Production of the Copepod Pseudodiaptomus forbesi *Is Not Enhanced by Ingestion of the Diatom* Aulacoseira granulata *During a Bloom*

Michelle Jungbluth, Calvin Lee, Cheryl Patel, Toni Ignoffo, Brian Bergamaschi & Wim Kimmerer

Estuaries and Coasts
Journal of the Coastal and Estuarine Research Federation

ISSN 1559-2723

Estuaries and Coasts
DOI 10.1007/s12237-020-00843-9

Springer

Estuaries and Coasts
https://doi.org/10.1007/s12237-020-00843-9

Production of the Copepod *Pseudodiaptomus forbesi* Is Not Enhanced by Ingestion of the Diatom *Aulacoseira granulata* During a Bloom

Michelle Jungbluth[1] · Calvin Lee[1] · Cheryl Patel[1] · Toni Ignoffo[1] · Brian Bergamaschi[2] · Wim Kimmerer[1]

Received: 19 June 2020 / Revised: 18 September 2020 / Accepted: 25 September 2020

Abstract

In 2016, a massive bloom of the chain-forming diatom *Aulacoseira granulata* occurred in the upper San Francisco Estuary, California, with chlorophyll concentrations up to 75 μg Chl L^{-1}. In this study, quantitative PCR was used to investigate consumption of the bloom organism by the numerically dominant zooplankter *Pseudodiaptomus forbesi* (Copepoda: Calanoida) and to estimate the contribution of the bloom to egg production. Copepods were collected on four transects during May and June 2016; egg production rates were somewhat elevated above previous rates measured in the estuary. Ingestion of *A. granulata* was highest on the first sampling day, just after the peak of the bloom, ranging from 175 to 945 cells copepod^{-1} day^{-1}. One month later ingestion rates dropped to 0–130 cells copepod^{-1} day^{-1}, despite continued dominance of *A. granulata* in the plankton. Ingestion of *A. granulata* provided from 0 to 21% (median 1%) of the estimated daily carbon required for growth and reproduction of *P. forbesi*. Although the copepods probably obtained nutrition from a microbial food web stimulated by the bloom, monitoring data showed little demographic response to this bloom. Thus, a massive diatom bloom in an unproductive estuary provided only a minor stimulus through an abundant consumer to the pelagic food web.

Keywords Food web · qPCR · Phytoplankton bloom

Introduction

Globally, productive fisheries have been linked to high biomass of large phytoplankton cells, principally diatoms, in estuarine and marine waters (Ryther 1969; Nixon 1988; Houde and Rutherford 1993; Woodworth-Jefcoats and Wren 2020). Phytoplankton blooms are common features of estuaries globally, arising through a variety of triggers generally involving resources becoming non-limiting followed by a rapid shift to a state where growth rates exceed mortality (Carstensen et al. 2015). In estuaries and some coastal waters, the timing and magnitude of blooms is highly variable (Cloern and Jassby 2008), and the fate of biomass produced during blooms varies with bloom timing and the responses of benthic and pelagic grazers (Ziemann et al. 1991; Conley and Malone 1992). The transfer of bloom-produced energy to higher pelagic trophic levels can also depend on the size and species identity of the mesozooplankton grazers (Runge 1985), and much of this energy may be routed through alternative pathways such as benthic and microzooplankton grazing (Cushing 1989; Kimmerer et al. 1994; Calbet et al. 2008; Lonsdale et al. 2009).

Diatom blooms, particularly those in the spring, can provide critical support for secondary production and growth of larval fishes (Ryther 1969; Turner 1984a; Mann 1993). However, the nutritional adequacy of diatoms for grazers remains in dispute despite decades of investigations (Irigoien et al. 2002; Ianora and Miralto 2010). Several decades of research have revealed immense complexity and nuance in diatom-copepod interactions and engendered intense disagreements. Diatoms are only one of many plankton that may sharply increase their biomass in response to favorable conditions (Barber and Hiscock 2006). Some diatoms produce

Communicated by David G. Kimmel

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12237-020-00843-9) contains supplementary material, which is available to authorized users.

✉ Michelle Jungbluth
mjungbluth@sfsu.edu

[1] Estuary & Ocean Science Center, San Francisco State University, 3150 Paradise Drive, Tiburon, CA 94920, USA

[2] California Water Science Center, US Geological Survey, 6000 J Street Placer Hall, Sacramento, CA 95819, USA

Published online: 10 October 2020

toxic substances that interfere with zooplankton reproduction by suppressing egg hatching (Ianora et al. 1996; Ban et al. 1997; Miralto et al. 1999; Ianora and Miralto 2010), or they may be nutritionally inadequate to support reproduction (Jones and Flynn 2005). However, experiments on copepods feeding in 12 diatom-rich areas showed no relationship between egg-hatching success and diatom biomass or its proportion of total phytoplankton biomass (Irigoien et al. 2002). Laboratory experiments show the effects of diatoms as a food source vary among copepod species, diatom species, and even different clones of the same diatom species (Ianora et al. 1995; Ask et al. 2006; Dutz et al. 2008; Amin et al. 2011; Lauritano et al. 2012). Thus, the suitability of diatoms in supporting copepod reproduction depends on many factors, may be species specific, and cannot be deduced without a very specific experimental approach.

In spring–summer of 2016 an exceptional diatom bloom occurred in the freshwater to brackish regions of the northern San Francisco Estuary (SFE), which are otherwise unproductive (Schraga et al. 2018). This bloom, produced by the chain-forming centric diatom *Aulacoseira granulata* (Syn. *Melosira granulata*; Guiry and Guiry 2019), persisted from March to September. Chlorophyll concentrations throughout the northern estuary exceeded 20 μg Chl L^{-1}, at the 99th percentile of measurements during 1988–2015 (Interagency Ecological Program 2020; Orsi and Mecum 1986).

Aulacoseira granulata is a cosmopolitan species found in waters with high concentrations of silica and phosphorus (Kilham 1971; Kilham et al. 1986; Guiry and Guiry 2019). Cells of *A. granulata* are 4–17 μm in diameter and up to 20 μm in length, while chains are up to 10 mm long. This species can be heavily silicified (Goldman and Horne 1983; Takano et al. 2004), and copepods can have difficulty consuming diatoms with thick frustules (Liu et al. 2016; Pančić et al. 2019). *Aulacoseira granulata* can form a resting stage in suboptimal conditions, remain viable for decades (Sicko-Goad et al. 1986), and survive through anoxic conditions (Lashaway and Carrick 2010). Blooms of *A. granulata* may also be stimulated allelopathically by exposure to another species. In a eutrophic river, growth of *A. granulata* was preceded by a bloom of the chlorophyte *Gloeocystis planctonica* and laboratory experiments confirmed the stimulation of *A. granulata* growth by *G. planctonica*-treated media (Poister et al. 2015).

The literature is inconsistent on consumption of *A. granulata* by zooplankton and its suitability to support secondary production. Chain-forming diatoms like *A. granulata* are often consumed by copepods at higher rates than single cells (Richman and Rogers 1969; Meyer et al. 2002), presumably due to greater ability of copepods to detect and handle large chains. Fecundity and egg-hatching success of the copepod *Eurytemora affinis* declined when they were fed *A.* (as *Melosira*) *granulata* (Ban et al. 1997), suggesting that it may not provide adequate nutrition as a sole food source (Jones and Flynn 2005) or may inhibit secondary production in some other way. *Aulacoseira granulata* was eaten at high rates by the copepod *Eudiaptomus japonicus* in Lake Biwa, Japan, despite low in situ abundance of the diatom (Kagami et al. 2002), whereas grazing on this diatom was not detected by mixed zooplankton in an Andean lake (Queimalinos et al. 1998). *Aulacoseira granulata*'s nutritional sufficiency for zooplankton is unknown, but prior research suggests that nutritional quality may not influence copepod feeding rates on diatoms as much as cell size, silica content, and chain length of the diatom species (Meyer et al. 2002; Bjærke et al. 2015; Liu et al. 2016).

Pseudodiaptomus forbesi is a member of a large genus of demersal copepods common in temperate to tropical estuarine and coastal waters (Walter 1987). *Pseudodiaptomus forbesi* was introduced to the SFE in 1987, probably in ballast water, and became abundant in 1988 (Orsi and Walter 1991). During late spring through autumn it is distributed throughout tidal freshwater and into brackish water (Kayfetz and Kimmerer 2017). Since its introduction, *P. forbesi* has been the most common food item consumed by planktivorous fish in this region (Nobriga 2002; Bryant and Arnold 2007; Slater and Baxter 2014). It consumes foods typical of grazing calanoid copepods, including diatoms, ciliates, and various flagellates (Bowen et al. 2015; Kayfetz and Kimmerer 2017), though cyanobacteria can be an important food source (Holmes 2018) and these copepods will eat the toxigenic *Microcystis aeruginosa* (Ger et al. 2018).

We capitalized on the bloom in the SFE to measure consumption of *Aulacoseira* by *Pseudodiaptomus forbesi*, the numerically dominant mesozooplankton species in the upper SFE. In a rapid-response study, we collected zooplankton in the field, determined abundance and reproductive rate, and used molecular analysis to quantify the bloom organism in the gut contents of copepods collected in and out of bloom areas. We then assessed the contribution of this consumption to the growth and reproduction of this copepod, using decades of monitoring and research for context.

Methods

Study Site

The San Francisco Estuary (SFE) is the largest estuary on the west coast of the United States, with a watershed area of ~ 200,000 km^2. The estuary has two main branches, the low-flow South San Francisco Bay and the river-dominated northern estuary linking Central San Francisco, San Pablo, and Suisun Bays and the California Delta (Fig. 1). Tides in the estuary are mixed diurnal and semidiurnal with a mean tidal range of ~ 1.25 m. The climate in the region is Mediterranean,

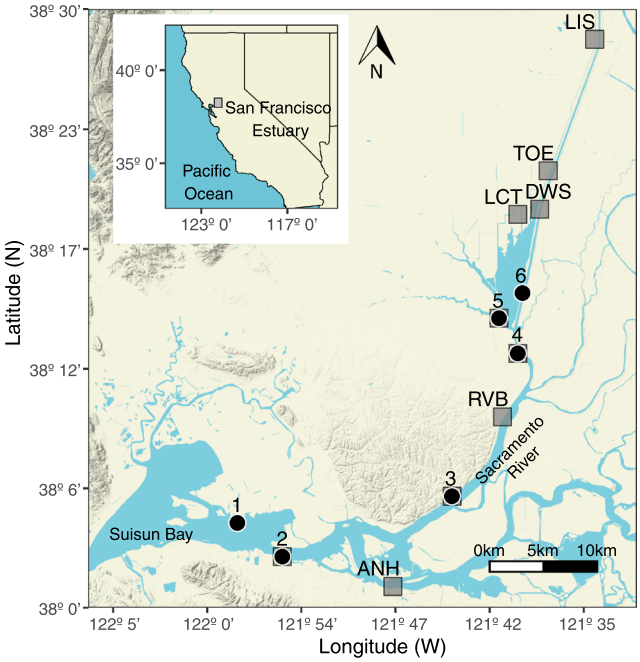

Fig. 1 Study area with sampling stations (black circles) visited during May–June 2016. Gray squares overlapping with black circles represent locations of USGS continuous-monitoring stations at many of our sample sites including Liberty Island (Station 5), Cache Slough Complex (Station 4), Decker Island (Station 3), and Mallard Island (Station 2), while single gray squares are USGS monitoring sites used for environmental data surrounding our study sites (LIS, TOE, DWS, LCT, RVB, ANH; USGS designations are noted in Table S1). The Deep Water Ship Channel (Station 6) and Snag Island (Station 1) were also sampled in the current study but did not overlap closely with USGS monitoring sites

with warm dry summers and cool wet winters. Freshwater flow through the estuary during 2016 was at the median of years 1980–2019, but 2016 was preceded by a 4-year drought with annual freshwater flows in 2014 and 2015 ranking 3rd and 8th of 40 years (https://data.cnra.ca.gov/dataset/dayflow, accessed 12 April 2020).

Our study focused on the northern estuary from the broad, shallow Suisun Bay (Station 1) up the deep, narrow tidal channel of the Sacramento River (stations 2 and 3), into the Cache Slough complex (stations 4–6; Fig. 1). Cache Slough links the Sacramento River to the flooded Liberty Island, the Sacramento Deep Water Ship Channel, and the Yolo Bypass, a seasonal floodplain. During high-flow periods in winter the Yolo Bypass and Cache Slough area are often flooded, while in summer these areas are reduced to narrow tidal riparian channels and freshwater wetlands.

Field Collection

Sampling was conducted on the Research Vessel *Mary Landsteiner* (US Geological Survey) on four dates in May and June 2016 to sample the extent of the bloom at six sites along the Sacramento River, from Suisun Bay to the Cache Slough complex (Fig. 1, Table S1). Sampling was conducted during the daytime, largely during ebb tide (Table 1). Salinity and temperature were recorded with a thermosalinograph (Sea-Bird Scientific SB45), at each station. Surface water for chlorophyll *a* analysis was collected in a 1-liter Nalgene bottle which was stored in a dark cooler until analysis.

Two zooplankton samples, one each for abundance and for molecular analysis, were collected at each station by gentle subsurface tows using a 150-μm mesh, 0.5-m-diameter ring net, fitted with a General Oceanics flowmeter with a low-speed rotor (Model 2030R). *Pseudodiaptomus forbesi* copepodites and adults migrate vertically on a tidal cycle in deeper channels, but is generally abundant through the water column on both flood and ebb (Kimmerer et al. 1998). This species is also demersal in shallow, clear waters (Kimmerer and Slaughter 2016). A previous study at station 5, the shallowest station, showed day/night differences in abundance of adult females in the 4-m water column (Kimmerer et al. 2018), but the mean proportions of *P. forbesi* females that were ovigerous did not differ between day and night during the three surveys of that study (Kimmerer unpublished). Thus, we are confident that our daytime sampling was unbiased with regard to the proportion of females that were ovigerous and, therefore, the egg production rate.

Volumes sampled were 1.7–12 m^3 for abundance and 1.2–5.7 m^3 for molecular analysis. Samples for microscopic analysis were preserved in ~4% v/v formaldehyde. Samples for molecular analysis were concentrated on a sieve, preserved with 95% non-denatured ethanol (EtOH), and stored in a −20 °C freezer until they were processed. All molecular samples received an ethanol exchange within 24 h of collection.

Monitoring Data

The SFE is rich in monitoring and other programs that provided valuable background information for our study. The U.S. Geological Survey (USGS) maintains continuous monitoring stations for chlorophyll fluorescence at 17 fixed stations across the upper San Francisco Estuary (U.S. Geological Survey 2020). We used data on fluorescence calibrated to chlorophyll concentration from nine stations across the range of our sampling stations (Fig. 1; Table S1). We also obtained chlorophyll data from discrete samples taken in the larger estuarine channels in our sampling region by the USGS (Cloern and Jassby 2012) and the California Interagency Ecological Program (IEP; Orsi and Mecum 1986; Sommer et al. 2007) for an analysis of how chlorophyll in 2016 compared to the prior three years. The additional USGS samples included all relevant stations from eastern Suisun Bay to Rio Vista (stations not shown; see U.S. Geological Survey 2020; Fig. 2 in Cloern and Jassby 2012). Interagency Ecological Program long-term monitoring stations used for this analysis include all frequently sampled stations in Suisun Bay and the south-central Delta (Fig. 1 in Kimmerer et al. 2019), and two

Table 1 Sampling dates and corresponding event numbers, site names, site numbers, sampling times, tidal stage (National Ocean and Atmospheric Administration, US Geological Survey tidal gauges), and environmental data where zooplankton samples were collected for our study. Environmental data collected at each station includes surface water temperature (°C), salinity (practical salinity units), and > 20 μm chlorophyll *a* mean and standard deviation (s.d.) (μg Chl L^{-1})

Event	Date	Site no.	Name	Time	Tide	Temp (°C)	Salinity	Chl *a* (μg L^{-1})	Chl *a* s.d.
I	20 May 2016	1	Snag Island	1500	Ebb	19.1	3.6	5.5	1
		2	Mallard Island	1415	Flood	19.3	1.9	14.3	–
		3	Decker Island	1255	Flood	20	0.1	7.3	0.1
		4	Cache Slough	1135	Peak ebb	19.2	0.1	5.3	1.6
		5	Liberty Island	900	Ebb	18.8	0.1	4.1	0.5
		6	Deep Water Ship Channel	1010	Ebb	20	0.1	8.2	0.8
II	25 May 2016	1	Snag Island	950	Ebb	18.6	3.0	0.5	0.2
		2	Mallard Island	1115	Peak ebb	18.8	0.7	4.9	1.5
		3	Decker Island	1310	Peak ebb	18.8	0.1	2.2	1.1
		4	Cache Slough	1425	Peak ebb	18.2	0.1	5	0.8
		5	Liberty Island	1618	Flood	18.8	0.1	3.9	3.4
		6	Deep Water Ship Channel	1519	Flood	20.3	0.1	2.5	0.1
III	9 June 2016	2	Mallard Island	1100	Ebb	20	2.2	1.3	0.6
		3	Decker Island	1300	Ebb	21.8	0.1	2.2	0.5
		4	Cache Slough	1420	Ebb	21.4	0.1	7.9	0.3
		5	Liberty Island	1555	Flood	20.9	0.1	7.3	1.7
		6	Deep Water Ship Channel	1525	Peak ebb	22.6	0.1	0.3	0
IV	28 June 2016	1	Snag Island	1320	Ebb	23.3	5.4	0.4	0.1
		2	Mallard Island	1240	Ebb	22.7	3.5	–	–
		3	Decker Island	1125	Ebb	22.9	0.1	18.2	1
		4	Cache Slough	838	Flood	23	0.1	6.9	0.9
		5	Liberty Island	1015	Flood	23.4	0.1	9.7	1.5
		6	Deep Water Ship Channel	935	Flood	23.2	0.1	9.7	0.6

stations defined by salinity of ~ 1 and 4. In addition, 36 phytoplankton samples were collected throughout the bloom period and analyzed for phytoplankton identification, abundance, and biovolume by BSA Environmental, Inc. These were used to determine the proportion of biovolume made up by *Aulacoseira* spp.

We used data on zooplankton abundance, also obtained from the IEP (Orsi and Mecum 1986), to determine if *P. forbesi* responded demographically to the bloom. The abundance of *P. forbesi* has followed a predictable pattern since its introduction, with a rapid spring increase followed by a plateau in summer and a gradual decline in autumn. Higher-than-average freshwater flow in winter depresses abundance and delays the spring increase, whereas the summer plateau is unaffected by interannual variability in flow (Kimmerer et al. 2017). Therefore, we focused this analysis on summer. The principal assumption was that any demographic response should be reflected in markedly higher abundance in 2016 than in other years, provided abundance in the preceding winter is accounted for. To obtain samples representative of the population, we used data from all stations at salinity < 1 (Practical Salinity Units) from 1994 through 2018 (20 total stations), then compared the pattern of seasonal abundance in 2016 with those in other years. To assess the response of the planktonic food web we also estimated total biomass of all post-naupliar copepods for the same region and time periods, using previously determined carbon masses (e.g., Kimmerer 2006; Kimmerer et al. 2017).

Laboratory Analyses

Samples were processed in the EOS Center laboratory at Tiburon, California. Duplicate subsamples of the water for chlorophyll *a* measurements were filtered onto GF/F (glass fiber filters; Whatman®, ~ 0.7 μm) or 5-μm Nuclepore® filters, or concentrated on a 20-μm nylon mesh sieve and backwashed onto a GF/F filter. We adjusted sample volumes depending on the amount of particulate matter: GF/F samples were 30–100 ml, 5-μm samples were 37–70 ml, and 20-μm samples were 100–200 ml. The 5-μm size fraction captures most particles on which copepods can feed efficiently, which would also include many single *Aulacoseira* cells (broken or

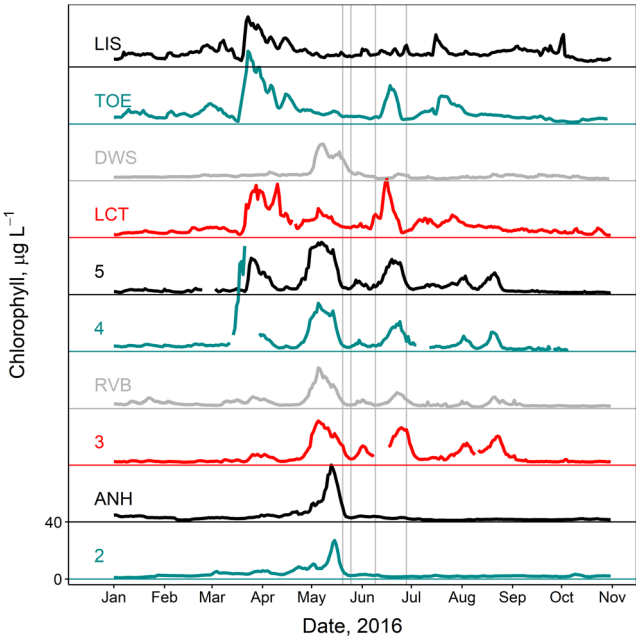

Fig. 2 Chlorophyll *a* time series from continuous-monitoring stations from January through October 2016. Abbreviated station names show the stations in approximate order from north (Lisbon Weir, top) to southwest (Mallard Slough, bottom; see Fig. 1 and Table S1). All plots are scaled from 0 to 40 µg Chl L^{-1} and horizontal lines show zero values, shifted upward for each station by 40 µg Chl L^{-1}. Vertical lines indicate sampling events I–IV. Colors are only to aid separation of plot lines

isolated cells < 20 µm), and the 20-µm fraction should include most of the *Aulacoseira* chains and little else, since the phytoplankton assemblage was dominated by *Aulacoseira* spp. chains at the time of our study. We extracted filters in 90% acetone and measured fluorescence using a Turner Designs 10-AU benchtop fluorometer calibrated with pure chlorophyll (Arar and Collins 1997). The samples were acidified with 10% hydrochloric acid.

Quantitative subsamples of preserved zooplankton samples were taken with a Stempel pipette, poured into a plastic tray, and counted under a dissecting microscope. We identified all organisms to the lowest feasible taxonomic level, and copepods to male, female with or without eggs, and copepodites. Nauplii were not counted. Our target for counting was 200 *P. forbesi* individuals or 1,000 total organisms, and actual counts were 159–1048 *P. forbesi* and 528–1078 organisms not including abundant rotifers.

For each sample, eggs were counted from up to 30 randomly selected ovigerous females of *P. forbesi* and in any loose egg sacs that were identifiable as those of *P. forbesi* (0–10%, median 0%, mean 3% of total eggs counted). The product of total ovigerous females in a sample times mean eggs per sac was added to the number in loose sacs, and that was divided by the total number of females to get the egg ratio for that sample. The egg production rate was determined by the egg ratio method (Edmondson et al. 1962) using a relationship of egg development time to temperature (Sullivan and Kimmerer 2013). There were > 30 total females in all but two samples: event I station 2 and event 4 station 2, which each had one and two ovigerous females, and nine and 13 non-ovigerous females, respectively. Egg production rates were placed in a longer-term context using a rectangular hyperbola previously fit to egg production rates measured in various studies in the northern estuary from 2006 through 2018, including this study (Gearty 2020):

$$E = C\,(S\mathrm{max})/(C + K). \quad (1)$$

where E is egg production rate and S_{max} is the maximum egg production rate (both eggs ♀$^{-1}$ day^{-1}), and C is chlorophyll concentration and K is half-saturation constant (both µg Chl L^{-1}). Fitted parameters with 95% confidence limits were $S_{max} = 3.6 \pm 0.8$ eggs ♀$^{-1}$ day^{-1} and $K = 3.4 \pm 2.0$ µg Chl L^{-1} with a residual standard error of 4.6 eggs ♀$^{-1}$ day^{-1}.

Aulacoseira-Specific Primers

To determine the DNA identity of the bloom-forming organism for species-specific primer design, we sequenced the bloom organism from an ethanol-preserved zooplankton sample taken at a station where the bloom was evident; few other phytoplankton taxa would have been collected in a towed 150 µm mesh net. We isolated 1 mL of algae from the sample and removed non-algal debris under a microscope. Ethanol was removed by vacuum centrifugation and the DNA was extracted following the DNeasy Blood and Tissue Kit (Qiagen; Total DNA from Animal Tissues spin-column protocol) with the following minor modifications: 3 µL of carrier RNA was added during the lysis step to increase recovery of DNA, and at the final step samples were eluted using 200 µL of nuclease-free water. All DNA extracts were stored in a -20 °C freezer until analysis.

General 18S rRNA primers 960F (Gast et al. 2004) and 18SCom1R (Zhang et al. 2005) (Integrated DNA Technologies) were used to amplify and sequence a 350-bp region of DNA from the algal DNA extracts. PCR reactions were run in 25 µL total volume, including 2.5 µL 10× buffer, 0.5 µL of 10 mM dNTPs, 2 µL of MgCl (25 mM), 0.5 µL of each primer, 1 µL bovine serum albumin (BSA; Ambion), 0.12 µL Taq Polymerase (New England Biolabs) and 16.88 µL nuclease-free water. PCR reactions were run with the following thermal cycling conditions (Bio-Rad S1000 Thermal Cycler): Initial denaturation at 94 °C for 3 min, followed by 33 cycles of denaturation at 94 °C for 1 min, annealing at 57.8 °C for 1 min, and extension at 72 °C for 1 min, with a final extension step at 72 °C for 1 min. PCR products were cleaned (ExoSAP-IT, Affymetrix), ligated, cloned, and sequenced using standard protocols (ABI 3500 Perkin-Elmer capillary sequencer; BigDye v3.1). Resultant DNA sequences were compared to those in the Genbank database using *blast-n.*

While there were no 100% matches in the Genbank database for the DNA sequences obtained, the closest hits were to diatom species, primarily *Aulacoseira granulata,* and other *Aulacoseira* species including *A. nyassensis* and *A. ambigua* (> 98% identity, 100% query coverage).

We designed a set of genus-specific primers to target a 158-bp region of the 18S rRNA gene from all *Aulacoseira* spp. with known 18S rRNA gene sequences based on the above results and alignment (Geneious v.11.1.2) with non-target species from the NCBI Genbank database. These *Aulacoseira*-specific primers are Aula18S_599F (5′–ATCTCTGCCCTCCTTGGTTG–3′) and Aula 18S_735R (5′–CAACGAAATAGTGCCAAAACCC–3′). We verified amplification of the target species using concentrates of *Aulacoseira* spp. from our zooplankton samples, tested against *P. forbesi* DNA to ensure non-amplification, and verified that our primers did not amplify a range of cultured algae including *Cryptomonas*, *Cyclotella*, *Chlamydamonas*, and *Melosira* sp.

Quantification of *Aulacoseira* Ingestion

For quantitative PCR (qPCR) analysis of *Aulacoseira* ingestion, groups of adult female *P. forbesi* were sorted from each ethanol-preserved zooplankton sample and cleaned of external debris, then rinsed with fresh ethanol three times to minimize any contamination from externally attached phytoplankton. We isolated five replicate subsamples of five copepods each from each sample, omitting sampling event III at stations 1 and 5 and sampling event IV at stations 1 and 3 which contained too few females. Each subsample was transferred into Buffer ATL while minimizing ethanol transfer and ground with a sterile pestle to rupture copepod exoskeletons. Total DNA was extracted using the DNeasy kit as described above, with an overnight incubation at 56 °C to maximize DNA lysis from copepod guts.

Aulacoseira was quantified from each DNA extract by qPCR. To generate a standard curve for qPCR, *A. granulata* cells were isolated from a concentrated, preserved zooplankton sample and diluted to a concentration of 10,000 cells mL^{-1}. This sample was extracted as described above, and ten-fold serial dilutions of this extract (50–0.005 cell μL^{-1}) were used in a standard curve for qPCR estimation of *A. granulata* cell numbers in copepod guts.

Quantitative PCR reactions to quantify *A. granulata* in copepods were run on an Applied Biosystems 7300 Real-Time PCR System. Triplicate qPCR reactions for each sample and standard were run with a 20 μL total volume, which included 10 μL of PowerUP SYBR Green master mix (Applied Biosystems), 0.75 μL each of *Aulacoseira* forward and reverse primer (10 μm concentration), 2 μL of BSA, 1.5 μL of PCR-grade water, and 5 μL of the template. Thermal cycling conditions included polymerase activation of 50 °C for 2 min and denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 52 °C for 15 s, and extension of 72 °C for 1 min. A melting curve analysis was performed at the end of each qPCR run.

To verify the presence of and estimate the abundance of *Aulacoseira* cells in the guts of *P. forbesi*, a tissue-digestion method was used to visually count and measure diatom frustules in the guts of individual copepods (Mayama et al. 2006). Briefly, individual adult female *P. forbesi* were isolated from a subset of samples rates taken on May 25, 2016, that had a range of estimated ingestion rates: stations 5 (high ingestion rate, $n = 5$), 4 (high, $n = 1$), 3 (medium, $n = 1$), and 1 (low, $n = 3$). Adult female *P. forbesi* individuals were sorted from each ethanol-preserved zooplankton sample and cleaned of external debris, then rinsed with fresh ethanol three times to minimize any contamination from externally attached phytoplankton. Each cleaned copepod was transferred individually into 1.5-mL microcentrifuge tubes, and 250-μL liquid drain cleaner (Drano™) was added. Tubes were incubated for 20 min with intermittent vortexing. To rinse, deionized water was added to each tube which was then vortexed to mix and centrifuged at 3000 RPM for 3 min. Then the fluid was gently poured off and the sample was rinsed three times with deionized water. Finally, a permanent slide was prepared using ZRAX high refractive index mounting medium. A slide was also made with field-collected *Aulacoseira* to view intact cells. Each slide was scanned under an inverted microscope at × 40 total magnification for diatom frustules and pieces of diatoms. Whole *Aulacoseira* frustules were counted and pieces of frustules were measured. If pieces were in a cluster, the area of the cluster was measured, and it was noted if there were one or multiple layers in the cluster. The number of cells in each single adult female copepod was estimated by calculating the area of frustule pieces on the slide divided by the surface area of a single whole cell.

Data Analysis

For each triplicate qPCR measurement, replicates with a cycle threshold (Ct) standard deviation (SD of Ct) value greater than 0.5 were considered outliers and excluded from further analysis. Only samples with Ct values within the lowest acceptable quantification standard (0.005 cell μL^{-1}) were included in the analysis, and all others were considered as lacking target DNA.

We divided the estimates of *Aulacoseira* cells per copepod by an assumed gut residence time of 30 min (Durbin et al. 2012) to obtain ingestion rates in cells per day. We estimated the mean carbon biomass of an *Aulacoseira* cell by measuring 30 individual cells and applying the calculation for carbon from the volume of a cylindrical diatom (Menden-Deuer and Lessard 2000), giving a mean of 35 pg C $cell^{-1}$. This was then

used to calculate the carbon content of ingested *Aulacoseira* cells.

To place ingestion in a bioenergetic context, we estimated the daily ingestion of carbon per copepod necessary to support observed reproductive rates, then estimated the daily percentage of that carbon provided through direct consumption of *Aulacoseira* as

$$C\% = \frac{100\, I\, E}{C\, P} \quad (2)$$

where $C\%$ is the percent of carbon required for egg production that is provided by consuming *Aulacoseira*, I is the ingestion rate of *Aulacoseira* (μg C day^{-1}), E is gross growth efficiency of 0.30 (Straile 1997), C is carbon per egg (0.06 μg, Kimmerer et al. 2014), and P is egg production rate (eggs ♀$^{-1}$ day^{-1}).

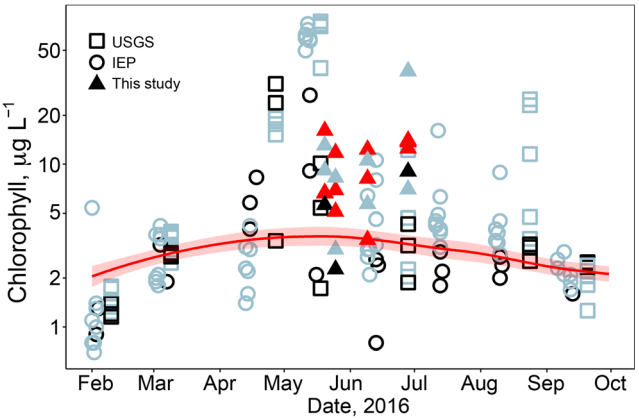

Fig. 3 Chlorophyll data (log scale) from discrete samples taken in 2016 by the USGS monitoring program (squares), the Interagency Ecological Program (circles), and our sampling transects (triangles), together with a loess curve fit to data on chlorophyll concentration vs. day from USGS data taken during pre-bloom years 2013–2015, with 95% confidence interval (shaded, N = 230). Colors indicate regions: red for the Cache Slough area, blue for the remainder of the Delta, and black for Suisun Bay

Results

The Bloom

Chlorophyll fluorescence underwent a series of ~ 8 peaks throughout spring and summer (Fig. 2). The bloom, defined here as above background levels of roughly 5 μg L^{-1}, apparently began in the Cache Slough Complex around 14 March, increasing in concentration daily and reaching a peak of 74 μg Chl L^{-1} at station 4 on 21 Mar 2016. The bloom encompassed much of the northern estuary by 22 Mar 2016, with a peak of 35 and 51 μg Chl L^{-1} at USGS stations LIS and TOE (Table S1) in the Yolo Bypass on 23 March, which diminished in amplitude at stations further south. A more persistent bloom then arose at station 5 on Liberty Island (Fig. 1) with a maximum of 37 μg Chl L^{-1} on 7 May, and blooms occurred across much of the estuary south of station 5 with generally decreasing amplitude and some delay in timing. A series of smaller blooms followed through June to August (Fig. 2). From March through September, the mean chlorophyll concentration in all sampling stations was 8 μg Chl L^{-1}.

We were unable to begin sampling until 20 May 2016, near the end of the second large chlorophyll peak (Fig. 2). Nevertheless, many of our zooplankton samples were awash with *Aulacoseira* chains, and chlorophyll concentrations in our samples ranged from 2 μg Chl L^{-1} outside the bloom areas to 37 μg Chl L^{-1}, with a median of 10 μg Chl L^{-1} (Fig. 3). Two routine monitoring programs found similar ranges of chlorophyll from April to August in the larger estuarine channels (Fig. 3).

We confirmed by microscopy that over the course of the study the dominant bloom organism in the size range > 20 μm continued to be *Aulacoseira* spp. at the core sampling stations (2–5). Samples from the bloom period analyzed for diatom biovolume showed that wherever biovolume rose to bloom levels (>5 μg L^{-1}), it was dominated by *Aulacoseira* spp. (Fig. S1a), with a median proportion of 89% of total diatom biovolume in 34 total samples (Fig. S1b). Cells of this genus were identified in 33 samples as *A. granulata*, with four samples containing diatoms identified as one of three other species (*A. lirata*, *A. islandica*, *A. italica*), and 22 samples in which *Aulacoseira* sp. was reported. Other phytoplankton taxa were present, and in most cases constituted only a small proportion of the total biovolume and abundance (Fig. S2a, b). Cyanobacteria were highly abundant at stations 3 and 5 throughout the study, but the cells were small and contributed very little to the total biovolume.

We put seasonal patterns of copepod abundance in the context of the bloom pattern to try to detect responses of *P. forbesi* to the bloom (Fig. 4). Bloom patterns were obtained from four continuous monitoring stations (Fig. 4a) closest to the center of the population for this copepod, in the central Delta (Fig. 1). Abundance of adult *P. forbesi* (Fig. 4b) throughout the Delta followed a similar seasonal trajectory to those seen in other years from 1994 to 2018, except that abundance in 2016 was higher in January–April than in other years, mostly before the bloom. From May on, abundance of *P. forbesi* adults was not markedly different in 2016 from that in other years. A similar overall pattern held for copepodites except for low values in Februrary (Fig. 4c).

Copepod egg production rates responded to bloom conditions, but with a lot of scatter (Fig. 5). Egg production was linearly related to chlorophyll in the > 20-μm size fraction, which was essentially all *Aulacoseira* biomass (Fig. 5a). Egg production rates frequently exceeded values predicted by a rectangular hyperbola previously fit to a larger set of data on egg production rate vs. total chlorophyll (Fig. 5b).

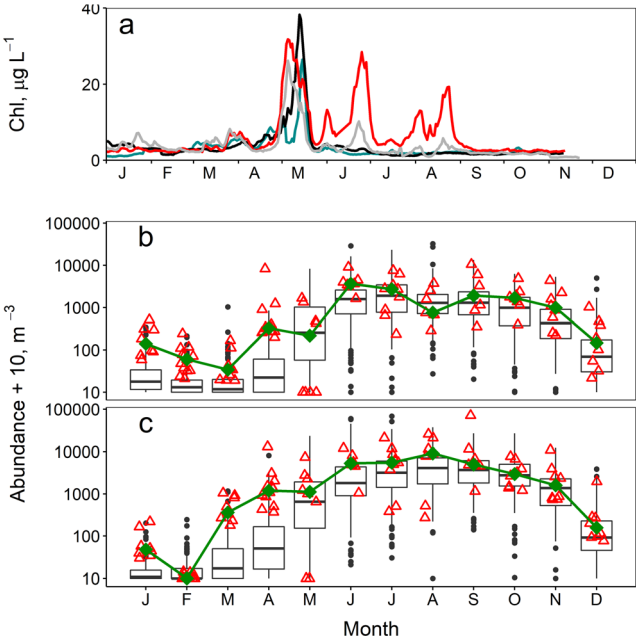

Fig. 4 Seasonal patterns of phytoplankton and *P. forbesi* abundance. **a** Chlorophyll concentration from four stations in the region of the copepod samples (stations 2, ANH, 3, RVB, Fig. 1) in 2016, color-coded as in Fig. 3. **b** Abundance of adult *P. forbesi* for 1994–2018, with 2016 data shown as triangles and diamonds representing medians, and all other years as boxplots showing quartiles (box), whiskers representing the most extreme value within 1.5 times the inter-quartile range of the box, and outliers as dots. **c** As in (**b**) for *P. forbesi* copepodites. Data for (**b**) and (**c**) from IEP

Copepod Gut Content Analysis

Sequencing of qPCR products verified amplification of *A. granulata* (100% identity, 100% query coverage) in the guts of the copepods. Quantitative PCR analysis of *P. forbesi* indicated that feeding on *A. granulata* was relatively high early in the study and declined over time, with an earlier decline of *A. granulata* in the guts in the more southerly areas (stations 1–4) than at northerly sites (Fig. 6). During the first sampling event, on 20 May, the mean of replicate measurements ranged from 4.4–13.2 cells copepod^{-1}, while 5 days later, the means ranged from 0.2–11.6 cells copepod^{-1}, and on 9 June and 28 June the means ranged from 0.1–1.8 cells copepod^{-1}.

Mean *P. forbesi* ingestion rates, determined from gut contents and assumed digestion times, ranged from 0 at station 2 on 9 June and station 4 on 28 June, to 548 cells copepod^{-1} day^{-1} at station 6 on 20 May (Fig. 6). The maximum ingestion rate from an individual measurement was 5118 cells copepod^{-1} day^{-1} at station 4 (Cache Slough) on 20 May.

Aulacoseira in gut contents of *P. forbesi*, and therefore the calculated ingestion rates, were unrelated to chlorophyll > 20 µm, despite the clear separation of gut-content data between the first two and last two sampling events (Fig. 7). During the two earlier sampling events, higher chlorophyll levels were associated with higher ingestion rates, whereas ingestion rates in the two later events were nearly zero even at chlorophyll

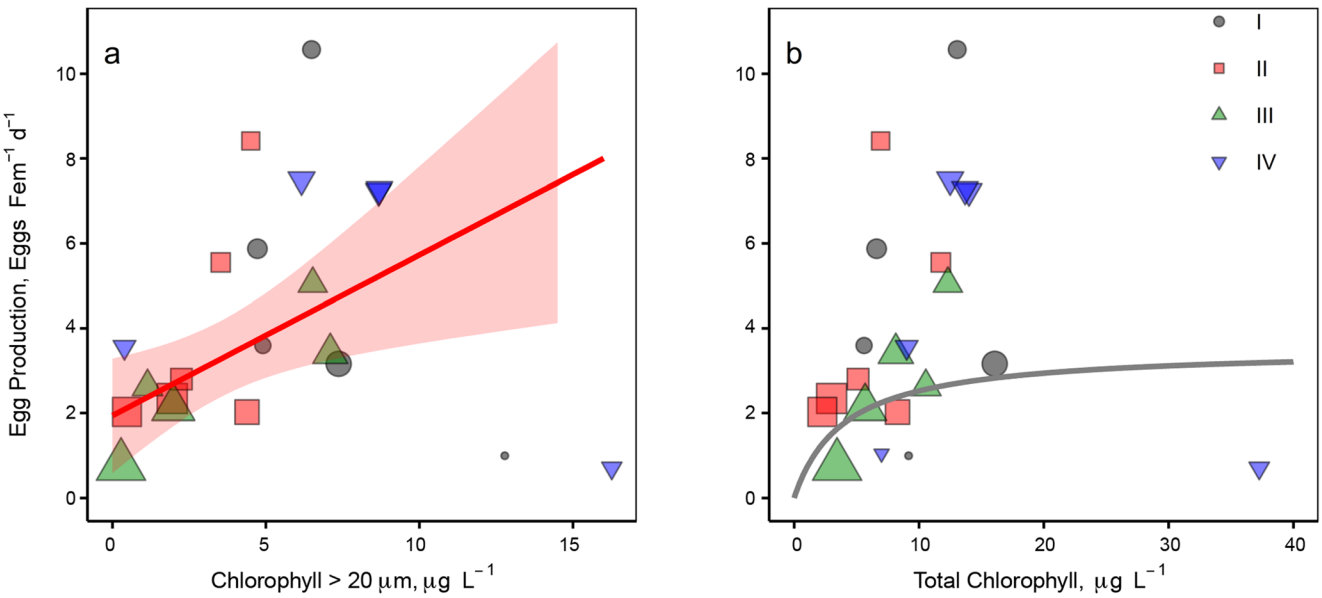

Fig. 5 Egg production rate of *Pseudodiaptomus forbesi* vs. **a** Chlorophyll concentration size-fractionated larger than 20 µm. **b** Chlorophyll concentration determined with GF/F filters. Shapes and colors of symbols indicate sample events I–IV with (I) 20 May 2016, (II) 25 May 2016, (III) 9 June 2016, and (IV) 28 June 2016. Areas of symbols represent total adult females counted (10–356). Line in (**a**) is a least-squares regression line with points weighted by the total number of females, with 95% confidence bands; EPR = 1.9 + (0.38 ± 0.14) Chl > 20, R^2 = 0.23, 19 df. Line in (**b**) is a rectangular hyperbola fit to 238 measurements of chlorophyll and egg production rate of *P. forbesi* from 2006 through 2018 (Gearty 2020); parameters are S_{max} = 3.6 ± 0.8 eggs ♀$^{-1}$ day^{-1} and K = 3.4 ± 2.0 µg Chl L^{-1} with a residual standard error of 4.6 eggs ♀$^{-1}$ day^{-1}.

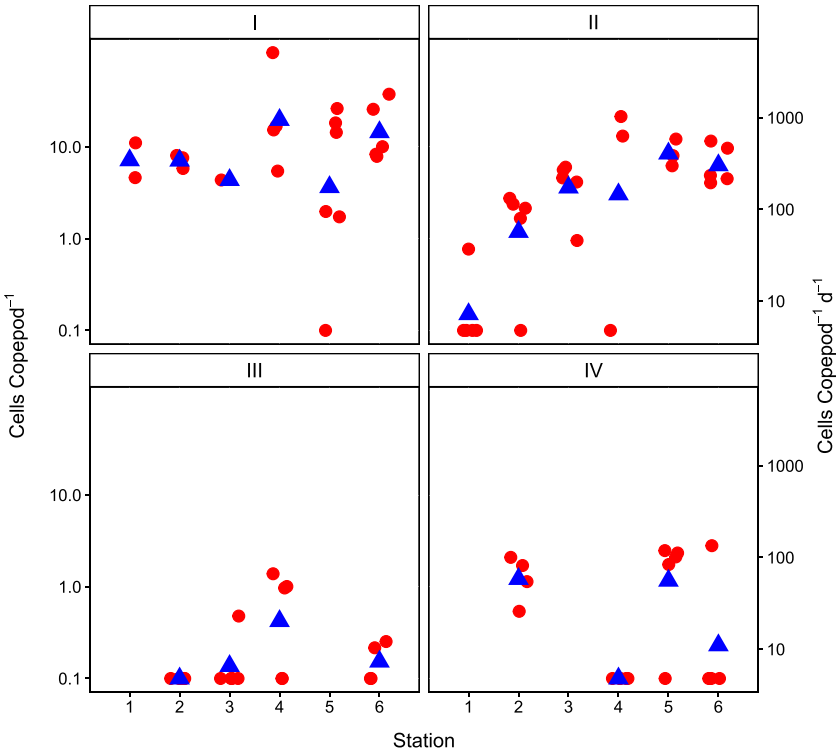

Fig. 6 Estimated number of cells of *Aulacoseira* sp. per individual *Pseudodiaptomus forbesi*, and calculated ingestion rate (right axis), by sample events I–IV and station. Sample events are (I) 20 May 2016, (II) 25 May 2016, (III) 9 June 2016, and (IV) 28 June 2016. Small circles are from individual qPCR measurements of 5 copepods sample^{-1}, and larger triangles are means by sampling event and station. Stations are arranged from southwest (left) to northeast (right): (1) Snag Island, (2) Mallard Island, (3) Decker Island, (4) Cache Slough, (5) Liberty Island, and (6) Deep Water Ship Channel

concentrations between 5 and 10 μg L^{-1} at the three northern stations (Fig. 7).

Egg production rates of *P. forbesi* were unrelated to ingestion rates of *A. granulata* (Fig. 8). Moreover, *Aulacoseira* carbon contributed only a small percentage to the estimated carbon required to support the observed egg production rates (Fig. 9). The median percentage of carbon required was 1% and the maximum of 21% occurred at a very low egg production rate. The apparent inverse relationship of egg production to the percent of required carbon is a consequence of Eqn. 1 and the lack of relationship between ingestion of *Aulacoseira* and egg production rate (Fig. 8).

The tissue digestion assessment to confirm ingestion of *Aulacoseira* cells found 0–108 diatom cells copepod^{-1} across stations, with a mean of 23 cells copepod^{-1} overall. While other diatoms may have been present in the guts at low numbers, most of the frustules were from *Aulacoseira* (>99%). In the "high" concentration samples (stations 4 and 5), the mean estimate was 31 *Aulacoseira* cells copepod^{-1}, with a maximum of 108, while in the low concentration sample (Station 1) only one of the three copepods contained 47 diatoms estimated from the frustules and fragments, and the single copepod from station 3 contained no diatoms.

Discussion

The egg-production rate of *P. forbesi* during spring 2016 included some unusually high values, with the highest single value and four individual values among the top 10 of 238 egg production rates from a variety of studies in the SFE (Gearty 2020). We did not conduct a formal test of the differences among these studies because of the temporally limited sampling in this study. Nevertheless, these results suggest that the reproductive rate of *P. forbesi* was at times elevated during this exceptional bloom.

However, our study provides multiple lines of evidence that reproductive rates of *P. forbesi* were only partially supported by ingestion of the diatom *A. granulata* during the bloom. The highest mean ingestion rate observed was 946 cells copepod^{-1} day^{-1}. We calculated that this supplied only ~ 21% of the daily carbon required for the observed egg production rate in that sample which, having a very low egg production rate, also had a relatively low carbon requirement (Fig. 9). Moreover, EPR was sometimes high at relatively low-chlorophyll levels (Fig. 5) even when ingestion of *Aulacoseira* was low (e.g., Fig. 8, sampling event IV). While the phytoplankton biovolume in our study was dominated by *Aulacoseira* spp. (Fig. S1), ingestion of *A. granulata* was unrelated to chlorophyll (either > 20 μm or total; Fig. 7). Though these results are not unprecedented (Hogfors et al. 2014), they do indicate a critical need to revise our view of what supports high copepod reproduction and growth, even during diatom blooms.

Copepod Diets

It was clear that *P. forbesi* consumed *Aulacoseira*, but that other foods must have been important as well. If copepods did not get much of their energetic support by consuming

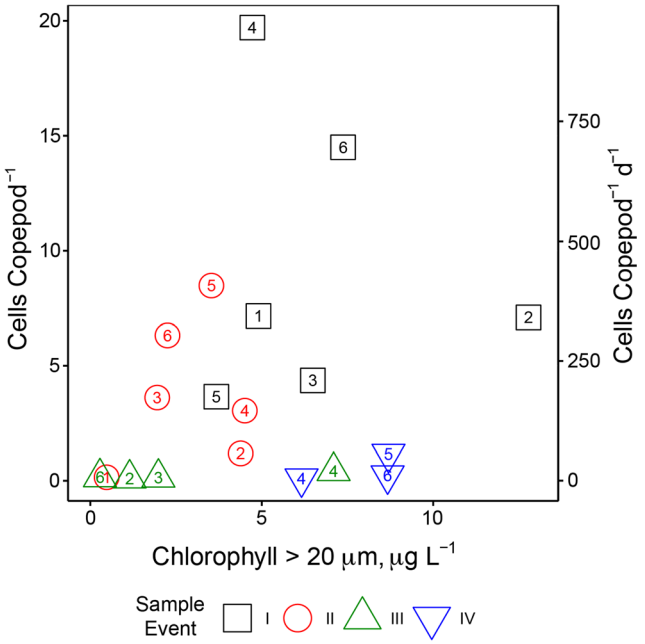

Fig. 7 Cells per copepod (left *y*-axis) and cells ingested copepod^{-1} day^{-1} (right *y*-axis) vs. chlorophyll concentration > 20 μm for corresponding sample event number (as in Fig. 5) and locations (numbers, as in Fig. 1)

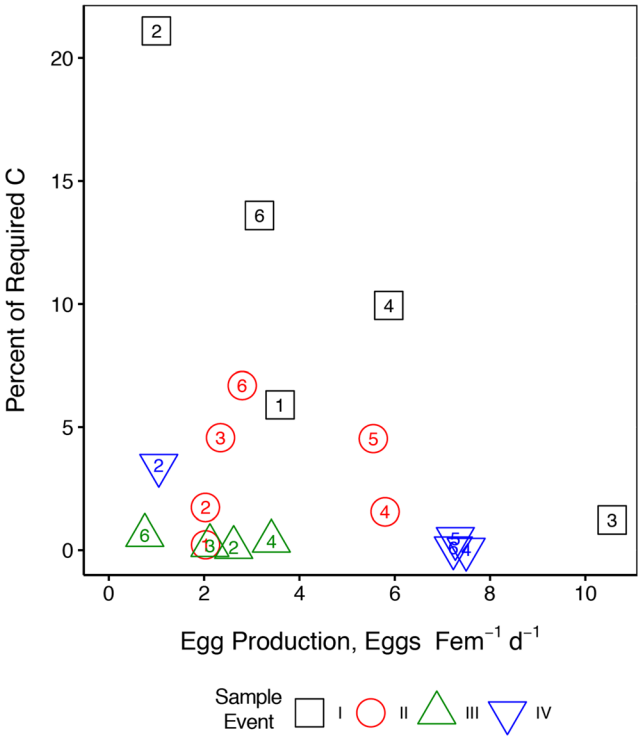

Fig. 9 Estimated consumption of *Aulacoseira* sp. as a percentage of estimated daily carbon requirement (C%, Eqn. 2) for *Pseudodiaptomus forbesi* vs. egg production rate. Symbols and numbers as in Fig. 7

Aulacoseira, what supported the apparent, though ephemeral, increase in egg production rate? Phytoplankton blooms are generally accompanied by blooms of bacteria and archaea, which transform and repackage organic matter produced by bloom organisms (Buchan et al. 2014). Phytoplankton blooms are also accompanied by rapid increases in abundance of microzooplankton grazers, which in turn may provide the bulk of the nutrition consumed by copepods (Turner and Granéli 1992). Thus, by increasing the rate of production of labile organic matter, phytoplankton blooms may support higher trophic levels through a variety of pathways other than direct consumption of the bloom organism. This may explain why egg production in some samples of *P. forbesi* was elevated in June 2016 (Fig. 5) after direct consumption of *Aulacoseira* had declined (Fig. 6).

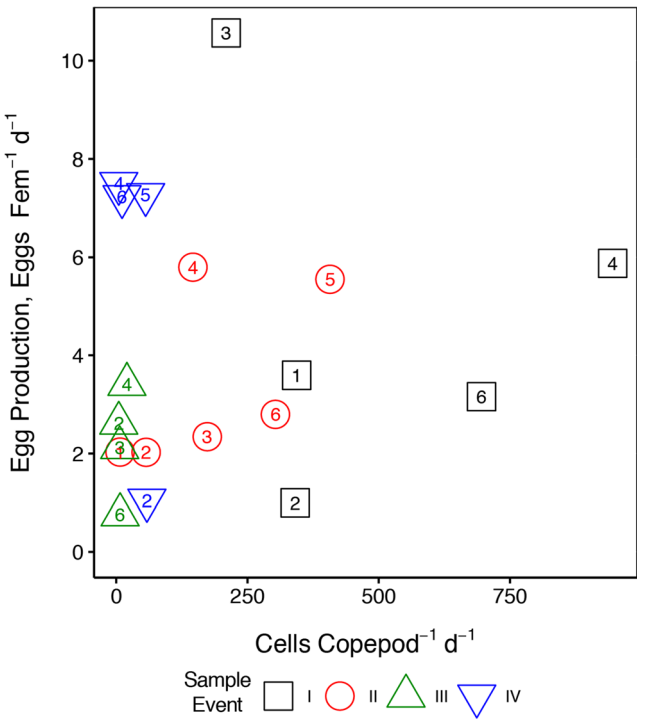

Fig. 8 Egg production rate vs. estimated ingestion rates of *Aulacoseira* sp. by *Pseudodiaptomus forbesi*. Symbols and numbers as in Fig. 7

While calanoid copepods are widely considered to feed on diatoms, particularly in productive systems (Fleming 1939; Turner 1984b; Koski and Riser 2006), evidence continues to mount that suggests other prey types can provide greater nutrition than diatoms and support higher rates of reproduction in the field (Kleppel 1993; Nejstgaard et al. 2001). Not only did our molecular analysis suggest low ingestion rates on *Aulacoseira* during our study period, but our tissue digestion experiment results also show low ingestion rates of any other species of diatoms present at the time of our study.

Blooms of microzooplankton often coincide with seasonal diatom blooms (Hansen 1991; Sherr and Sherr 2007) and can be an important food source for copepods (Sherr and Sherr 2007; Calbet and Saiz 2005; Gifford et al. 2007); however, only the phytoplankton were quantified in the current study. The other phytoplankton that contributed the most to biovolume during our study include the flagellates *Strombomonas* sp. (Euglenophyta; stations 3 and 5) and

Rhodomonas lacustris (Cryptophyta; Station 3) both at stations along the Sacramento River (Figure S3a, b) where flagellates can be abundant (Kress 2012). Heterotrophic ciliates can be abundant in the late spring around the downstream stations (e.g., Bouley and Kimmerer 2006). Calanoid copepods are omnivores, obtaining at least some of their food from microzooplankton (Bowen et al. 2015; Kayfetz and Kimmerer 2017; Yeh et al. 2020; Yi et al. 2017), plant detritus (Heinle et al. 1977; Harfmann et al. 2019), and even fungi (Yeh et al. 2020; Yi et al. 2017). Chytrid fungi have been found to be high-nutrient (e.g., PUFAs and cholesterols) food for other zooplankton and are implicated in mediating carbon transfer from diatoms to higher trophic levels (Kagami et al. 2011). Given the low rate of direct feeding on *A. granulata*, *P. forbesi* must have consumed alternative food during our study, but additional studies targeting alternative prey in the samples would be needed to identify what they were.

While we do not have much evidence for other taxa that contributed to the copepod diet in our study, cyanobacteria may have been one important food source for *P. forbesi* on some sampling dates. In our study cyanobacteria made up >95% of the total phytoplankton abundance (Fig. S2), but < 5% of the biovolume (Fig. S1 b). Prior work in the Cache Slough Complex (stations 4–6) has shown high levels of cyanobacteria DNA and negligible levels of cryptophyte DNA in the guts of *P. forbesi*, the opposite of what we expected based on their respective abundance and presumed nutritional value (Holmes 2018; Kimmerer et al. 2018). Other sequencing-based studies have found cyanobacteria (*Synechococcus*) to be highly abundant in the guts of *C. finmarchicus* (Yeh et al. 2020). Moreover, the growth rate of *P. forbesi* was weakly but positively related to cyanobacterial biovolume in the Yolo Bypass during 2015–2017 (Owens et al. 2019). Though often considered a poor food source, cyanobacteria can be ingested by copepods and can support growth and reproduction (Hogfors et al. 2014; Engstrom-Ost et al. 2014; Ger et al. 2018). Ingestion of cyanobacteria may depend on what other prey taxa are available; in some cases, cyanobacteria seem to be avoided or rejected by copepods (e.g., Meyer-Harms et al. 1999). However, copepods may shift phenotypes and develop traits allowing them to feed on cyanobacteria (Ger et al. 2016). During periods of high cyanobacteria abundance, it is possible that *P. forbesi* may exploit aggregations of cyanobacteria as a nutritional source or consume them indirectly, such as through secondary predation (DeMott and Moxter 1991; Sheppard and Harwood 2005; King et al. 2008).

Variability in Ingestion of *Aulacoseira*

The highest ingestion rates we observed on *A. granulata* occurred on the first sampling date, 20 May 2016, during the decline of the bloom, when the maximum percent carbon ingested was 24% of the daily carbon needs of *P. forbesi*. Our ingestion rate estimates were also more variable in earlier stages of the bloom (evident in sampling event I and II, Fig. 6), which may reflect heterogeneity in feeding success of individual *P. forbesi* on long *Aulacoseira* chains and individual cells. The decline in ingestion rates observed over the course of our study started at the southernmost stations (Fig. 6), which are also the most influenced by tidal flow. Senescent diatom blooms may, however, be more bioavailable than blooms in the exponential growth phase (Barofsky et al. 2010; Ray et al. 2016). The observed decline in ingestion of *A. granulata* may be due, in part, to changes in palatability of the diatom over the course of the bloom, or from prey switching in the later bloom stages.

Our results show that the diatom *A. granulata* was not the primary source of food for *P. forbesi* during the period of the bloom studied here, despite dominating the phytoplankton assemblage at all study sites (Fig. S1), though ingestion rates on *A. granulata* may have been higher prior to the start of our study. The observed lack of a strong relationship between ingestion of *Aulacoseira* and chlorophyll levels (Fig 7) is unlikely to be from a mismatch in volumes or locations sampled, as both chlorophyll and zooplankton were collected with surface samples. Chlorophyll concentration is also a proxy for phytoplankton biomass (e.g., Cullen 1982; Fennel and Boss 2003; Jakobsen and Markager 2016) and does not account for the availability and nutritive value of that biomass to grazers. Given the overwhelming biovolume of *Aulacoseira* in the phytoplankton (Fig. S1), we assume that low consumption by *P. forbesi* could reflect the mechanical challenge for this species to consume this silica-rich diatom (Liu et al. 2016) or the poor nutritive value of diatoms relative to other potential prey (Jones and Flynn 2005).

Ingestion rate estimates based on our tissue digestion experiments were within the range we would expect based on our qPCR measurements containing five copepods sample^{-1} assuming the diatom frustules remain in the gut longer than labile DNA. Copepods macerate their prey (Mauchline 1998a) making the labile organic matter inside, including DNA, more prone to digestion and degradation. The silica-based diatom frustules themselves are not digested completely in copepod guts and remain visible in fecal pellets (e.g., Turner 1984b). Studies of DNA degradation in copepod guts suggest prey DNA is digested rapidly (Durbin et al. 2008; Nejstgaard et al. 2008) and reaches near-zero levels within 30 min of ingestion (Durbin et al. 2012). While exact gut evacuation rates for this species are not known, gut evacuation rates in other calanoid copepod species are largely between 30-60 mins (Mauchline 1998b).

Fate of the Bloom

The bloom injected a massive stimulus in terms of organic carbon into the northern SFE. We estimated phytoplankton

gross primary production for station 5 using a model for turbid estuaries (Jassby 2008). Inputs to the model are chlorophyll concentration (Fig. 2), with values above 5 μg L^{-1} denoting elevated conditions, extinction coefficient which we estimated from turbidity data obtained from the same station, and incident photosynthetically active radiation (PAR), estimated as in Jassby (2008). Estimated gross primary production (GPP) during 2016 roughly doubled from 180 to 370 mg C m^{-2} day^{-1}.

Where did the excess productivity go? There is evidence that even after phytoplankton blooms, particulate organic carbon and particulate organic matter in the northern SFE is generally less labile (i.e., low in PUFAs and higher in saturated fatty acids) than comparable sites in other estuaries, and thus may not be readily available to heterotrophic organisms or higher trophic levels (Canuel 2001). In fact, it is likely that much of it sank to the bottom and was subsequently buried, consumed and metabolized by the benthos, or remineralized by heterotrophic microbes. We did not see evidence of an immediate life history response by the benthic organisms (bivalve larvae, gastropod larvae, others) nor by other pelagic zooplankton taxa during our study (Fig. S4). There was also no clear response in the abundance of benthic invertebrates in 2016 at sites around our study region (Palmieri 2020). In the ocean, heterotrophic bacteria remineralize roughly one-half of the carbon produced by autotrophs and bacterial production correlates with chlorophyll *a* during phytoplankton blooms (Cole et al. 1988; Ducklow et al. 1993; Buchan et al. 2014). Thus, it is likely that much of the excess production was remineralized by bacteria and archaea and respired to CO_2, transferred via microbial biomass up the food web, or cycled within the microbial loop (Coffin and Sharp 1987).

If we assume that much of this extra productivity eventually entered the pelagic food web, the response of the pelagic ecosystem seems paltry. Not only was *P. forbesi* abundance unresponsive, but total zooplankton biomass at salinity < 1 was unresponsive to the bloom as well (Fig. S5a). The median biomass in 2016 was higher than the grand median from the other years through April, but beginning in May the median biomass in 2016 was generally consistent with values from other years. Likewise, median biomass of small fishes at salinity < 1 was generally no higher in 2016 than in previous years, and in May, June, and November it was zero (Fig. S5b).

Implications for the Estuary

The upper (northern) San Francisco Estuary has been a high-nutrient-low-chlorophyll region since the mid-1980s (Wilkerson et al. 2006); silicate is rarely a limiting nutrient (Cloern et al. 1983; Kimmerer 2005). Reasons for this situation are complex (Dahm et al. 2016). This region, notably Suisun Bay and the western California Delta (Fig. 1), was once characterized by summer-long diatom blooms with typical summer chlorophyll values of ~ 20–30 μg L^{-1} (Alpine and Cloern 1992), and blooms in the southern Delta were often dominated by diatoms identified as *Melosira* spp. (Lehman 1992). The regular occurrence of high-biomass chlorophyll blooms ended abruptly with the 1987 invasion of the clam *Potamocorbula amurensis*, which reduced phytoplankton biomass ~ five-fold (Alpine and Cloern 1992), eliminated summer-long diatom blooms (Kimmerer 2005), and, through size-selective grazing, reduced the proportion of biomass in large cells (Kimmerer et al. 2012). Since 1987, summer phytoplankton biomass and productivity in brackish regions of the estuary have been persistently low, with ~ half of the biomass and productivity in cells smaller than 5 μm (Kimmerer et al. 2012). Occasional, brief blooms have occurred in springs of some years, which have been attributed to late seasonal development of clam populations (Dugdale et al. 2016). An additional cause proposed for the low phytoplankton biomass is the nutrient environment which may inhibit rapid growth of diatoms (Wilkerson et al. 2006; Dugdale et al. 2007). However, calculated grazing by clams and micro- and mesozooplankton combined was sufficient to inhibit blooms most of the time, and phytoplankton growth minus grazing was positively related to the month-by-month change in chlorophyll concentration (Kimmerer and Thompson 2014).

Low phytoplankton productivity since 1987 has degraded the food environment for several estuarine fishes (Feyrer et al. 2003). Abundance of fishes in the deep channels of the estuarine low-salinity zone declined either immediately or within a few years after the clam invasion. The fish species that declined included some that are mainly planktivorous throughout life, e.g., the endangered delta smelt *Hypomesus transpacificus* (Nobriga 2002; Slater and Baxter 2014), threatened longfin smelt *Spirinchus thaleichthys* (Feyrer et al. 2003), and northern anchovy *Engraulis mordax* (Kimmerer 2006), as well as some that are planktivorous only during early life, such as striped bass *Morone saxatilis* (Sommer et al. 2011).

Management of the San Francisco Estuary centers on the conflict between environmental protection and extraction of freshwater from the estuary and its watershed for human uses. This conflict is sharpest for the endangered and declining delta smelt (Sommer et al. 2007). Delta smelt are heavily dependent on copepods, notably *P. forbesi*, which itself has declined in the main summer habitat of delta smelt as a result of excessive mortality of nauplii (Kayfetz and Kimmerer 2017; Kimmerer et al. 2019). Delta smelt are also highly vulnerable to losses from the population to massive water diversion facilities in the southern Delta (Kimmerer 2008). Because of the value of the diverted water, proposals and plans have been growing for remedial actions to enhance phytoplankton production thereby stimulating production of food for delta smelt. Our results suggest that blooms of *A. granulata* are unlikely to directly provide the wished-for stimulus to higher trophic levels through *P. forbesi*.

Acknowledgments BSA Inc. analyzed the phytoplankton samples for counts and biovolume by taxon. Funding was provided by Contract 2284 from the Delta Stewardship Council and Contract 17-11 from the State and Federal Contractors Water Association, both to San Francisco State University. Thanks to the Carpenter Lab at the Estuary & Ocean Science Center for help with tissue digestion and diatom slide protocols, and A. Slaughter for helpful discussion that improved data interpretation. Thank you also to two helpful reviewers for constructive feedback that improved this manuscript. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

References

Alpine, Andrea E., and James E. Cloern. 1992. Trophic interactions and direct physical effects control phytoplankton biomass and production in an estuary. *Limnology and Oceanography* 37 (5): 946–955. https://doi.org/10.4319/lo.1992.37.5.0946.

Amin, Roswati Md, Marja Koski, Ulf Båmstedt, and Charles Vidoudez. 2011. Strain-related physiological and behavioral effects of Skeletonema marinoi on three common planktonic copepods. *Marine Biology* 158 (9): 1965–1980. https://doi.org/10.1007/s00227-011-1706-7.

Arar, E.J., and G.B. Collins. 1997. *Method 445.0 in vitro determination of chlorophyll a and pheophytin in marine and freshwater algae by fluorescence*. Washington: U.S. Environmental Protection Agency.

Ask, Jenny, Marko Reinikainen, and Ulf Bamstedt. 2006. Variation in hatching success and egg production of Eurytemora affinis (Calanoida, Copepoda) from the Gulf of Bothnia, Baltic Sea, in relation to abundance and clonal differences of diatoms. *Journal of Plankton Research* 28 (7): 683–694.

Ban, Syuhei, Carolyn Burns, Jacques Castel, Yannick Chaudron, Epaminondas Christou, Ruben Escribano, Serena Fonda Umani, Stephane Gasparini, Francisco Guerrero Ruiz, and Monica Hoffmeyer. 1997. The paradox of diatom-copepod interactions. *Marine Ecology Progress Series* 157: 287–293.

Barber, R.T., and M.R. Hiscock. 2006. A rising tide lifts all phytoplankton: growth response of other phytoplankton taxa in diatom-dominated blooms: a rising tide raises all phytoplankton. *Global Biogeochemical Cycles* 20: n/a. https://doi.org/10.1029/2006GB002726.

Barofsky, A., P. Simonelli, C. Vidoudez, C. Troedsson, J.C. Nejstgaard, H.H. Jakobsen, and G. Pohnert. 2010. Growth phase of the diatom Skeletonema marinoi influences the metabolic profile of the cells and the selective feeding of the copepod Calanus spp. *Journal of Plankton Research* 32 (3): 263–272. https://doi.org/10.1093/plankt/fbp121.

Bowen, A., G. Rollwagen-Bollens, S.M. Bollens, and J. Zimmerman. 2015. Feeding of the invasive copepod Pseudodiaptomus forbesi on natural microplankton assemblages within the lower Columbia River. *Journal of Plankton Research* 37: 1089–1094. https://doi.org/10.1093/plankt/fbv078.

Bryant, M.E., and Arnold, J.D.. (2007). Diets of age-0 striped bass in the San Francisco Estuary, 1973-2002. *California Fish and Game* 93.

Buchan, Alison, Gary R. LeCleir, Christopher A. Gulvik, and José M. González. 2014. Master recyclers: features and functions of bacteria associated with phytoplankton blooms. *Nature Reviews Microbiology* 12 (10): 686–698. https://doi.org/10.1038/nrmicro3326.

Bjærke, Oda, Per R. Jonsson, Asraful Alam, and Erik Selander. 2015. Is chain length in phytoplankton regulated to evade predation? *Journal of Plankton Research* 37: 1110–1119. https://doi.org/10.1093/plankt/fbv076.

Calbet, A., and E. Saiz. 2005. The ciliate-copepod link in marine ecosystems. *Aquatic Microbial Ecology* 38: 157–167. https://doi.org/10.3354/ame038157.

Calbet, A., I. Trepat, R. Almeda, V. Saló, E. Saiz, Movilla Ji, M. Alcaraz, L. Yebra, and R. Simó. 2008. Impact of micro- and nanograzers on phytoplankton assessed by standard and size-fractionated dilution grazing experiments. *Aquatic Microbial Ecology* 50: 145–156. https://doi.org/10.3354/ame01171.

Canuel, E. A. 2001. Relations between river flow, primary production and fatty acid composition of particulate organic matter in San Francisco and Chesapeake Bays: a multivariate approach. *Organic Geochemistry*: 21.

Carstensen, Jacob, Riina Klais, and James E. Cloern. 2015. Phytoplankton blooms in estuarine and coastal waters: seasonal patterns and key species. *Estuarine, Coastal and Shelf Science* 162: 98–109. https://doi.org/10.1016/j.ecss.2015.05.005.

Cloern, J., A. Alpine, B. Cole, R. Wong, J. Arthur, and M. Ball. 1983. River discharge controls phytoplankton dynamics in the northern San Francisco Bay estuary. *Estuarine, Coastal and Shelf Science* 16 (4): 415–429.

Cloern, J.E., and A.D. Jassby. 2008. Complex seasonal patterns of primary producers at the land-sea interface. *Ecology Letters* 11 (12): 1294–1303. https://doi.org/10.1111/j.1461-0248.2008.01244.x.

Cloern, J.E., and A.D. Jassby. 2012. Drivers of change in estuarine-coastal ecosystems: discoveries from four decades of study in San Francisco Bay. *Reviews in Geophysics* 50: 1–33.

Coffin, R.B., and J.H. Sharp. 1987. Microbial trophodynamics in the Delaware Estuary. *Marine Ecology Progress Series* 41: 253–266. https://doi.org/10.3354/meps041253.

Cole, J.C., S. Findlay, and M.L. Pace. 1988. Bacterial production in fresh and saltwater ecosystems: a cross-system overview. *Marine Ecology Progress Series* 43: 1–10. https://doi.org/10.3354/meps043001.

Conley, D.J., and T.C. Malone. 1992. Annual cycle of dissolved silicate in Chesapeake Bay - implications for the production and fate of phytoplankton biomass. *Marine Ecology Progress Series* 81: 121–128.

Cullen, John J. 1982. The deep chlorophyll maximum: comparing vertical profiles of chlorophyll a. *Canadian Journal of Fisheries and Aquatic Sciences* 39. NRC Research Press: 791–803.

Cushing, D.H. 1989. A difference in structure between ecosystems in strongly stratified waters and in those that are only weakly stratified. *Journal of Plankton Research* 11 (1): 1–14.

Dahm, C.N., Parker, A.E., Adelson, A.E., Christman, M.A., and Bergamaschi, B.A.. 2016. Nutrient dynamics of the Delta: effects on primary producers. *San Francisco Estuary & Watershed Science* 14.

DeMott, W.R., and F. Moxter. 1991. Foraging on cyanobacteria by copepods: responses to chemical defenses and resource abundance. *Ecology* 72 (5): 1820–1834.

Ducklow, H.W., D.L. Kirchman, H.L. Quinby, C.A. Carlson, and H.G. Dam. 1993. Stocks and dynamics of bacterioplankton carbon during the spring bloom in the eastern North Atlantic Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography* 40 (1-2): 245–263. https://doi.org/10.1016/0967-0645(93)90016-G.

Dugdale, Richard C., Frances P. Wilkerson, Victoria E. Hogue, and Albert Marchi. 2007. The role of ammonium and nitrate in spring bloom development in San Francisco Bay. *Estuarine, Coastal and Shelf Science* 73 (1-2): 17–29. https://doi.org/10.1016/j.ecss.2006.12.008.

Dugdale, Richard C., Frances P. Wilkerson, and Alexander E. Parker. 2016. The effect of clam grazing on phytoplankton spring blooms in the low-salinity zone of the San Francisco Estuary: a modelling approach. *Ecological Modelling* 340: 16. https://doi.org/10.1016/j.ecolmodel.2016.08.018.

Durbin, E.G., M.C. Casas, and T.A. Rynearson. 2012. Copepod feeding and digestion rates using prey DNA and qPCR. *Journal of Plankton Research* 34 (1): 72–82. https://doi.org/10.1093/plankt/fbr082.

Durbin, Edward G., Maria C. Casas, Tatiana A. Rynearson, and David C. Smith. 2008. Measurement of copepod predation on nauplii using qPCR of the cytochrome oxidase I gene. *Marine Biology* 153 (4): 699–707. https://doi.org/10.1007/s00227-007-0843-5.

Dutz, Jörg, Marja Koski, and Sigrún H. Jónasdóttir. 2008. Copepod reproduction is unaffected by diatom aldehydes or lipid composition. *Limnology and Oceanography* 53 (1): 225–235.

Edmondson, W.T., G.C. Anderson, and G.W. Comita. 1962. Reproductive rate of copepods in nature and its relation to phytoplankton population. *Ecology* 43 (4): 625–634.

Engstrom-Ost, Jonna, Towe Holmborn, Andreas Brutemark, Hedvig Hogfors, Anu Vehmaa, and Elena Gorokhova. 2014. The effects of short-term pH decrease on the reproductive output of the copepod Acartia bifilosa - a laboratory study. *Marine and Freshwater Behaviour and Physiology* 47 (3): 173–183. https://doi.org/10.1080/10236244.2014.919096.

Fennel, Katja, and Emmanuel Boss. 2003. Subsurface maxima of phytoplankton and chlorophyll: steady-state solutions from a simple model. *Limnology and Oceanography* 48 (4): 1521–1534. https://doi.org/10.4319/lo.2003.48.4.1521.

Feyrer, F., B. Herbold, S.A. Matern, and P.B. Moyle. 2003. Dietary shifts in a stressed fish assemblage: consequences of a bivalve invasion in the San Francisco Estuary. *Environmental Biology of Fishes* 67 (3): 277–288.

Fleming, Richard H. 1939. The control of diatom populations by grazing. *ICES Journal of Marine Science* 14. Oxford University Press: 210–227.

Gast, R.J., M.R. Dennett, and D.A. Caron. 2004. Characterization of Protistan Assemblages in the Ross Sea, Antarctica, by denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology* 70 (4): 2028–2037. https://doi.org/10.1128/AEM.70.4.2028-2037.2004.

Gearty, A.J.. (2020). Mechanisms controlling productivity of a calanoid copepod in northern San Francisco Estuary. M.S. Thesis, San Francisco: San Francisco State University.

Ger, K.A., P. Urrutia-Cordero, P.C. Frost, L.A. Hansson, O. Sarnelle, A.E. Wilson, and M. Lurling. 2016. The interaction between cyanobacteria and zooplankton in a more eutrophic world. *Harmful Algae* 54: 128–144. https://doi.org/10.1016/j.hal.2015.12.005.

Ger, Kemal Ali, Timothy G. Otten, Rita DuMais, Toni Ignoffo, and Wim Kimmerer. 2018. In situ ingestion of Microcystis is negatively related to copepod abundance in the upper San Francisco Estuary. *Limnology and Oceanography* 63 (6): 2394–2410. https://doi.org/10.1002/lno.10946.

Gifford, Scott M., Gretchen Rollwagen-Bollens, and Stephen M. Bollens. 2007. Mesozooplankton omnivory in the upper San Francisco Estuary. *Marine Ecology Progress Series* 348: 33–46. https://doi.org/10.3354/meps07003.

Goldman, C.R., and Horne, A.J.. (1983). *Limnology*. International Student Edition. McGraw-Hill Education.

Guiry, M.D., and Guiry, G.M.. (2019). Aulacoseira granulata (Ehrenberg) Simonsen. *AlgaeBase*.

Hansen, Per J. 1991. Quantitative importance and trophic role of heterotrophic dinoflagellates in a coastal pelagial food web. *Marine Ecology Progress Series* 73: 253–261. https://doi.org/10.3354/meps073253.

Harfmann, Jennifer, Tomofumi Kurobe, Brian Bergamaschi, Swee Teh, and Peter Hernes. 2019. Plant detritus is selectively consumed by estuarine copepods and can augment their survival. *Scientific Reports* 9 (1): 9076. https://doi.org/10.1038/s41598-019-45503-6.

Heinle, D.R., R.P. Harris, J.F. Ustach, and D.A. Flemer. 1977. Detritus as food for estuarine copepods. *Marine Biology* 40 (4): 341–353. https://doi.org/10.1007/BF00395727.

Hogfors, Hedvig, Nisha H. Motwani, Susanna Hajdu, Rehab El-Shehawy, Towe Holmborn, Anu Vehmaa, Jonna Engstrom-Ost, Andreas Brutemark, and Elena Gorokhova. 2014. Bloom-forming Cyanobacteria support copepod reproduction and development in the Baltic Sea. *PLoS One* 9 (11): e112692. https://doi.org/10.1371/journal.pone.0112692.

Holmes, A.. 2018. High-throughput sequencing reveals unexpected phytoplankton prey of an estuarine copepod. M.S. Thesis, San Francisco: San Francisco State University.

Houde, E.D., and E.S. Rutherford. 1993. Recent trends in estuarine fisheries - predictions of fish production and yield. *Estuaries* 16 (2): 161–176.

Ianora, A., and A. Miralto. 2010. Toxigenic effects of diatoms on grazers, phytoplankton and other microbes: a review. *Ecotoxicology* 19 (3): 493–511. https://doi.org/10.1007/s10646-009-0434-y.

Ianora, A., S.A. Poulet, and A. Miralto. 1995. A comparative study of the inhibitory effect of diatoms on the reproductive biology of the copepod Temora stylifera. *Marine Biology* 121 (3): 533–539.

Ianora, A., S.A. Poulet, A. Miralto, and R. Grottoli. 1996. The diatom Thalassiosira rotula affects reproductive success in the copepod Acartia clausi. *Marine Biology* 125 (2): 279–286.

Interagency Ecological Program. (2020). Environmental monitoring program: phytoplankton. California Department of Water Resources. Interagency Ecological Program (IEP).

Irigoien, Xabier, Roger P. Harris, Hans M. Verheye, Pierre Joly, Jeffrey Runge, Michel Starr, David Pond, Robert Campbell, Rachael Shreeve, Peter Ward, Amy N. Smith, Hans G. Dam, William Peterson, Valentina Tirelli, Marja Koski, Tania Smith, Derek Harbour, and Russell Davidson. 2002. Copepod hatching success in marine ecosystems with high diatom concentrations. *Nature* 419 (6905): 387–389. https://doi.org/10.1038/nature01055.

Jakobsen, Hans H., and Stiig Markager. 2016. Carbon-to-chlorophyll ratio for phytoplankton in temperate coastal waters: seasonal patterns and relationship to nutrients. *Limnology and Oceanography* 61 (5): 1853–1868. https://doi.org/10.1002/lno.10338.

Jassby, A.D. 2008. Phytoplankton in the upper San Francisco Estuary: recent biomass trends, their causes and their trophic significance. *San Francisco Estuary and Watershed Science* 6 (1): 2.

Jones, Ruth H., and Kevin J. Flynn. 2005. Nutritional status and diet composition affect the value of diatoms as copepod prey. *Science* 307 (5714): 1457–1459. https://doi.org/10.1126/science.1107767.

Kagami, Maiko, Nico R. Helmsing, and Ellen van Donk. 2011. Parasitic chytrids could promote copepod survival by mediating material transfer from inedible diatoms. *Hydrobiologia* 659 (1): 49–54. https://doi.org/10.1007/s10750-010-0274-z.

Kagami, Maiko, Takehito Yoshida, Tek Gurung, and Jotaro Urabe. 2002. Direct and indirect effects of zooplankton on algal composition in in situ grazing experiments. *Oecologia* 133 (3): 356–363. https://doi.org/10.1007/s00442-002-1035-0.

Kayfetz, K., and Wim J. Kimmerer. 2017. Abiotic and biotic controls on the copepod Pseudodiaptomus forbesi in the upper San Francisco Estuary. *Marine Ecology Progress Series* 581. Marine Ecology Progress Series: 85–101.

Kilham, Peter. 1971. A hypothesis concerning silica and the freshwater planktonic diatoms1. *Limnology and Oceanography* 16 (1): 10–18. https://doi.org/10.4319/lo.1971.16.1.0010.

Kilham, Peter, Susan S. Kilham, and Robert E. Hecky. 1986. Hypothesized resource relationships among African planktonic diatoms. *Limnology and Oceanography* 31 (6): 1169–1181. https://doi.org/10.4319/lo.1986.31.6.1169.

Kimmerer, W.J. 2005. Long-term changes in apparent uptake of silica in the San Francisco estuary. *Limnology and Oceanography* 50 (3): 793–798.

Kimmerer, W.J. 2008. Losses of Sacramento River Chinook salmon and delta smelt to entrainment in water diversions in the Sacramento-San Joaquin Delta. *San Francisco Estuary and Watershed Science* 6: 2.

Kimmerer, W.J., J.R. Burau, and W.A. Bennett. 1998. Tidally-oriented vertical migration and position maintenance of zooplankton in a temperate estuary. *Limnology and Oceanography* 43 (7): 1697–1709. https://doi.org/10.4319/lo.1998.43.7.1697.

Kimmerer, W.J., A.E. Parker, U. Lidström, and E.J. Carpenter. 2012. Short-term and interannual variability in primary production in the low-salinity zone of the San Francisco Estuary. *Estuaries and Coasts* 35 (4): 913–929. https://doi.org/10.1007/s12237-012-9482-2.

Kimmerer, Wim J., Ellen Gartside, and James J. Orsi. 1994. Predation by an introduced clam as the likely cause of substantial declines in zooplankton of San Francisco Bay. *Marine Ecology Progress Series* 113: 81–93 JSTOR.

Kimmerer, Wim J., Ignoffo, T. R., Bemowski, B, Modéran, J, Holmes, A, and Bergamaschi, B.A.. 2018. Zooplankton dynamics in the cache slough complex of the Upper San Francisco Estuary. *San Francisco Estuary and Watershed Science* 16. https://doi.org/10.15447/sfews.2018v16iss3art4.

Kimmerer, Wim J., Edward S. Gross, Anne M. Slaughter, and John R. Durand. 2019. Spatial subsidies and local mortality of an estuarine copepod revealed using a box model. *Estuaries and Coasts* 42 (1): 218–236. https://doi.org/10.1007/s12237-018-0436-1.

Kimmerer, Wim J., Toni R. Ignoffo, Karen R. Kayfetz, and Anne M. Slaughter. 2017. Effects of freshwater flow and phytoplankton biomass on growth, reproduction, and spatial subsidies of the estuarine copepod Pseudodiaptomus forbesi. *Hydrobiologia* 807: 113–130. https://doi.org/10.1007/s10750-017-3385.

Kimmerer, Wim J., Toni R. Ignoffo, Anne M. Slaughter, and Alison L. Gould. 2014. Food-limited reproduction and growth of three copepod species in the low-salinity zone of the San Francisco Estuary. *Journal of Plankton Research* 36 (3): 722–735. https://doi.org/10.1093/plankt/fbt128.

Kimmerer, Wim J., and Janet K. Thompson. 2014. Phytoplankton growth balanced by clam and zooplankton grazing and net transport into the low-salinity zone of the San Francisco Estuary. *Estuaries and Coasts* 37 (5): 1202–1218.

Kimmerer, W J., and Slaughter, A. M.. 2016. Fine-scale distributions of Zooplankton in the Northern San Francisco Estuary. *San Francisco Estuary and Watershed Science* 14. https://doi.org/10.15447/sfews.2016v14iss3art2.

Kimmerer, Wim J. 2006. Response of anchovies dampens effects of the invasive bivalve Corbula amurensis on the San Francisco Estuary foodweb. *Marine Ecology Progress Series* 324: 207–218. https://doi.org/10.3354/meps324207.

King, R.A., D.S. Read, M. Traugott, and W.O.C. Symondson. 2008. Molecular analysis of predation: a review of best practice for DNA-based approaches. *Molecular Ecology* 17 (4): 947–963. https://doi.org/10.1111/j.1365-294X.2007.03613.x.

Kleppel, Gs. 1993. On the diets of calanoid copepods. *Marine Ecology Progress Series* 99: 183–195. https://doi.org/10.3354/meps099183.

Koski, Marja, and Christian Wexels Riser. 2006. Post-bloom feeding of *Calanus finmarchicus* copepodites: selection for autotrophic versus heterotrophic prey. *Marine Biology Research* 2 (2): 109–119. https://doi.org/10.1080/17451000600684367.

Kress, E.S. 2012. Phytoplankton abundance and community structure in the Sacramento and San Joaquin rivers. Master's thesis, San Francisco: San Francisco State University.

Lashaway, A.R., and H.J. Carrick. 2010. Effects of light, temperature and habitat quality on meroplanktonic diatom rejuvenation in Lake Erie: implications for seasonal hypoxia. *Journal of Plankton Research* 32 (4): 479–490. https://doi.org/10.1093/plankt/fbp147.

Lauritano, Chiara, Ylenia Carotenuto, Antonio Miralto, Gabriele Procaccini, and Adrianna Ianora. 2012. Copepod population-specific response to a toxic diatom diet Edited by Myron Peck. *PLoS One* 7 (10): e47262. https://doi.org/10.1371/journal.pone.0047262.

Lehman, P.W. 1992. Environmental factors associated with long-term changes in chlorophyll concentration in the Sacramento-San Joaquin Delta and Suisun Bay, California. *Estuaries* 15 (3): 335–348.

Liu, Hongbin, Mianrun Chen, Feng Zhu, and Paul J. Harrison. 2016. Effect of Diatom Silica content on copepod grazing, growth and reproduction. *Frontiers in Marine Science* 3. https://doi.org/10.3389/fmars.2016.00089.

Lonsdale, D.J., R.M. Cerrato, R. Holland, A. Mass, L. Holt, R.A. Schaffner, J. Pan, and D.A. Caron. 2009. Influence of suspension-feeding bivalves on the pelagic food webs of shallow, coastal embayments. *Aquatic Biology* 6: 263–279. https://doi.org/10.3354/ab00130.

Mann, K.H. 1993. Physical oceanography, food chains, and fish stocks: a review. *ICES Journal of Marine Science* 50 (2): 105–119. https://doi.org/10.1006/jmsc.1993.1013.

Mauchline, J. 1998a. Gut Food and Feeding. In *Advances in Marine Biology*, ed. J. H. S. Blaxter, Southward, A. J., and P. A. Tyler, 33:139–172. The biology of calanoid copepods. San Diego: Academic Press.

Mauchline, J. 1998b. Physiology. In *Advances in marine biology: the biology of calanoid copepods*, ed. J. H. S. Blaxter, A. J. Southward, and P. A. Tyler, 33:176–219. San Diego: Academic Press.

Mayama, S., Katoh, K., Omori, H., and Seino, S.. 2006. Diatoms part 2: Preparation of slides. Science and Education. *Diatom Project*.

Menden-Deuer, Susanne, and Evelyn J. Lessard. 2000. Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnology and Oceanography* 45 (3): 569–579. https://doi.org/10.4319/lo.2000.45.3.0569.

Meyer, B., X. Irigoien, M. Graeve, R.N. Head, and R.P. Harris. 2002. Feeding rates and selectivity among nauplii, copepodites and adult females of Calanus finmarchicus and Calanus helgolandicus. *Helgoland Marine Research* 56 (3): 169–176. https://doi.org/10.1007/s10152-002-0105-3.

Meyer-Harms, Bettina, Xabier Irigoien, Robert Head, and Roger Harris. 1999. Selective feeding on natural phytoplankton by Calanus finmarchicus before, during, and after the 1997 spring bloom in the Norwegian Sea. *Limnology and Oceanography* 44 (1): 154–165. https://doi.org/10.4319/lo.1999.44.1.0154.

Miralto, A., G. Barone, G. Romano, S.A. Poulet, A. Ianora, G.L. Russo, I. Buttino, G. Mazzarella, M. Laabir, M. Cabrini, and M.G. Giacobbe. 1999. The insidious effect of diatoms on copepod reproduction. *Nature* 402 (6758): 173–176. https://doi.org/10.1038/46023.

Nejstgaard, Jens C., Bent H. Hygum, Lars-Johan Naustvoll, and Ulf Båmstedt. 2001. Zooplankton growth, diet and reproductive success compared in simultaneous diatom- and flagellate-microzooplankton-dominated plankton blooms. *Marine Ecology Progress Series* 221: 77–91. https://doi.org/10.3354/meps221077.

Nejstgaard, Jens C., Marc E. Frischer, Paolo Simonelli, Christofer Troedsson, Markus Brakel, Filiz Adiyaman, Andrey F. Sazhin, and L. Felipe Artigas. 2008. Quantitative PCR to estimate copepod feeding. *Marine Biology* 153 (4): 565–577. https://doi.org/10.1007/s00227-007-0830-x.

Nixon, S.W. 1988. Physical energy inputs and the comparative ecology of lake and marine ecosystems. *Limnology and Oceanography* 33: 1005–1025.

Nobriga, Matthew L. 2002. Larval delta smelt diet composition and feeding incidence: environmental and ontogenetic influences. *California Fish & Game* 88: 149–164.

Orsi, J., and W. Mecum. 1986. Zooplankton distribution and abundance in the Sacramento-San Joaquin Delta in relation to certain

environmental factors. *Estuaries* 9 (4): 326–339. https://doi.org/10.2307/1351412.

Orsi, J.J., and T.C. Walter. 1991. Pseudodiaptomus forbesi and P. marinus (Copepoda: Calanoida), the latest copepod immigrants to California's Sacramento-San Joaquin Estuary. In *Proceedings of the fourth international conference on Copepoda*, ed. S. Nishida Uye and J.S. Ho, 553–562. Hiroshima: Bulletin of the Plankton Society of Japan.

Owens, S., T.R. Ignoffo, J. Frantzich, A. Slaughter, and W. Kimmerer. 2019. High growth rates of a dominant calanoid copepod in the northern San Francisco Estuary. *Journal of Plankton Research* 41 (6): 939–954. https://doi.org/10.1093/plankt/fbz064.

Palmieri, B.. 2020. *2012 to 2016 EMP benthic invertebrate summary report*. Environmental Monitoring Program Report. Department of Water Resources.

Pančić, Marina, Rocio Rodriguez Torres, Rodrigo Almeda, and Thomas Kiørboe. 2019. Silicified cell walls as a defensive trait in diatoms. *Proceedings of the Royal Society B: Biological Sciences* 286 (1901): 20190184. https://doi.org/10.1098/rspb.2019.0184.

Poister, David, Alison Schaefer, Andrew Baert, John Tracey, and Katelyn Richards. 2015. Stimulated rejuvenation of dormant *Aulacoseira granulata* (Bacillariophyta) by *Gloeocystis planctonica* (Chlorophyta) in a eutrophic river Edited by P. Kroth. *Journal of Phycology* 51 (2): 332–342. https://doi.org/10.1111/jpy.12277.

Queimalinos, Claudia P., Beatriz E. Modenutti, and Esteban G. Balseiro. 1998. Phytoplankton responses to experimental enhancement of grazing pressure and nutrient recycling in a small Andean lake. *Freshwater Biology* 40 (1): 41–49. https://doi.org/10.1046/j.1365-2427.1998.00326.x.

Ray, Jessica L., Katrine S. Skaar, Paolo Simonelli, Aud Larsen, Andrey Sazhin, Hans H. Jakobsen, Jens C. Nejstgaard, and Christofer Troedsson. 2016. Molecular gut content analysis demonstrates that Calanus grazing on Phaeocystis pouchetii and Skeletonema marinoi is sensitive to bloom phase but not prey density. *Marine Ecology Progress Series* 542: 63–77. https://doi.org/10.3354/meps11560.

Richman, Sumner, and Jane N. Rogers. 1969. The feeding of Calanus Helgolandicus on synchronously growing populations of the marine diatom Ditylum Brightwellii1. *Limnology and Oceanography* 14 (5): 701–709. https://doi.org/10.4319/lo.1969.14.5.0701.

Runge, Jeffrey A. 1985. Relationship of egg production of Calanus pacificus to seasonal changes in phytoplankton availability in Puget Sound, Washington. *Limnology and Oceanography* 30 (2): 382–396. https://doi.org/10.4319/lo.1985.30.2.0382.

Ryther, J.H. 1969. Photosynthesis and fish production in the sea. *Science* 166 (3901): 72–76.

Schraga, T.S., Nejad, E.S., Martin, C.A., and Cloern, J.E.. 2018. *USGS measurements of water quality in San Francisco Bay (CA), beginning in 2016*. U.S. Geological Survey Data Release ver. 3.0, March 2020.

Sheppard, S.K., and J.D. Harwood. 2005. Advances in molecular ecology: tracking trophic links through predator-prey food-webs. *Functional Ecology* 19 (5): 751–762. https://doi.org/10.1111/j.1365-2435.2005.01041.x.

Sherr, Evelyn B., and Barry F. Sherr. 2007. Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. *Marine Ecology Progress Series* 352: 187–197. https://doi.org/10.3354/meps07161.

Sicko-Goad, L., E.F. Stoermer, and G. Fahnenstiel. 1986. Rejuvenation of Melosira granulata (Bacillariophyceae) resting cells from the anoxic sediments of Douglas Lake, Michigan. 1. Light microscopy and 14C uptake. *Journal of Phycology* 22 (1): 22–28. https://doi.org/10.1111/j.1529-8817.1986.tb02510.x.

Slater, S., and Baxter, R.. 2014. Diet, prey selection, and body condition of age-0 delta smelt, Hypomesus transpacificus, in the Upper San Francisco Estuary. *San Francisco Estuary and Watershed Science* 12. https://doi.org/10.15447/sfews.2014v12iss3art1.

Sommer, Ted, Chuck Armor, Randall Baxter, Richard Breuer, Larry Brown, Mike Chotkowski, Steve Culberson, Fredrick Feyrer, Marty Gingras, Bruce Herbold, Wim Kimmerer, Anke Mueller-Solger, Matt Nobriga, and Kelly Souza. 2007. The collapse of pelagic fishes in the upper San Francisco Estuary: El Colapso de los Peces Pelagicos en La Cabecera Del Estuario San Francisco. *Fisheries* 32 (6): 270–277. https://doi.org/10.1577/1548-8446(2007)32[270:TCOPFI]2.0.CO;2.

Sommer, Ted, Francine Mejia, Kathryn Hieb, Randall Baxter, Erik Loboschefsky, and Frank Loge. 2011. Long-term shifts in the lateral distribution of age-0 striped bass in the San Francisco Estuary. *Transactions of the American Fisheries Society* 140 (6): 1451–1459. https://doi.org/10.1080/00028487.2011.630280.

Straile, Dietmar. 1997. Gross growth efficiencies of protozoan and metazoan zooplankton and their dependence on food concentration, predator-prey weight ratio, and taxonomic group. *Limnology and Oceanography* 42 (6): 1375–1385.

Sullivan, L.J., and W.J. Kimmerer. 2013. Egg development times of Eurytemora affinis and Pseudodiaptomus forbesi (Copepoda, Calanoida) from the upper San Francisco Estuary with notes on methods. *Journal of Plankton Research* 35 (6): 1331–1338. https://doi.org/10.1093/plankt/fbt076.

Takano, Keishi, Seiki Igarashi, and Shuji Hino. 2004. Seasonal changes in silicon content of diatoms estimated from the ratio of particulate silicon to diatom volume under silicon sufficiency in diatom-rich Lake Barato. *Limnology* 5 (2): 115–120. https://doi.org/10.1007/s10201-004-0117-6.

Turner, J. T. (1984a). *The feeding ecology of some zooplankters that are important prey items of larval fish*. 7. NOAA Technical Report. National Marine Fisheries Service.

Turner, Jefferson T. 1984b. Zooplankton feeding ecology: contents of fecal pellets of the copepods Acartia tonsa and Labidocera aestiva from continental shelf waters near the mouth of the Mississippi River. *Marine Ecology* 5 (3): 265–282. https://doi.org/10.1111/j.1439-0485.1984.tb00125.x.

Turner, Jefferson T., and Edna Granéli. 1992. Zooplankton feeding ecology: grazing during enclosure studies of phytoplankton blooms from the west coast of Sweden. *Journal of Experimental Marine Biology and Ecology* 157 (1): 19–31.

U.S. Geological Survey. 2020. USGS Water Data for the Nation. U.S. Government. *National Water Information System: Web Interface*. May 27.

Walter, T.C. 1987. Review of the taxonomy and distribution of the demersal copepod genus Pseudodiaptomus (Calanoida: Pseudodiaptomidae) from southern Indo-Pacific waters. *Australian Journal of Marine and Freshwater Research* 38: 363–396.

Wilkerson, F.P., R.C. Dugdale, V.E. Hogue, and A. Marchi. 2006. Phytoplankton blooms and nitrogen productivity in San Francisco Bay. *Estuaries and Coasts* 29 (3): 401–416.

Woodworth-Jefcoats, Phoebe A., and Johanna L.K. Wren. 2020. Toward an environmental predictor of tuna recruitment. *Fisheries Oceanography* 29 (5): 436–441. https://doi.org/10.1111/fog.12487.

Yeh, Heidi D., Jennifer M. Questel, Kendra R. Maas, and Ann Bucklin. 2020. Metabarcoding analysis of regional variation in gut contents of the copepod Calanus finmarchicus in the North Atlantic Ocean. *Deep Sea Research Part II: Topical Studies in Oceanography* 51: 104738. https://doi.org/10.1016/j.dsr2.2020.104738.

Yi, Xiaoyan, Yousong Huang, Yunyun Zhuang, Hongju Chen, Feifei Yang, Weimin Wang, Donghui Xu, Guangxing Liu, and Huan

Zhang. 2017. In situ diet of the copepod Calanus sinicus in coastal waters of the South Yellow Sea and the Bohai Sea. *Acta Oceanologica Sinica* 36 (6): 68–79. https://doi.org/10.1007/s13131-017-0974-6.

Zhang, Huan, Debashish Bhattacharya, and Senjie Lin. 2005. Phylogeny of dinoflagellates based on mitochondrial cytochrome B and nuclear small subunit rDNA sequence comparisons 1: Phylogeny of dinoflagellates. *Journal of Phycology* 41 (2): 411–420. https://doi.org/10.1111/j.1529-8817.2005.04168.x.

Ziemann, D.A., L.D. Conquest, M. Olaizola, and P.K. Bienfang. 1991. Interannual variability in the spring phytoplankton bloom in Auke Bay, Alaska. *Marine Biology* 109 (2): 321–334.