Improved marine-derived POM availability and increased pH related to freshwater influence in an inland sea

Alexander T. Lowe,[*,1,2] **Emily A. Roberts,**[1,2] **Aaron W. E. Galloway**[3]
[1]Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington
[2]Department of Biology, University of Washington, Seattle, Washington
[3]Oregon Institute of Marine Biology, University of Oregon, Charleston, Oregon

Abstract

Rapid changes, including warming and freshening, are occurring in coastal marine ecosystems worldwide. These environmental changes have the potential to alter ecosystem energetics by influencing availability of food sources and organism physiology. We investigated the influence of oceanographic variability on food availability and quality to benthic and pelagic suspension-feeders using detailed observations of phytoplankton, particulate organic matter (POM) detritus, and diverse biomarkers (fatty acids and carbon, nitrogen, and sulfur stable isotopes) along a salinity gradient in the San Juan Archipelago, Washington, U.S.A. We tested the hypothesis that freshwater input from riverine discharge would cause significant changes to oceanographic conditions and reduce food quality (indicated by essential fatty acids; EFA), owing to greater contribution of terrestrial organic matter. Contrary to our expectations, availability of high-quality marine-derived POM increased with freshwater input (reduced salinity). Phytoplankton biomass and biomarker composition responded to oceanographic change similarly across tidal and seasonal scales. Using a meta-analysis spanning a range of spatial and temporal scales, we found that chlorophyll *a*, temperature, dissolved oxygen (DO) and pH were consistently and significantly higher at reduced salinity. The increase of DO and pH corresponding to higher phytoplankton biomass in low salinity water signifies an important feedback of biological activity on seawater chemistry. This analysis supports the use of salinity as an indicator of processes controlling food availability and oceanographic conditions in this region. Collectively, these results highlight the importance of ecosystem connectivity in coastal environments and produce hypotheses for expected changes related to altered river discharge dynamics.

Coastal marine ecosystems worldwide are experiencing environmental changes, including regional warming and freshening (Rhein et al. 2013). Rising air temperatures have led to changes in the timing of snowmelt and river discharge to estuaries globally (van Vliet et al. 2013). The warming and freshening of coastal waters represent potential physiological stressors to marine organisms (Crain et al. 2008), and may also exacerbate the rate of ocean acidification owing to changes in the buffering capacity of seawater and delivery and decomposition of allochthonous carbon (Borges and Abril 2011). Variability of environmental conditions in coastal marine ecosystems exerts a strong influence on ecological communities through physical interactions of organisms with the environment (Cloern 2001) and food availability (Menge et al. 2003). Recent studies have shown that the availability of high quality food can offset impacts of environmental stressors, such as the effect of low pH on larval oysters (Hettinger et al. 2013) and mussels (Melzner et al. 2011). Food availability is thus a major factor determining resilience of heterotrophic organisms to environmental stressors (Sara et al. 2011; Thomsen et al. 2013).

Food availability, defined here as the abundance and quality of energetic sources to suspension-feeders, in coastal marine ecosystems is composed of many sources of primary production including autochthonous and allochthonous organic matter (Miller et al. 2013; Lowe et al. 2014). The quality of a food source is a function of both the biochemical composition and ease of consumption (reviewed in Shin et al. 2003; Müller-Navarra 2008; Wagner et al. 2013). The biochemical composition reflects particulate organic matter (POM) composition and the environment in which primary production occurs; whereas the size and shape of the POM and

*Correspondence: alowe70@uw.edu

morphology of the feeding appendages of the suspension-feeder largely determine ease of consumption. The combination of these factors drives the amount of energy and essential building blocks such as amino acids, sterols and fatty acids (FA) available to suspension-feeders for growth and reproduction (Martin-Creuzburg et al. 2009).

FA are structurally diverse molecules that play critical roles in trophic interactions and physiological processes, including as precursors to anti-inflammatory eicosanoids, maintenance of membrane fluidity, and energy storage (reviewed in Dalsgaard et al. 2003). The study of FA production in marine ecosystems is critical as certain classes of polyunsaturated FA are synthesized almost entirely by phytoplankton (Dalsgaard et al. 2003; Gladyshev et al. 2013), and are considered essential nutrients for invertebrates, fish and humans (Arts et al. 2001). Long-chain (e.g., $\geq C_{20}$) ω-3 and ω-6 essential FA (EFA) cannot be synthesized de novo by heterotrophs, yet are critical to the growth and reproduction of diverse metazoans (Brett et al. 2009; Parrish 2009). Moreover, environmental availability of EFA is likely to regulate efficiency of energetic trophic transfer (Brett and Müller-Navarra 1997; Gladyshev et al. 2011), and ecosystem-scale community structure (Litzow et al. 2006). The dynamics of EFA concentrations in aquatic ecosystems are therefore important indicators of food quality through time (Galloway and Winder 2015) and over large spatial scales (Budge et al. 2014).

Changing environmental conditions influence food availability and quality via effects on prey physiology (Galloway and Winder 2015) and large-scale effects on the sources available (Polis et al. 1997; Menge et al. 2003). Identification of environmental drivers of primary productivity is essential for predicting biological responses to global change stemming from natural and anthropogenic forcings (Behrenfeld et al. 2006). While the underlying mechanisms controlling ecosystem-scale primary productivity are complex, rapid non-linear increases in primary productivity are often associated with a change in a single environmental variable (Cloern 1996; Dierssen et al. 2002; Taylor and Ferrari 2011). These environmental variables may prove to be important indicators of ecosystem change (Melillo et al. 2014).

In estuarine and brackish environments, changes of salinity due to freshwater input can have cascading effects on water column stratification, temperature and nutrient availability. In British Columbia, Canada, the timing of peak discharge from the Fraser River, an undammed river draining a 217,000 km^2 watershed, has advanced in response to warming air temperatures (Morrison et al. 2002). The freshwater discharge of approximately 9000 m^3/s from the Fraser River drives much of the seasonal variation in surface oceanographic conditions in the northern Salish Sea (Waldichuk 1957). Long-term salinity records in the Salish Sea have shown resultant trends of decreasing average salinity and increasing temperature in this region (Cummins and Masson 2014; Riche et al. 2014) mirroring patterns of oceanographic

change observed globally (van Vliet et al. 2013). However, linking these environmental changes to ecosystem processes can be difficult owing to the complexity of biological responses, and is therefore an important area of future research.

We compiled a multivariate dataset in the Salish Sea of high-resolution POM composition, a suite of stable isotope (SI) and FA biomarkers, and multiple physical oceanographic factors to determine environmental drivers of food availability. The study area focused on the San Juan Archipelago (SJA), a dynamic tidally mixed region along a salinity gradient within the central Salish Sea (Fig. 1; Banas et al. 2015). Suspension feeders in this region potentially gain energy from diverse oceanic, terrestrial and riverine organic matter sources. We tested the hypothesis that variability of oceanic and freshwater influence drives major changes in the food sources available to benthic suspension feeders. Principally, we predicted that increased freshwater input would bring greater terrestrial organic matter, and thus lower quality food, into the system (e.g., Yamanaka et al. 2013). Further, we tested the hypothesis that a salinity threshold, defined by the halocline differentiating oceanic and river-influenced water masses, could be used as an indicator of food availability over time. We conducted a meta-analysis to evaluate the use of the salinity threshold as an indicator of oceanographic conditions, food availability and feedbacks of primary production on water chemistry. These results were compared to a multi-decadal salinity dataset from the Salish Sea to investigate the effects of climate change on this ecosystem over the last seven decades.

Methods

Oceanographic influence on POM availability

The inland temperate marine waters of the San Juan Archipelago, Washington, U.S.A. (SJA) lie at the junction between the Strait of Juan de Fuca water mass, characterized by oceanic water (salinity = 32–33), and the Strait of Georgia, characterized by strong influence of freshwater discharge primarily from the Fraser River (salinity < 28.5; Masson and Cummins 2000). Fraser River discharge causes drops in salinity to ∼5 in the Strait of Georgia, whereas salinity generally does not decrease below 24 in the SJA. Channels of the SJA are regions of intense vertical mixing, with mixing down to a sill depth of approximately 100 m and flux of water to the depths of the Strait of Georgia and also out into the Strait of Juan de Fuca (Waldichuk 1957). This intense vertical mixing could limit the photosynthetically active radiation (PAR) essential for photosynthesis (Mackas and Harrison 1997).

Seasonal POM samples (herein referred to as POM-SEAS) were collected during Summer 2010, 2011, Winter 2010, 2011, and Spring 2010 (Fig. 1). Three sites (PIL, PTC and SKP) were sampled on each of 4 d evenly distributed over 2 weeks (a neap-spring tide cycle) per season. This sampling regime was designed to sample at multiple temporal and spatial scales to capture the range of natural oceanographic variability occurring
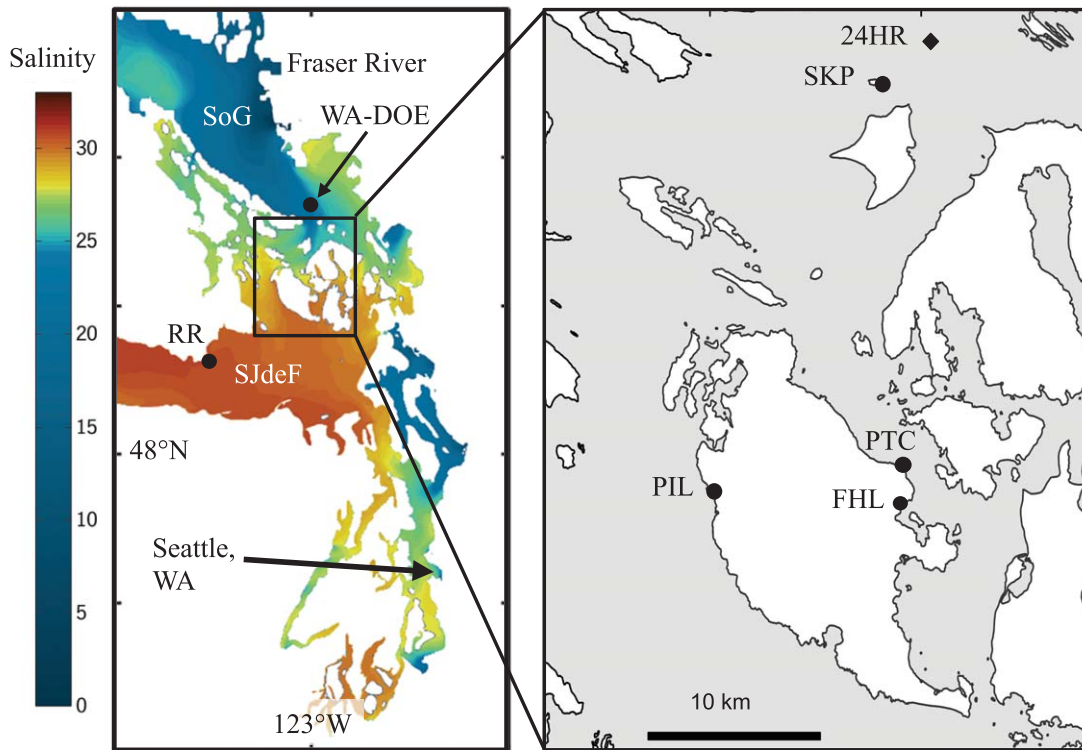
Fig. 1. Regional map of the study area in the Salish Sea in Washington State, U.S.A., and British Columbia, Canada. Data was gathered from sites located along the salinity gradient from the Fraser River in the Strait of Georgia (SoG) to Race Rocks Lighthouse (RR) in the Strait of Juan de Fuca (SJdeF). POM sampling occurred at SKP, PIL, PTC and 24HR in the San Juan Archipelago (SJA) shown in the inset. Data sources, sites and collection methods are described in Table 1. Colormap of a representative salinity gradient during peak Fraser River discharge is reproduced from Banas et al. (2015).

along the salinity gradient (Table 1). Sampling effort was equally distributed among ebb, slack and flood tides at each site. During Spring 2012, POM samples were collected every 2 h over a 24 h period (POM-24HR) at one site to investigate changes in POM composition related to tide stage and mixing of Juan de Fuca and Strait of Georgia water masses. POM samples were collected via ∼10 min diagonal tows (20 μm net) from ∼11 m to the surface and pre-filtered through a 190 μm screen to remove zooplankton. Surface temperature, salinity, and chlorophyll *a* (Chl *a*) measurements were taken at 1 m depth immediately after each tow. Temperature and salinity were measured with a YSI 55. Chl *a* was determined from water samples using a spectrofluorometric method (Jeffrey and Humphrey 1975). Water temperature was measured hourly at PIL, PTC and SKP using HOBO U24 data loggers (Onset Computer Corp.) at 6 m depth from August 2011 to 2012.

POM samples were filtered onto GF/F filters and the material was lyophilized for biomarker analysis. Briefly, 2 mg and 8 mg of POM material were ground and packaged for carbon and nitrogen, and sulfur isotopic analysis, respectively. Isotope analysis was conducted at the Washington State University Isotope Core Lab and reported in standard isotopic notation (δ^{13}C, δ^{15}N, and δ^{34}S). FA were extracted from 10 mg of dry POM material using a modified-Folch method

(Taipale et al. 2011). FA methyl esters were analyzed with an 85-min GC-FID method designed to separate 16 and 18 monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) (Taipale et al. 2011). FA concentrations (mg FAME g POM^{-1}) were quantified with a regression generated by dilutions of a Nu-Check 569B standard (Lowe et al. 2014). Eicosapenaenoic acid (20:5ω3, EPA), docosahexaenoic acid (22:6ω3, DHA), and arachidonic acid (20:4ω6, ARA) were summed to represent EFA.

Prior to filtration, a 3-mL aliquot of each sample was collected for visual analysis of POM composition using a compound microscope. Diatoms and dinoflagellates were identified to the lowest possible taxonomic unit and detritus was identified as organic, inorganic, biogenic inorganic (diatom frustules or spicules lacking organic matter) and unidentifiable small detritus. Further visual identification of the sources of detritus (i.e., kelp, phytoplankton, plant) was not possible. Using a point count method, the relative area of each POM category was determined by counting the particles beneath each intersection of a 10 × 10 gradicule. The relative proportion, or bio-area of each category was calculated from three replicate counts of 100 particles for each sample. The bio-area of phytoplankton and detritus was significantly correlated to Chl *a* ($r^2 = 0.85$) and variation in biomarker

Table 1. Temporal and spatial scales, variables measured and sources of the data used in this analysis. Names of each dataset correspond to locations shown in Fig. 1 and abbreviations used throughout the text. See Methods for links to and further descriptions of the data.

Name	Source	Location	Collection Date	Timescale	Variables	Analysis
POM-24HR	Current study	San Juan Archipelago: POM-24HR	Spring 2012	Hourly	Salinity, temperature, Chl *a*, POM composition, POM SI, POM FA, POM C:N	Drivers of POM dynamics
POM-SEAS	Current study	San Juan Archipelago: SKP, PIL, PTC	Summer, Winter 2010,2011; Spring 2011	Daily, fortnightly, seasonal, annual	Salinity, temperature, Chl *a*, POM composition, POM SI, POM FA, POM C:N	Drivers of POM dynamics
SJA-PIL	Current study	San Juan Archipelago: PIL	Summer 2014	Hourly, daily	Salinity, temperature, Chl *a*, pH, dissolved oxygen	Meta-analysis
FHL	Friday Harbor Labs- Ocean Acidification Environmental Lab	San Juan Archipelago: FHL	Summer 2013, Spring 2014	Hourly, daily, fortnightly, seasonal	Salinity, temperature, pH	Meta-analysis
WA-DOE	Washington Dept. Ecology	Strait of Georgia	1989–2014	Seasonal, annual, decadal	Salinity, temperature, Chl *a*, pH, dissolved oxygen	Meta-analysis
RR	Canada Dept. Fisheries and Oceans	Race Rocks Lighthouse, Strait of Juan de Fuca, BC	1942–2012	Daily, fortnightly, seasonal, annual, decadal	Salinity, temperature	Meta-analysis, Long-term trends

signatures, indicating this method accurately described the composition of the POM samples (see Lowe et al. 2014 for further details of the method). The combination of analyses allowed us to investigate environmental drivers of food source (diatom and dinoflagellate abundance, δ^{13}C and δ^{34}S), abundance (Chl *a*, total phytoplankton bio-area and total FAME concentration [mg g POM^{-1}]) and quality (C : N, δ^{15}N, and EFA concentration [mg g POM^{-1}]) of the POM.

Statistical analysis of POM and oceanographic conditions

The influence of oceanographic conditions on food availability (Chl *a*, POM community composition, MSI and FA) was investigated using analysis of covariance. Independent, continuous variables included temperature and salinity measured at the time POM samples were collected. The season of sampling was a categorical covariate (Spring, Summer or Winter). Proportional EFA and bio-area data were arcsin-square root transformed, while Chl *a* data were log-transformed to meet assumptions of normality prior to all analyses.

A posteriori comparisons of POM-SEAS and POM-24HR data relative to water mass were conducted using Welch's *t*-tests for univariate data and PERMANOVA for multivariate data (POM composition, FA, and δ^{13}C, δ^{34}S and δ^{15}N). For this analysis, the data sets were separated by salinity > 30 and < 30 (indicating freshwater influence). Statistical analyses were conducted using R (R Development Core Team 2013) or PRIMER 6 (Anderson et al. 2008). An increase of pH

observed in low salinity water in the FHL dataset may have been due to equilibration of super-saturated water with atmospheric CO_2 in the stratified surface layer. To test this assumption we conducted a Two-way ANOVA of pH vs. salinity (above and below 30) and time of day (day = 9:00–18:00 local time; night = 18:00–9:00 local time) to test for effects of photosynthesis vs. physical processes related to stratification.

Salinity as a biological indicator: meta-analysis of regional datasets

We hypothesized that a small change in salinity resulting from freshwater influence would result in large changes in food availability, owing to the different oceanographic conditions and POM sources available in oceanic and riverine water masses. We predicted that a salinity threshold defined by the halocline separating the river-influenced, stratified surface layer from the uniformly mixed oceanic water column would be a useful indicator of the integrated biological processes related to a water mass. The salinity threshold was empirically determined for the POM sampling. Because of the difference in water column density along the salinity gradient, we used the median observed salinity at RR and WA-DOE, respectively, to differentiate river-influenced (low salinity) from oceanic water masses (high salinity) at these ends of the salinity gradient. Data used to test the "salinity threshold" hypothesis were gathered from multiple

sources spanning different spatial and temporal scales (Fig. 1; Table 1), including continuous sampling instruments installed at PIL (referred to as SJA-PIL), observations from the Friday Harbor Laboratories (FHL), Washington state Department of Ecology (WA-DOE) and Race Rocks (RR) Lighthouse (Table 1).

The meta-analysis of multiple oceanographic and food quality parameters compared means of univariate data below and above the salinity threshold with random effects models in the "meta" package in R (Schwarzer 2015). The random effects model was used to account for large differences in sample collection methodology and random error among datasets. "Meta" calculates the effect size as the mean difference of a parameter between two treatments (e.g., above and below the salinity threshold), divided by the pooled standard deviation of all samples. Effect size is a standard meta-analysis method appropriate for comparing effects of disparate biological and environmental variables related to a common treatment (Gates 2002). The treatment effect, defined as the difference of the mean value between the two treatments, is presented for ease of interpretation.

We restricted the analysis to data collected < 10 m depth between April and September owing to confounding seasonal effects on productivity and freshwater influence, and to be consistent with POM sampling. To control for autocorrelation among sequential observations in the high resolution monitoring datasets, observations were randomly subsampled from the entire dataset according to the salinity (above or below the threshold). Autocorrelation among time points can violate the assumption of independence and lead to spurious conclusions regarding treatment effects in ANOVA. Within each dataset, a bootstrapped mean and standard deviation was generated using the number of transitions from one salinity regime to the other over the time series (i.e., above to below salinity threshold) as the sample size to randomly select observations associated with low and high salinity measurements. This effectively separated randomly selected observations in time. This reduced the number of observations from 12780 to 1630 (RR), 1961 to 120 (FHL) and 3834 to 16 (SJA-PIL). Incidentally, results from the subsampled dataset did not differ from those using all available data.

Description of data sources used in meta-analysis

SJA-PIL

Salinity, temperature (°C), pH (total scale), dissolved oxygen (DO, mg L^{-1}), and Chl *a* (μg L^{-1}) measurements were collected at 1 min increments at PIL on the West side of San Juan Island from 31 August to 03 September 2014 using a YSI 6600 for salinity, temperature, DO and Chl *a*. A Honeywell Durafet III sensor calibrated to the total scale was used to measure pH. The instruments were attached to the substrate at approximately 4 m depth to replicate conditions experienced by shallow subtidal benthic organisms. We targeted this depth and season to contrast spring observations. Sampling provided high-resolution temporal data from one site (Table 1).

FHL

Salinity and temperature were measured hourly by an SBE Microcat at the Friday Harbor Laboratories on the east side of San Juan Island (live data at http://depts.washington.edu/fhl/wx.html). The pH of seawater inflow to the pump house was measured at approximately 1.7 m below MLLW at 15 min intervals by a field-effect Honeywell Durafet III sensor calibrated to the total scale (NIST pH standards followed by tris buffer; DelValls and Dickson 1998). Water samples for carbonate chemistry were taken at discrete weekly time points and were used for verification of Durafet measurements (sample chemistry published in Murray et al. 2015). Water samples were analyzed for alkalinity and dissolved inorganic carbon at the FHL Ocean Acidification Environmental Laboratory (Dickson et al. 2007; Riebesell et al. 2010), and used to calculate pH in CO2calc (ver. 2013; Murray et al. 2015). Error between Durafet pH and calculated sample pH was 0.06 pH units (root mean square error, $n = 4$), and sensor was 0.04 pH units (RMS error in tris buffer, $n = 8$), and was considered sufficient to capture dynamics in pH (range 8.07–7.72 in Summer 2013, Spring 2014).

WA-DOE

Salinity, temperature (°C), pH, DO (mg L^{-1}), and Chl *a* (μg L^{-1}) data were collected at 1 m depth near Patos Island during long-term marine water quality sampling conducted by the Washington Department of Ecology (http://www.ecy.wa.gov). The median salinity ($S = 27.8$) was used as the salinity threshold for this site. Sampling occurred semi-monthly from 1989 through 2014, providing low temporal resolution data from one site.

RR

Salinity and temperature (°C) data were obtained from the Canadian Department of Fisheries and Oceans (http://www.racerocks.com/racerock/data/seatemp/seatemp.htm). Race Rocks lies ocean-ward of the western limit of the intense mixing region characteristic of the SJA (Fig. 1), and thus represents the high salinity endpoint of the expected gradient of riverine influence (Masson and Cummins 2000). Daily measurements were made during daytime high tide from 1942 to 2012. This dataset represents the longest continuous monitoring dataset in the region, and allows investigation of long-term environmental variability at one site. The median salinity ($S = 31.25$) was used as the salinity threshold for this site.

Results

Oceanographic influence on POM availability

Temperature and salinity in the SJA exhibited large variability on daily and seasonal scales. The daily variation in oceanographic conditions was greatest during summer and varied with tide phase (Fig. 2). Daily fluctuations of
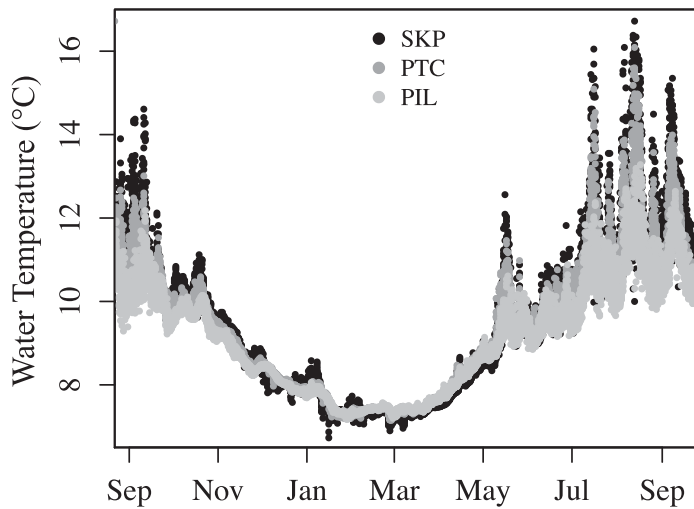
Fig. 2. Hourly water temperature measured from August 2011 to September 2012 at ~6 m depth at PIL, PTC, and SKP in the San Juan Archipelago. Patterns of salinity showed inverse relationship with low salinities coinciding with high temperature.

temperature and salinity, related to tidal mixing and advection of water masses were as large as 7°C (Fig. 2) and 10 ppt, similar to the observed seasonal range in the SJA (10°C and 12 ppt). Across seasons, temperature and salinity were strongly negatively correlated (linear regression, $n = 66$, r^2-*adj* = 0.42, $p < 0.0001$). Low temperatures (~8°C) were associated with higher salinity ($S = 30$–31), while warm temperatures (~14°C) were associated with freshwater influence (S ~27).

Phytoplankton bio-area correlated significantly with salinity and temperature (Fig. 3b,c; Table 2) and was greater at warmer temperatures and lower salinity. Phytoplankton abundance differed significantly among seasons (Table 2), yet phytoplankton abundance was highly variable at smaller time scales and exhibited a similar range over a single tide series as over a year of sampling (Fig. 3a). Diatoms were the dominant taxonomic group in nearly all samples (Fig. 4b); dinoflagellates were observed in greater abundance only at intermediate salinity and higher temperatures (Fig. 4c). Detrital particles were numerically abundant in all seasons and proportionally dominant in winter. These changes in species composition contributed to the variation in overall phytoplankton bio-area in relation to salinity and temperature. Other food source ($\delta^{13}C$ and $\delta^{34}S$), abundance (Chl *a*, total phytoplankton bio-area and total FA concentration [mg g POM^{-1}]) and quality (C : N, $\delta^{15}N$, and EFA concentration [mg g POM^{-1}]) parameters were consistently and significantly correlated to salinity and season, with few interactions (Table 2).

Across seasons, phytoplankton biomass was significantly higher and composition was significantly different when freshwater influence increased (Fig. 4a–c). Phytoplankton bio-area increased relative to detritus and was dominated by fewer taxa leading to significant differences in POM composition at lower salinities (Table 3). These community differences led to an

increase in the concentration of total EFA (Fig. 4d) and to enrichment of $\delta^{13}C$ (Fig. 4e) and $\delta^{34}S$ (Fig. 4f) at low salinity. For example, EFA concentration (mg g POM^{-1}) was on average ~6 times higher below a salinity of 30 than above 30 (average ± SE mg EFA g dry POM^{-1}: $< 30 = 3.80 ± 0.92$, $> 30 = 0.68 ± 0.22$; Fig. 4d). $\delta^{15}N$ differed significantly, however these differences were small and the value was generally indicative of primary producers. Similar to POM composition, multivariate analyses of SI and FA showed significant differences related to a change in salinity. The enrichment of $\delta^{13}C$ and $\delta^{34}S$ and increase in marine phytoplankton-associated EFA strongly suggest a greater importance of marine-derived sources in the lower salinity water mass.

Measurements of pH in the surface water (~2 m depth) at FHL showed a strong negative correlation to salinity (Fig. 5). The highest pH values (>8.0) were only observed during spring and summer and when salinity was less than 30. pH was significantly higher during the day ($F_{1,1957} = 26.80$, $p < 0.0001$) and in low salinity ($F_{1,1957} = 639.71$, $p < 0.0001$) with no interaction ($F_{1,1957} = 0.95$, $p = 0.33$).

Salinity as a biological indicator: meta-analysis of regional datasets

Analysis of regional POM showed a strong effect of salinity during the spring (POM-24HR; Table 3) and when all data were combined (POM-SEAS; $p = 0.0002$). While sampling structured to target different water masses is sparse in the SJA, we were able to compare observations from multiple sources spanning hourly to decadal timescales (Table 1) to test the hypothesis that a salinity threshold, defined by the halocline differentiating oceanic from river-influence water masses, can be used as a biological indicator of food availability and oceanographic conditions.

Significant differences of oceanographic conditions and food availability parameters were observed in relation to the salinity threshold at all timescales (random effects meta-analysis: $p < 0.0001$; Table 4). On average, the decreased salinity corresponded to a 1.13° increase of temperature ($p < 0.0001$), a Chl *a* increase of 1.9 μg L^{-1} ($p = 0.0038$) and elevated DO and pH (1.31 mg L^{-1} DO and 0.08 pH, respectively; $p < 0.0001$).

Days below the salinity threshold at RR showed considerable interannual variation, ranging from 13 d in 1944 to 352 d in 1997 (Fig. 6). Observations of low salinity have increased significantly since 1942 at RR (linear regression: $n = 71$, r^2-*adj.* = 0.092, $p = 0.006$). On average the frequency of low salinity events has increased by 12.5 d per decade (Fig. 6). The majority of the increase in frequency has occurred in spring (linear regression: $n = 71$, r^2-*adj.* = 0.132, $p = 0.001$). The frequency of observations below the salinity threshold has increased by 5% per decade in spring, with smaller increases of frequency in summer (2.7% per decade) and fall (1.8% per decade), however summer and fall increases were not significant (r^2-*adj.* = 0.035, $p = 0.063$, and r^2-*adj.* = 0.007, $p = 0.226$, respectively). Variation within seasons was highest in summer (variance = 0.42) and similar in spring (variance = 0.27) and fall (variance = 0.24).
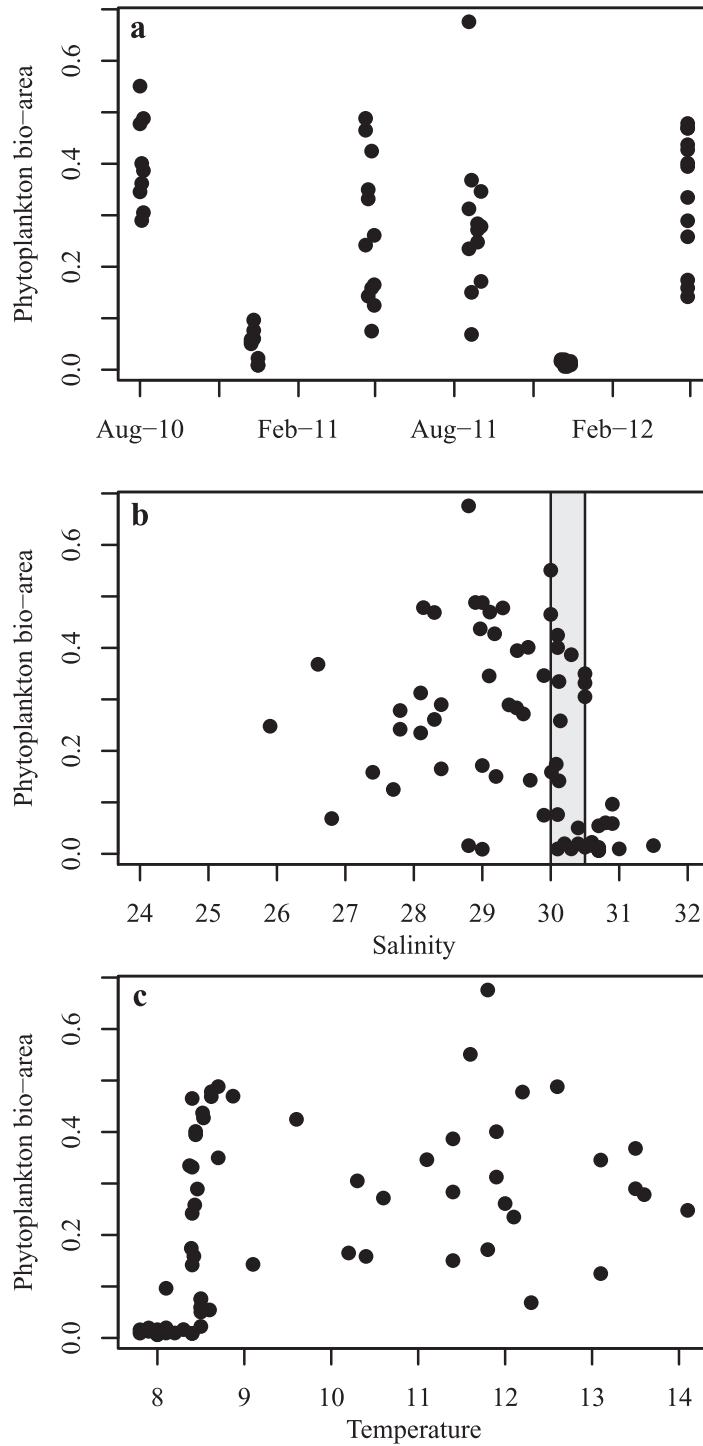
Fig. 3. Total phytoplankton bio-area in relation to (**a**) season, (**b**) salinity, and (**c**) water temperature (°C) in SJA. Shading in panel b indicates salinity change differentiating well-mixed oceanic water mass from the river-influenced water mass used to define the salinity threshold ($S = 30$).

Discussion

Oceanographic influence on POM availability

We found that the composition and biochemical makeup of the POM varied considerably at multiple temporal and spatial scales, yet the variation in composition was consistently related to salinity changes. The data support the hypothesis that freshwater influence drives major changes to food availability in the SJA. Contrary to our predictions, however, the food sources available at low salinity represented a greater availability of high-quality marine

Table 2. Results of Ancova analyses of food source, availability and quality in relation to water temperature, salinity and season. Significant effects indicated in bold.

Food source

Source	Diatom bio-area				Dinoflagellate bio-area				$\delta^{13}C$				$\delta^{34}S$			
	df	MS	F	p	df	MS	F	p	df	MS	F	p	df	MS	F	p
Salinity (S)	1	27.85	49.01	**0.000**	1	5.24	24.82	**0.000**	1	11.05	144.76	**0.000**	1	0.58	37.01	**0.000**
Temperature (T)	1	1.05	1.84	0.183	1	1.66	7.87	**0.007**	1	0.29	3.85	0.063	1	0.26	16.26	**0.000**
Season	2	11.03	19.42	**0.000**	2	23.54	111.47	**0.000**	2	8.98	117.74	**0.000**	2	0.97	61.56	**0.000**
S*T	1	0.02	0.04	0.852	1	0.93	4.39	**0.041**	1	0.06	0.75	0.395	1	0.05	3.07	0.086
S*Season	2	0.62	1.09	0.348	2	0.00	0.01	0.989	2	0.03	0.42	0.659	2	0.01	0.94	0.399
T*Season	2	2.41	4.24	**0.022**	2	1.01	4.79	**0.012**	2	0.02	0.24	0.792	2	0.06	3.64	**0.033**
S*T*Season	2	0.58	1.02	0.372	2	0.88	4.15	**0.021**	2	0.20	2.62	0.095	2	0.02	1.51	0.230
Residuals	37	0.57			53	0.21			22	0.08			54	0.02		

Availability

Source	Chl *a* ($\mu g\ L^{-1}$)				All phytoplankton bio-area				Total FA (mg g POM^{-1})			
	df	MS	F	p	df	MS	F	p	df	MS	F	p
Salinity (S)	1	11.05	144.76	**0.000**	1	0.58	37.01	**0.000**	1	27.85	49.01	**0.000**
Temperature (T)	1	0.29	3.85	0.063	1	0.26	16.26	**0.000**	1	1.05	1.84	0.183
Season	2	8.98	117.74	**0.000**	2	0.97	61.56	**0.000**	2	11.03	19.42	**0.000**
S*T	1	0.06	0.75	0.395	1	0.05	3.07	0.086	1	0.02	0.04	0.852
S*Season	2	0.03	0.42	0.659	2	0.01	0.94	0.399	2	0.62	1.09	0.348
T*Season	2	0.02	0.24	0.792	2	0.06	3.64	**0.033**	2	2.41	4.24	**0.022**
S*T*Season	2	0.20	2.62	0.095	2	0.02	1.51	0.230	2	0.58	1.02	0.372
Residuals	22	0.08			54	0.02			37	0.57		

Quality

Source	EFA (mg g POM^{-1})				$\delta^{15}N$				C:N			
	df	MS	F	p	df	MS	F	p	df	MS	F	p
Salinity (S)	1	12.98	47.41	**0.000**	1	2.32	3.78	0.057	1	5.24	24.82	**0.000**
Temperature (T)	1	1.16	4.24	**0.047**	1	2.74	4.47	**0.039**	1	1.66	7.87	**0.007**
Season	2	3.88	14.18	**0.000**	2	15.90	25.98	**0.000**	2	23.54	111.47	**0.000**
S*T	1	0.02	0.06	0.804	1	8.80	14.39	**0.000**	1	0.93	4.39	**0.041**
S*Season	2	0.37	1.34	0.275	2	2.35	3.84	**0.027**	2	0.00	0.01	0.989
T*Season	2	0.77	2.80	0.074	2	1.34	2.20	0.121	2	1.01	4.79	**0.012**
S*T*Season	2	0.02	0.09	0.918	2	0.48	0.79	0.459	2	0.88	4.15	**0.021**
Residuals	37	0.27			54	0.61			53	0.21		
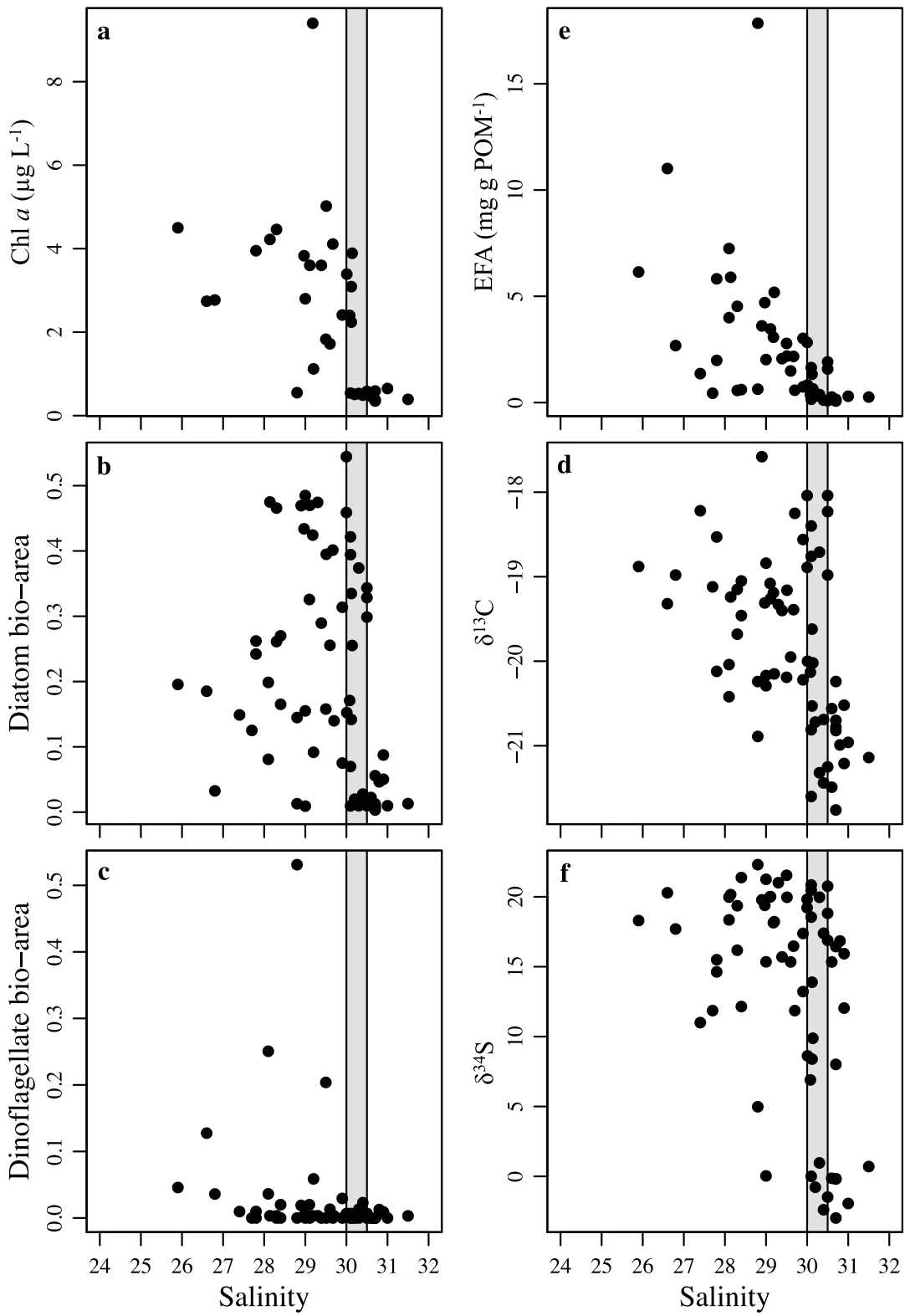
Fig. 4. Relationship to salinity of food abundance indicators: (**a**) Chl *a*, (**b**) Diatom bio-area (proportional composition), (**c**) Dinoflagellate bio-area, and food quality indicator: (**d**) EFA concentration (mg g POM^{-1}), and food source indicators: (**e**) δ^{13}C and (**f**) δ^{34}S. Shading as in Fig. 3b.

Table 3. Comparison of environmental and biological parameters measured above and below the salinity threshold during POM sampling in the SJA (see Table 1 for definitions). *p*-values from Welch's *t*-tests or PERMANOVA (multivariate, mv) analyses in right column, significant differences in bold. Isotope signatures (SI) in standard δ notation. Phytoplankton in percent bio-area.

Source	Parameter	Mean < 30	*n*	Mean > 30	*n*	*p*-value
POM-24HR	Salinity	29.03	8	30.09	5	
	Temperature (°C)	8.56	8	8.40	5	**0.017**
	Chl *a* (μg L^{-1})	5.67	8	2.53	5	**0.005**
	All phytoplankton	42.10%	8	21.36%	5	**0.003**
	δ^{13}C	−19.33	8	−20.06	5	**0.005**
	δ^{34}S	18.65	8	9.54	5	**<0.0001**
	POM community	mv	8	mv	5	**0.001**
	SI	mv	8	mv	5	**0.003**
	EFA	mv	8	mv	5	**0.035**
POM-SEAS	Salinity	28.50	26	30.50	27	
	Temperature (°C)	11.29	26	8.74	27	**<0.0001**
	Chl *a* (μg L^{-1})	2.43	10	0.49	11	**<0.0001**
	All phytoplankton	25.76%	26	14.03%	27	**0.003**
	δ^{13}C	−19.42	26	−20.26	27	**0.005**
	δ^{34}S	16.13	26	9.96	27	**0.005**
	POM community	mv	26	mv	27	**0.004**
	SI	mv	26	mv	27	**<0.0001**
	EFA	mv	21	mv	15	**0.001**

phytoplankton as indicated by changes in FA composition, enrichment of δ^{13}C and δ^{34}S and an increase in phytoplankton abundance. The empirical pattern of increased phytoplankton biomass at lower salinity is evidence for either (1) a spatial subsidy of high quality POM derived from the Strait of Georgia, and/or (2) greater primary productivity in the shallow mixed layer of the river-influenced water mass. These mechanisms are non-exclusive and likely both contribute to the observed patterns; the higher variability of phytoplankton abundance at low salinity may be a result of the mixing of oceanic and river-influenced water masses, yet some level of primary productivity may be necessary to maintain algal biomass levels in the river-influenced water mass since mesograzers are also associated with blooms in the Strait of Georgia (Harrison et al. 1983). Low abundance of phytoplankton in winter was expected owing to limited light availability and intense buoyancy flux of tidally flushed water in the SJA region (Mackas and Harrison 1997). Yet low phytoplankton abundance was also observed during spring and summer when environmental conditions should promote greater primary productivity. For example, the range of Chl *a* over a single tide cycle during May (POM-24HR) was as large as the range observed among seasons. Similarly broad changes of EFA concentration, C : N and SI values were observed at different tide stages, with a sixfold increase of EFA and enrichment of δ^{13}C and δ^{34}S occurring at lower salinities. During these low salinity events, the fresh, warm water extended to depths greater than 20 m, influencing benthic organisms throughout the photic zone (A. Lowe, unpubl. data; Masson and Peña 2009; Bashevkin et al. 2016).

The high pH associated with greater freshwater input is paradoxical, as the reduced buffering capacity of lower salinity water, and the increased temperature associated with lower salinity, should reduce pH (Cai et al. 2010). However, the pattern of high pH in low salinity was consistent across scales. The strong positive correlation of higher pH with phytoplankton biomass in lower salinity water provides evidence for an important biological feedback on the chemical environment in the SJA. We hypothesize that phytoplankton productivity modulates pH by drawing down carbon dioxide and outweighs physical influences on carbonate chemistry. This is supported by the observation that pH recorded at FHL was higher during the day, and in lower salinity water mass, with no statistical interaction between these factors. Conversely, heterotrophic activity in the water column may contribute to the low pH observed at higher salinity. Bacterial FA in the SJA were proportionally more abundant when phytoplankton decreased (Lowe et al. 2014) and paralleled observations of the microbially dominated "Blue" phase in the Tyrrhenian Sea (D'Alelio et al. 2014), suggesting the greater microbial activity in high salinity water contributes to the reduced pH (e.g., Wallace et al. 2014). Simultaneous measurements of water column primary productivity and changes to pH are still needed, but this observation adds to
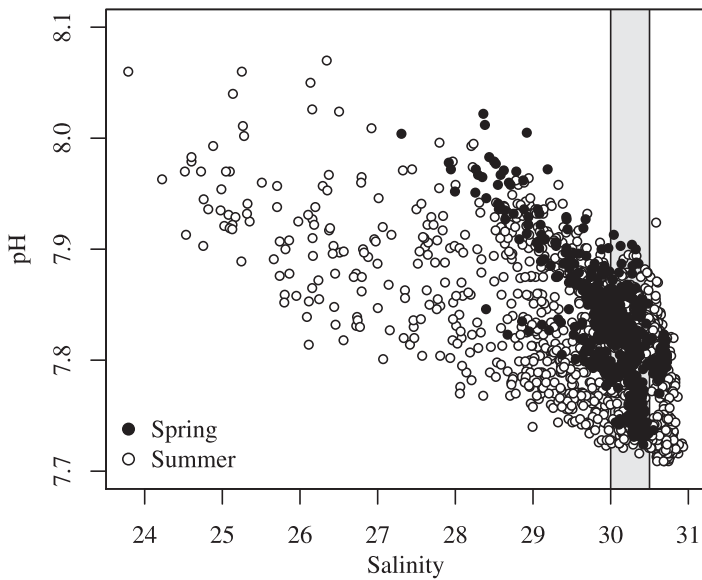
Fig. 5. Variation of pH in relation to salinity measured at 15 min intervals with a Honeywell Durafet III at the Friday Harbor Labs during spring 2014 (solid) and summer 2013–2014 (hollow). Shading as in Fig. 3b.

multiple studies suggesting this biological feedback may have significant ecosystem consequences.

Environmental conditions affect the FA content of algae (Dalsgaard et al. 2003; Galloway and Winder 2015) leading to declines in EFA and food quality under increased $pCO2$ (Rossoll et al. 2012), high temperature and low salinity (Galloway and Winder 2015). However, growing evidence suggests the plasticity in community composition in response to environmental changes observed in this study will be of greatest importance for determining energy cycling (D'Alelio et al. 2014), effects on consumers (Rossoll et al. 2013) and the abundance of EFA synthesized by photoautotrophs (Galloway and Winder 2015). Indeed, POM composition varied greatly with oceanographic conditions in this study, and changes in the phytoplankton species composition can explain more than 70% of the variation in POM fatty acid composition (Lowe et al. 2014). Variability in EFA composition matters to a wide range of consumers as long-chain ($\geq C_{20}$) EFA such as EPA, DHA, ARA, and ω-3:ω-6 FA ratios are critical for zooplankton (Jónasdóttir and Kiorboe 1996; Park et al. 2002; Brett et al. 2009) and fish (Watanabe et al. 1983; Copeman et al. 2002). These differences in EFA concentrations related to oceanographic conditions potentially have large consequences for community structure (Litzow et al. 2006; Budge et al. 2014).

The availability of EFA in high quality POM may interact with exposure to environmental stressors to determine resilience of marine organisms (e.g., Hettinger et al. 2013; Thomsen et al. 2013). Organisms can accumulate or synthesize many FA to mitigate for exposure to environmental stressors (Pernet et al. 2007), and may benefit from the greater availability of EFA and important precursor FA during low salinity events. However, organisms that exhibit increased metabolism or reduced activity when exposed to low salinity for long periods may be physiologically or behaviorally isolated from this resource (Shumway 1979, 1983; Pia et al. 2012). Many stressors including pH, temperature, food availability and salinity are correlated in coastal environments and our results may be used to design more realistic experiments investigating the role of EFA in responses to multiple stressors. The SJA represent a natural laboratory for studying these interactive effects; previous research has identified high variability of EFA composition of benthic suspension-feeders along the salinity gradient that may be a result of the timing and duration of exposure to fluctuating oceanographic conditions and the concomitant changes to food availability (Galloway et al. 2013). An important area of future research will be to determine if the timing of biologically-driven pH changes in coastal environments exerts selective pressure on species traits, particularly for the phenology of spawning or larval release, which often synchronize with phytoplankton blooms (Starr et al. 1991) and may indirectly benefit from the improvement of carbonate chemistry associated with phytoplankton blooms.

Salinity as a biological indicator: meta-analysis of regional datasets

The meta-analysis of datasets covering multiple temporal and spatial scales revealed a consistent relationship among biological and oceanographic variables related to the salinity threshold along the estuarine gradient from the Strait of Georgia to the Strait of Juan de Fuca. Significant differences in phytoplankton biomass (Chl *a*), feedbacks of primary productivity (DO and pH increases) and temperature were observed across disparate datasets (Table 4). The changes in temperature, Chl *a*, and pH related to the salinity threshold were robust even across the large interannual variation observed at RR and WA-DOE. Diatom and dinoflagellate biomass above and below the salinity threshold were significantly different, and although pico-phytoplankton were not quantified as part of POM samples (nets were > 20 μm), a negative correlation between the abundance of the cyanobacteria *Synecococcus* spp. and salinity has been previously reported in the SJA, suggesting this negative relationship with salinity exists across photosynthesizing taxa (Brown 2013). Similar observations of increased phytoplankton-derived biomass and FA were related to a slight decrease in salinity from river discharge in the Tyrrhenian Sea (D'Alelio et al. 2014), Monterey Bay, U.S.A. (Fischer et al. 2014) and the Columbia River, U.S.A. (Brodeur and Morgan 2015), demonstrating the generality of this pattern.

In coastal marine ecosystems, episodic pulses of freshwater discharge greatly influence environmental conditions

Table 4. Results of meta-analysis of environmental and food availability parameters above and below the salinity threshold from available datasets spanning multiple scales. Positive treatment effective (TE) indicates higher value in low salinity vs. high salinity water. Calculation of mean above and below salinity threshold, Treatment effect size (±SE) and *p*-value conducted with "meta" package in R (Schwarzer 2014). SJA-POM food availability data from 24-h sampling in the SJA (this study). See methods for descriptions of other data sources.

Parameter	Units	Dataset	n Low (High)	TE ± SE	Overall *p*-value
Temperature	°C	WA-DOE	67 (39)	2.79 ± 0.496	
		SJA-PIL	8 (8)	0.75 ± 0.042	
		FHL	60 (60)	1.18 ± 0.018	
		RR	815 (815)	0.50 ± 0.002	
		POM-24HR	8 (5)	0.16 ± 0.052	
		POM-SEAS	6 (26)	1.39 ± 0.763	
		Mean		**1.13 ± 0.229**	**<0.0001**
Chl *a*	log(μg/L)	WA-DOE	31 (10)	0.22 ± 0.534	
		SJA-PIL	8 (8)	0.17 ± 0.12	
		POM-24HR	8 (5)	0.44 ± 0.152	
		Mean		**0.28 ± 0.269**	**0.0038**
Dissolved oxygen	mg/L	WA-DOE	37 (20)	1.55 ± 0.606	
		SJA-PIL	8 (8)	1.07 ± 0.081	
		Mean		**1.31 ± 0.344**	**<0.0001**
pH	Total scale	WA-DOE	59 (37)	0.10 ± 0.057	
		SJA-PIL	8 (8)	0.08 ± 0.007	
		FHL	60 (60)	0.06 ± 0.001	
		Mean		**0.08 ± 0.022**	**<0.0001**
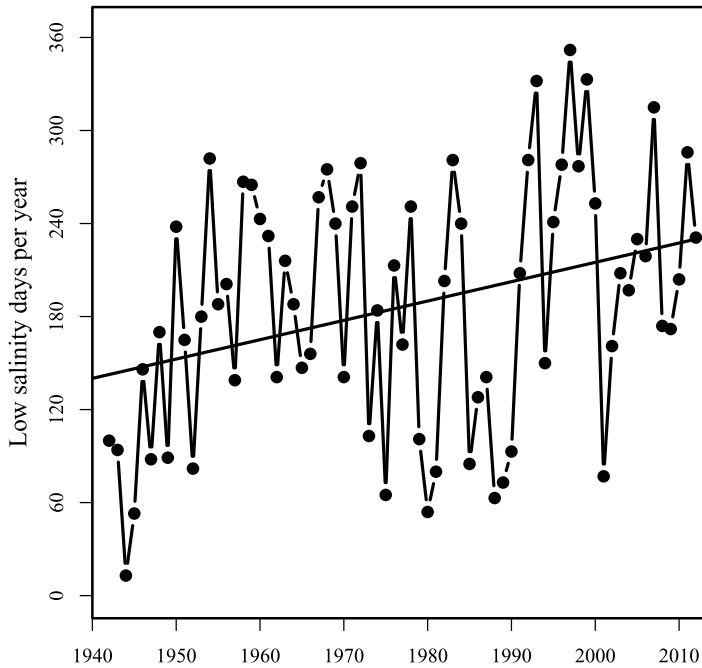
Fig. 6. Annual salinity at Race Rocks, Canada plotted as days per year below the salinity threshold from 1942 to 2012. Days below the salinity threshold have increased significantly since 1942 (12.5 d decade^{-1}; linear regression, $n = 71$, $p = 0.006$).

(LeBlond et al. 1994; Cloern 1996) and primary productivity (Masson and Peña 2009; D'Alelio et al. 2014; Fischer et al. 2014). Regions that experience brief estuarine plumes can be highly productive because tidal mixing can enhance seawater nutrients (Yin et al. 1995). The Fraser River contributes nitrogen to brackish surface water in the Strait of Georgia, but may be of less importance in the SJA water that is rarely nutrient limited (>5uM/L nitrate + nitrite; Mackas and Harrison 1997; Murray et al. 2015). While a change in salinity of this magnitude does not necessarily induce physiological responses from marine phytoplankton, the low water density associated with warmer and lower-salinity water acts to maintain stratification in an otherwise well-mixed water column (Cokelet and Stewart 1985). A change in mixed layer depth in the SJA from 100 m to < 20 m as a result of freshwater input has a large effect on the integrated mixed layer PAR. Given previous measurements of light attenuation with water depth (attenuation coefficient $k = 0.14$ to 0.32 m^{-1} from Brown 2013, % irradiation = exp^{-k}*depth), phytoplankton travelling in a mixed layer of 20 m would have 5 times greater integrated mixed layer PAR than those travelling to 100 m depth. Thus, the stratification alleviates light limitation by prolonging phytoplankton residence time in the high nutrient surface water (Taylor and Ferrari 2011; Murray et al. 2015).

The stark differences in food availability and quality observed across a small change of salinity suggest that

salinity, a commonly measured variable in many long-term datasets, is a valuable indicator of the scalable processes leading to improved food availability in this region. Identifying biological indicators and the range of biological responses to them allows for the generation of testable hypotheses regarding long-term environmental changes (Melillo et al. 2014). This method could be applied to similar environmental thresholds in many coastal ecosystems with freshwater driven patterns of productivity including areas of brackish water (LeBlond et al. 1994; Cloern 1996) and polar regions with significant glacial meltwater (Dierssen et al. 2002). As with many biological indicators, there may be location specific limitations to this method dependent on biological responses to the environmental conditions. Observations in this study were restricted to the SJA, between the Strait of Georgia and the Strait of Juan de Fuca, and the oceanography in these deeper bodies of water may be different than in SJA channels. In the Strait of Georgia, very low salinity ($\sim$5) may cause physiological stress and the relationship between salinity and primary production may be quite different in this range of salinities. However, the timing of the spring phytoplankton bloom can be predicted by wind-driven vertical mixing (Yin et al. 1997; Collins et al. 2009) and salinity could still be an indicator of the processes leading to stratification and enhanced primary productivity.

We used long-term observations from Race Rocks, British Columbia, to generate hypotheses about oceanographic conditions and food availability in the SJA over time. Patterns of primary productivity in the Strait of Georgia have been linked to variability of environmental conditions (Harrison et al. 1983; Allen and Wolfe 2013), however direct long-term observations are limited. The frequency of low salinity events has increased significantly over the last 71 yr in this region (Fig. 6) in response to altered air temperatures and precipitation regimes in the Rocky and Cascade Mountain Ranges (Morrison et al. 2002; Stewart et al. 2004). Using this salinity threshold, we predict that the surface brackish water mass from the Strait of Georgia may more frequently extend further south to the SJA before becoming mixed by tides and wind (Waldichuk 1957). This could cause more frequent or longer of pulses of food availability in the SJA. These pulses of improved food availability have likely occurred earlier in the spring and later into fall as the number of days per year below the salinity threshold has increased. The salinity threshold can be used to generate testable hypotheses that potentially link observations currently lacking explanations. For example, the observed decrease in average DO concentration in Strait of Georgia deep water (Masson and Cummins 2007) may be a function of the predicted lengthening of the growing season. The majority of primary productivity in the northern Salish Sea is remineralized in situ (Johannessen et al. 2003) such that greater diatom abundance in the surface layer would result in more biomass export to and oxygen con-

sumption during decomposition in deep water (e.g., Keeling et al. 2010).

The relationship between riverine discharge, oceanographic conditions, phytoplankton concentration, and carbonate chemistry described here has major implications for the study of coastal ecosystems. These observations support a view that physiological explanations alone are not sufficient to explain the influence of oceanographic variability on POM availability and quality; physiological models of phytoplankton primary productivity may fail to predict local responses to enhanced freshening and stratification related to climate change if they do not incorporate stratification dynamics (Sarmiento et al. 2004). The increased pH in the surface layer was associated with biological activity in this study and parallels patterns related to density stratification and primary productivity observed in the Southern Salish Sea (Reum et al. 2014); similarly, biological activity and riverine inputs drive variation of pH in Willapa Bay, Washington, that are decoupled from oceanic dynamics (Ruesink et al. 2015). Thus future studies should strive to incorporate both physiological and oceanographic processes into investigations of response of organisms to environmental change. The observation of greater food availability, quality and higher DO and pH associated with freshwater discharge in coastal systems highlights the profound influence of and connectivity to inland climate and dynamics of human development hundreds of miles away. It also suggests that long-term changes to river hydrology resulting from predicted deglaciation (e.g., Clarke et al. 2015) or river diversion will have dramatic impacts on environmental conditions and food availability in the SJA that will be mirrored in many coastal regions around the world.

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Acknowledgments