Journal of
Plankton Research

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J. Plankton Res. (2020) 42(2): 221–237. First published online March 5, 2020 doi:10.1093/plankt/fbaa010

ORIGINAL ARTICLE

Biotic vs. abiotic forcing on plankton assemblages varies with season and size class in a large temperate estuary

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Received November 6, 2019; editorial decision February 10, 2020; accepted February 10, 2020

Corresponding editor: Xabier Irigoien

Large river estuaries experience multiple anthropogenic stressors. Understanding plankton community dynamics in these estuaries provides insights into the patterns of natural variability and effects of human activity. We undertook a 2-year study in the Columbia River Estuary to assess the potential impacts of abiotic and biotic factors on planktonic community structure over multiple time scales. We measured microplankton and zooplankton abundance, biomass and composition monthly, concurrent with measurements of chlorophyll *a*, nutrient concentrations, temperature and salinity, from a dock in the lower estuary. We then statistically assessed the associations among the abundances of planktonic groups and environmental and biological factors. During the late spring high flow period of both years, the lower estuary was dominated by freshwater and low salinity-adapted planktonic taxa, and zooplankton grazers were more strongly associated with the autotroph-dominated microplankton assemblage than abiotic factors. During the early winter period of higher salinity and lower flow, nutrient (*P*) availability exerted a strong influence on microplankton taxa, while only temperature and upwelling strength were associated with the zooplankton assemblage. Our results indicate that the relative influence of biotic (grazers) and abiotic (salinity, flow, nutrients and upwelling) factors varies seasonally and inter-annually, and among different size classes in the estuarine food web.

KEYWORDS: Columbia River Estuary; plankton community structure; biotic vs. abiotic forcing

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INTRODUCTION

Large river estuaries are characterized by the dynamic interplay of physical, chemical, geological and biological processes, often occurring within the context of increasing human impacts, such as eutrophication, habitat disturbance, modification of river flow through impoundments and diversions, introduction of non-native taxa and climate change (Kennish, 2002; Martínez *et al.*, 2007; Robins *et al.*, 2016). Planktonic organisms are the dominant primary and secondary producers in pelagic estuarine ecosystems, with generation times of days to weeks, and often the 'first responders' to these highly variable conditions. Thus, the manner in which different components of estuarine plankton communities vary across time may provide important insights into patterns of natural variability and the effects of human activity on the base of these increasingly stressed food webs (Richardson and Schoeman, 2004; Richardson, 2008).

Changes in plankton community structure may directly impact the viability and productivity of many other ecologically, socially and commercially important estuarine populations, as plankton comprise the dominant prey resource for early life history stages of fish (both resident and transient migrators) and certain benthic invertebrates (e.g. bivalve mollusks) (Richardson, 2008; Bollens *et al.*, 2010; Cloern *et al.*, 2014). In addition, anthropogenic impacts on estuarine systems can be manifested in changes in abundance and diversity of plankton. For instance, eutrophication, climate warming and human-mediated variations in flow regime have been associated with increased frequency and magnitude of harmful algal blooms in river estuaries (Carstensen *et al.*, 2007; Bricker *et al.*, 2008; Roy *et al.*, 2016). Similarly, large river estuaries with major international ports are particularly at risk of introduction of invasive aquatic species through ballast water release (Bollens *et al.*, 2002, 2012; Dexter and Bollens, 2019). Indeed, the impacts of human activity have their largest effects on the top and the bottom of food webs, and are often cascaded upward and downward through the plankton in pelagic systems (Wollrab *et al.*, 2012).

Both biotic (e.g. predation, competition, etc.) and abiotic (e.g. temperature, salinity, turbidity, mixing, etc.) factors influence the structure and abundance of the many assemblages within planktonic communities, especially in estuaries, regardless of whether they are the result of natural variability or anthropogenic drivers. However, the relative impacts of these concurrently acting factors, and how their influence may vary over time, is not well studied or understood. [Note: We are using an ecological nomenclature modified from Stroud *et al.* (2015) to define *community* as 'a group of interacting species populations occurring together in space' and *assemblage* as 'a taxonomically related and comparably sized group of species populations that occur together in space'].

For example, a range of published studies describe how environmental factors drive changes in abundance, composition and successional patterns in large estuaries, but these have typically focused on only one size class or functional group of plankton, such as the phytoplankton, e.g. Rhode River (Gallegos *et al.*, 2010), Schelde Estuary (Muylaert *et al.*, 2000), Coruna Estuary (Bode *et al.*, 2017); the microzooplankton, e.g. Bay of Biscay (Dupuy *et al.*, 2011) or the meso- and microzooplankton, e.g. St. Lawrence Estuary (Laprise and Dodson, 1994), San Francisco Estuary (SFE) (Bollens *et al.*, 2011), Willapa Bay (Graham and Bollens, 2010). Only a very small number of studies report temporal changes across multiple assemblages from picoplankton up to microzooplankton, e.g. in the Cochin estuary (Sooria *et al.*, 2015) and Bahia Blanca estuary (Barría de Cao *et al.*, 2011). In both of these cases, the investigators associated environmental conditions with the abundances of individual assemblages, but did not assess the biotic factors that might also have influenced the distribution and abundance of those assemblages.

The Columbia River Estuary (CRE) is the downstream terminus of the Columbia-Snake River system, whose watershed spans parts of seven states in the US Pacific Northwest and two provinces in western Canada. The CRE is increasingly impacted by human activity, with dramatic increases in population within the watershed over the past 30 years, and extensive impoundments that have changed the flow regime and associated transport of nutrients and other dissolved components downstream (Wise *et al.*, 2007). The CRE is also the gateway through which several species of endangered Pacific salmonids migrate between the ocean and upstream spawning grounds throughout the Columbia River Basin, and where juvenile salmonids feed on planktonic and emergent prey at key stages in their life histories (Kirn *et al.*, 1985; Goertler *et al.*, 2016). Yet, over the past half century, only a handful of published studies have examined the dynamics of the plankton community in the CRE (Haertel and Osterberg, 1967; Haertel *et al.*, 1969; Neitzel *et al.*, 1982; Jones *et al.*, 1990; Simenstad *et al.*, 1990a; Bollens *et al.*, 2012; Breckenridge *et al.*, 2015; Dexter *et al.*, 2015). Despite its ecological and economic importance to the region, the CRE has been relatively poorly studied compared to other large river estuaries, particularly with regard to dynamic environmental influences on the biological communities residing in and/or transiting through its waters.

To explore the interactions and relative influence of biotic and abiotic factors on the estuarine plankton

community over a range of temporal scales, and specifically to address the current knowledge gap regarding such processes in the CRE, we sampled the plankton across a wide size (~2 μm to 2 mm) and functional (primary producers, primary and secondary consumers) range, and measured a variety of environmental factors, at a single location in the lower CRE on a monthly basis over a 2-year period (2005–2006). Using these data, our goal was to address three inter-related research questions:

1. What are the biotic (primary producers, consumers) and abiotic (river flow, salinity, temperature, etc.) forcing factors influencing the abundance and composition of planktonic assemblages in the lower CRE?
2. How do the relative impacts of biotic and abiotic factors vary over seasonal and inter-annual time periods in the lower CRE?
3. How does the influence of these biotic and abiotic factors on the plankton community in the lower CRE compare to other large river estuaries?

METHOD

Study site

The Columbia River is 1954 km long with an average outflow of 5500 m^3s^{-1} that drains an area of 660 480 km^2 (Simenstad *et al*., 1990a), and thus is the second largest river entering the Pacific Ocean along the west coast of North America. The CRE is a mesotidal river-dominated system that is also influenced by seasonal periods of coastal upwelling (Jay and Smith, 1990; Simenstad *et al*., 1990a).

River discharge peaks twice each year, with high flows in April through June due to snowmelt, and a second, smaller period of high flow in November through March due to winter rainfall (Hickey *et al*., 1998; Chawla *et al*., 2008). Salinity intrusion increases during periods of low river flow, and may extend 20–44 km upstream from the mouth of the river (Chawla *et al*., 2008). Although Columbia River discharge increases with rainfall and snowmelt, the ~214 impoundments of the river have reduced seasonal variation in flow (Payne *et al*., 2004). In addition, the CRE (like other estuaries along the US Pacific Northwest coastline) is affected by the frequency, intensity and duration of upwelling events that advect coastally derived nutrients and plankton into the estuary (Roegner *et al*., 2011a, b). Commercial fisheries for steelhead trout, chinook, coho, chum and sockeye salmon exist in the Columbia River, with several fish stocks under the protection of the US Endangered Species Act (Simenstad *et al*., 1990b; Keefer *et al*., 2004; Weitkamp *et al*., 2012).

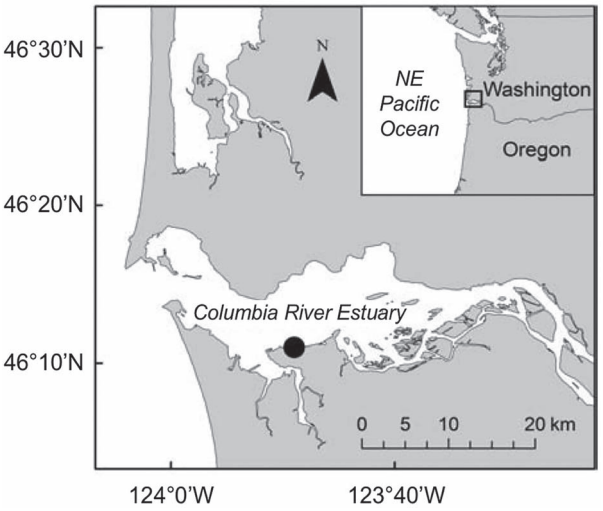

Fig. 1. Map of the CRE. Filled circle is the location of sampling.

Sample collection

Samples were collected in the third week of each month during 2005 and 2006 from a pier located at 46°11′25″N 123°49′28″W, which is on the southern bank of the Columbia River in the city of Astoria, OR, ~20 km from the river mouth (Fig. 1). This pier extends ~40 m from the shore, and at the point of collection water depth varied between 4.0 and 6.5 m. This site experiences tidally variable intrusion of saline waters, which are either well mixed or strongly stratified depending on hydrological conditions (Breckenridge *et al*., 2015).

On each sampling date, sampling was conducted during daylight hours. Temperature and salinity profiles were recorded from the surface to the bottom using a YSI 85 probe, and relative water clarity was estimated by measuring the Secchi depth. Surface water for microplankton (unicellular protists and cyanobacteria colonies ~15–200 μm in size), nanoplankton (cells ~5–15 μm in size), chlorophyll *a* and nutrient (NO_3, NO_2, PO_4, SiO_2) measurements was collected via triplicate bucket samples. Microplankton were collected in 200-mL water subsamples, preserved in 5% acid Lugols solution and stored in opaque jars until analysis. Nanoplankton subsamples were collected in 100-mL opaque bottles, preserved in a 1% glutaraldehyde solution, then stained and filtered as described below, and frozen until analysis. In addition, subsamples were taken for later laboratory analyses of chlorophyll *a* and nutrient concentrations. Metazoan planktonic organisms ~75 μm to 2 mm in size were collected via triplicate vertical tows taken from 0.5 m above the bottom to the surface with a 0.5-m diameter mouth, 75-μm mesh net, with attached flowmeter (General Oceanics Inc.). The average water volume sampled

per tow was 0.79 m^3. Samples were preserved in a 10% buffered formalin solution for later taxonomic processing. For the purposes of this project, we refer to all metazoan organisms from these net tows as 'zooplankton' to distinguish them from unicellular protists and cyanobacteria, acknowledging that the microplankton also includes some heterotrophic and mixotrophic consumer taxa. As such, zooplankton is used here only as a size (>75 μm) and taxonomic (Kingdom Animalia) classification.

In the laboratory, subsamples for chlorophyll *a* were filtered through GF/F filters (Whatman Inc.), and the filters wrapped in foil and immediately frozen. Within 1 week of collection, thawed filters were placed in vials containing 20 mL of 90% acetone for 24 h. The concentration of chlorophyll *a* suspended in the acetone after incubation was measured on a Turner Model 10 AU fluorometer, using the acidification method (Strickland and Parsons, 1972). Nutrient subsamples were sent to the Marine Chemistry Laboratory at the University of Washington for analysis following the protocols of the World Ocean Circulation Experiment hydrographic program.

Taxonomic processing and data preparation

Taxonomic processing of microplankton samples (protists and cyanobacteria) was conducted by settling 1–10 mL of Lugol's-preserved water overnight in Utermohl chambers, and then examining settled samples with an Olympus CK-40 inverted microscope at 200–400×. All individuals were identified to genus (and species when possible) using Patterson and Hedley (1992) and Wehr *et al*. (2015), and sized using an ocular micrometer. Biovolume was calculated based on geometric shape (Hillebrand *et al*., 1999) and carbon biomass was then estimated using the algorithms of Menden-Deuer and Lessard (2000).

For nanoplankton sample processing, a 20-mL aliquot was removed from each glutaraldehyde-preserved sample, stained with 4′,6-diamidino-2-phenylindole, filtered onto 1.0-μm black polycarbonate filters and mounted on glass slides (Sherr *et al*., 1983). The slides were kept frozen until analysis. To enumerate the nanoplankton, a minimum of 100 cells between 2 and 20 μm were counted using an epifluorescence microscope at 400–450× magnification under blue light. Cells were sized and nanoplankton carbon biomass was estimated from biovolume as described above for microplankton.

An aliquot of 2–10 mL (1–5% of the entire sample) was subsampled from each formalin-preserved zooplankton sample, and a minimum of 200 non-naupliar organisms were identified and enumerated using a Leica MZ6 stereomicroscope (Leica Microsystems). Identification to the genus or species level was made for most rotifers and microcrustaceans using Thorp and Covich (2010). Counts of individual taxa were converted to density (individuals m^{-3}) by dividing the counts by the total volume of water sampled. Three replicate samples were processed for each date. Nauplii and eggs were excluded from all statistical analyses, and rare taxa (those present in less than 5% of samples) were aggregated into higher taxonomic groups to keep the total number of taxa to a manageable number.

Calculation of predictor variables

A suite of physical and biological variables were evaluated as predictors of microplankton and zooplankton assemblage structure. Profiles of water column temperature and salinity were collected at 1-m intervals from surface to bottom on each sampling date, and from those measurements several derived variables (i.e. surface value, bottom value, stratification and mean value) were calculated. River flow was calculated as the average discharge ($ft^3\ s^{-1}$) over the 14 days prior to sampling. River discharge data were downloaded from the US Geological Survey National Water Information System (https://waterdata.usgs.gov/nwis/uv/?referred_module=sw). Coastal upwelling was included both as the index value from the day prior to our sampling, and as the 14-day average prior to our sampling. Upwelling values (Bakun index values) were downloaded from NOAA/NMFS/PFEL for coordinates at 45°N (http://www.pfeg.noaa.gov/products/las/docs/global_upwell.htmL).

In addition, sampling date was included as a predictor variable in the form of a 'circularized' month, calculated by applying a cosine transformation to the month value. Light availability, as solar radiation in Wh m^{-2} h^{-1} measured at Cannon Beach, OR (~40 km south of our CRE sampling site), was obtained from the University of Oregon's Solar Radiation Monitoring Laboratory (http://solardat.uoregon.edu/SolarData.htmL). Finally, we calculated two tidal indicators. An 'Ebb vs. Flood' index was calculated as $(S_t - PT_t)/(FT_t - PT_t)$, where S_t was the time when the samples were taken, PT_t was the time of the previous tide change and FT_t was the time of the following tide change. When the sample was collected on an ebb tide, we multiplied this index by −1. Values of 1 and −1 indicate the end of flood and the end of ebb, respectively, and a value of zero indicates a tide change. We created a similar but continuous index of high vs. low water, calculated as $(S_t - PT_t)/(FT_t - PT_t)*2–1$ for samples collected during flood tides, and as $1 - (S_t - PT_t)/(FT_t - PT_t)*2–1$ for samples collected during ebb tides. This created an index where 1 indicates the highest water level and −1 indicates the lowest water value for any given tide.

Biological predictors of community composition included water column chlorophyll *a* concentration, nanoplankton biomass and abundances or biomass of higher order taxonomic groupings of microplankton and zooplankton. Microplankton predictors of zooplankton assemblage structure included the biomass of dinoflagellates, flagellates, ciliates, diatoms, chlorophytes and filamentous bacteria. Diatoms were further divided into size classes of 5–19, 20–49, 50–149 and >150 μm. Zooplankton predictors of microplankton assemblage structure included the Log_{10} $(x+1)$ abundances of rotifers, cladocerans, amphipods, calanoid copepods, harpacticoid copepods, cyclopoid copepods, total copepod nauplii, total copepodites, total adult copepods and total zooplankton. Note that we also examined microplankton predictors calculated as the Log_{10} $(x+1)$ abundances of these categories when testing the associations of zooplankton assemblages with biotic and abiotic factors. However, the results did not differ substantively from those using microplankton predictors as biomass. Thus, we only report associations with zooplankton assemblages using microplankton biomass, as these may better reflect the energy and material pathways through the lower planktonic food web.

Statistical analyses

We conducted a suite of non-parametric statistical analyses of community data following the general strategy outlined by Field *et al*. (1982) and Clarke (1993). This approach is largely robust to the temporal autocorrelation inherent in time-series data, and does not assume linearity of relationships or normality of data. Our approach consisted of non-metric multidimensional scaling (NMDS), hierarchical agglomerative clustering and correlating environmental vectors across ordination space. We also tested for significant ($P < 0.05$) differences in biodiversity (calculated as Shannon's H) and abundance or biomass among clusters via Kruskal–Wallis one-way ANOVA on ranks. Statistical analyses were performed separately for the microplankton and zooplankton assemblages.

Ordination of assemblage data was conducted through NMDS (Kruskal, 1964) of untransformed abundances for 80 (microplankton) and 60 taxa (zooplankton). Both ordinations were constructed using the Bray–Curtis measure of dissimilarity, with ties in the dissimilarity matrix treated according to Kruskal's primary approach (no penalties for ties). NMDS structure and dimensionality were validated via the examination of Shepard plots, and the Dexter *et al*. (2018) permutation test, which tests a null hypothesis that observed NMDS stress values could arise from stochastic sampling effects rather than strong species associations. Hierarchical agglomerative clustering of assemblage data was conducted using the flexible beta clustering algorithm (Kaufman and Rousseeuw, 1990) operating upon the Bray–Curtis measure of dissimilarity. Values of beta for the clustering algorithm were set to 0.6 for zooplankton as well as microplankton data (Milligan, 1989).

The strength of correlation between environmental predictor variables and NMDS ordination scores was assessed via the 'ordisurf' function, which allows for non-linear correlations based upon a generalized additive model with penalized splines (Wood, 2003). For all ordinations, sufficiently linear predictor variables were overlain as arrows indicating the direction and strength of correlation with ordination axes, generated via the 'envfit' function and assessed through permutation testing. Predictor variables with strongly non-linear correlations were visualized as topographic isoclines on separate ordination plots, and are provided in the supplemental figures. All multivariate analyses were conducted using the vegan package (Oksanen *et al*., 2017) for R version 3.2.2 (R Core Team, 2015).

RESULTS

Environmental conditions

There were substantial seasonal and inter-annual differences in the environmental conditions of the CRE during our study period from January 2005 to December 2006. Water column temperatures were consistently highest in summer/autumn and lowest in winter of both years; however, waters were generally ~2°C warmer during each month in 2005 compared to 2006. Also, the period of warmest temperatures (>15°C) was longer in 2005 (May–October) than in 2006 (June–September) (Fig. 2A). Seasonal and inter-annual differences in water column salinity were also pronounced. During 2005, salinity was >5 throughout the winter (January–April), then was <3 throughout the late spring and summer, followed by a pulse of high salinity (12) in October. In 2006, the seasonal pattern was nearly reversed: salinity was mostly <2 during the winter and early spring (January–June), and >5 from July to November, with a high salinity pulse of 9–10 during September–October (Fig. 2B).

Microplankton

Composition and phenology of microplankton assemblages

Across our 2-year study period, the overall abundance of the microplankton assemblage was dominated by diatoms (75% of the abundance on average), with the three most abundant diatom taxa (*Aulacoseira granulata*, small

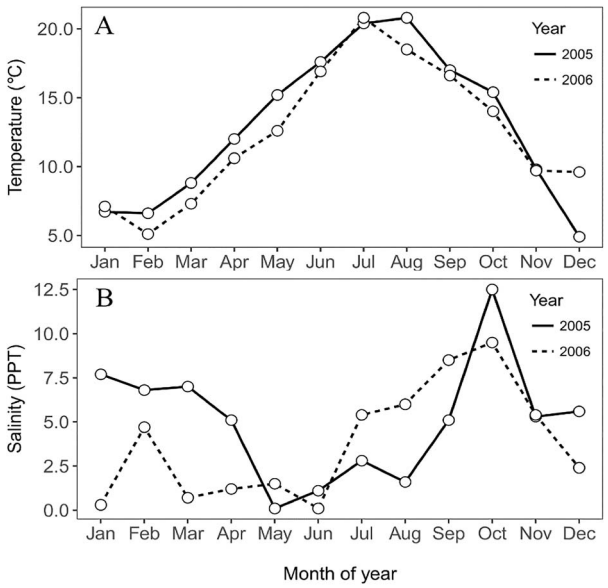

Fig. 2. Mean water column temperature (**A**) and salinity (**B**) in the lower CRE over the sampling period. Solid lines represent data from 2005, and dashed lines represent data from 2006.

Table I: Top 15 most abundant microplankton taxa

Taxon	% of total abundance
Aulacoseira granulata	21.5
Centric diatoms (small)	19.8
Asterionella sp.	15.2
Mesodinium rubrum	8.8
Fragilaria sp.	6.6
Pennate diatoms (small)	5.7
Chlorophytes	3.0
Centric diatoms (medium)	2.9
Ankistrodesmus sp.	2.7
Synedra sp.	2.1
Centric diatoms (large)	1.7
Skeletonema sp.	1.6
Heterotrophic dinoflagellate 'A'	1.2
Micractinium sp.	1.2
Filamentous bacteria	0.9

centric diatoms and *Asterionella* sp.) comprising 56.4% of total microplankton abundance. The remainder of the microplankton assemblage consisted of chlorophytes and other green algae (9.0%), the mixotrophic ciliate *Mesodinium rubrum* (=*Myrionecta rubra*) (8.8%), heterotrophic dinoflagellates (1.2%) and filamentous cyanobacteria (0.7%) (Table I).

Each of the most abundant microplankton taxa showed considerable seasonal variation in abundance, usually with a single distinct peak once per year, although the timing and magnitude of these peaks also varied between years. Most notably, *Asterionella* sp. reached maximum abundance between February and April, and *A. granulata* peaked during June to July of both 2005 and 2006. *M. rubrum* was relatively low in abundance throughout 2005, but exhibited a substantial bloom from August to October 2006. This resulted in two distinct peaks in total chlorophyll *a* biomass during 2006, one in July to August and one in October, in addition to the diatom-dominated spring peak in March (Fig. 3).

We also examined the temporal pattern of microplankton biomass for each major taxonomic category within the assemblage over our 2-year study period. The results show a similar pattern of diatom dominance as observed by abundance, especially from early spring to late summer in both 2005 and 2006, yet also illustrate a substantial bloom of *M. rubrum* ciliates during late summer and autumn of 2006 (Fig. 4). Also notable is the inter-annual difference in timing and magnitude of biomass peaks for several microplankton groups, with the biomass of flagellates, chlorophytes and filamentous bacteria substantially higher in 2006 vs. 2005 (Fig. 4).

To analyze the temporal patterns of abundance and composition, microplankton taxa were assigned to three distinct assemblage sub-groups through hierarchical agglomerative clustering (Table II). The composition of these clusters was highly robust to alternative clustering approaches (Supplementary Fig. S1). There were strong differences between these assemblage clusters in terms of phenology, species composition, abundance and diversity over the sampling period. A clear pattern of seasonal succession among the identified clusters is indicated on the histogram of occurrence (Fig. 5).

Microplankton cluster 1. This cluster occurred from January through July 2005 and from February through July 2006 (Fig. 5). The three most abundant taxa (small centric diatoms, *A. granulata* and *Asterionella* sp.) comprised 62% of total abundance within this cluster (Table II), while the 10 most abundant taxa comprised 91% of total cluster abundance. Median abundance of micro-cluster 1 was 416 526 individuals m^{-3} with a 50% interquartile range of 338 438–515 500 individuals m^{-3}. Median diversity as measured by Shannon's H for micro-cluster 1 was 2.0 with a 50% interquartile range of 1.8–2.1.

Microplankton cluster 2. This group appeared in August 2005 and persisted through January 2006, but did not appear again until November 2006 and persisted through the end of sampling in December 2006 (Fig. 5). The three most abundant taxa (*A. granulata*, small centric diatoms, and *Ankistrodesmus* sp.) comprised 44% of total abundance within this cluster, while the 10 most abundant taxa comprised 78% of total cluster abundance (Table II). Total abundance of micro-cluster 2 was more than an order of magnitude lower than the other assemblage clusters. Median abundance of micro-cluster 2 was 28 282 individuals m^{-3} with a 50% interquartile range

Table II: The top 10 most abundant taxa for each of the microplankton assemblage clusters

Micro-cluster 1	% of total abundance	Micro-cluster 2	% of total abundance	Micro-cluster 3	% of total abundance
Centric diatoms (small)	22.8	*Aulacoseira granulata*	18.5	*Mesodinium rubrum*	49.6
Aulacoseira granulata	20.5	Centric diatoms (small)	15.0	*Aulacoseira granulata*	21.3
Asterionella sp.	18.9	*Ankistrodesmus* sp.	10.7	Chlorophytes	7.8
Fragilaria sp.	8.5	*Fragilaria* sp.	8.2	Heterotrophic dinoflagellate 'A'	5.7
Pennate diatoms (small)	7.8	*Mesodinium rubrum*	5.6	*Ankistrodesmus* sp.	3.4
Centric diatoms (medium)	3.7	Chlorophytes	5.2	Centric diatoms (small)	1.4
Synedra sp.	2.4	Heterotrophic dinoflagellate 'A'	4.5	*Amphora* sp.	1.4
Ankistrodesmus sp.	2.3	*Asterionella* sp.	4.0	Filamentous bacteria	1.2
Skeletonema sp.	2.3	Centric diatoms (large)	3.3	Pennate diatoms (small)	1.2
Centric diatoms (large)	2.1	Pennate diatoms (small)	3.2	*Fragilaria* sp.	0.8

of 25 361–41 736 individuals m^{-3}. Median diversity as measured by Shannon's H for micro-cluster 2 was significantly higher than the other clusters, at 2.4 with a 50% interquartile range of 2.3–2.7.

Microplankton cluster 3. Micro-cluster 3 was observed only from August to October 2005 (Fig. 5). This cluster was dominated by a very large abundance of the bloom-forming mixotrophic ciliate *M. rubrum*, accounting for 49.6% of the total abundance for this cluster. The three most abundant taxa in micro-cluster 3 (*M. rubrum*, *A. granulata* and chlorophytes) comprised 79% of total abundance, while the 10 most abundant taxa comprised 94% of total cluster abundance (Table II). Total abundance of micro-cluster 3 was the same order of magnitude as micro-cluster 1. Median abundance of assemblage micro-cluster 3 was 332 969 individuals m^{-3} with a 50% interquartile range of 319 712–389 873 individuals m^{-3}. Median diversity as measured by Shannon's H for micro-cluster 3 was significantly lower than either of the other microplankton clusters, at 1.6 with a 50% interquartile range of 1.5–2.7 ($X^2 = 12.4$, df = 2, $P = 0.002$). There were also significant differences in total abundance between clusters ($X^2 = 15.7$, df = 2, $P = 3.4 \times 10^{-4}$).

Environmental correlates with the microplankton assemblage

A 2D NMDS ordination of the microplankton assemblage data resulted in a stress value of 0.07, and the three microplankton clusters were plotted as an interpretive overlay on the NMDS ordination (Fig. 6). Results from 1000 independent permutations of the microplankton assemblage matrix using the Dexter *et al.* (2018) permutation test allowed us to reject the hypothesis that the observed stress value of 0.07 could arise from stochastic sampling effects ($z = -17.3$; $P < 0.001$).

Strong correlations were observed between ordination structure and several biological factors (rotifers, cyclopoids and chlorophyll), physical factors (month, light, river flow, salinity and temperature) and chemical factors (phosphorus). These environmental correlates are plotted as vectors on the ordination of microplankton assemblage data (Fig. 6). Salinity and river flow showed a strong but highly non-linear correlation and thus are visualized as topographic isoclines in separate plots (Supplementary Figs S2 and S3). In general, high flow/low salinity conditions were strongly associated with micro-cluster 1, which strongly contrasts with the low flow/high salinity regime associated with micro-clusters 2 and 3.

A complete listing of the environmental variables significantly correlated with microplankton taxa, and their association scores, is presented in Table III. In general, points situated on the right side of the NMDS ordination (micro-clusters 1 and 3; Fig. 7) were associated with increasing abundances of cyclopoid copepods and rotifers, elevated chlorophyll concentrations, and, to a lesser extent, increasing light levels. Points situated on the left side of the ordination (micro-cluster 2; Fig. 6) are associated with increasing concentrations of phosphorus. The spread of points along ordination axis 2 is associated with seasonal environmental changes (i.e. temperature and light availability).

Zooplankton

Composition and phenology of the zooplankton assemblage

Across the period of our study, the three most abundant zooplankton taxa were copepod nauplii, small unidentified rotifers and *Asplanchna* spp. (Rotifera). These three taxa comprised 74.2% of all zooplankton abundance, while the remainder of the zooplankton assemblage consisted of a relatively diverse set of copepod, cladoceran and rotifer taxa (Table IV). Of particular note, the invasive calanoid copepod, *Pseudodiaptomus forbesi,* comprised 4.0% of total abundance.

Table III: Environmental correlates of microplankton ordination scores. These were assessed via the 'ordisurf' function in the R package 'vegan,' which allows for non-linear correlations based upon a generalized additive model with penalized splines

Variable	Deviance explained (%)	*F* statistic	Estimated deg. of freedom	Uncorrected *P*-value	Corrected *P*-value
Salinity (mean)	81.2	6.55	7.6	8.0E-05	0.002*
Light	67.8	3.74	4.7	2.1E-04	0.002*
Chlorophyll	72.9	4.42	6.0	2.9E-04	0.002*
Cyclopoid copepods	63.9	3.21	4.3	3.3E-04	0.002*
Rotifera	63.8	3.20	4.3	3.4E-04	0.002*
Month (circularized)	68.1	3.65	5.2	4.4E-04	0.002*
Phosphorus	60.5	2.73	4.2	0.001	0.004*
Temperature (mean)	66.1	3.24	5.3	0.001	0.004*
River flow	68.3	3.25	6.4	0.004	0.011*
Nitrogen	33.1	0.99	1.6	0.012	0.031*
Upwelling	34.3	1.00	2.0	0.014	0.034*
Zooplankton	29.7	0.83	1.6	0.019	0.043*
Nanoplankton biomass	39.6	1.12	3.0	0.021	0.044*
Cladocera	35.4	0.95	2.6	0.023	0.046*
Copepod nauplii	27.3	0.72	1.5	0.027	0.047*
Calanoid copepods	27.0	0.71	1.5	0.029	0.047*
Copepodites	22.5	0.54	1.4	0.051	0.073
Silica	31.2	0.72	2.7	0.073	0.100

Asterisks indicate *P*-value < 0.05.

Table IV: Top 15 most abundant zooplankton taxa from 2005 to 2006

Taxon	% of total abundance
Copepod nauplii	40.7
Small unidentified rotifers	22.0
Asplanchna spp.	11.5
Coullana canadensis	4.1
Pseudodiaptomus forbesi	4.0
Keratella spp.	3.4
Eurytemora affinis	3.0
Polychaete larvae	2.7
Brachionus spp.	2.3
Cirripedia larvae	1.8
Bosmina longirostris	0.9
Polyarthra spp.	0.8
Pseudobradya spp.	0.5
Kellicottia spp.	0.4
Diacyclops thomasi	0.3

As observed in the microplankton assemblage, the abundances of many zooplankton taxa were highly variable seasonally and between years (Fig. 7). For instance, *P. forbesi* showed a distinct peak in August and September of both years, and the abundance was more than seven times higher in 2005 (Fig. 7A). Similarly, the abundance of the harpacticoid copepod *Coullana canadensis* was somewhat higher in autumn 2006 than the rest of that year, but spiked upward by as much as 10-fold during three different months in 2005 (Fig. 7B). *Asplanchna* spp. abundance was generally low during 2005, but peaked sharply in April 2005 (Fig. 7C).

Hierarchical agglomerative clustering of zooplankton data defined two assemblage clusters that differed with respect to phenology, total abundance and species composition.

Zooplankton cluster 1. This cluster was observed in January and February 2005 and again from November 2005 to January 2006. This cluster was absent for 9 months, and then re-emerged in November 2006 (Fig. 8). The three most abundant taxa in zoop-cluster 1 (Polychaete larvae, Rotifera and Cirripedia larvae) comprised 81% of total abundance within this group, while the 10 most abundant taxa comprised 94% of total zoop-cluster abundance (Table V). Within zoop-cluster 1, median abundance was 2 137 individuals m^{-3} with a 50% interquartile range of 1 489–3 536 individuals m^{-3}. Median diversity as measured by Shannon's H for group 1 was 1.3 with a 50% interquartile range of 1.0–1.9.

Zooplankton cluster 2. Zoop-cluster 2 was present during March–October 2005, and from February to October 2006 (Fig. 8). Total abundance within zoop-cluster 2 was approximately an order of magnitude greater than zoop-cluster 1. The three most abundant taxa (Rotifera, *Asplanchna* spp. and *C. canadensis*) comprised 65% of total abundance in this cluster, while the 10 most abundant taxa comprised 94% of total zoop-cluster abundance (Table V). The invasive calanoid copepod *P. forbesi* comprised 6.9% of total cluster abundance, but in some months (e.g. September 2005) comprised greater than 90% of total assemblage abundance. Within zoop-cluster 2, median abundance was 17 217 individuals m^{-3} with a 50% interquartile range of 11 040–32 149 individuals m^{-3} (Fig. 10A). Median diversity as measured by Shannon's H for zoop-cluster 2 was 1.4 with a 50%

Table V: The top 10 most abundant taxa for each of the two zooplankton assemblage clusters

Zoop-cluster 1	% of total abundance	Zoop-cluster 2	% of total abundance
Polychaete larvae	57.7	Rotifera	37.9
Rotifera	19.6	*Asplanchna* spp.	20.1
Cirripedia larvae	3.6	*Coullana canadensis*	7.1
Eurytemora affinis	3.2	*Pseudodiaptomus forbesi*	6.9
Pseudobradya spp.	2.9	*Keratella* spp.	6.0
Nematoda	1.7	*Eurytemora affinis*	5.1
Asplanchna spp.	1.6	*Brachionus* spp.	4.0
Paracalanus parvus	1.5	Cirripedia larvae	3.1
Brachionus spp.	1.2	Polychaete larvae	2.7
Acartia tonsa	1.1	*Bosmina longirostris*	1.6

Table VI: Environmental correlates of zooplankton ordination scores. These were assessed via the 'ordisurf' function in the R package 'vegan,' which allows for non-linear correlations based upon a generalized additive model with penalized splines

Variable	Deviance explained (%)	*F* statistic	Estimated deg. of freedom	Uncorrected *P*-value	Corrected *P*-value
Month (circularized)	71.5	4.30	5.4	2.0E-04	0.002*
Temperature (mean)	68.4	3.75	5.1	4.3E-04	0.003*
Upwelling	55.9	2.14	4.3	0.004	0.018*
Nitrogen	51.7	1.75	4.3	0.013	0.043*
Nanoplankton	50.0	1.65	4.1	0.013	0.043*
Chlorophytes	23.8	0.59	1.5	0.043	0.125

Asterisks indicate P-values < 0.05.

interquartile range of 1.2–1.6. Levels of diversity between zoop-clusters were not significantly different as assessed via Kruskal–Wallis one-way ANOVA on ranks ($X^2 = 0.081$, df = 1, $P = 0.775$), while the abundance of zoop-cluster 2 taxa was significantly higher than the abundance of taxa in zoop-cluster 1 ($X^2 = 12.9$, df = 1, $P = 3.3 \times 10^{-4}$).

Environmental correlates with the zooplankton assemblage

A 2D NMDS ordination of zooplankton assemblage data was produced with a resultant stress value of 0.16, and the two zooplankton clusters are plotted as an interpretive overlay on the corresponding NMDS ordination (Fig. 9). Results from 1000 independent permutations of the zooplankton assemblage matrix using the Dexter *et al.* (2018) permutation test allowed us to reject the hypothesis that the observed stress value of 0.16 could arise from stochastic sampling effects ($z = -7.5$; $P < 0.001$). As the boundary between clusters was sensitive to changes in the clustering algorithm (Supplementary Fig. S4), we regarded points in the center of the ordination as transitional between the two zooplankton clusters.

Strong correlations were observed between ordination structure in the zooplankton and several biological factors (nanoplankton biomass, chlorophyte biomass), physical factors (upwelling, temperature) and chemical factors (nitrogen). These environmental correlates are plotted as vectors on the ordination of zooplankton assemblage data (Fig. 9). In cases where environmental correlates were significant but strongly non-linear (e.g. nitrogen and nanoplankton), such associations are instead visualized as topographic isoclines (Supplementary Figs S5 and S6). A complete listing of the significantly correlated environmental variables and their association scores is presented in Table VI.

The ordination illustrates a strong signal of seasonality, with samples positioned on the left showing a strong association with winter, and samples on the right strongly associated with increasing temperatures and upwelling. The relationship of nitrogen and nanoplankton to the zooplankton ordination scores is substantially non-linear across ordination space (Supplementary Figs S5 and S6), but a general trend of decrease in both factors can be observed from the top to the bottom of ordination axis 2 (Fig. 9).

DISCUSSION

Our goals in this 2-year study were to quantify the strength and temporal variability of the biotic and abiotic environmental factors influencing planktonic community structure in the lower CRE, the second largest river estuary on the Pacific coast of North America. Such comprehensive assessments of estuarine plankton abundance

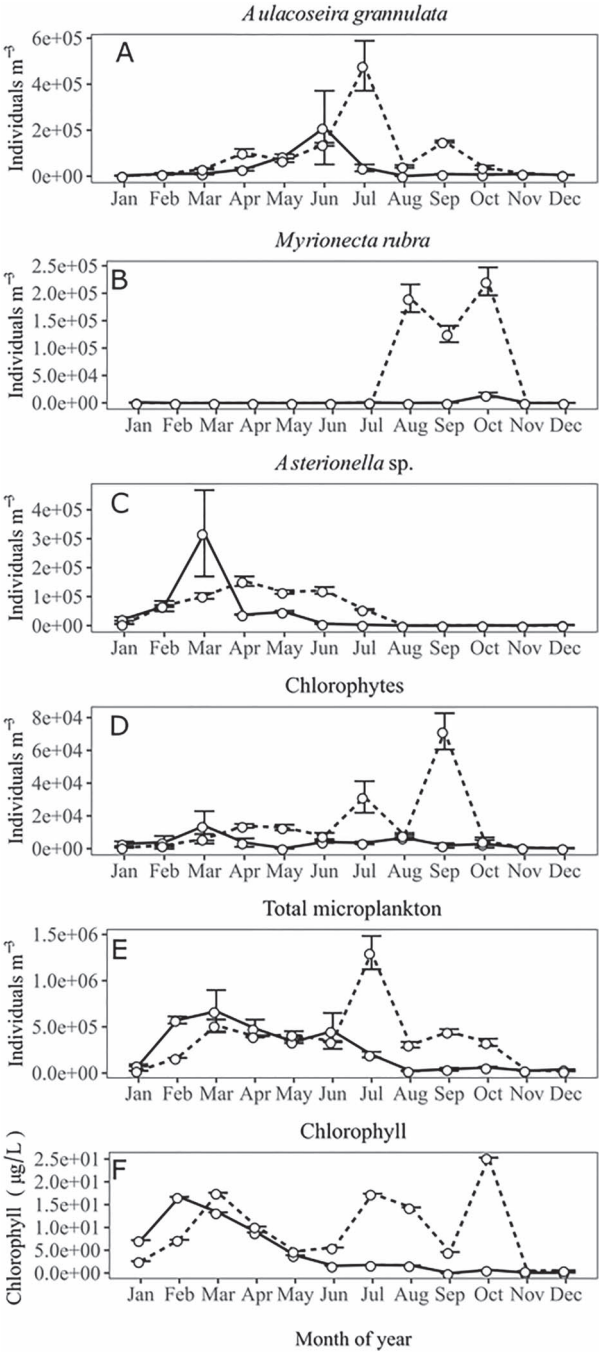

Fig. 3. Patterns of microplankton abundance. Mean (±standard error) abundance of *A. granulata* (**A**), *M. rubrum* (**B**), *Asterionella* spp. (**C**), Chlorophytes (**D**), total microplankton (**E**) and mean water column chlorophyll *a* biomass (**F**). Solid lines represent data from 2005, and dashed lines represent data from 2006.

and composition across numerous size and functional groupings, as well as detailed measurements over a wide range of associated environmental conditions, are somewhat rare in the literature. Yet these investigations allow for identification of important large-scale ecological patterns and associations that may point to underlying biotic and abiotic processes that structure estuarine planktonic communities.

Temporal patterns in plankton abundance and composition

Our observation of a distinct seasonal succession in microplankton and zooplankton assemblages in the lower CRE generally aligns with observations reported from other estuarine systems. For instance, diatoms dominated the microplankton in the CRE during spring and summer, shifting to either the chlorophytes or the photosynthetic ciliate *M. rubrum* in late summer and early autumn. In the Schelde estuary in Belgium, diatoms dominated most of the year, but shifted from large centric diatoms (*Actinocyclus normannii* and *Stephanodiscus hantzschii*) in late winter to smaller, chain-forming species (*A. granulata*) along with chlorophytes in summer (Muylaert *et al*., 2006), a seasonal pattern and set of taxa very similar to those in the CRE.

For zooplankton, a mix of omnivorous, predatory, and small algivorous rotifer species were dominant in the lower CRE during spring, while small rotifers mostly dominated during summer and early autumn. Several other large estuaries have high abundances of rotifers during non-winter, e.g. SFE (Bollens *et al*., 2011) and show a temporal dominance shift from algivorous taxa (*Brachionus* spp. and *Synchaeta* spp.) in spring to smaller, more heat-tolerant taxa (e.g. *Trichocerca* spp.) in summer, including Elbe Estuary (Holst *et al*., 1998), Chesapeake Bay (Park and Marshall, 2000) and Schelde Estuary (Lionard *et al*., 2005).

In the CRE, the Asian calanoid copepod *P. forbesi* appeared in large numbers during late summer/autumn and shifted the overall zooplankton assemblage to one dominated by crustaceans. *P. forbesi* is invasive and highly abundant seasonally in the lower Columbia River (Cordell *et al*., 2008; Dexter *et al*., 2015, 2020; Emerson *et al*., 2015), and in the SFE (Orsi and Walter, 1991; Kimmerer *et al*., 1998; Bollens *et al*., 2011; Bollens *et al*., 2012; Kayfetz and Kimmerer, 2017).

Plankton abundances and diversity in the lower CRE from late winter to early summer were consistent from 2005 to 2006, but differed substantially between years during the late summer and autumn. In July 2006, *Asterionella* sp. diatoms were nearly three times more abundant than the rest of the year, and in August 2006 *M. rubrum* created a 'red water' bloom in the lower estuary, which disappeared in November. Large late summer blooms of *M. rubrum* are common in temperate estuaries, most likely due to warm temperatures, high salinity and stratification that reduces turbulence and allows cells to accumulate (Crawford *et al*., 1997; Johnson *et al*., 2013). The bloom

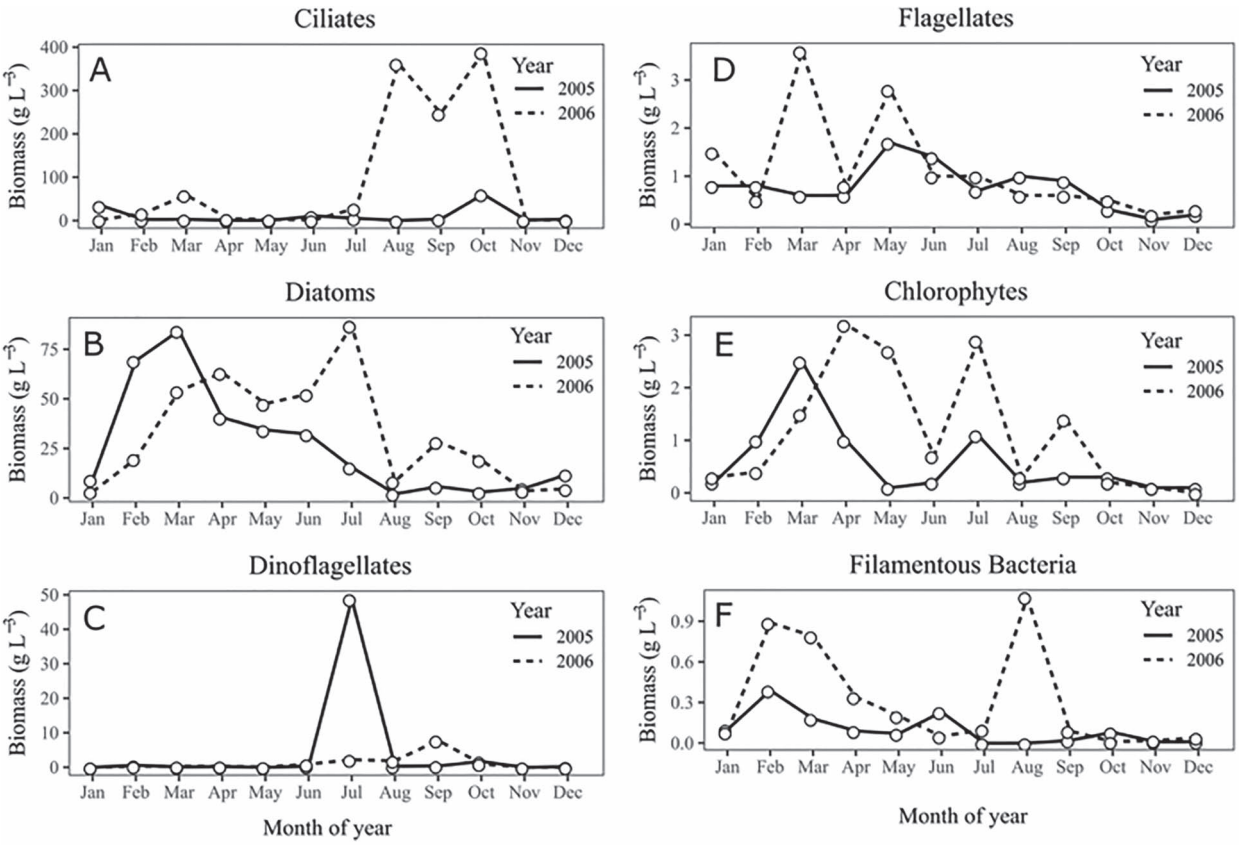

Fig. 4. Biomass of microplankton taxonomic groups. Ciliates (**A**), diatoms (**B**), dinoflagellates (**C**), flagellates (**D**), chlorophytes (**E**) and filamentous bacteria (**F**). Solid lines represent data from 2005, and dashed lines represent data from 2006.

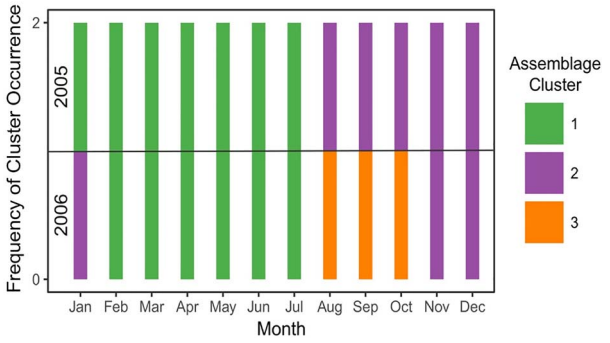

Fig. 5. Pattern of microplankton assemblage cluster occurrence. The upper portion represents monthly occurrence in 2005 and the lower portion represents monthly occurrence in 2006.

of *M. rubrum* we observed during 2006 was strongly associated with warm temperatures that persisted over a 5-month period from July to November, conditions similar to those reported during other *M. rubrum* blooms in the lower CRE (Herfort *et al*., 2011, 2012).

On the other hand, abundances of both the invasive copepod *P. forbesi* and the harpacticoid copepod *C. canadensis* were substantially higher in autumn 2005 than in 2006. Breckenridge *et al*. (2015) noted the same pattern in *P. forbesi* abundance in the CRE from samples collected in the main channel of the lower estuary. However, in an analysis of a 8.5-year data set of zooplankton abundance at a tidally-influenced fresh water location in the upper CRE, Dexter *et al*. (2015) found higher overall abundances of *P. forbesi* and only minimal interannual variation in the magnitude of maximum abundance. Possible explanations for the low abundance of *P. forbesi* during autumn 2006 include lower temperatures in the estuary than in 2005 and very high river flow rates during spring 2006 that may have flushed overwintering stages out of the estuary into the coastal ocean (Breckenridge *et al*., 2015).

Biotic and abiotic factors influencing plankton community structure

Our results illustrate a strong seasonal nature to the associations between the plankton assemblages and their bio-physical environment in the lower CRE, summarized

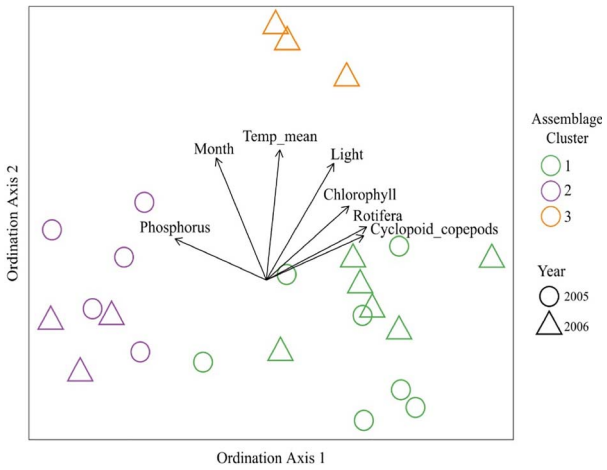

Fig. 6. The NMDS ordination of microplankton assemblage data with several interpretive overlays. Environmental correlates are plotted as linear vectors, and points are colored by cluster identity. Note that salinity and river flow showed non-linear correlations with microplankton clusters and are visualized as topographic isoclines in separate plots (Supplementary Figs S2 and S3). High flow/low-salinity conditions were strongly associated with micro-cluster 1, while a low flow/high-salinity regime was associated with micro-clusters 2 and 3.

in Figure 10. From January to June in 2005 and 2006, as river flow increased to its maximum, we observed strong associations of diatoms (mainly *Asterionella* sp.) with rotifers and cyclopoid copepods (primarily *Diacyclops thomasi*). This was not surprising, given that these zooplankton groups are associated with low-salinity environments and are common grazers of phytoplankton in estuaries (Sellner *et al.*, 1993; Rollwagen-Bollens and Penry, 2003; Lionard *et al.*, 2005; Gifford *et al.*, 2007; Chang *et al.*, 2010). There was also a significant, but non-linear, association of the spring microplankton assemblage with low salinity and high river flow, as well as an association with light availability, the latter likely due to co-variation with season (circularized month) and thus a typical response of temperate phytoplankton with the onset of spring. Also during this period, zooplankton abundance was strongly associated with warming temperatures and increasing upwelling strength, and somewhat with nanoplankton biomass.

The association of the zooplankton with upwelling index during this spring-to-summer transition likely represents an increasing marine influence on the estuarine system, as river flow decreased and temperature increased. Indeed, the CRE is located immediately adjacent to a strong coastal upwelling system that brings deep, nutrient-rich and often oxygen-poor waters onto the continental shelf and directly into the estuary during summer months when river flow is low (Roegner *et al.*, 2011a, b). Other investigators have observed the phytoplankton community in the lower CRE to be strongly influenced by coastal upwelling, with riverine taxa

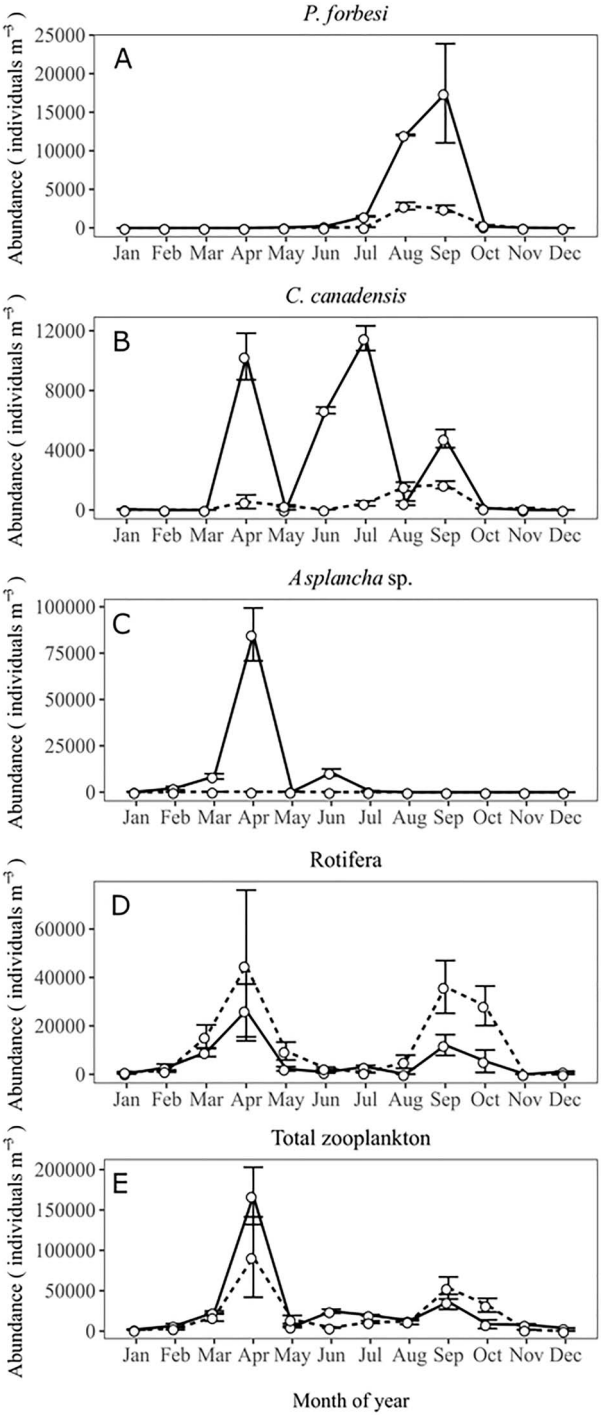

Fig. 7. Patterns of zooplankton abundance. Mean (±standard error) abundance of *P. forbesi* (**A**), *C. canadensis* (**B**), *Asplanchna* sp. (**C**), small rotifers (**D**) and total zooplankton (**E**). Solid lines represent data from 2005, and dashed lines represent data from 2006.

dominating during springtime high river flow and marine taxa advected into the estuary during summertime low river flow periods (Roegner *et al.*, 2011a). Our finding that zooplankton community composition also varies

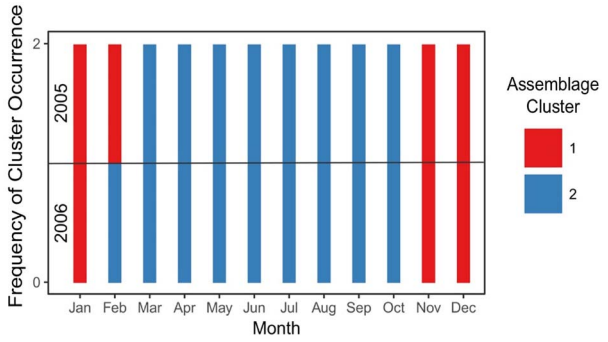

Fig. 8. Pattern of zooplankton assemblage cluster occurrence. The top section represents monthly occurrence in 2005 and the lower section represents monthly occurrence in 2006.

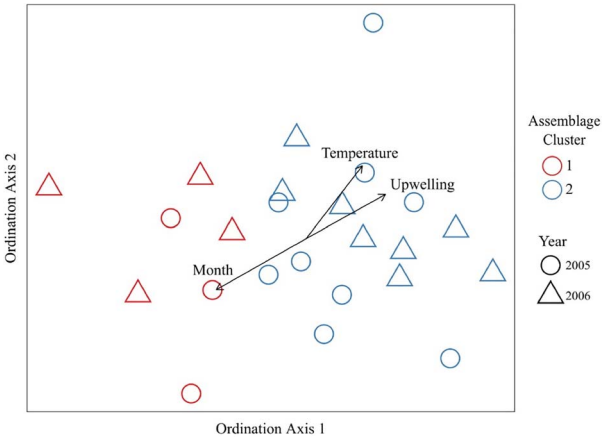

Fig. 9. The NMDS ordination of zooplankton assemblage data with several interpretive overlays. Environmental correlates are plotted as linear vectors, and points are colored by cluster identity.

with upwelling strength further illustrates the influence of this important, physical process on Northeast Pacific estuaries. We did not observe significant associations between tidal stage and any of the microplankton or zooplankton assemblage clusters in the CRE, as has been documented in other estuaries (e.g. Schlacher and Wooldridge, 1995; da Costa *et al*., 2013; Ricardo *et al*., 2014), but our monthly sampling frequency was unlikely to have allowed for assessment of tidal effects (Marques *et al*., 2009).

By contrast, during winter months (November to January), during lower river flow and higher salinity, microplankton abundance (mostly the diatoms *A. granulata* and *Ankistrodesmus* sp.) was relatively low and the assemblage was most closely associated with inorganic phosphate concentrations, while the zooplankton winter assemblage (dominated by polychaete larvae and mixed rotifer taxa) was associated with colder winter temperatures. Under these conditions, the lack of an association of diatoms with grazers was likely due to the zooplankton community being dominated by coastal polychaete larvae that were flushed into the estuary during low upwelling periods and low river flow. These larvae do not typically survive well on a diet of chain-forming diatoms such as *A. granulata* (Leung and Cheung, 2017).

Overall, our findings from a single location sampled monthly for 2 years are consistent with a cross-channel study of the lower CRE by Breckenridge *et al*. (2015), who also observed distinct plankton assemblages associated with late summer, low-flow periods dominated by *M. rubrum* and *P. forbesi*, and spring, high-flow periods dominated by *Aulacoseira* sp. diatoms and the native copepod *Eurytemora affinis*. This suggests that our sampling program is generally indicative of the lower CRE as a whole. Our results reported here further extend our understanding of the system, suggesting that when the Columbia River experiences high flow, biotic factors strongly influence microplankton assemblage structure, but when river flow is low and there is more marine influence, abiotic factors are more important to planktonic primary producers (Fig. 10). Like many large field studies of planktonic community dynamics, we did not sample larger predators, and thus cannot assess their biotic and abiotic impacts on zooplankton abundance and diversity. However we did find that, unlike the microplankton, the zooplankton assemblage was most associated with abiotic factors regardless of river flow (Fig. 10).

Because previous studies of estuarine zooplankton have dealt mainly with abiotic factors, it is somewhat difficult to compare these with our results about the effects of environmental factors on the plankton in the CRE. Some authors have concluded that including biotic factors in their investigation of the effects of physical forces on plankton communities could have helped to improve the sometimes low explanatory power of their statistical analyses (e.g. Selleslagh *et al*., 2012). Our results indicating a strong association of abiotic factors (temperature, upwelling index) with the zooplankton, dominated at most times of year by rotifers, do align with observations from other river estuaries. For example, rotifer abundance was found to be highly correlated with physico-chemical factors (temperature, salinity and Secchi depth) in the Pearl River Estuary in China (Wang *et al*., 2009) and the Bahia Blanca Estuary in Argentina (Barría de Cao *et al*., 2011) throughout the annual cycle.

However, our finding that biotic factors were strongly associated with the largely autotrophic microplankton assemblage in the CRE, especially during high flow and low salinity, has not been reported in studies of other estuaries. Most investigations reported the strong influence of physical factors, such as light availability and temperature, e.g. Schelde Estuary (Muylaert *et al*., 2000), river discharge, e.g. Sheldt Estuary (Naithani *et al*., 2016)

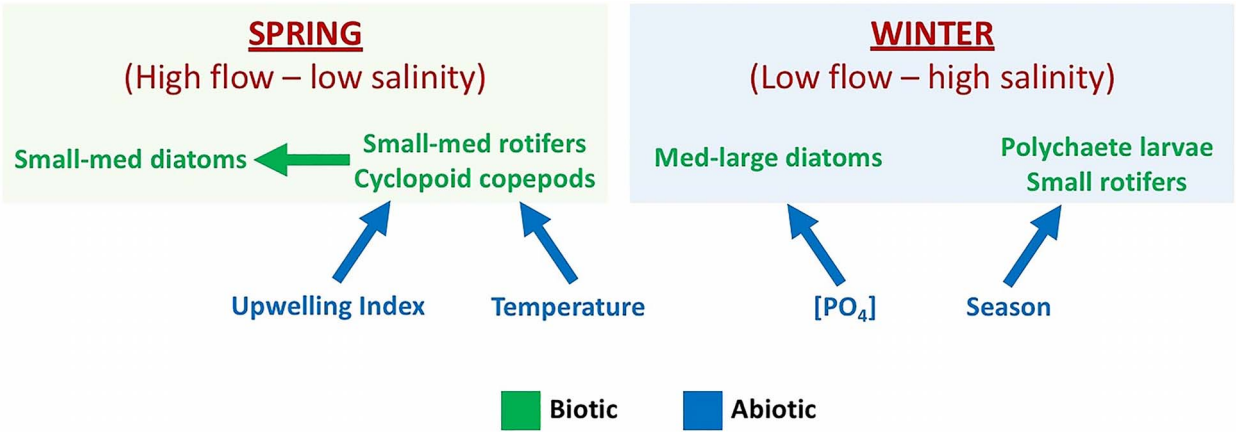

Fig. 10. Illustration of the seasonal variability in the associations of biotic and abiotic factors with microplankton and zooplankton assemblages in the CRE.

or the combination of upwelling index and river flow, e.g. Coruna Estuary (Bode *et al*., 2017), on the temporal variability of phytoplankton. However, since these studies did not explicitly test the influence of biotic factors on these communities, we cannot assess their importance in these estuaries relative to abiotic factors.

Finally, while our study did not measure the trophic relationships among planktonic taxa, our results provide an interesting frame through which to consider potential relationships between the physical environment and trophic interactions within plankton assemblages in the lower CRE. We observed seasonal differences in assemblage structure to be most pronounced among the microplankton, particularly autotrophic taxa (especially diatoms, *M. rubrum* and chlorophytes). During spring and early summer, when river flows were high and salinity low, the microplankton assemblage was most associated with their potential grazers, e.g. the significant associations between microplankton abundance and rotifers (mainly the omnivorous *Asplanchna* spp.) and *D. thomasi*, a cyclopoid copepod known to consume diatoms and ciliates in the lower Columbia River and associated tidal floodplain lakes (Rollwagen-Bollens *et al*., 2013). However, during autumn and winter, when flows were lower and salinity higher, microplankton abundance and diversity was strongly associated with dissolved phosphorus concentrations.

The results of other studies examining the relationship between environmental factors and biotic factors in estuarine plankton communities are mixed. In the Bahia Blanca Estuary, Guinder *et al*. (2017) concluded that grazing by an invasive copepod was the strongest control on phytoplankton blooms there. Conversely, York *et al*. (2014) found that the effect of copepod grazers was minimal on primary producers in the upper reaches of the SFE and concluded that grazing influences were not important to structuring the phytoplankton assemblage. Rollwagen-Bollens *et al*. (2006) showed similar results in the lower SFE, where grazing by the abundant copepod taxon *Acartia* spp. had a greater impact on heterotrophic protist grazers than on autotrophic plankton. And in the York River Estuary in Chesapeake Bay, the investigators observed a lack of grazing effects on the phytoplankton (Sin *et al*., 2006).

Both terrestrial and aquatic ecologists have begun to converge on the notion that the relative influence of top-down (i.e. predation, grazing) vs. bottom-up (i.e. nutrient availability) drivers is strongly case dependent, and may vary with scale, abiotic factors and the particular taxa present (Hansson *et al*., 2004). Our findings generally supports this conclusion, with the relative influence of biotic and abiotic drivers in the CRE varying with season and size class of plankton (which roughly approximated trophic level in our study).

CONCLUSION

The lower CRE is a highly dynamic system with a strong seasonal signal in both its physical-hydrologic regime and its planktonic community structure. When river flow is high during the late spring, the lower estuary is dominated by low salinity-adapted plankton and the abundance of zooplankton grazers has as great or greater an influence on the microplankton assemblage than abiotic factors or resource availability. Conversely, when river flow is low in early winter, nutrient (*P*) availability exerts a strong influence on the microplankton, and the zooplankton assemblage in the lower CRE is most influenced by temperature and upwelling strength. These results indicate

that seasonally variable environmental conditions modulate the relative importance of biotic and abiotic factors in structuring plankton communities in this large river estuary. We recommend that more process-oriented studies be undertaken in the future to examine specific mechanisms likely to underlie the associations of coastal upwelling, river flow, nutrient limitation and grazing with plankton community structure.

SUPPLEMENTARY DATA

Supplementary data can be found online at http://plankt.oxfordjournals.org.

ACKNOWLEDGEMENTS

We wish to thank Angela Gibson, Olga Kalata and Joanne Breckenridge for assistance with microscopical analyses, taxonomic identifications, statistical analyses and data processing. In addition, space used during manuscript preparation was generously provided to G.R.B. and S.B. by the Oceans Institute and the School of Civil, Environmental and Mining Engineering at the University of Western Australia.

FUNDING

This work was supported by Washington State University to S.B. for field collections and sample analyses; manuscript preparation was supported by Washington Sea Grant #R/OLWD-2 to G.R.B. and S.B. and EPA STAR Grant #FP 917809-01-0 to E.D. and S.B.

REFERENCES

Barría de Cao, M. S., Piccolo, M. C. and Perillo, G. M. (2011) Biomass and microzooplankton seasonal assemblages in the Bahía Blanca estuary, Argentinean coast. *J. Mar. Biol. Assoc. U. K.*, **91**, 953–959.

Bode, A., Varela, M., Prego, R., Rozada, F. and Santos, M. D. (2017) The relative effects of upwelling and river flow on the phytoplankton diversity patterns in the ria of a Coruña (NW Spain). *Mar. Biol.*, **164**, 93.

Bollens, S., Breckenridge, J., Cordell, J., Rollwagen-Bollens, G. and Kalata, O. (2012) Invasive copepods in the lower Columbia River estuary: seasonal abundance, co-occurrence and potential competition with native copepods. *Aq. Invasions*, **7**, 101–109.

Bollens, S. M., Breckenridge, J. K., Vanden Hooff, R. C. and Cordell, J. R. (2011) Mesozooplankton of the lower San Francisco estuary: spatio-temporal patterns, ENSO effects and the prevalence of non-indigenous species. *J. Plankton Res.*, **33**, 1358–1377.

Bollens, S. M., Cordell, J. R., Avent, S. and Hooff, R. (2002) Zooplankton invasions: a brief review, plus two case studies from the Northeast Pacific Ocean. *Hydrobiologia*, **480**, 87–110.

Bollens, S. M., vanden Hooff, R., Butler, M., Cordell, J. R. and Frost, B. W. (2010) Feeding ecology of juvenile Pacific salmon (*Oncorhynchus* spp.) in a Northeast Pacific fjord: diet, availability of zooplankton, selectivity for prey, and potential competition for prey resources. *Fish. Bull.*, **108**, 393–407.

Breckenridge, J. K., Bollens, S. M., Rollwagen-Bollens, G. and Roegner, G. C. (2015) Plankton assemblage variability in a river-dominated temperate estuary during late spring (high-flow) and late summer (low-flow) periods. *Estuaries Coast*, **38**, 93–103.

Bricker, S. B., Longstaff, B., Dennison, W., Jones, A., Boicourt, K., Wicksb, C. and Woernerc, J. (2008) Effects of nutrient enrichment in the nation's estuaries: a decade of change. *Harmful Algae*, **8**, 21–32.

Carstensen, J., Henriksen, P. and Heiskanen, A.-S. (2007) Summer algal blooms in shallow estuaries: definition, mechanisms, and link to eutrophication. *Limnol. Oceanogr.*, **52**, 370–384.

Chang, K.-H., Doi, H., Nishibe, Y. and Nakano, S. (2010) Feeding habits of omnivorous Asplanchna: comparison of diet composition among *Asplanchna herricki*, *A. priodonta* and *A. girodi* in pond ecosystems. *J. Limnol.*, **69**, 209.

Chawla, A., Jay, D. A., Baptista, A. M., Wilkin, M. and Seaton, C. (2008) Seasonal variability and estuary–shelf interactions in circulation dynamics of a river-dominated estuary. *Estuaries Coast*, **31**, 269–288.

Clarke, K. R. (1993) Non-parametric multivariate analyses of changes in community structure. *Aust. J. Ecol.*, **18**, 117–143.

Cloern, J. E., Foster, S. Q. and Kleckner, A. E. (2014) Phytoplankton primary production in the world's estuarine-coastal ecosystems. *Biogeosciences*, **11**, 2477–2501.

Cordell, J. R., Bollens, S. M., Draheim, R. and Sytsma, M. (2008) Asian copepods on the move: recent invasions in the Columbia–Snake River system. *USA. ICES J. Mar. Sci.*, **65**, 753–758.

DA Costa, K. G., Bezerra, T. R., Monteiro, M. C., Vallinoto, M., Berrêdo, J. F., DA Costa, R. M. and LCC, P. (2013) Tidal-induced changes in the zooplankton community of an Amazon estuary. *J. Coast. Res.*, **289**, 756–765.

Crawford, D. W., Purdie, D. A., Lockwood, A. P. M. and Weissman, P. (1997) Recurrent red-tides in the Southampton water estuary caused by the phototrophic ciliate *Mesodinium rubrum*. *Estuar. Coast. Shelf Sci.*, **45**, 799–812.

Dexter, E. and Bollens, S. (2019) Zooplankton invasions in the early 21st century: a global survey of recent studies and recommendations for future research. *Hydrobiologia*.

Dexter, E., Bollens, S. M., Rollwagen-Bollens, G., Emerson, J. and Zimmerman, J. (2015) Persistent vs. ephemeral invasions: 8.5 years of zooplankton community dynamics in the Columbia River. *Limnol. Oceanogr.*, **60**, 527–539.

Dexter, E., Rollwagen-Bollens, G. and Bollens, S. M. (2018) The trouble with stress: a flexible method for the evaluation of nonmetric multidimensional scaling. *Limnol. Oceanogr.: Methods*, **16**, 434–443.

Dexter, E., Bollens, S. M., Cordell, J. and Rollwagen-Bollens, G. (2020) Zooplankton invasion on a grand scale: insights from a 20-year time-series across 38 Northeast Pacific estuaries. *Ecosphere*, In Press.

Dupuy, C., Talarmin, A., Hartmann, H. J., Delmas, D., Courties, C. and Marquis, E. (2011) Community structure and grazing of the nano-microzooplankton on the continental shelf of the Bay of Biscay. *Estuar. Coast. Shelf Sci.*, **95**, 1–13.

Emerson, J., Bollens, S. and Counihan, T. (2015) Seasonal dynamics of zooplankton in Columbia–Snake River reservoirs, with special emphasis on the invasive copepod *Pseudodiaptomus forbesi*. *Aq. Invasions*, **10**, 25–40.

Field, J., Clarke, K. and Warwick, R. (1982) A practical strategy for analysing multispecies distribution patterns. *Mar. Ecol. Prog. Ser.*, **8**, 37–52.

Gallegos, C. L., Jordan, T. E. and Hedrick, S. S. (2010) Long-term dynamics of phytoplankton in the Rhode River, Maryland (USA). *Estuaries Coast*, **33**, 471–484.

Gifford, S., Rollwagen-Bollens, G. and Bollens, S. (2007) Mesozooplankton omnivory in the upper San Francisco estuary. *Mar. Ecol. Prog. Ser.*, **348**, 33–46.

Goertler, P. A. L., Simenstad, C. A., Bottom, D. L., Hinton, S. and Stamatiou, L. (2016) Estuarine habitat and demographic factors affect juvenile Chinook (*Oncorhynchus tshawytscha*) growth variability in a large freshwater tidal estuary. *Estuaries Coast*, **39**, 542–559.

Graham, E. S. and Bollens, S. M. (2010) Macrozooplankton community dynamics in relation to environmental variables in Willapa Bay, Washington, USA. *Estuaries Coast*, **33**, 182–194.

Guinder, V. A., Molinero, J. C., López Abbate, C. M., Berasategui, A. A., Popovich, C. A., Spetter, C., Morcovecchio, J. and Freije, H. (2017) Phenological changes of blooming diatoms promoted by compound bottom-up and top-down controls. *Estuaries Coast*, **40**, 95–104.

Haertel, L. and Osterberg, C. (1967) Ecology of zooplankton, benthos and fishes in the Columbia River estuary. *Ecology*, **48**, 459–472.

Haertel, L., Osterberg, C., Curl, H. and Park, P. K. (1969) Nutrient and plankton ecology of the Columbia River estuary. *Ecology*, **50**, 962–978.

Hansson, L.-A., Gyllstrom, M., Stahl-Delbanco, A. and Svensson, M. (2004) Responses to fish predation and nutrients by plankton at different levels of taxonomic resolution. *Freshwater Biol.*, **49**, 1538–1550.

Herfort, L., Peterson, T. D., Campbell, V., Futrell, S. and Zuber, P. (2011) *Myrionecta rubra* (*Mesodinium rubrum*) bloom initiation in the Columbia River estuary. *Estuar. Coast. Shelf Sci.*, **95**, 440–446.

Herfort, L., Peterson, T. D., Prahl, F. G., McCue, L. A., Needoba, J. A., Crump, B., Reogner, G. C., Campbell, V. *et al.* (2012) Red waters of *Myrionecta rubra* are biogeochemical hotspots for the Columbia River estuary with impacts on primary/secondary productions and nutrient cycles. *Estuaries Coast*, **35**, 878–891.

Hickey, B. M., Pietrafesa, L. J., Jay, D. A. and Boicourt, W. C. (1998) The Columbia River plume study: subtidal variability in the velocity and salinity fields. *J. Geophys. Res.: Oceans*, **103**, 10339–10368.

Hillebrand, H., Dürselen, C.-D., Kirschtel, D., Pollingher, U. and Zohary, T. (1999) Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.*, **35**, 403–424.

Holst, H., Zimmermann, H., Kausch, H. and Koste, W. (1998) Temporal and spatial dynamics of planktonic rotifers in the Elbe estuary during spring. *Estuar. Coast. Shelf Sci.*, **47**, 261–273.

Jay, D. A. and Smith, J. D. (1990) Circulation, density distribution and neap-spring transitions in the Columbia River estuary. *Prog. Oceanogr.*, **25**, 81–112.

Johnson, M. D., Stoecker, D. K. and Marshall, H. G. (2013) Seasonal dynamics of *Mesodinium rubrum* in Chesapeake Bay. *J. Plankton Res.*, **35**, 877–893.

Jones, K. K., Simenstad, C. A., Higley, D. L. and Bottom, D. L. (1990) Community structure, distribution, and standing stock of benthos, epibenthos, and plankton in the Columbia River estuary. *Prog. Oceanogr.*, **25**, 211–241.

Kaufman, L. and Rousseeuw, P. (1990) *Finding Groups in Data: An Introduction to Cluster Analysis*, Wiley, Hoboken, NJ, USA.

Kayfetz, K. and Kimmerer, W. (2017) Abiotic and biotic controls on the copepod *Pseudodiaptomus forbesi* in the upper San Francisco estuary. *Mar. Ecol. Prog. Ser.*, **581**, 85–101.

Keefer, M. L., Peery, C. A., Jepson, M. A., Tolotti, K. R., Bjornn, T. C. and Stuehrenberg, L. C. (2004) Stock-specific migration timing of adult spring–summer Chinook salmon in the Columbia River basin. *N. Amer. J. Fish. Manage.*, **24**, 1145–1162.

Kennish, M. J. (2002) Environmental threats and environmental future of estuaries. *Environ. Conserv.*, **29**, 78–107.

Kimmerer, W. J., Burau, J. R. and Bennett, W. A. (1998) Tidally oriented vertical migration and position maintenance of zooplankton in a temperate estuary. *Limnol. Oceanogr.*, **43**, 1697–1709.

Kirn, R. A., Ledgerwood, R. O. and Jensen, A. L. (1985) Diet of subyearling Chinook salmon (*Oncorhynchus tshawytscha*) in the Columbia River estuary and changes effected by the 1980 eruption of Mount St. Helens. *Northwest Sci.*, **60**, 191–196.

Kruskal, J. B. (1964) Multidimensional scaling by optimizing goodness of fit to a nonmetric hypothesis. *Psychometrika*, **29**, 1–27.

Laprise, R. and Dodson, J. J. (1994) Environmental variability as a factor controlling spatial patterns in distribution and species diversity of zooplankton in the St. Lawrence estuary. *Mar. Ecol. Prog. Ser.*, **107**, 67–81.

Leung, J. Y. S. and Cheung, N. K. M. (2017) Feeding behaviour of a serpulid polychaete: turning a nuisance species into a natural resource to counter algal blooms? *Mar. Pollut. Bull.*, **115**, 376–382.

Lionard, M., Azémar, F., Boulêtreau, S., Muylaert, K., Tackx, M. and Vyverman, W. (2005) Grazing by meso- and microzooplankton on phytoplankton in the upper reaches of the Schelde estuary (Belgium/the Netherlands). *Estuar. Coast. Shelf Sci.*, **64**, 764–774.

Marques, S. C., Azeiteiro, U. M., Martinho, F., Viegas, I. and Pardal, M. A. (2009) Evaluation of estuarine mesozooplankton dynamics at a fine temporal scale: the role of seasonal, lunar and diel cycles. *J. Plankton Res.*, **31**, 1249–1263.

Martínez, M. L., Intralawan, A., Vázquez, G., Pérez-Maqueo, O., Sutton, P. and Landgrave, R. (2007) The coasts of our world: ecological, economic and social importance. *Ecol. Econ.*, **63**, 254–272.

Menden-Deuer, S. and Lessard, E. J. (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnol. Oceanogr.*, **45**, 569–579.

Milligan, G. (1989) A study of the beta-flexible clustering method. *Multivar. Behav. Res.*, **24**, 163–176.

Muylaert, K., Declerck, S., Van Wichelen, J., De Meester, L. and Vyverman, W. (2006) An evaluation of the role of daphnids in controlling phytoplankton biomass in clear water versus turbid shallow lakes. *Limnol - Ecol Manage Inland Waters*, **36**, 69–78.

Muylaert, K., Sabbe, K. and Vyverman, W. (2000) Spatial and temporal dynamics of phytoplankton communities in a freshwater tidal estuary (Schelde, Belgium). *Estuar. Coast. Shelf Sci.*, **50**, 673–687.

Naithani, J., DE Brye, B., Buyze, E., Vyverman, W., Legat, V. and Deleersnijder, E. (2016) An ecological model for the Scheldt estuary and tidal rivers ecosystem: spatial and temporal variability of plankton. *Hydrobiologia*, **775**, 51–67.

Neitzel, D., Page, T. and Hanf, R. (1982) Mid-Columbia River microflora. *J. Freshwater Ecol.*, **1**, 495–505.

Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, B., Simpson, G. L. *et al.* (2017) Vegan: community ecology package.

Orsi, J. J. and Walter, T. C. (1991) *Pseudodiaptomus forbesi* and *P. marinus* (Copepoda Calanoida), the latest copepod immigrants to California's Sacramento - San Joaquin estuary. *Bull. Plankton Soc. Japan*, **1991**, 553–562.

Park, G. S. and Marshall, H. G. (2000) The trophic contributions of rotifers in tidal freshwater and estuarine habitats. *Estuar. Coast. Shelf Sci.*, **51**, 729–742.

Patterson, D. J. and Hedley, S. (1992) *Free-Living Freshwater Protozoa: A Colour Guide*, Wolfe Publishing, Boca Raton, FL, USA.

Payne, J. T., Wood, A. W., Hamlet, A. F., Palmer, R. N. and Lettenmaier, D. P. (2004) Mitigating the effects of climate change on the water resources of the Columbia River basin. *Clim. Change*, **62**, 233–256.

R Core Team (2015) *R: The R Project for Statistical Computing*.

Ricardo, G. F., Davis, A. R., Knott, N. A. and Minchinton, T. E. (2014) Diel and tidal cycles regulate larval dynamics in salt marshes and mangrove forests. *Mar. Biol.*, **161**, 769–784.

Richardson, A. J. (2008) In hot water: zooplankton and climate change. *ICES J. Mar. Sci.*, **65**, 279–295.

Richardson, A. J. and Schoeman, D. S. (2004) Climate impact on plankton ecosystems in the Northeast Atlantic. *Science*, **305**, 1609–1612.

Robins, P. E., Skov, M. W., Lewis, M. J., Giménez, L., Davies, A. G., Malham, S. K., Neill, S. P., JE, M. D. *et al.* (2016) Impact of climate change on UK estuaries: a review of past trends and potential projections. *Estuar. Coast. Shelf Sci.*, **169**, 119–135.

Roegner, G. C., Needoba, J. A. and Baptista, A. M. (2011a) Coastal upwelling supplies oxygen-depleted water to the Columbia River estuary. *PLoS One*, **6**, e18672.

Roegner, G. C., Seaton, C. and Baptista, A. M. (2011b) Climatic and tidal forcing of hydrography and chlorophyll concentrations in the Columbia River estuary. *Estuaries Coast*, **34**, 281–296.

Rollwagen-Bollens, G., Bollens, S., Gonzalez, A., Zimmerman, J., Lee, T. *et al.* (2013) Feeding dynamics of the copepod *Diacyclops thomasi* before, during and following filamentous cyanobacteria blooms in a large, shallow temperate lake. *Hydrobiologia*, **705**, 101–118.

Rollwagen-Bollens, G. C., Bollens, S. M. and Penry, D. L. (2006) Vertical distribution of micro-and nanoplankton in the San Francisco estuary in relation to hydrography and predators. *Aquat. Microb. Ecol.*, **44**, 143–163.

Rollwagen-Bollens, G. C. R. and Penry, D. L. (2003) Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA). *Mar. Ecol. Prog. Ser.*, **257**, 139–158.

Roy, E. D., Smith, E. A., Bargu, S. and White, J. R. (2016) Will Mississippi River diversions designed for coastal restoration cause harmful algal blooms? *Ecol. Eng.*, **91**, 350–364.

Schlacher, T. A. and Wooldridge, T. H. (1995) Small-scale distribution and variability of demersal zooplankton in a shallow, temperate estuary: tidal and depth effects on species-specific heterogeneity. *Cah. Biol. Mar.*, **36**, 211–227.

Selleslagh, J., Lobry, J., N'Zigou, A. R., Bachelet, G., Blanchet, H., Chaalali, A., Sautour, B. and Boët, P. (2012) Seasonal succession of estuarine fish, shrimps, macrozoobenthos and plankton: Physico-chemical and trophic influence. The Gironde estuary as a case study. *Estuar. Coast. Shelf Sci.*, **112**, 243–254.

Sellner, K. G., Brownlee, D. C., Bundy, M. H., Brownlee, S. G. and Braun, K. R. (1993) Zooplankton grazing in a Potomac River cyanobacteria bloom. *Estuaries*, **16**, 859–872.

Sherr, E., Caron, D. and Sherr, B. (1983) Staining of heterotrophic protists for visualization via epifluorescence microscopy. In Kemp, P. (ed.), *Handbook of methods in Aquat. Microb. Ecol*, Lewis Publishers, Boca Raton, pp. 213–227.

Simenstad, C., Small, L., McIntire, C., Jay, D. and Sherwood, C. (1990a) Columbia River estuary studies - an introduction to the estuary, a brief-history, and prior studies. *Prog. Oceanogr.*, **25**, 1–13.

Simenstad, C. A., Small, L. F. and David McIntire, C. (1990b) Consumption processes and food web structure in the Columbia River estuary. *Prog. Oceanogr.*, **25**, 271–297.

Sin, Y., Wetzel, R. L., Lee, B.-G. and Kang, Y. H. (2006) Integrative ecosystem analyses of phytoplankton dynamics in the York River estuary (USA). *Hydrobiologia*, **571**, 93–108.

Sooria, P. M., Jyothibabu, R., Anjusha, A., Vineetha, G., Vinita, J., Raj, L., Paul, M. and Jagadeesan, L. (2015) Plankton food web and its seasonal dynamics in a large monsoonal estuary (cochin backwaters, India)-significance of mesohaline region. *Environ. Monit. Assess.*, **187**, 427.

Strickland, J. and Parsons, T. (1972) A practical handbook for seawater analysis. Bulletin no. 167. Fisheries research board of Canada.

Stroud, J. T., Bush, M. R., Ladd, M. C., Nowicki, R. J., Shantz, A. A. and Sweatman, J. (2015) Is a community still a community? Reviewing definitions of key terms in community ecology. *Ecol. Evol.*, **5**, 4757–4765.

Thorp, J. H. and Covich, A. P. (2010) *Ecology and Classification of North American Freshwater Invertebrates*, 3rd edn, Academic Press, San Diego, CA, USA.

Wang, Q., Yang, Y. and Chen, J. (2009) Impact of environment on the spatio-temporal distribution of rotifers in the tidal Guangzhou segment of the Pearl River estuary, China. *Int. Rev. Hydrobiol.*, **94**, 688–705.

Wehr, J. D., Sheath, R. G. and Kociolek, J. P. (2015) *Freshwater Algae of North America: Ecology and Classification*, 2nd edn, Edit. Elsevier Academic Press, London.

Weitkamp, L. A., Bentley, P. J. and Litz, M. N. (2012) Seasonal and interannual variation in juvenile salmonids and associated fish assemblage in open waters of the lower Columbia River estuary. *Fish. Bull.*, **110**, 426–450.

Wise, D., Rinella, F., Rinella, J., Fuhrer, G., Embrey, S. *et al.* (2007) *Nutrient and Suspended-Sediment Transport and Trends in the Columbia River and Puget Sound Basins, 1993–2003. No. 2007–5186*, Scientific Investigations Report. U.S. Geological Survey.

Wollrab, S., Diehl, S. and De Roos, A. M. (2012) Simple rules describe bottom-up and top-down control in food webs with alternative energy pathways a Beckerman, Ed. *Ecol. Lett.*, **15**, 935–946.

Wood, S. N. (2003) Thin plate regression splines. *J. R. Stat. Soc. Series B Stat. Methodology*, **65**, 95–114.

York, J. K., McManus, G. B., Kimmerer, W. J., Slaughter, A. M. and Ignoffo, T. R. (2014) Trophic links in the plankton in the low salinity zone of a large temperate estuary: top-down effects of introduced copepods. *Estuaries Coast*, **37**, 576–588.