served as a proxy for all of its strongly correlated variables. All strongly correlated variables retained and removed are listed in Table A1.

Non-metric multidimensional scaling (NMDS) ordination (Kruskal, 1964) was used to visualize differences in zooplankton assemblages and relationships with explanatory variables (Clarke, 1993). NMDS projects multidimensional space into reduced dimensions, where samples that are closer to one another in space are more similar in composition. We assessed NMDS goodness of fit using traditional stress values, where values less than 0.2 are generally considered useable for inference (Clarke, 1993), and we also compared observed values to the distribution of stress values produced by ecological null model simulations, following methods in Dexter et al. (2018b). Biological gradients, including log (abundance), taxa richness and Shannon-Wiener diversity were plotted as vectors on the NMDS to further show assemblage structure. The BIOENV non-parametric correlation procedure was used to identify the best subset of explanatory variables that have maximum

Spearman rank correlation with zooplankton assemblage dissimilarities (Clarke and Ainsworth, 1993). For this analysis, we used the Gower distance metric for our explanatory variables as it can handle both numeric and factor variables (Gower, 1971). Variables from the best-fit model were then plotted as vectors on the NMDS. All NMDS, ecological null model simulations, BIOENV, PERMANOVA, and 'betadisper' models were run using 1000 permutations and implemented in the R *vegan* package (Oksanen et al., 2019). All statistical analyses were run in R version 3.5.0 (R Core Team, 2018).

3. Results

3.1. Environmental conditions and landscape characteristics

Water temperatures in the CRE ranged from 2.4 to 23.4 °C over the study period and followed the expected seasonal pattern, with temperatures highest in summer, lowest in winter, and moderate during spring and autumn (Fig. 2A). This pattern was similar in both years and for all sites, except that peak summer temperature (August) was consistently

lower at Ilwaco. Across the four sites sampled in both years, three-way aligned rank ANOVA found no significant differences in temperature by year ($F_{1,62} = 0.01$, $p = 0.91$), or site ($F_{3,62} = 0.07$, $p = 0.97$), but did find a strongly significant seasonal effect ($F_{3,62} = 107.36$, $p < 0.001$). In Year 2, which included Kelso, there was also no effect of site on temperature ($F_{4,39} = 0.13$, $p = 0.97$).

River discharge ranged from 2591–14,946 m³ s⁻¹ and was highest during the spring freshet (March–June) and lowest from July–October (Fig. 2B). Three-way parametric ANOVA revealed significantly different discharge by year ($F_{1,32} = 5.75$, $p = 0.02$), season ($F_{3,32} = 40.43$, $p < 0.001$) and station ($F_{1,32} = 12.35$, $p = 0.001$). Flow was higher in Year 1 than Year 2, particularly during early spring (mean \pm SE; Year 1: 7735 \pm 783, Year 2: 6512 \pm 598), and the downstream station (Beaver Army Terminal) had greater average discharge than upstream (Vancouver) (downstream: 8019 \pm 774, upstream: 6227 \pm 579).

Saline water was only detected at Ilwaco and salinity was strongly negatively correlated with discharge (Pearson $r = -0.79$). Highest salinities were measured from July–November, and lowest from February–June (Fig. 2C). There was no difference in salinity between years

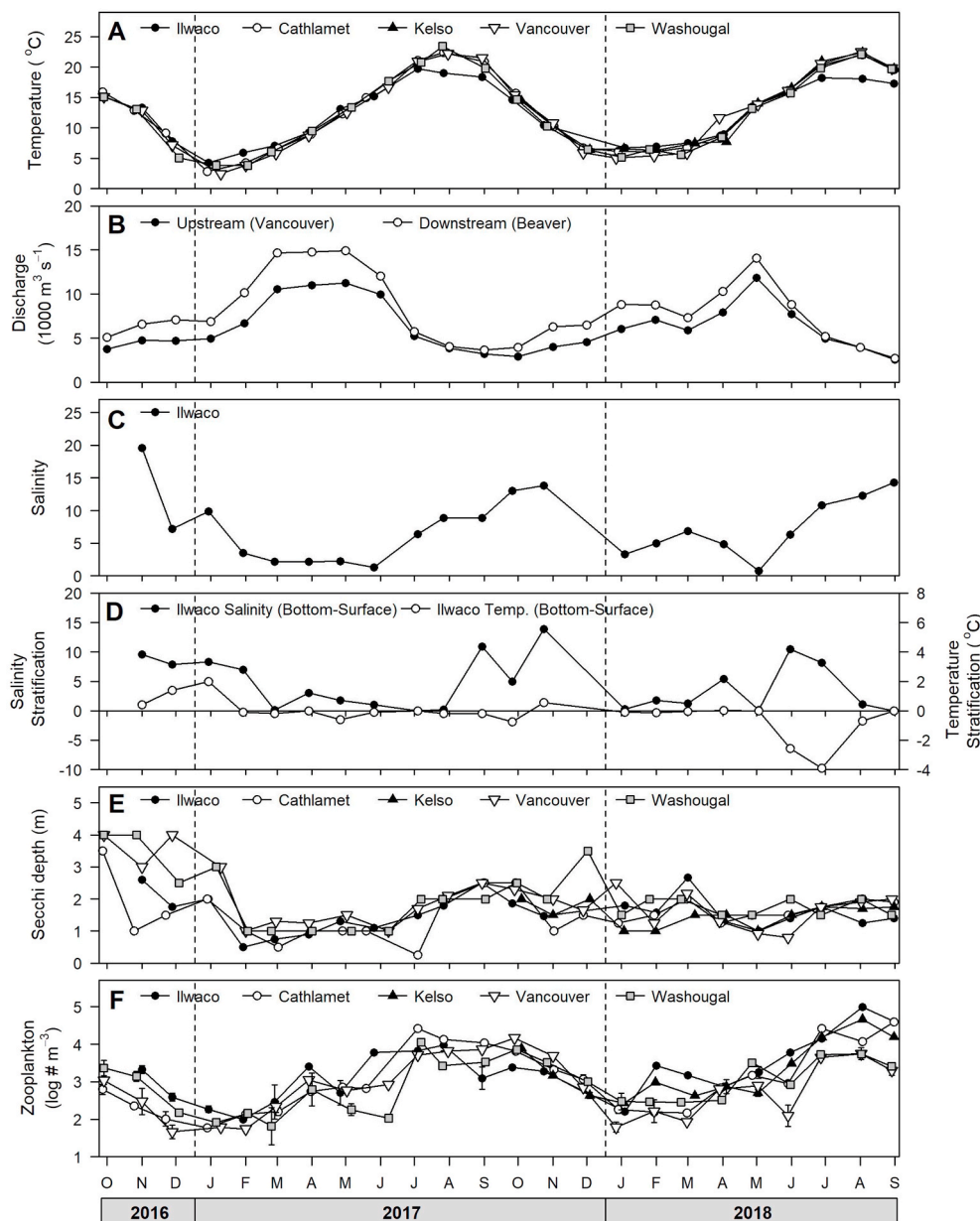


Fig. 2. (A) Monthly mean river temperature, (B) river discharge, (C) salinity, (D) salinity and temperature stratification, (E) Secchi depth, and (F) mean (\pm SE) zooplankton abundance plotted on a log 10 scale for sites sampled Oct. 2016–Sep. 2018. Temperature and salinity were taken as the average of the top 4 m of the water column. Salinity was only detected at Ilwaco. Monthly average river discharge is displayed for USGS gauges upstream at Vancouver, WA (used for Vancouver and Washougal sites) and downstream at Beaver Army Terminal near Quincy, OR (used for Kelso, Cathlamet and Ilwaco sites). Temperature and salinity stratification are only displayed for Ilwaco and were calculated as the bottom measurement minus the surface measurement. Vertical dashed lines separate calendar years.

(one-way ANOVA $F_{1,20} = 0.65$, $p = 0.43$) but salinity did differ seasonally (Welch's ANOVA $F_{3,8.6} = 10.07$, $p = 0.004$). Salinity stratification at Ilwaco tended to be highest during summer and autumn, and lowest in spring (Fig. 2D), but did not differ significantly by season ($F_{3,14} = 1.98$, $p = 0.16$) or year ($F_{1,14} = 0.02$, $p = 0.90$). Temperature stratification at Ilwaco was very weak, except during summer 2018, when surface waters were up to 4 °C warmer than bottom waters, and during December 2016–January 2017 when surface waters were 2 °C colder than bottom waters (Fig. 2D). There was no observable temperature stratification at any of the four upstream sites.

Water transparency in Year 1 was greatest from October–January and lowest from February–July, however this pattern was not consistent in Year 2 (Fig. 2E). In Year 2 Secchi depths in autumn were greatly reduced with a less pronounced seasonal pattern (Fig. 2E). Finally, the primary differences in land cover among sites included increased development in the watershed at Vancouver, a higher proportion of mixed forest at Kelso, and higher proportions of woody wetlands and grasslands at Ilwaco (Fig. A1).

3.2. Zooplankton total abundance and dominant taxa

A total of 64,171 non-naupliar zooplankton specimens were identified and enumerated from 210 samples, representing 78 taxa (Table A2).

Zooplankton average monthly abundance was highly variable, ranging from 21 to 98,175 individuals m^{-3} (low: Vancouver, December 2016, high: Ilwaco, August 2018). Peak densities were observed from July–October, with a lower magnitude peak also seen in spring (Fig. 2F). Lowest densities occurred from December–February. Zooplankton total abundance (log-transformed) for sites sampled both years was significantly greater in Year 2 than Year 1 (three-way ANOVA $F_{1,62} = 19.10$, $p < 0.001$). In Year 1, abundance differed by season ($F_{3,31} = 42.05$, $p < 0.001$), with seasons ranked summer > spring = autumn > winter (Tukey's HSD $p < 0.01$ for all pairwise comparisons except spring - autumn: $p = 0.56$). In Year 2, abundance also differed by season ($F_{3,39} = 47.14$, $p < 0.001$), but abundance was greater in autumn than spring (ranks: summer > autumn > spring > winter; Tukey's HSD $p < 0.05$ for all pairwise comparisons). Abundance did not vary by site in Year 1, but it did in Year 2 ($F_{4,39} = 4.06$, $p = 0.008$), with abundance at Vancouver significantly less than at Ilwaco (Tukey's HSD $p = 0.004$).

Zooplankton taxa composition varied seasonally at all sites (Fig. 3). At tidal freshwater sites, spring assemblages were dominated by rotifers and native cyclopoid copepods. During summer and autumn, *Bosmina* spp. (cladocerans) and *P. forbesi* (non-native calanoid copepod) were numerically dominant. In winter, assemblages were comprised of rotifers, native cyclopoids, calanoid copepods and a diverse set of cladocerans. Ilwaco's zooplankton assemblage was predominately comprised

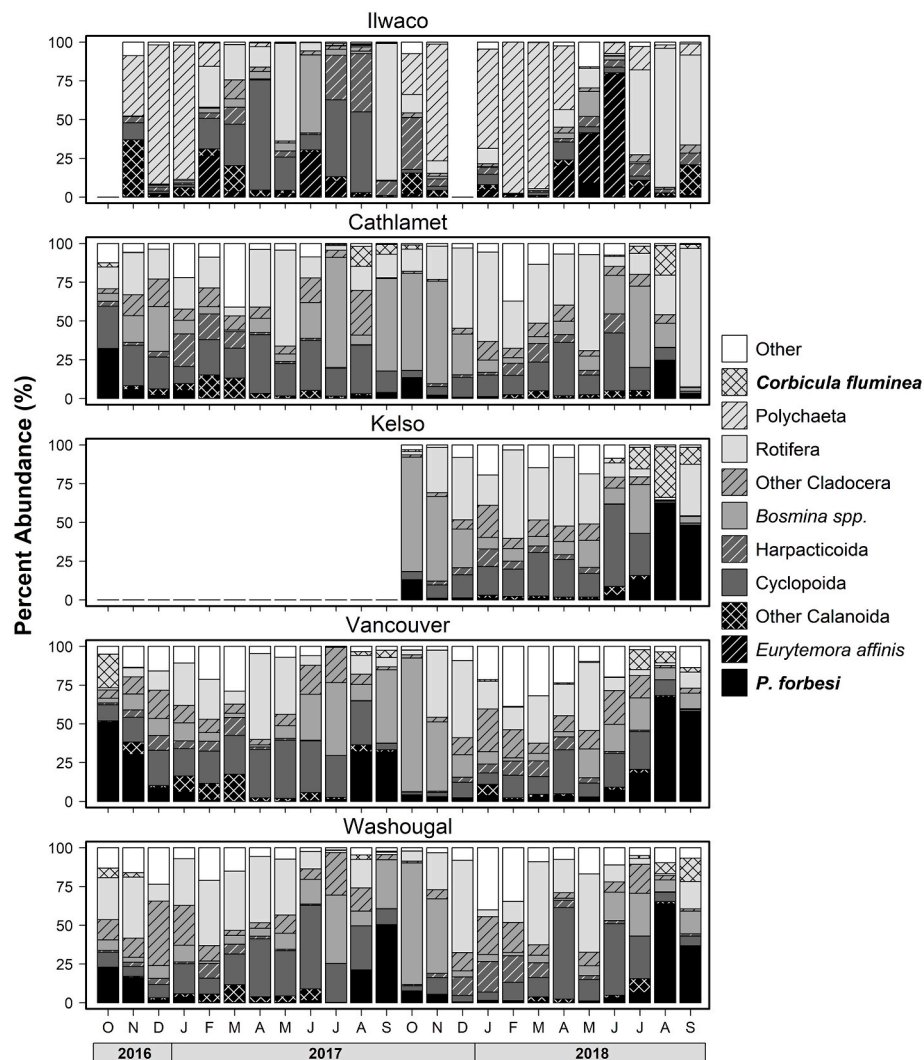


Fig. 3. Percent abundance of dominant zooplankton taxa sampled October 2016–September 2018 at Ilwaco (marine-influenced) and freshwater sites (Cathlamet, Kelso, Vancouver, Washougal). Less abundant taxa have been aggregated into coarser taxonomic groupings, and juvenile and adult copepods have been combined. Non-native taxa are in bold. Blanks indicate sampling was not conducted.

of estuarine and marine taxa. From late autumn to winter, polychaete larvae dominated Ilwaco's assemblage, while *Eurytemora affinis*, a native calanoid copepod, comprised a large proportion of the assemblage from February–July, particularly in Year 2. In Year 1, when there was higher spring discharge, the Ilwaco spring assemblage more closely resembled that of the freshwater sites. During summer, Ilwaco was characterized by large proportions of cyclopoids, harpacticoids, *E. affinis*, and particularly in late summer, *Synchaeta* sp. (marine rotifer) (Fig. 3).

3.3. Zooplankton taxa diversity

For sites sampled both years, taxa richness did not vary by year ($F_{1,62} = 0.041$, $p = 0.84$) or season ($F_{1,3} = 1.66$, $p = 0.18$), but did differ among sites ($F_{3,62} = 27.02$, $p < 0.001$) (Fig. 4A). Ilwaco had lower richness than all freshwater sites (Tukey's HSD $p < 0.001$ for all pairwise comparisons). Variation among freshwater sites sampled both years was not significant, but in Year 2, Cathlamet had lower richness than Kelso (Tukey's HSD $p = 0.01$). Shannon-Wiener diversity (H) showed more intra-annual variability than richness, but generally was lowest during late summer/early autumn and highest in winter and spring (Fig. 4B). Diversity (H) varied by season ($F_{3,62} = 8.54$, $p < 0.001$) and site ($F_{3,62} = 15.97$, $p < 0.001$). Similar to richness, Ilwaco had significantly lower diversity (H) than freshwater sites (Tukey's HSD $p < 0.001$ all pairwise comparisons) and there were no differences among freshwater sites. In Year 2, Kelso had similar diversity (H) to the other freshwater sites. In both years Ilwaco had a two-month period of very low diversity (H) during winter.

3.4. Zooplankton assemblage structure and associations with explanatory variables

For sites sampled both years, PERMANOVA found a weak year effect ($R^2 = 0.029$, $F_{1,62} = 5.96$, $p = 0.001$), and stronger season ($R^2 = 0.24$, $F_{3,62} = 16.15$, $p = 0.001$) and site effects ($R^2 = 0.23$, $F_{3,62} = 15.67$, $p = 0.001$) on the zooplankton assemblage, as well as weak year \times season and season \times site interaction effects. Multivariate dispersions (spread of the data points) were significantly different by season and site, indicating that groups may vary at least in part due to differences in dispersion. Within PERMANOVAs run on each season separately, there was always a weak year effect ($R^2 = 0.07$ – 0.11 , $p < 0.05$), and stronger site effect ($R^2 = 0.33$ – 0.45 , $p = 0.001$). PERMANOVAs for Year 2 resulted in similar seasonal effects to those seen across both years ($R^2 = 0.26$, $F_{3,39} = 12.94$, $p = 0.001$), but stronger site effects within seasons

($R^2 = 0.55$ – 0.72 , $p < 0.01$). Across both sampling years, Ilwaco's assemblage differed from all other sites in each season but showed the greatest similarity to freshwater sites in spring (Table 2). Similarities among freshwater sites sampled both years were also generally greatest in spring, with significant differences for freshwater site pairs only seen during autumn and winter (Table 2). Results for PERMANOVA, dispersion tests and post hoc comparisons are summarized in Tables A3, A4 and A5, respectively.

Cluster analysis identified seven distinct zooplankton assemblages across all sampling locations in the CRE. The first break on the dendrogram separated all Ilwaco samples from freshwater samples. Samples were then further divided into four clusters for freshwater sites (F-1, F-2, F-3, F-4) and three clusters at Ilwaco (I-1, I-2, I-3), with breaks corresponding loosely with season (Fig. 5). At freshwater sites, F-1 was characterized by low abundance and high diversity and was predominantly seen during winter (Table 3). This cluster was comprised of

Table 2

Seasonal average Bray-Curtis similarity index (similarity = $1 - \text{Bray-Curtis dissimilarity}$) of zooplankton assemblages for pairs of sampling sites. Values are shown for sites sampled in both years. Similarity index values range from 0 to 1, with 0 indicating no overlap and 1 perfect assemblage overlap. Statistically different pairs of sites ($p < 0.05$) are in bold and specific p-values are reported in Table A5.

	Ilwaco	Cathlamet	Vancouver
Spring			
Cathlamet	0.54		
Vancouver	0.49	0.68	
Washougal	0.49	0.66	0.62
Summer			
Cathlamet	0.27		
Vancouver	0.25	0.55	
Washougal	0.26	0.56	0.62
Autumn			
Cathlamet	0.25		
Vancouver	0.25	0.54	
Washougal	0.24	0.52	0.58
Winter			
Cathlamet	0.35		
Vancouver	0.33	0.59	
Washougal	0.32	0.60	0.62

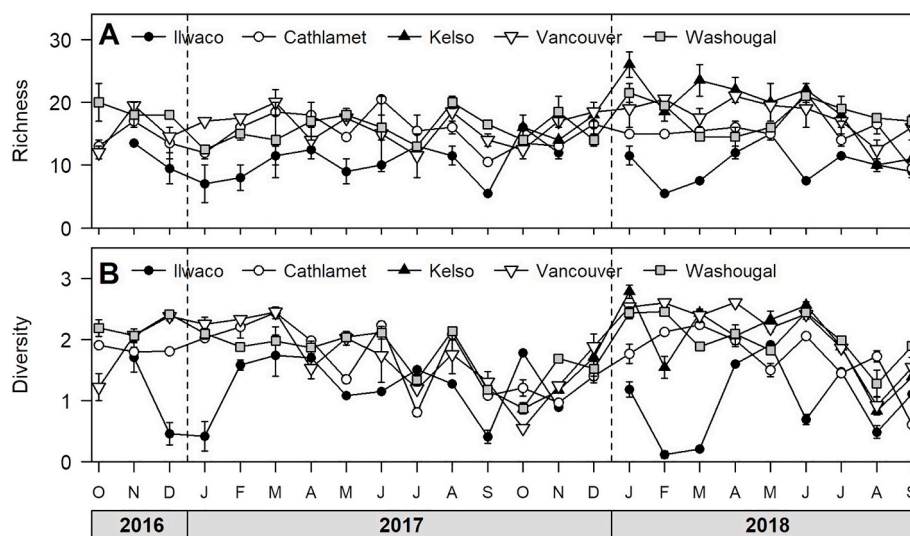
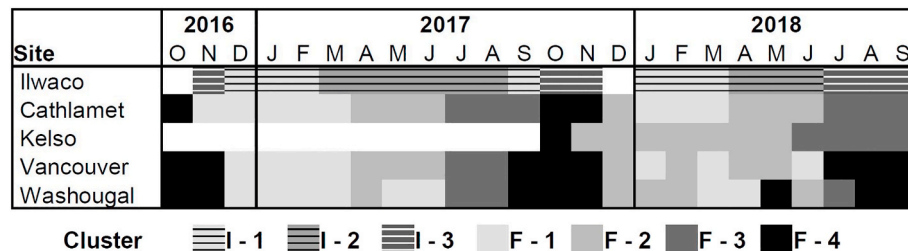


Fig. 4. (A) Taxa richness and (B) Shannon-Wiener diversity index for zooplankton sampled October 2016–September 2018. Vertical dashed lines separate calendar years.

Table 3Mean (SE) of zooplankton abundance, taxa richness, taxa diversity (*H*), temperature, salinity and discharge for zooplankton assemblage clusters.

Cluster	Zooplankton abundance (#m ⁻³)	Taxa richness	Taxa diversity	Temp. (°C)	Salinity	Discharge (m ³ s ⁻¹)
F-1: Freshwater Winter	144 (15.5)	19.5 (0.5)	2.25 (0.05)	7.2 (0.9)	0 (0)	7775 (523)
F-2: Freshwater Winter/Spring	748 (66.5)	22.2 (0.6)	2.05 (0.09)	9.9 (0.7)	0 (0)	9363 (598)
F-3: Freshwater Summer	15,932 (3370)	18.9 (1.0)	1.56 (0.15)	20.9 (0.4)	0 (0)	4617 (383)
F-4: Freshwater Autumn/Invaded	4370 (747)	19.0 (0.6)	1.47 (0.11)	16.1 (0.9)	0 (0)	4323 (447)
I-1: Ilwaco Winter	942 (375)	10.4 (1.0)	0.66 (0.22)	8.2 (1.8)	6.4 (1.0)	7531 (787)
I-2: Ilwaco Spring/Summer	3920 (1145)	14.6 (0.8)	1.48 (0.15)	13.6 (1.5)	3.9 (0.9)	11,055 (1368)
I-3: Ilwaco Summer/Autumn	26,536 (15,567)	14.3 (1.3)	1.27 (0.21)	15.3 (1.3)	14.0 (1.2)	4783 (613)

**Fig. 5.** Spatial and temporal occurrence of zooplankton assemblage clusters. Pattern fill indicates clusters seen at Ilwaco (I), solid fill indicates clusters seen at tidal freshwater sites upriver (F). White cells indicate that the site was not sampled in that month.

cyclopoids, *Asplanchna* sp. and cladocerans (Fig. 6), and had one significant indicator taxon, *Collembola* (springtails) (Table A6). F-2 had similar composition to F-1 but was characterized by higher abundance and indicated by three cladoceran taxa. This cluster was seen during spring of Year 1, but its occurrence was extended in Year 2 (November–June). Cluster F-3 occurred during summer and was characterized by high abundance. It was comprised of large proportions of *Bosmina* spp., *P. forbesi* I–V, cyclopoids and Brachionidae, and had 18 significant indicator taxa, of which veligers of *C. fluminea* (non-native Asian clam) were strongest. F-4 was predominately seen from August–November and had the highest proportions of non-native *P. forbesi*. Both juvenile and adult *P. forbesi* were notable indicator taxa of F-4.

Cluster I-1 at Ilwaco was mostly a winter cluster (Fig. 5) and was characterized by low abundance, low diversity, and high proportions of polychaete larvae and *Synchaeta* sp. (Table 3, Fig. 6). Polychaete larvae (pre-chaetiger stage) were the only significant indicator of I-1 (Table A6). Cluster I-2 occurred during spring and summer at Ilwaco when salinity was low and discharge high, and was comprised of *E. affinis*, freshwater cyclopoids and *Bosmina* spp. I-2 had 3 indicator taxa, notably adult and juvenile *E. affinis*. The final cluster at Ilwaco, I-3, occurred from July–November, when temperature and salinity were both highest, and had the highest overall abundance and contained the most marine and coastal upwelling associated taxa. Cluster I-3 was dominated by *Synchaeta* sp. and polychaete larvae, and had 18 significant indicators, the strongest of which were *Acartia* spp., *Podon* sp. and Appendicularia.

NMDS ordination of all zooplankton samples (all sites, sampled in any year) produced a two-dimensional solution with an observed stress value (stress = 0.139) that was significantly lower than the distribution of values produced by null model simulations ($Z = -40.22$, $p < 0.001$) (Fig. 7A). BIOENV analysis identified temperature and estuarine zone (i. e. river mouth or tidal freshwater) (Spearman $r = 0.71$) as the best subset of explanatory variables that explained the variability of zooplankton assemblages (Table A7). The NMDS showed distinct separation in ordination space of samples by estuarine zone, however there was little variation evident among freshwater sites. Cluster I-2 was located closest in ordination space to the freshwater clusters.

The NMDS ordination of the tidal freshwater data set also produced a two-dimensional solution (stress = 0.127, $Z = -42.10$, $p < 0.001$) (Fig. 7B). For freshwater sites, BIOENV identified temperature and season as the best subset of explanatory variables (Spearman $r = 0.598$)

(Table A7). Temperature explained much of the variability among freshwater zooplankton clusters, however no spatial or landscape variables were significantly correlated with zooplankton assemblages (Table A8). NMDS of the Ilwaco samples produced a two-dimensional solution (stress = 0.118, $Z = -10.19$, $p < 0.001$) (Fig. 7C), and BIOENV selected temperature and salinity as the best subset of explanatory variables (Spearman $r = 0.677$) (Table A7). Because salinity was highly negatively correlated with discharge ($r = -0.79$) and we are unable to separate the effects of these variables, we consider both salinity and discharge, in addition to temperature, as important for structuring zooplankton assemblages at Ilwaco.

3.5. Non-native zooplankton distribution and dynamics

Non-native taxa comprised the greatest percentage of the zooplankton assemblage from summer to early autumn, frequently accounting for over 50% and up to 95% of abundance during this period, while absent or at very low abundance the remainder of the year (Fig. 8A). Ilwaco had the lowest proportion of non-native taxa, followed by Cathlamet. We encountered five non-native zooplankton species in the CRE: *P. forbesi*, *C. fluminea*, *B. coregoni*, *Limnithona tetraspina* and *Sinocalanus doerri*. *P. forbesi* and *C. fluminea* veligers comprised the majority of non-native abundance and were many orders of magnitude more abundant than the other non-native species (Fig. 8). Peak abundance of *P. forbesi* adults, *P. forbesi* I–V, and *C. fluminea* all occurred at Kelso in August 2018 (Fig. 8B–D). *B. coregoni* was encountered at all sites and was often present year-round at low abundance, with peaks seen in spring and summer at Cathlamet and Vancouver (Fig. 8E). *L. tetraspina* was infrequently detected, and only seen at Cathlamet, Kelso and Vancouver (Fig. 8F–G). Only three *S. doerri* specimens were encountered, two from a Cathlamet sample collected in December 2016, and one from a June 2017 Ilwaco sample.

4. Discussion

4.1. Longitudinal zooplankton variability

Zooplankton assemblages in the CRE during our two-year study period, October 2016–September 2018, were distinctly different at tidal freshwater sites (Cathlamet, Kelso, Vancouver, Washougal) than at our estuarine mixing zone site near the mouth of the river (Ilwaco). There

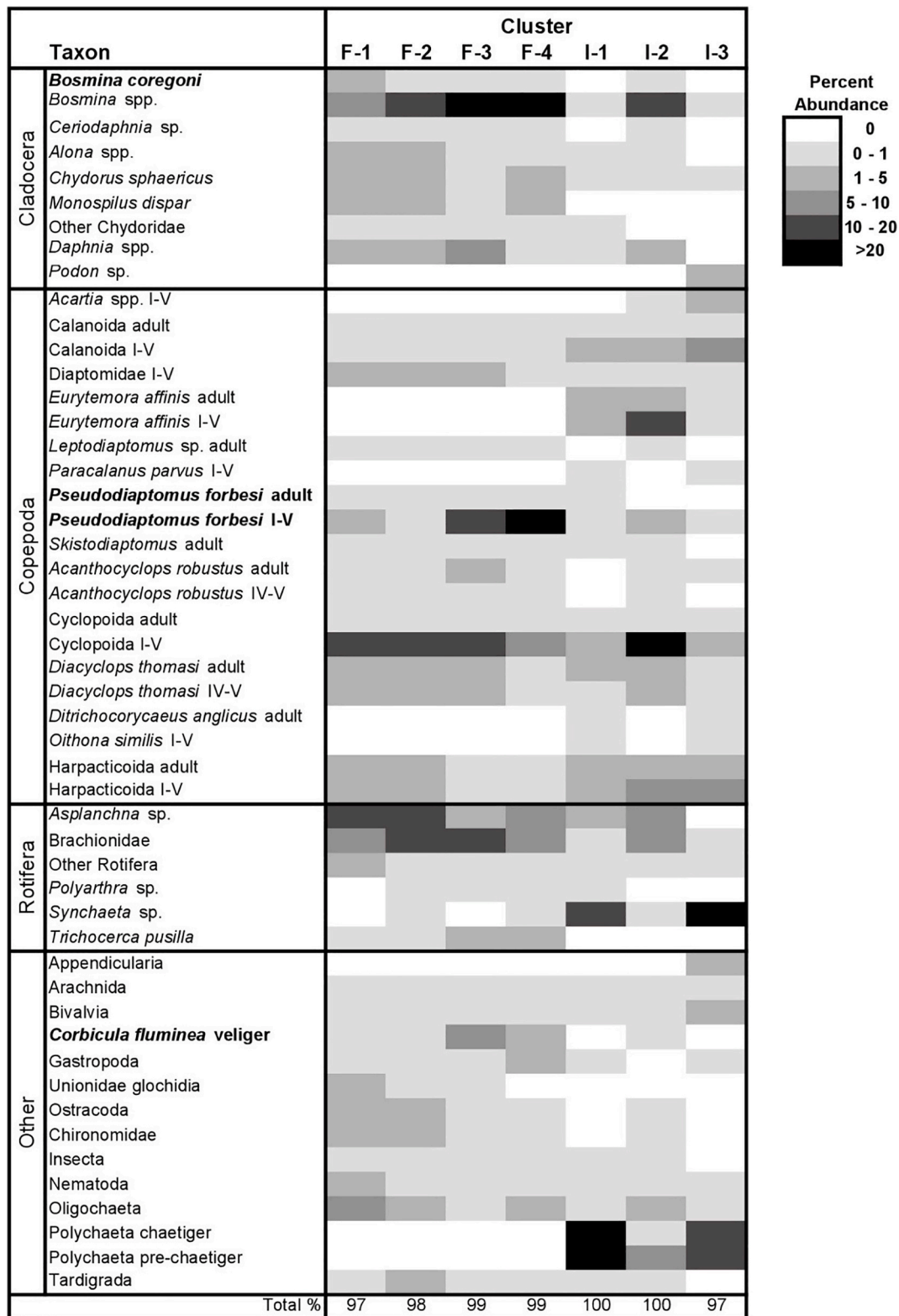


Fig. 6. Heatmap of percent abundance by cluster for the top 50 zooplankton taxa. Clusters F-1 through F-4 are freshwater clusters seen at Cathlamet, Kelso, Vancouver and Washougal sites, while clusters I-1, I-2 and I-3 were seen at Ilwaco. “Total %” indicates the cumulative percentage of the top 50 taxa. Non-native taxa are listed in bold.

were higher proportions of estuarine and marine taxa at Ilwaco than at our tidal freshwater sites, likely related to Ilwaco being a saline site in close proximity to the coastal ocean. We also saw lower taxa richness and diversity (H) at Ilwaco relative to our freshwater sites, however

taxonomic resolution was not uniform and may have confounded our results. Specifically, Ilwaco had higher proportions of polychaete larvae and harpacticoid copepods, taxa which were not identified to the genus or species level.

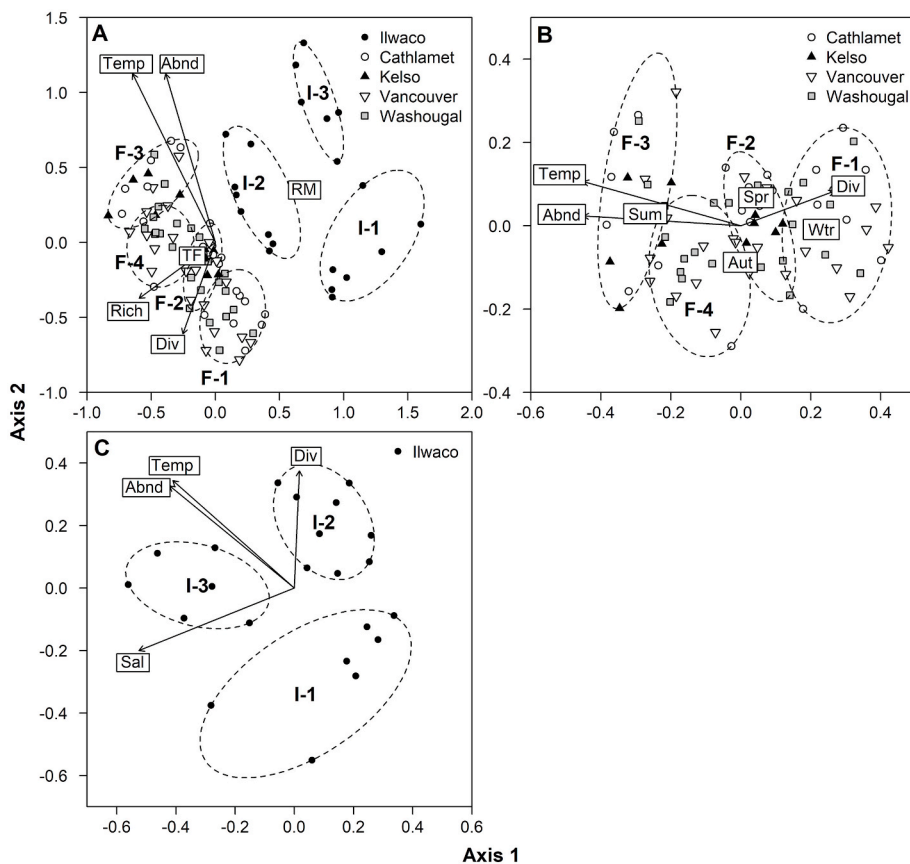


Fig. 7. NMDS ordinations of zooplankton samples. Cluster membership is shown via dashed ellipses. The best subset of explanatory variables (BIOENV) are plotted as vectors for numeric variables and centroids for factor variables. Vector length corresponds to the magnitude of the Spearman correlation, and the vector points in the direction of maximum correlation. Significant ($p < 0.05$) correlations are shown for biological variables. (A) NMDS ordination for all sites, best subset included estuarine zone ($r^2 = 0.405$) and temperature ($r^2 = 0.678$), (B) NMDS ordination for freshwater sites, best subset included temperature ($r^2 = 0.730$) and season ($r^2 = 0.582$), (C) NMDS ordination for Ilwaco samples, best subset included temperature ($r^2 = 0.699$) and salinity ($r^2 = 0.769$). Acronyms: Temp = Temperature, Sal = Salinity, RM = River Mouth, TF = Tidal Freshwater, Abnd = Log (abundance), Rich = Richness, Div = Diversity (H'), Spr = Spring, Sum = Summer, Aut = Autumn, Wtr = Winter.

Jones et al. (1990) also detected assemblage differences between regions of the CRE classified in their study as Tidal-Fluvial (rkm ~29–37), Estuarine Mixing (rkm ~16–29) and Plume and Ocean (rkm < 16). They attributed assemblage differences to variation in salinity, circulation and sedimentation processes within these regions (Jones et al., 1990). Li et al. (2006) likewise found spatially heterogeneous zooplankton assemblages in the Pearl River Estuary, China, related to position relative to the salinity gradient. We do not have adequate sampling sites to speak to differences in assemblage along the full salinity gradient, however prior studies in the CRE (Haertel and Osterberg, 1967; Jones et al., 1990; Morgan et al., 1997; Breckenridge et al., 2015) and other estuaries (e.g., Li et al., 2006; Marques et al., 2006; Bollens et al., 2011) have clearly demonstrated that zooplankton assemblages vary spatially along the salinity gradient in response to tidal exchanges, physiological tolerances of taxa, behavioral adaptations and biotic factors (e.g., zooplankton prey and predators). The biodiversity of other aquatic organisms (microplankton, macrozoobenthos and macrophytes) similarly vary along the salinity gradient, and locations of maximum richness differ for organisms of different size, mobility and life histories due to differences in their abilities to capitalize on unstable environments along the salinity gradient (Telesh et al., 2013, 2015).

Spatial variability of zooplankton assemblage structure has also been associated with proximity to the river mouth or coastal ocean in California estuaries (Mission Bay: Elliott and Kaufmann, 2007; San Francisco Estuary: Bollens et al., 2011; Bollens et al., 2014) and zooplankton abundance, biomass and diversity have been associated with river flow and tidal circulation patterns in the Mondego estuary, Portugal (Marques et al., 2007).

While differences between estuarine zones (i.e. river mouth and tidal freshwater) were prominent in our study, we observed only intermittent and weak differences in zooplankton assemblage structure and no differences in abundance among our four tidal freshwater sites. We also

saw no significant correlations between either rkm or watershed land cover (WBD HUC10 scale) and zooplankton assemblage structure for these sites. We had expected to encounter longitudinal variability because our tidal freshwater sites spanned over 130 rkm (rkm 64–197) and tributary input, human development and land use vary considerably along this stretch of the estuary. Our findings are notable because these factors have previously been associated with zooplankton spatial variability in riverine and lake systems (as described below) but have rarely been assessed in tidal freshwaters.

Specifically, in contrast to our findings, Dickerson et al. (2010) observed longitudinal variability of zooplankton assemblages in the Missouri River, USA, with distance to the nearest upstream reservoir explaining the largest amount of the variation in zooplankton community structure. Longitudinal variability has additionally been seen in lowland rivers of Belgium, where zooplankton abundance has been observed to increase downstream (Viroux, 2002), and where assemblages were found to differ above and below tributaries (Viroux, 1999). Similarly, in the Ohio River, USA, abundances for most zooplankton taxa are greater below than above tributaries (Thorpe et al., 1994). The CRE's largest tributary, the Willamette River, enters the CRE just downstream of our Vancouver site, yet there were no marked differences in zooplankton densities between sites upstream and downstream of the confluence. It is possible that either the mass differential between the Columbia River and the Willamette is so great as to make the Willamette's contribution negligible, or the effects of tributary input may only be detectable over a much smaller spatial extent than our study was able to capture.

One of the ways development can affect plankton populations is through excess nutrient loadings associated with municipal and industrial discharges. For example, in the Long Island Sound Estuary, USA, zooplankton and phytoplankton biomasses vary along a west-east eutrophication gradient (Capriulo et al., 2002). While development in

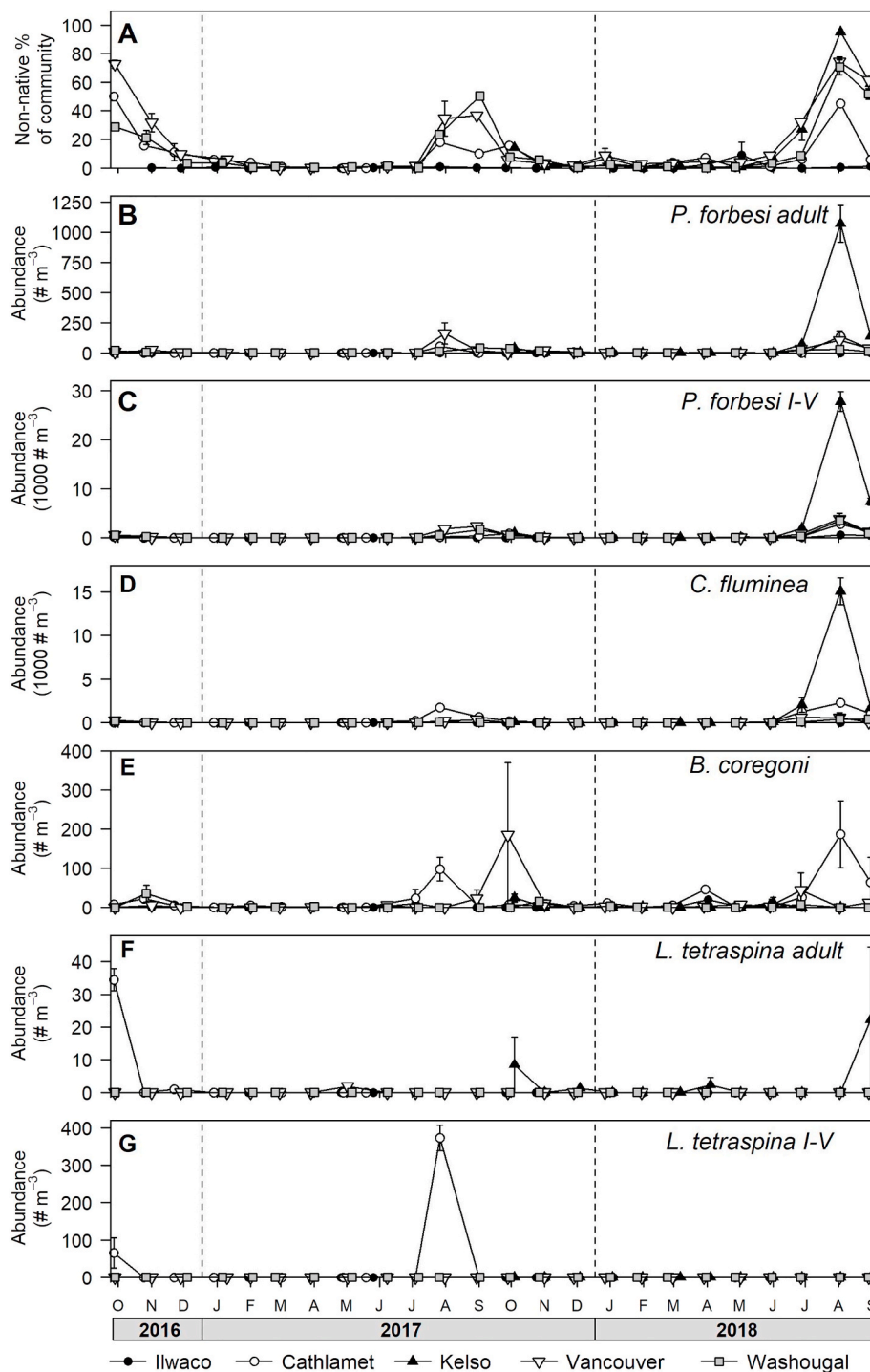


Fig. 8. Spatial and temporal patterns of non-native zooplankton in the Columbia River Estuary. (A) Non-native taxa as a percentage of the total non-naupliar zooplankton assemblage, (B) abundance of *P. forbesi* adult copepods, (C) *P. forbesi* I-V copepodites, (D) *C. fluminea* veligers, (E) *B. coregoni*, (F) *L. tetraspina* adult copepods, and (G) *L. tetraspina* I-V copepodites. Note that panels C and D are on a scale of 1000's of individuals m⁻³. Error bars are one standard error. Vertical dashed lines mark breaks between years.

the CRE is concentrated around Portland, OR (Portland Metropolitan area 2019 population: ~2.5 million; [US Census Bureau, 2020](#)), and the Willamette River, which drains the Portland area, has elevated nutrient levels relative to the mainstem Columbia ([Prah et al., 1998](#)), we saw little evidence that development impacts zooplankton assemblages in the CRE. [Lawrence et al. \(2004\)](#) observed that calanoid copepods in the Waquoit Bay, MA estuarine system were uncoupled from land-derived nitrogen loading, and suggested the lack of a relationship may be due to the short residence time of the system (1.5–2.3 days) relative to copepod generation times. It is possible that the short residence time of the CRE similarly limits the effects of nutrient loadings associated with human development and other land use.

In contrast, [Dodson et al. \(2005\)](#) found that small lakes in Wisconsin, USA that were surrounded by agricultural or urban commercial land use had decreased zooplankton richness in comparison to reference prairie lakes. Zooplankton richness has similarly been observed to be lower in depressional wetlands surrounded by agriculture ([Dodson and Lillie, 2001](#)). These studies demonstrate that land use affects zooplankton in some lentic systems, but our results suggest it may not structure riverine or estuarine zooplankton as strongly, presumably because zooplankton in these environments are not occupying fixed locations on the landscape. Perhaps using a larger spatial scale for riverine systems would yield different results.

[Pace et al. \(1992\)](#) examined spatial differences in zooplankton along

160 rkm of the freshwater reach of the Hudson River Estuary (HRE), and in contrast to our findings observed heterogeneous zooplankton assemblages. However, the HRE has a much higher average residence time (126 days) than the CRE (~3–23 days, see Methods for calculation), which may partially account for these different findings. Higher residence times would decrease the flushing rate and potentially increase the amount of time plankton are exposed to local conditions. It is logical to presume that if plankton experience local environmental conditions for extended periods (multiple generations), greater spatial heterogeneity may develop.

Low residence times in river-dominated estuaries, such as the CRE, may also have a homogenizing effect on zooplankton assemblages by increasing advection and dispersion of organisms. We observed greater similarity of CRE zooplankton assemblages among sites during the spring high-flow period, when water residence times were at their lowest. High flows have similarly been observed to decrease beta-diversity of zooplankton, phytoplankton, fish and macrophytes through increased connectivity, reduced habitat heterogeneity and increased exchanges of organisms between water bodies in Neotropical and north temperate river-floodplain systems (Thomaz et al., 2007; Bozelli et al., 2015) and among connected ponds and lakes in Brazil (Lopes et al., 2014).

Our dock-based sampling program was designed to assess zooplankton assemblage variability along the longitudinal gradient of the CRE; however, cross-channel or other spatial variability may exist that was not captured by our sampling design. There are no published studies that examine cross-channel zooplankton assemblage variability for the CRE, but in the lower estuary, zooplankton assemblage composition is similar between a dock station at Astoria (Bollens et al., 2012; Rollwagen-Bollens et al., 2020) and mid-channel shipboard samples (Breckenridge et al., 2015), which suggests that for the CRE, dock sampling is generally indicative of the channel assemblage. This may not be the case for all systems. For example, zooplankton distribution has been found to vary laterally across the St. Lawrence (Casper and Thorp, 2007) and Ohio Rivers, USA (Thorp et al., 1994). Our dock sampling is also likely less representative of zooplankton assemblages found in alternative habitats (e.g. tidal flat, slope, channel bottom, slough), evidenced by prior work in the lower CRE (Jones et al., 1990) and Fraser River Estuary (FRE), Canada (Breckenridge et al., 2020) that saw variability of assemblage composition and abundance between channels and alternative habitats.

4.2. Temporal zooplankton variability

Zooplankton in the CRE exhibited strong seasonal variability of abundance, assemblage structure and taxa diversity (H'), and weak interannual variability of abundance and assemblage structure. Strong seasonal dynamics have previously been observed in the lower CRE (Haertel and Osterberg, 1967; Jones et al., 1990; Bollens et al., 2012; Breckenridge et al., 2015; Rollwagen-Bollens et al., 2020), and at a single site in the tidal freshwater reach (Dexter et al., 2015, 2020b), as well as in many other temperate latitude estuaries (Ambler et al., 1985; Gewant and Bollens, 2005; Graham and Bollens, 2010; Dexter et al., 2020a). Our observations of lower zooplankton abundance in Year 1 relative to Year 2 and weak differences in zooplankton assemblage structure between years may have been related to freshwater flow, which was significantly higher in Year 1. During spring high flows at Ilwaco in Year 1, salinity was lower and freshwater taxa comprised a higher proportion of its assemblage than in Year 2. Interannual zooplankton assemblage variability has been similarly linked to freshwater flow and its effect on salinity in the San Francisco Estuary (SFE) (Bollens et al., 2014).

The zooplankton taxa and abundances that we observed at our tidal freshwater sites were consistent with observations in 2005–2013 from Vancouver, WA made by Dexter et al. (2015) and in run-of-river reservoirs upstream in 2009–2011 (Emerson et al., 2015). Small bodied

organisms, predominately rotifers and small cyclopoids, were important components of the assemblage during the spring high flow period. This finding agrees with traditional understanding that the short life cycles and high reproductive rates characteristic of small bodied organisms ("r-selectors") enable these taxa to respond more rapidly and better capitalize on seasonally dynamic environments than larger bodied organisms ("k-selectors"), which profit from environments that are comparatively more stable (MacArthur, 1972). In the tidal freshwater reach of the CRE, the period with greatest stability is likely the low flow period in late summer - autumn, when larger bodied copepods were prevalent.

Peak abundance of zooplankton at our freshwater sites occurred during summer, coinciding with highest temperatures, as was the case further upstream on the Columbia River (Emerson et al., 2015; Dexter et al., 2020b). The majority of the peak was comprised of non-native taxa (*P. forbesi* and *C. fluminea* veligers), species whose abundances have been found to be strongly positively correlated with temperature (Dexter et al., 2015, 2020b; Hassett et al., 2017). It is probable that the abundance peak observed in summer was driven by improved conditions for these taxa; however, the peak was also associated with low discharge and consequently higher residence times, which may have allowed for increased build-up of standing crop through reduced advection. Interestingly, the calanoid copepod *E. affinis* was not detected at any of our freshwater sites, despite being present in the lower estuary and having invaded freshwater systems elsewhere in the U.S. (Lee, 1999; Beaver et al., 2019).

At the mouth of the Columbia River in Ilwaco, the zooplankton assemblage alternated between a mix of predominately estuarine and freshwater taxa (spring and early summer), marine taxa (summer and autumn) and estuarine taxa (winter). Taxa of the marine group have previously been associated with coastal upwelling, which brings cold-water taxa of northern origin to the Washington and Oregon coasts (Peterson and Miller, 1977; Keister and Peterson, 2003). Upwelling-favorable winds typically occur in this region during summer and result in intrusion of high-salinity water and marine-derived chlorophyll into the CRE (Roegner et al., 2011). Zooplankton taxa encountered at Ilwaco were largely consistent with those collected nearby in Baker Bay in epibenthic sampling during 1980–1981 (Jones et al., 1990).

4.3. Distribution and dynamics of non-native zooplankton

We encountered five non-native zooplankton species which had previously been documented in the CRE, including three copepod species (*P. forbesi*, *Limnithona tetraspina* and *Sinocalanus doerri*), one cladoceran (*Bosmina coregoni*) and veliger larvae of the Asian clam (*C. fluminea*). Of our five sites, Ilwaco at the river mouth had the lowest percentages of non-native taxa, followed by Cathlamet, which is the most downstream tidal freshwater site. The three most upstream sites all had similar proportions of non-native taxa. The most abundant non-native zooplankters in the estuary, *P. forbesi* and *C. fluminea* (planktonic as larvae), have established reproducing populations in the river (Emerson et al., 2015; Hassett et al., 2017; Dexter et al., 2020b), therefore the increased proportion of non-native taxa at upstream sites is likely related to the physiological tolerances and habitat preferences of these taxa, as well as advection from upstream, rather than from frequent reintroduction.

P. forbesi and *C. fluminea* veligers were widely distributed within the tidal freshwater reach and frequently accounted for over 50% of total zooplankton abundance during summer and early autumn, consistent with findings of prior studies in the CRE and further upstream (Bollens et al., 2012; Dexter et al., 2015, 2020b; Emerson et al., 2015). At our Vancouver and Washougal sites, *P. forbesi* peak timing and magnitude (~1500–4000 individuals m^{-3}) was comparable to previous observations at the Vancouver dock (Dexter et al., 2015, 2020b), and in Bonneville and John Day Reservoirs upstream (Emerson et al., 2015). Peak

magnitude at Kelso in Year 2 was considerably higher ($\sim 28,000$ individuals m^{-3}) than was observed elsewhere in the estuary. Kelso was only sampled in Year 2 and it is unclear if this trend would be consistent across years. *P. forbesi* similarly peaks in freshwaters of the SFE during summer and autumn (Kimmerer et al., 2018).

P. forbesi was only rarely encountered at Ilwaco near the river mouth. When *P. forbesi* was abundant elsewhere in the estuary (July–October), salinity at Ilwaco ranged from 6.4 to 14.4 (mean 10.6). As this is near the upper limit of its preferred salinity range (<11) (Orsi and Walter, 1991), salinity may partially explain its low abundance. In a survey of 38 Northeast Pacific estuaries, *P. forbesi* was only encountered over a narrow salinity range (0–7.3) and was most common at <1 (Dexter et al., 2020a). *P. forbesi* is similarly uncommon at salinity >2 in the upper SFE, though it formerly occupied more brackish waters (Kayfetz and Kimmerer, 2017). On the opposite side of the CRE at Astoria, OR (rkm 20), *P. forbesi* was abundant during late summer sampling in 2005–2006 (Bollens et al., 2012; Rollwagen-Bollens et al., 2020) when July–October salinities ranged from 1.8 to 12.8 (mean 6.7). Lower abundances of *P. forbesi* at Ilwaco relative to Astoria may be due to the slightly higher salinities seen at Ilwaco. It is also possible that *P. forbesi* is less suited to or less readily advected to Ilwaco than Astoria, due to some other site or circulation difference. The Ilwaco site is located inside a shallow peripheral bay (Baker Bay) surrounded by tide flats, while the Astoria site is alongside the main navigation channel.

Cathlamet had lower proportions of non-native taxa, particularly *P. forbesi*. *P. forbesi* was detected downstream from Cathlamet at Astoria in high numbers (Bollens et al., 2012), suggesting *P. forbesi* does not increase linearly along the estuarine gradient. No variables sampled in our study explain this pattern, however, qualitatively river velocity at our Cathlamet site appeared lower than at the other tidal freshwater sites.

L. tetraspina, though highly abundant in the low salinity regions of the SFE (Bouley and Kimmerer, 2006), was only present at low abundance in the CRE. It has been suggested that *L. tetraspina* may be less suited to the CRE than the SFE due to its small body size and because the CRE is a higher energy system (Cordell et al., 2008). *S. doerri* was also rarely encountered and was present at much lower abundance than in previous CRE studies (Cordell et al., 2008).

We did not detect *P. inopinus*, which has now been absent from the CRE since 2004, although it remains the dominant late summer zooplankton in many Washington and Oregon estuaries to the north and south, respectively (Cordell et al., 2007; Dexter et al., 2020a).

Due to their high abundance, it is likely that *P. forbesi* and *C. fluminea* are having effects on the CRE food web during summer and autumn, since these non-native taxa are likely competing with native taxa for food resources. For instance, we observed *P. forbesi* to co-occur with native cyclopoids, including *D. thomasi*. *P. forbesi* has been known to consume diatoms, ciliates, flagellates and dinoflagellates (Bowen et al., 2015) and its diet strongly overlaps with *D. thomasi* (Brandl, 2005; Rollwagen-Bollens et al., 2013). There is evidence that *C. fluminea* veligers do feed (King et al., 1986; Bollens et al., unpublished data), however their diet and interactions with native taxa in the CRE are as yet unknown.

4.4. Environmental forcing

Different environmental variables were associated with zooplankton assemblages at our river mouth site (Ilwaco) than at tidal freshwater sites in the CRE. At Ilwaco, according to BIOENV analysis, salinity and discharge, which were negatively correlated, and temperature best explained the variation in zooplankton assemblages. At tidal freshwater sites, temperature and season best explained the variability in zooplankton assemblage structure. The fact that “season” was included in addition to temperature suggests that this factor captured variation in assemblages over and above seasonal differences in temperature, possibly related to discharge, chlorophyll *a*, or some combination of

biotic or abiotic factors that vary seasonally. Our results contribute to a large body of literature supporting the strong impacts of temperature and salinity on estuarine zooplankton (e.g. Miller, 1983; Laprise and Dodson, 1994; Li et al., 2006; Bollens et al., 2011).

In addition to homogenizing zooplankton assemblages, as described above, high flows and low residence times may limit the build-up of zooplankton standing stock. The CRE residence time is short relative to the generation time of most freshwater and estuarine zooplankton groups, particularly during the spring high-flow period. For comparison, generation times at 5–20 °C for calanoid copepods are approximately 8–97 days, cyclopoid copepods 15–118 days, cladocerans 8–100 days, and rotifers 3–26 days, with shorter generation times within groups related to higher temperatures and smaller body sizes (Gillooly, 2000). We observed reduced abundance in the year with higher discharge, and low water residence time has similarly been linked to reduced zooplankton abundance, biomass and productivity in other systems (Pace et al., 1992; Bum and Pick, 1996; Doubek et al., 2019; Breckenridge et al., 2020). It would follow that high-energy, river-dominated estuaries like the CRE with low residence times would likely have lower zooplankton abundances when compared to estuaries with higher residence times. This idea has been explored by Breckenridge et al. (2020) in the FRE to the north. They compared zooplankton abundances in the FRE, an undammed, snow-melt dominated system with a low residence time (6–30 h) to abundances seen in the literature from well-studied estuaries around the world, and found that FRE abundances are on the low end of published values. They further posited that channelization, which reduces residence time, may reduce zooplankton production, with implications for higher trophic levels. The average annual zooplankton abundance inclusive of nauplii seen in the CRE during our study (7250 ± 1907 individuals m^{-3}) was nearly three times higher than the average for channel sites of the FRE (2506 ± 240 individuals m^{-3}), yet still on the lower end of the spectrum of published values (Breckenridge et al., 2020).

Freshwater discharge, in addition to affecting zooplankton through impacts on residence time, likely affected seasonal zooplankton assemblage composition at Ilwaco through its impact on salinity. Distinct high-flow and low-flow assemblages have been observed in the lower CRE (Breckenridge et al., 2015; Rollwagen-Bollens et al., 2020), and other estuaries, including the SFE (Bollens et al., 2011), Darwin Harbour, Australia (Duggan et al., 2008) and Guadiana Estuary, Portugal (Chícharo et al., 2006). One taxon that appears to do particularly well during high freshwater flows is the tidally vertically-migrating *E. affinis* (Morgan et al., 1997), which was highly abundant in our sampling at Ilwaco during spring and early summer and has been positively correlated with discharge in the SFE (Kimmerer, 2002), Chesapeake Bay (Kimmel and Roman, 2004) and in past CRE studies (Jones et al., 1990; Breckenridge et al., 2015).

We examined a number of explanatory variables; however, a considerable amount of variation remains unexplained. Notably, we did not measure nutrient concentrations, site-specific river velocity, biotic variables (e.g., zooplankton prey or predators) nor assess coastal upwelling, and it was not logistically possible to standardize our sampling within the tidal cycle. Prior work at a single station in the lower CRE (Astoria, OR) found that zooplankton assemblage structure was seasonally correlated with nanoplankton biomass, upwelling strength and nitrogen concentration in addition to temperature (Rollwagen-Bollens et al., 2020), and it is likely these variables may account for some of our unexplained variation.

5. Conclusions

This study was the first to examine zooplankton year-round along the 234 rkm length of the CRE. We found that assemblage structure differed weakly by year over the two-year duration of the study but differed strongly by season, with non-native zooplankton taxa most abundant at tidal freshwater sites during late summer - early autumn. Taxa

composition, richness and diversity (H) at tidal freshwater sites differed from those observed at the mouth of the river, though there was little variation among the four freshwater sites. Non-native taxa, notably *P. forbesi* and veligers of *C. fluminea*, were widely distributed within the freshwater reach and frequently comprised over 50% of total zooplankton abundance at tidal freshwater sites during August–October. Our results reaffirm that temperature, salinity and discharge structure zooplankton assemblages in estuaries, and suggest that for large river-dominated systems with low residence times, such as the CRE, spatial factors (rkm, tributary input, human development, land use) may have little effect on zooplankton within the tidal freshwater reach.

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CRediT authorship contribution statement

Kristin A. Connelly: Formal analysis, Writing - original draft,

Visualization. **Gretchen Rollwagen-Bollens:** Conceptualization, Methodology, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Stephen M. Bollens:** Conceptualization, Methodology, Writing - review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendices.

Table A.1

Strong ($r > 0.7$) pairwise correlations (Pearson) between numeric explanatory variables within the all sites, freshwater and Ilwaco data sets. The variable retained and included in analyses is listed under “Retained explanatory variable” and the highly correlated variables removed are listed under “Strongly correlated variable(s).” Retained variables used in BIOENV models and displayed as vectors on NMDS plots should be considered a proxy for the variable plus all strongly correlated variables. Variables with no strong correlation are listed as n/a.

Retained explanatory variable	Strongly correlated variable(s)	Pearson's correlation (r)
All sites explanatory data set		
Developed medium intensity (%)	Developed open space %	0.924
	Developed low intensity %	0.999
	Developed high intensity %	0.997
	Barren land %	−0.701
	Deciduous forest %	−0.748
	Evergreen forest %	−0.861
	Shrub/scrub %	−0.751
	Pasture/hay %	0.970
Mixed forest (%)	n/a	n/a
Woody wetlands (%)	Grassland/herbaceous %	0.763
	Emergent herbaceous wetlands %	0.852
River kilometer	Cultivated crops %	0.976
	Grassland/herbaceous %	−0.962
	Emergent herbaceous wetlands %	−0.803
	Open water	0.777
Secchi depth (m)	n/a	n/a
Temperature (°C)	n/a	n/a
Discharge (m ³ s ^{−1})	n/a	n/a
Day of year	n/a	n/a
Freshwater explanatory data set		
Developed medium intensity %	Developed open space %	0.942
	Developed low intensity %	0.999
	Developed high intensity %	0.998
	Deciduous forest %	−0.931
	Evergreen forest %	−0.854
	Shrub/scrub %	−0.760
	Pasture/hay %	0.966
Barren land %	Open water %	0.817
	Developed open space %	−0.862
	Developed high intensity %	−0.700
	Evergreen forest %	0.774

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Table A.1 (continued)

Retained explanatory variable	Strongly correlated variable(s)	Pearson's correlation (r)
Mixed forest %	Pasture/hay %	−0.754
Woody wetlands %	Emergent herbaceous wetland %	0.769
	Deciduous forest %	−0.818
	Evergreen forest %	−0.856
	Pasture/hay %	0.794
River kilometer	Open water %	0.818
	Deciduous forest %	−0.720
	Shrub/scrub %	−0.898
	Grassland/herbaceous %	−0.985
	Cultivated crops	0.953
Temperature (°C)	n/a	n/a
Discharge (m ³ s ^{−1})	n/a	n/a
Secchi depth (m)	n/a	n/a
Day of year	n/a	n/a
Ilwaco explanatory data set		
Salinity	Discharge (m ³ s ^{−1})	−0.785
Temperature (°C)	n/a	n/a
Secchi depth (m)	n/a	n/a
Temperature stratification (°C)	n/a	n/a
Salinity stratification	n/a	n/a
Day of year	n/a	n/a

Table A.2

Zooplankton taxa encountered in Columbia River Estuary samples collected Oct. 2016–Sep. 2018. The number of specimens for each taxon, percentage of total specimens, and frequency of occurrence are provided. Taxa are ranked by total number and non-native taxa are in bold. For multivariate assemblage analyses, rare taxa present in <5% of samples at all sites were aggregated (Other Cladocera: *Macrothrix* sp., *Scapholoberis* sp., *Holopedium gibberum*, *Simocephalus* sp.; Other Chydoridae: *Pleuroxus* sp., *Graptoloberis testudinaria*; Calanoida: *Epischura* sp. Adult, *Epischura* sp. I–V, *Sinocalanus doerri* adult, *Calanus* sp. adult; Cyclopoida: *Eucyclops* sp., *Thermocyclops* sp., *Microcyclops vericans*, *Ditrichocorycaeus anglicus* I–V) or removed (Isopoda, fish egg, unknown), however these taxa were retained for diversity analyses. Nauplii of *P. forbesi* (n = 8033), other copepods (n = 11,919) and Cirripedia (n = 699) were counted but excluded from all statistical analyses.

	Taxon	Total number	Percent of specimens (%)	Frequency (%)
Cladocera	<i>Bosmina</i> spp.	11,401	17.77	94.29
	<i>Daphnia</i> spp.	1504	2.34	58.10
	<i>Chydorus sphaericus</i>	895	1.39	68.10
	<i>Alona</i> spp.	580	0.90	52.38
	<i>Monospilus dispar</i>	419	0.65	48.10
	<i>Bosmina coregoni</i>	327	0.51	36.67
	<i>Ceriodaphnia</i> sp.	266	0.41	33.81
	Other Chydoridae	86	0.13	23.81
	<i>Podon</i> sp.	79	0.12	4.29
	<i>Moina</i> sp.	76	0.12	7.14
	<i>Alonella</i> sp.	48	0.07	9.52
	<i>Diaphanosoma</i> sp.	45	0.07	11.90
	<i>Ilyocryptus</i> sp.	31	0.05	10.48
	<i>Eurycercus</i> sp.	29	0.05	7.62
	<i>Evadne nordmanni</i>	28	0.04	2.86
	<i>Leydigia</i> sp.	25	0.04	6.19
	Other Cladocera	22	0.03	9.05
	<i>Camptocerus</i> sp.	16	0.02	3.81
	<i>Sida crystallina</i>	15	0.02	6.19
	<i>Leptodora kindtii</i>	9	0.01	3.33
Copepoda	Cyclopoida I–V	8588	13.38	96.67
	<i>Pseudodiaptomus forbesi</i> I–V	5448	8.49	58.10
	Harpacticoida adult	1282	2.00	73.33
	Harpacticoida I–V	1269	1.98	74.29
	<i>Eurytemora affinis</i> I–V	1089	1.70	14.29
	Diaptomidae I–V	820	1.28	61.43
	<i>Diacyclops thomasi</i> adult	777	1.21	62.38
	<i>Diacyclops thomasi</i> IV–V	590	0.92	53.81
	Calanoida I–V	546	0.85	37.62
	<i>Acanthocyclops robustus</i> adult	325	0.51	39.52
	<i>Eurytemora affinis</i> adult	271	0.42	12.86
	<i>Pseudodiaptomus forbesi</i> adult	260	0.41	40.00
	<i>Acanthocyclops robustus</i> IV–V	126	0.20	21.90
	Cyclopoida adult	124	0.19	27.62
	<i>Skistodiaptomus</i> adult	116	0.18	22.38
	<i>Acartia</i> spp. I–V	90	0.14	6.19
	<i>Leptodiaptomus</i> sp. adult	84	0.13	18.57
	<i>Limnithona tetraspina</i> I–V	80	0.12	2.38
	<i>Paracalanus parvus</i> I–V	76	0.12	2.86
	Calanoida adult	52	0.08	12.38
	<i>Limnithona tetraspina</i> adult	49	0.08	4.76
	<i>Eucyclops</i> sp. adult	43	0.07	13.33

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Table A.2 (continued)

	Taxon	Total number	Percent of specimens (%)	Frequency (%)
Rotifera	<i>Microcyclops rubellus</i> adult	39	0.06	9.05
	<i>Microcyclops rubellus</i> I–V	32	0.05	8.57
	<i>Oithona similis</i> I–V	30	0.05	2.86
	<i>Paracalanus parvus</i> adult	26	0.04	2.38
	<i>Acartia</i> spp. adult	23	0.04	3.33
	<i>Ditrichocorycaeus anglicus</i> adult	22	0.03	2.38
	<i>Oithona similis</i> adult	12	0.02	2.38
	<i>Mesocyclops</i> sp. adult	11	0.02	4.76
	<i>Paracyclops</i> sp. adult	5	0.01	1.43
	Brachionidae	6104	9.51	80.95
	<i>Asplanchna</i> sp.	5811	9.06	85.71
	<i>Synchaeta</i> sp.	2157	3.36	9.05
	<i>Trichocerca pusilla</i>	545	0.85	30.95
	Other Rotifera	242	0.38	20.48
	<i>Polyarthra</i> sp.	93	0.14	6.67
Other	Polychaeta pre-chaetiger	3307	5.15	14.29
	Polychaeta chaetiger	1631	2.54	15.71
	Oligochaeta	1589	2.48	78.57
	<i>Corbicula fluminea veliger</i>	1543	2.40	33.33
	Ostracoda	661	1.03	56.19
	Chironomidae	531	0.83	60.48
	Tardigrada	388	0.60	55.24
	Nematoda	381	0.59	58.57
	Gastropoda	302	0.47	31.90
	Arachnida	172	0.27	35.71
	Bivalvia	116	0.18	13.81
	Insecta	79	0.12	21.43
	Unionidae glochidia	61	0.10	6.67
	Collembola	54	0.08	14.29
	Amphipoda	54	0.08	13.81
	Appendicularia	43	0.07	4.29
	Larval fish	38	0.06	3.33
	Hydra	30	0.05	8.57
	Chaetognatha	27	0.04	1.43
	Mysida	3	0.00	1.43
	Cirripedia cyprid	3	0.00	1.43
Total		64,171	100	

Table A.3

PERMANOVA results for comparisons of zooplankton assemblages by sampling year, season and site. PERMANOVA was run using Type 1 sequential sums of squares. Variables are listed in order of entrance to the model. Asterisk indicates the result may be influenced by significant heterogeneity of group dispersions.

Variable	df	SS	MS	F	R ²	p (perm)
Both years: Inter-annual, seasonal and spatial patterns						
Zooplankton~Year*Season*Site						
Year	1	0.490	0.490	5.964	0.029	0.001
Season	3	3.984	1.328	16.152	0.240	0.001*
Site	3	3.866	1.289	15.674	0.233	0.001*
Year:Season	3	0.614	0.205	2.491	0.037	0.001*
Year:Site	3	0.377	0.125	1.526	0.023	0.068
Season:Site	9	1.522	0.169	2.057	0.092	0.001*
Year:Season:Site	9	0.677	0.075	0.915	0.041	0.653
Residuals	62	5.098	0.082		0.307	
Total	93	16.628			1.000	
Spring, all sites: Zooplankton~Year*Site						
Year	1	0.206	0.206	3.471	0.100	0.002
Site	3	0.667	0.222	3.743	0.325	0.001
Year:Site	3	0.231	0.077	1.297	0.112	0.181
Residuals	16	0.951	0.059		0.463	
Total	23	2.056			1.000	
Summer, all sites: Zooplankton~Year*Site						
Year	1	0.306	0.306	3.139	0.078	0.019
Site	3	1.778	0.593	6.081	0.453	0.001
Year:Site	3	0.282	0.094	0.963	0.072	0.503
Residuals	16	1.559	0.097		0.397	
Total	23	3.925			1.000	

(continued on next page)

Table A.3 (continued)

Variable	df	SS	MS	F	R ²	p (perm)
Autumn, all sites: Zooplankton~Year*Site						
Year	1	0.381	0.381	3.985	0.111	0.002
Site	3	1.490	0.497	5.198	0.434	0.001
Year:Site	3	0.222	0.074	0.774	0.065	0.722
Residuals	14	1.337	0.096		0.390	
Total	21	3.429			1.000	
Winter, all sites: Zooplankton~Year*Site						
Year	1	0.212	0.212	2.711	0.065	0.021
Site.Code	3	1.454	0.485	6.202	0.449	0.001
Year:Site.Code	3	0.319	0.106	1.361	0.099	0.168
Residuals	16	1.250	0.078		0.386	
Total	23	3.234			1.000	
Year 2 (includes Kelso): Seasonal and spatial patterns						
Zooplankton~Season*Site						
Season	3	2.371	0.790	12.940	0.256	0.001
Site	4	2.992	0.748	12.250	0.323	0.001*
Season:Site	12	1.517	0.126	2.070	0.164	0.001*
Residuals	39	2.382	0.061		0.257	
Total	58	9.262			1.000	
Spring, all sites: Zooplankton~Site						
Site	4	0.654	0.164	3.000	0.545	0.001
Residuals	10	0.545	0.055		0.455	
Total	14	1.200			1.000	
Summer, all sites: Zooplankton~Site						
Site	4	1.548	0.387	5.808	0.699	0.002
Residuals	10	0.666	0.067		0.301	
Total	14	2.214			1.000	
Autumn, all sites: Zooplankton~Site						
Site	4	0.983	0.246	3.428	0.604	0.004
Residuals	9	0.645	0.072		0.396	
Total	13	1.627			1.000	
Winter, all sites: Zooplankton~Site						
Site	4	1.324	0.331	6.302	0.716	0.001
Residuals	10	0.525	0.053		0.284	
Total	14	1.849			1.000	

Table A.4

Results of tests for homogeneity of multivariate group dispersions for PERMANOVA models. Statistically heterogeneous dispersions are in bold. Group dispersions were not significantly different for season specific PERMANOVAs (not shown).

Variable	df	F	p	Pairwise comparison	p	Pairwise comparison	p
Both years: Inter-annual, seasonal and spatial patterns							
Zooplankton~Year*Season*Site							
Year	1, 92	0.4096	0.514	–	–	–	–
Season	3, 90	2.6313	0.058	Aut-Spr	0.013	Spr-Sum	0.009
				Aut-Sum	0.909	Spr-Wtr	0.062
				Aut-Wtr	0.537	Sum-Wtr	0.448
Site	3,90	5.4311	0.003	I–C	0.02	V–C	0.418
				I–V	0.001	V–W	0.859
				I–W	0.001	C–W	0.321
Year 2 (includes Kelso): Seasonal and spatial patterns							
Zooplankton~Season*Site							
Season	3, 55	0.9541	0.449	–	–	–	–
Site	4, 54	2.6011	0.046	I–C	0.173	V–C	0.234
				I–K	0.032	V–K	0.768
				I–V	0.007	C–W	0.269
				I–W	0.003	K–W	0.903
				K–C	0.435	V–W	0.754

Table A.5

Results of pairwise PERMANOVA post hoc tests to assess statistical differences between sites. Statistically significant differences are in bold. Asterisk indicates the result may be influenced by significant heterogeneity of multivariate group dispersions.

Variable	Pairwise comparison	p	Pairwise comparison	p	Pairwise comparison	p
Both years: Inter-annual, seasonal and spatial patterns						
Zooplankton~Year*Season*Site						
Season	Aut-Spr	0.001*	Aut-Wtr	0.001	Spr-Sum	0.001*
	Aut-Sum	0.001	Sum-Wtr	0.001	Spr-Wtr	0.001
Site	I-C	0.001*	I-W	0.001*	V-C	0.092
	I-V	0.001*	C-W	0.066	V-W	0.263
Spring: Zooplankton~Year*Site						
Site	I-C	0.004	I-W	0.006	V-C	0.299
	I-V	0.003	C-W	0.389	V-W	0.236
Summer: Zooplankton~Year*Site						
Site	I-C	0.003	I-W	0.003	V-C	0.070
	I-V	0.005	C-W	0.094	V-W	0.871
Autumn: Zooplankton~Year*Site						
Site	I-C	0.003	I-W	0.002	V-C	0.464
	I-V	0.003	C-W	0.042	V-W	0.620
Winter: Zooplankton~Year*Site						
Site	I-C	0.001	I-W	0.003	V-C	0.045
	I-V	0.002	C-W	0.012	V-W	0.150
Year 2 (includes Kelso): Seasonal and spatial patterns						
Zooplankton~Season*Site						
Season	Aut-Spr	0.001	Aut-Wtr	0.001	Spr-Sum	0.001
	Aut-Sum	0.008	Sum-Wtr	0.001	Spr-Wtr	0.002
Site	I-C	0.001	C-K	0.226	K-V	0.058
	I-K	0.001*	C-V	0.054	K-W	0.263
	I-V	0.001*	C-W	0.109	V-W	0.069
	I-W	0.001*				
Spring: Zooplankton~Site						
Site	I-C	0.024	C-K	0.460	K-V	0.191
	I-K	0.030	C-V	0.134	K-W	0.604
	I-V	0.014	C-W	0.488	V-W	0.126
	I-W	0.033				
Summer: Zooplankton~Site						
Site	I-C	0.006	C-K	0.457	K-V	0.221
	I-K	0.005	C-V	0.188	K-W	0.306
	I-V	0.005	C-W	0.315	V-W	0.490
	I-W	0.006				
Autumn: Zooplankton~Site						
Site	I-C	0.011	C-K	0.618	K-V	0.539
	I-K	0.021	C-V	0.520	K-W	0.319
	I-V	0.017	C-W	0.384	V-W	0.700
	I-W	0.021				
Winter: Zooplankton~Site						
Site	I-C	0.014	C-K	0.021	K-V	0.031
	I-K	0.005	C-V	0.050	K-W	0.182
	I-V	0.008	C-W	0.057	V-W	0.078
	I-W	0.005				

Table A.6

Significant ($p < 0.05$) indicator taxa and indicator values for zooplankton clusters. Indicator values range from 0 to 1, with 1 being the strongest indicator value, which would signify that a taxon was only seen in one cluster and was present in all of its samples. Non-native taxa are in bold.

Cluster	Taxa	Indicator Value	Cluster	Taxa	Indicator Value
Freshwater clusters			Ilwaco clusters		
F-1	Collembola	0.20	I-1	Polychaeta pre-chaetiger	0.34
F-2	<i>Alona</i> spp.	0.35	I-2	<i>Eurytemora affinis</i> adult	0.51
	<i>Chydorus sphaericus</i>	0.28		<i>Eurytemora affinis</i> I-V	0.39
	<i>Ilyocryptus</i> sp.	0.23		Nematoda	0.22
F-3	<i>Corbicula fluminea</i> veliger	0.57	I-3	<i>Acartia</i> spp. I-V	0.95
	<i>Diaphanosoma</i> sp.	0.50		<i>Podon</i> sp.	0.83
	<i>Acanthocyclops robustus</i> IV-V	0.46		Appendicularia	0.83

(continued on next page)

Table A.6 (continued)

Cluster	Taxa	Indicator Value	Cluster	Taxa	Indicator Value
Freshwater clusters			Ilwaco clusters		
	<i>Trichocerca pusilla</i>	0.40		<i>Acartia</i> spp. adult	0.83
	<i>Acanthocyclops robustus</i> adult	0.39		Bivalvia	0.70
	<i>Mesocyclops</i> sp. adult	0.37		<i>Evadne nordmanni</i>	0.67
	<i>Daphnia</i> spp.	0.32		<i>Synchaeta</i> sp.	0.66
	<i>Diacyclops thomasi</i> IV-V	0.29		<i>Paracalanus parvus</i> I-V	0.64
	Chironomidae	0.29		Polychaeta chaetiger	0.57
	<i>Leptodiaptomus</i> spp. adult	0.29		<i>Oithona similis</i> I-V	0.45
	<i>Bosmina</i> spp.	0.26		Calanoida I-V	0.42
	<i>Ceriodaphnia</i> sp.	0.25		Chaetognatha	0.33
	<i>Leptodora kindtii</i>	0.24		Cirripedia cyprid	0.33
	Brachionidae	0.24		Harpacticoida I-V	0.32
	Diaptomidae I-V	0.23		<i>Oithona similis</i> adult	0.30
	<i>Asplanchna</i> sp.	0.23		Harpacticoida adult	0.30
	Cyclopoida I-V	0.23		Other Rotifera	0.25
	<i>Microcyclops rubellus</i> I-V	0.21		<i>Paracalanus parvus</i> adult	0.23
F-4	<i>Monospilus dispar</i>	0.51			
	Gastropoda	0.38			
	<i>Pseudodiaptomus forbesi</i> adult	0.38			
	<i>Pseudodiaptomus forbesi</i> I-V	0.35			
	Tardigrada	0.33			
	Other Chydoridae	0.26			
	Ostracoda	0.24			
	Oligochaeta	0.23			

Table A.7

Parameters and Spearman correlation coefficients for the best fit BIOENV model for each number of parameters. Models are presented for the three explanatory data sets (all sites, freshwater sites, Ilwaco). The overall best fit model for each data set is in bold.

No. parameters	Parameters in best fit model	Spearman Correlation
All sites		
1	Estuarine zone	0.5835
2	Temperature, Estuarine zone (best fit model)	0.7118
3	Temperature, Julian day, Estuarine zone	0.7092
4	Woody wetlands, Temperature, Season, River zone	0.7055
5	Woody wetlands, Secchi, Temperature, Day of year, Estuarine zone	0.6963
6	Woody wetlands, Secchi, Temperature, Discharge, Day of year, Estuarine zone	0.6719
7	Woody wetlands, River KM, Secchi, Temperature, Day of year, Season, Estuarine zone	0.6602
8	Woody wetlands, River KM, Secchi, Temperature, Discharge, Day of year, Season, Estuarine zone	0.6334
9	Woody wetlands, River KM, Secchi, Temperature, Discharge, Day of year, Season, Water year, Site, Estuarine zone	0.6122
10	Woody wetlands, River KM, Secchi, Temperature, Discharge, Day of year, Season, Water year, Site, Estuarine zone	0.5824
11	Mixed forest, Woody wetlands, River KM, Secchi, Temperature, Discharge, Day of year, Season, Water year, Site, Estuarine zone	0.546
12	Developed medium intensity, Mixed forest, Woody wetlands, River KM, Secchi, Temperature, Discharge, Day of year, Season, Water year, Site, Estuarine zone	0.5061
Freshwater sites		
1	Temperature	0.5823
2	Temperature, Season (best fit model)	0.5979
3	Temperature, Secchi, Season	0.5918
4	Temperature, Secchi, Day of year, Season	0.5509
5	Temperature, Discharge, Secchi, Day of year, Season	0.5151
6	River KM, Temperature, Discharge, Secchi, Day of year, Season	0.4897
7	River KM, Temperature, Discharge, Secchi, Day of year, Season, Water year	0.4737
8	Woody wetlands, River KM, Temperature, Discharge, Secchi, Day of year, Season, Water year	0.4409
9	Barren land, Woody wetlands, River KM, Temperature, Discharge, Secchi, Day of year, Season, Water year	0.3986
10	Barren land, Mixed forest, Woody wetlands, River KM, Temperature, Discharge, Secchi, Day of year, Season, Water year	0.3669
11	Developed medium intensity, Barren land, Mixed forest, Woody wetlands, River KM, Temperature, Discharge, Secchi, Day of year, Season, Water year	0.3286
12	Developed medium intensity, Barren land, Mixed forest, Woody wetlands, River KM, Temperature, Discharge, Secchi, Day of year, Season, Water year, Site	0.2976
Ilwaco sites		
1	Salinity	0.4963
2	Salinity, Temperature (best fit model)	0.6768
3	Salinity, Temperature, Season	0.6597
4	Salinity, Temperature, Day of year, Season	0.6265
5	Salinity, Temperature, Secchi, Day of year, Season	0.6044
6	Salinity, Temperature, Secchi, Temperature stratification, Day of year, Season	0.5775
7	Salinity, Temperature, Secchi, Temperature stratification, Salinity stratification, Day of year, Season	0.5362
8	Salinity, Temperature, Secchi, Temperature stratification, Salinity stratification, Day of year, Season, Water year	0.5009

Table A.8

Spearman correlation values (r^2) and permutation p-values (perm p) between explanatory variables and zooplankton. BIOENV best fit model parameters for each data set are in bold. Collinear explanatory variables removed from analysis are listed in [Table A2](#).

Category	Explanatory Variable	r^2	perm p
All sites			
Environment	Temperature	0.6778	0.001
Environment	Discharge	0.1717	0.001
Environment	Secchi depth	0.0416	0.109
Landscape	Woody wetlands %	0.659	0.001
Landscape	Devel. medium intensity %	0.1184	0.004
Landscape	Mixed forest %	0.0596	0.032
Spatial	River KM	0.4966	0.001
Spatial	Site (factor)	0.4198	0.001
Spatial	Estuarine zone (factor)	0.4052	0.001
Temporal	Season (factor)	0.3173	0.001
Temporal	Day of year	0.2153	0.001
Temporal	Year (factor)	0.0171	0.149
Biological	Log (Abundance)	0.8699	0.001
Biological	Richness	0.3027	0.001
Biological	Diversity	0.2679	0.001
Freshwater sites			
Environment	Temperature	0.7301	0.001
Environment	Discharge	0.4022	0.001
Environment	Secchi depth	0.2653	0.001
Landscape	Woody wetlands %	0.0401	0.185
Landscape	Devel. medium intensity %	0.0366	0.217
Landscape	Mixed forest %	0.031	0.278
Landscape	Barren land %	0.0012	0.942
Spatial	River KM	0.0393	0.186
Spatial	Site (factor)	0.0357	0.412
Temporal	Season (factor)	0.5823	0.001
Temporal	Day of year	0.2887	0.001
Temporal	Year (factor)	0.0461	0.034
Biological	Log (Abundance)	0.9411	0.001
Biological	Diversity	0.361	0.001
Biological	Richness	0.0307	0.293
Ilwaco			
Environment	Salinity	0.7685	0.001
Environment	Temperature	0.6989	0.001
Environment	Secchi depth	0.3692	0.009
Environment	Temperature stratification	0.2084	0.084
Environment	Salinity stratification	0.2954	0.028
Temporal	Day of year	0.4897	0.005
Temporal	Season (factor)	0.5408	0.001
Temporal	Year (factor)	0.0367	0.461
Biological	Log (Abundance)	0.7374	0.001
Biological	Diversity	0.3655	0.014
Biological	Richness	0.2349	0.072

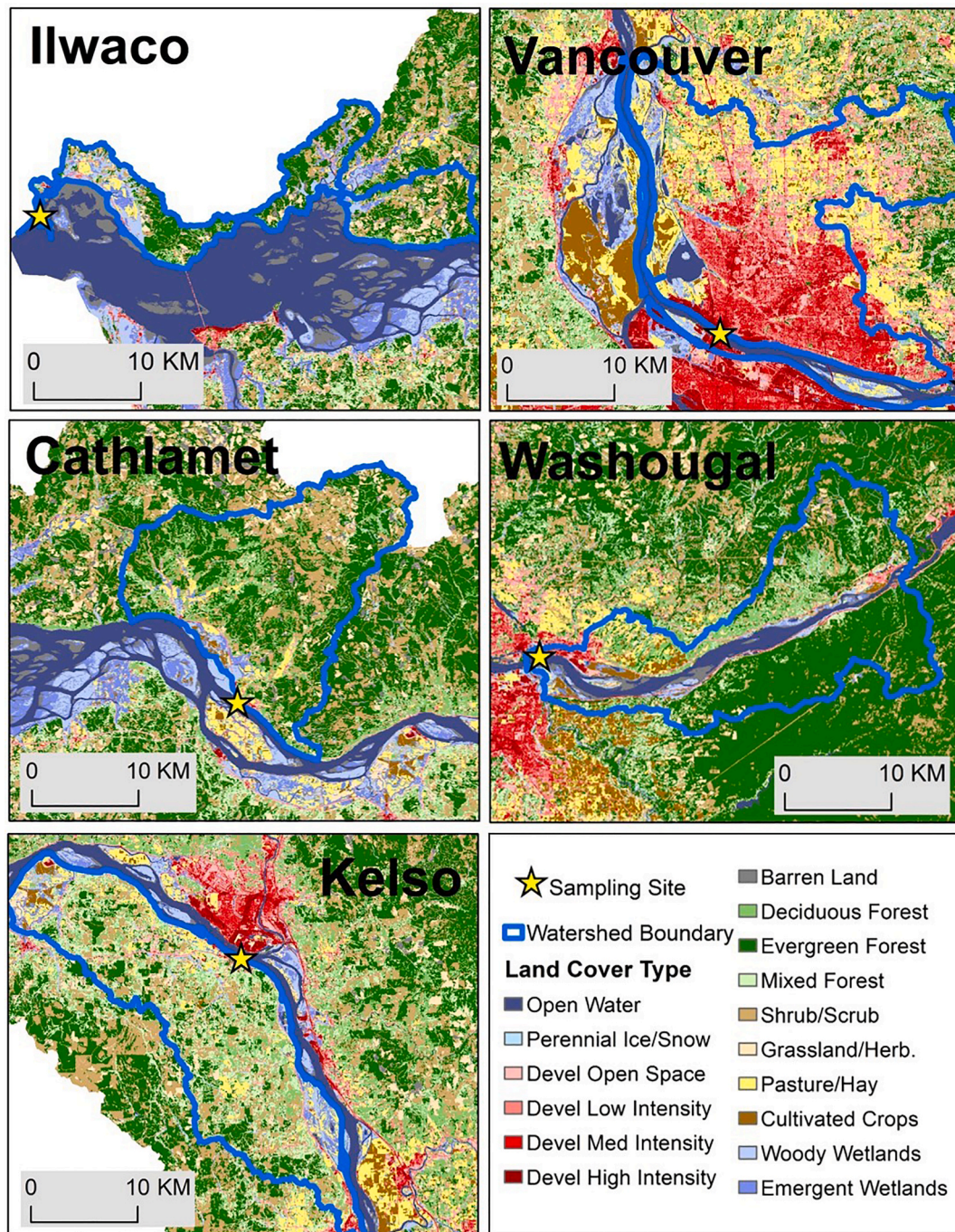


Fig. A.1. Land cover for watersheds containing sampling sites at the USGS Watershed Boundary Dataset HUC10 scale. Land cover is from the National Land Cover Database 2011 produced by the Multi-Resolution Land Characteristics Consortium.

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