



# Abiotic and biotic controls on the copepod *Pseudodiaptomus forbesi* in the upper San Francisco Estuary

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**ABSTRACT:** Salinity is a key control on species distribution in estuaries, but interspecific interactions can shift distributions of estuarine species away from physiologically optimal salinities. The distribution of the introduced calanoid copepod *Pseudodiaptomus forbesi* in the upper San Francisco Estuary (SFE) shifted from brackish to fresh water in 1993 following the introductions of 2 brackish-water copepods, the small but numerically dominant *Limnithona tetraspina* (Cyclopoida) and the predatory *Acartiella sinensis* (Calanoida). The nearly simultaneous timing of these introductions complicated interpretation of the temporal change in distribution of *P. forbesi*. Although *P. forbesi* is now uncommon at salinity >~2, which might be interpreted as the result of salinity stress, short-term experiments showed high survival of adults up to salinity ~8 and of nauplii to salinity of at least 14, and reproduction was highest at salinity 5. Feeding experiments showed some overlap in diets of *P. forbesi* and *L. tetraspina*, but *P. forbesi* consumed a broader range of prey than *L. tetraspina*. Furthermore, feeding rates of the *L. tetraspina* population appeared insufficient to reduce prey availability to *P. forbesi*. Previous reports of high consumption of nauplii by *A. sinensis* and the clam *Potamocorbula amurensis* suggest that these interspecific interactions are important in constraining the distribution of *P. forbesi* in the upper SFE. Thus, we interpret the temporal shift in distribution of *P. forbesi* as due mainly to the introduction of the predatory copepod, whose high abundance may have been facilitated by the availability of a common alternative prey, *L. tetraspina*.

**KEY WORDS:** Competition · Distribution · Facilitation · Low-salinity zone · Food web · *Limnithona tetraspina* · Salinity tolerance

## INTRODUCTION

Salinity is the main physical control on species distributions in estuaries. Many estuarine species are abundant only within a limited range of salinity (Jeffries 1962, Bulger et al. 1993), and salinity tolerance is traditionally considered to be the determining factor in the distributions of pelagic species (Soetaert & Van Rijswijk 1993, Telesh & Khlebovich 2010). Although the effects of salinity through tolerance of a species may seem obvious, they may obscure the

importance of interspecific interactions. Food availability and the distribution of predators and competitors often covary with salinity, and exert bottom-up and top-down controls on a species. These other factors may be more important determinants of habitat suitability than salinity alone (Rippingale & Hodgkin 1975, Maciej Gliwicz 2002, Cloern & Dufford 2005).

Zooplankton play a key role in pelagic food webs, transferring energy from phytoplankton and microbes to higher trophic levels (Runge 1988). Few studies have examined how interspecific interactions shape

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zooplankton distribution in the field, and the available literature is almost entirely from lakes (DeMott 1989, Urabe 1990, Shurin et al. 2001). In open systems such as estuaries, it is difficult to infer interspecific interactions from field data, except when perturbations cause changes in species composition. Such cases occur when new species are introduced. Species introductions act as natural experiments, wherein the niche occupied by native or naturalized species may be studied before and after an introduction. Ecosystem changes resulting from species introductions may be reflected in changes to the distribution or abundance of native or naturalized species (Alpine & Cloern 1992, Shiganova 1998, Kimmerer 2006, Lehtiniemi & Gorokhova 2008).

The San Francisco Estuary (SFE) is a highly invaded ecosystem (Cohen & Carlton 1998, Orsi & Ohtsuka 1999). Frequent introductions in this system have been associated with substantial changes in abundance and species composition of the native and naturalized species. Invasive species have altered productivity and food web efficiency (Alpine & Cloern 1992, Kimmerer et al. 1994, Greene et al. 2011, Kimmerer & Thompson 2014) and have been linked to declines in native fish populations (Bennett & Moyle 1996, Sommer et al. 2007).

The invasion of the clam *Potamocorbula amurensis* in 1986 into the SFE had particularly notable effects on the lower food web (Carlton et al. 1990). Grazing by this clam has been implicated in eliminating summertime phytoplankton blooms (Alpine & Cloern 1992) and in declines of primary consumers (Kimmerer et al. 1994, Kimmerer & Orsi 1996, Kimmerer & Lougee 2015). By consuming a large proportion of the phytoplankton and microzooplankton production in the system, *P. amurensis* competes with suspension-feeding zooplankton (Greene et al. 2011, Kimmerer & Thompson 2014), and the clam also directly consumes copepod nauplii (Kimmerer & Lougee 2015).

The copepod fauna of the upper SFE consists of a small number of species, mostly introduced from East Asia (Orsi & Ohtsuka 1999). The copepod *Pseudodiaptomus forbesi*, first reported in the upper SFE in 1987, probably arrived in ballast water from its native waters in China (Orsi & Walter 1991). Since 1989, it has been the most abundant calanoid copepod in the upper SFE from spring through autumn and is an important food source for larval and juvenile fish, including the endangered delta smelt *Hypomesus transpacificus* (Nobriga 2002, Hobbs et al. 2006, Slater & Baxter 2014). Three additional copepod species were introduced from East Asia in 1993, 2 of

which established populations in brackish waters of the upper SFE: the small cyclopoid *Limnithona tetraspina* and the predatory calanoid *Acartiella sinensis* (Orsi & Ohtsuka 1999). Shortly after its introduction, *L. tetraspina* became the most numerically abundant copepod in the upper SFE (Bouley & Kimmerer 2006).

Historical records from long-term zooplankton monitoring (Orsi & Mecum 1986) indicate a shift in the distribution of *P. forbesi* to a lower salinity around 1993 (Kimmerer et al. 1998, Slaughter et al. 2016). This shift in distribution did not co-occur with any long-term changes in hydrology (Cloern & Jassby 2012), but it was concurrent with the introduction of the 2 new brackish-water copepod species (Orsi & Ohtsuka 1999). Thus, we hypothesized that these introduced copepod species may have altered the distribution of *P. forbesi* through predation or by competition for a limited food supply. Alternative explanations for the shift of *P. forbesi* to lower salinity include a change in salinity tolerance or an increase in food availability which could provide the energy necessary to tolerate fresh water, both of which have been observed in the species complex *Eurytemora affinis* (Lee 1999, Lee et al. 2013).

The aim of this study was to investigate controls on the distribution of *P. forbesi* in the upper SFE, including salinity tolerance and potential interspecific interactions with *L. tetraspina*. A sister study investigated predation by *A. sinensis* on *P. forbesi* and *L. tetraspina* (Slaughter et al. 2016). The results of these studies together help us narrow the possible explanations for the upstream shift in distribution of *P. forbesi*. We conducted 3 sets of experiments for this study (1) to determine the effects of salinity on survival and reproduction of *P. forbesi*, (2) to assess the potential for competition for food between *P. forbesi* and *L. tetraspina* by determining feeding rates on natural prey, and (3) to assess the potential for predation of *L. tetraspina* on *P. forbesi* nauplii. To further evaluate potential causes of the upstream shift, we calculated predation mortality to the *P. forbesi* population due to *P. amurensis* and *A. sinensis*.

## MATERIALS AND METHODS

### Study area

The upper SFE includes the brackish-water region of Suisun Bay and Marsh and the largely freshwater delta of the Sacramento and San Joaquin Rivers. This region is strongly tidally influenced and vertically

well-mixed and is characterized by high nutrients, high turbidity, and low productivity (Jassby & Cloern 2000, Kimmerer et al. 2012).

We define the low-salinity zone (LSZ) as the region of the estuary with salinity of  $\sim 0.5$ –5 (practical salinity scale), essentially the oligohaline zone of the Venice classification system. The position of the LSZ has been used as a tool for managing freshwater outflow and diversions, and as a habitat index for several pelagic species (Jassby et al. 1995, Kimmerer 2004). This region encompasses most of the summer–autumn habitat for the endangered, endemic delta smelt (Bennett 2005).

### Monitoring data

We obtained historical abundance data for *Pseudodiaptomus forbesi* and *Limnoithona* spp. from the Interagency Ecological Program (IEP) zooplankton monitoring survey ([www.water.ca.gov/bdma/meta/zooplankton.cfm](http://www.water.ca.gov/bdma/meta/zooplankton.cfm)). Temperature and conductivity were measured at every station (Orsi & Mecum 1986) and conductivity was converted to salinity (practical salinity scale).

Two species of *Limnoithona* occur in the estuary, but adults have been routinely identified to species in the monitoring data only since 2007. *L. tetraspina* dominates its congener by at least an order of magnitude, and is most abundant in the LSZ compared to *L. sinensis*, which is primarily found in fresh water (Bouley & Kimmerer 2006). Therefore, we treated counts of *Limnoithona* spp. from the LSZ before 2007 as *L. tetraspina* for our calculations. Nauplii of *Limnoithona* are not reliably identifiable in mixed samples, as their morphology has not been described (Fofonoff et al. 2017), so we used counts of unidentified nauplii as a proxy for *L. tetraspina* nauplii. This is a close approximation because during spring–autumn, *L. tetraspina* is 1 to 2 orders of magnitude more abundant than any other copepod species in the LSZ, and the nauplii of every other abundant copepod are identified to species.

The shift in distribution of *P. forbesi* was quantified using abundance data from June–October 1989–2016. The annual median abundance in fresh water (salinity,  $S < 0.5$ ) was calculated, and the seaward limit of the distribution was calculated for each year as the maximum salinity at which abundance was at least as great as 10, 25, or 100% of the freshwater median. The time series of each of these values was analyzed to identify a single change point using the changepoint package in R (Killick & Eckley 2014).

The means and confidence intervals of the pre- and post-change data were then determined. This method removes the effect of uneven sampling in salinity space, which would bias estimates based on presence or absence in the samples.

During 1994, the zooplankton monitoring program was reduced to about 40% of its previous level of sampling effort. Stations were removed from the program or replaced with nearby stations, stations were added, and sampling effort decreased from twice to once monthly. Because these changes coincided in time with the change of interest, it was essential to take the changes in monitoring into account when analyzing the data. We used 4 different versions of the dataset to ensure the analysis was robust, progressively eliminating stations to try to minimize bias due to changing sampling effort. Below, we present results of analysis with ca. half of the stations removed and 1 of each pair of surveys in each month before 1994 selected at random (Dataset 2), which reduced the total number of samples from 3958 to 2373 with between 73 and 98 samples  $\text{yr}^{-1}$ . Results using the full data set or other reductions were similar (see the Supplement at [www.int-res.com/articles/suppl/m581p085\\_supp.pdf](http://www.int-res.com/articles/suppl/m581p085_supp.pdf)).

### Sampling and laboratory conditions

Laboratory studies were conducted in the summer and autumn when freshwater inputs are low, water temperatures are high ( $\sim 18$ – $22^\circ\text{C}$ ; Kimmerer 2004), and *P. forbesi* is abundant. Copepods and surface water for experiments were collected at several stations in the upper estuary (Table 1). Temperature and salinity were measured *in situ* with a handheld meter (YSI Model 30). Copepods were collected by a gentle subsurface tow of a 150  $\mu\text{m}$  mesh, 0.5 m diameter plankton net equipped with a non-filtering plastic cod end to minimize damage to organisms. Tow contents were diluted in 20 l insulated coolers of surface water for transport back to the Romberg Tiburon Center ( $37.889^\circ\text{N}$ ,  $122.447^\circ\text{W}$ ), where all experiments were conducted. Additional surface water for experiments was collected with a clean bucket and transported in insulated coolers.

All experiments and acclimations were conducted in a temperature-controlled room maintained at  $19^\circ\text{C}$  on a 14:10 h light:dark cycle, which approximately mimics the summer conditions in this estuary. Field water used in experiments was filtered by siphoning through a submerged mesh screen affixed to a PVC pipe to remove particles larger than the specified

Table 1. Field and laboratory conditions for experiments. Copepods were collected at 4 stations in the Upper Estuary: Martinez Pier in the low-salinity zone, Antioch at the confluence of the 2 rivers, Rio Vista in the Sacramento River, and Twitchell Island in the San Joaquin River. Field and laboratory salinity (Sal, practical salinity scale) and temperature (Temp, °C), number of copepods per replicate (N), number of replicates (Reps) per treatment, incubator volume (Vol, ml), and mode of analysis on FlowCAM for grazing experiments. Dates are given as mm.dd.yy

Expt	Date	Sample site	Lat (°N)	Long (°W)	Species	Field Sal	Field Temp	Laboratory Sal	Laboratory Temp	N	Reps	Vol	FlowCAM mode
<b>Acute salinity tolerance</b>													
	06.20.12	Antioch	38.021	121.820	<i>Pseudodiaptomus forbesi</i>	0.7	21.4	0–16	19	10	3	15	–
<b>Effects of salinity on reproduction</b>													
	05.21.13	Rio Vista	38.159	121.687	<i>P. forbesi</i>	0.1	20.4	0–12	19	1	24–29	15	–
<b>Grazing experiments</b>													
1	08.30.11	Antioch	38.021	121.820	<i>P. forbesi</i>	0.4	21.3	0.4	19	6	4	175	Fluorescence
2	09.07.11	Antioch	38.021	121.820	<i>P. forbesi</i>	0.1	21.4	0.1	19	6	4	175	Fluorescence
	09.07.11	Martinez Pier	38.028	122.139	<i>Limnoithona tetraspina</i>	7.4	20.5	0.1	19	50	4	175	Fluorescence
3	09.13.11	Antioch	38.021	121.820	<i>P. forbesi</i>	0.1	20.5	0.1	19	6	4	175	Fluorescence
	09.13.11	Martinez Pier	38.028	122.139	<i>L. tetraspina</i>	6.6	19.8	0.1	19	50	4	175	Fluorescence
4	09.27.11	Rio Vista	38.159	121.687	<i>P. forbesi</i>	0.1	20.0	0.1	19	6	4	175	Auto-image
5	10.04.11	Twitchell Is.	38.095	121.668	<i>P. forbesi</i>	0.1	18.4	0.1	19	6	4	175	Auto-image
6	10.04.11	Antioch	38.021	121.820	<i>P. forbesi</i>	0.3	20.0	0.3	19	6	4	175	Auto-image
<b>Predation by <i>L. tetraspina</i> on <i>P. forbesi</i></b>													
	Autumn 2012	Laboratory cultures			0.1–1.1		2	19	20	7	15	–	

mesh size, or by vacuum pump through a 47 mm Whatman GF/F filter (effective pore size 0.7 µm) to remove all particles.

### Acute salinity tolerance

Acute salinity tolerance (salinity shock) experiments were conducted with copepods collected in late June 2012 from Antioch (S = 0.7; Table 1). Within 4 h of collection, adult female *P. forbesi* (both ovigerous and non-ovigerous) were sorted from the tow contents under a dissecting microscope and trans-

ferred to a 4 l plastic beaker containing GF/F-filtered water from the collection site. Copepods were given 48 h to acclimate to laboratory conditions before being sorted into treatments. During this time, copepods were gently aerated and fed once daily in excess (>500 µg C l<sup>-1</sup>) a diet (Table 2) based on past experience culturing *P. forbesi* (Sullivan & Kimmerer 2013).

After the acclimation period, adults were filtered onto a 200 µm mesh sieve, and the filtrate, containing nauplii that had hatched during the 48 h acclimation, was concentrated on a 53 µm mesh. Copepods were washed from the sieves into petri dishes with GF/F-

Table 2. Live algal cultures and cryopreserved algae fed to *Pseudodiaptomus forbesi* in salinity-tolerance experiments. Details on culture media are provided in the 'Materials and methods'

Experiment	Treatment	Cultured or cryopreserved food and supplier	
Acute salinity tolerance	Prior to experiment	<i>Selenastrum capricornutum</i>	UTEX 1648
		<i>Chlamydomonas reinhardtii</i>	UTEX 90
		<i>Scenedesmus obliquus</i>	UTEX 393
		Shellfish Diet®	Reed Mariculture
		Nannochloropsis	Reed Mariculture
Effects of salinity on reproduction	All treatments	<i>Selenastrum capricornutum</i>	UTEX 1648
		<i>Cyclotella meneghiniana</i>	UTEX LB FD257
		Shellfish Diet®	Reed Mariculture
		Nannochloropsis	Reed Mariculture
	Salinities 0.1, 2, 5	<i>Cryptomonas ovata</i>	UTEX LB 35
	Salinities 8, 12	<i>Rhodomonas salina</i>	CCMP 1419

filtered water from the collection site. Non-ovigerous adult female *P. forbesi* were sorted under a dissecting microscope into 30 groups of 10 individuals, and each group was kept in a 5 ml holding well. Only females were used because tolerance to salinity shock may differ between sexes (Roddie et al. 1984). Nauplii were sorted as described for adults. Once all copepods had been sorted, they were transferred by pipette into glass vials containing 15 ml of water at 10 treatment salinities: 0, 0.7, 2, 4, 6, 8, 10, 12, 14, and 16. Each treatment was replicated 3 times with adults and 3 times with nauplii. The 0 salinity treatment was deionized water, and the 0.7 salinity treatment was GF/F-filtered water from the field collection site. The other treatments were prepared by mixing GF/F-filtered water from the collection site with GF/F-filtered water collected from Central San Francisco Bay ( $S = 26$ ).

Copepods were incubated for 48 h, during which time they were not fed. At the end of the 48 h, copepods were stained with the vital stain Neutral Red (dimethyl diaminophenazine chloride). To each vial we added 0.15 ml of a 0.1% solution (for a final concentration of 1:100 000), allowed the copepods 20 min to take up the stain, then killed and preserved them in a 4% formaldehyde solution (Fleming & Coughlan 1978). Copepods were later counted under a dissecting microscope and identified as having survived or not survived the treatment, based on uptake of the vital stain (Dressel et al. 1972). To calculate survival rates by salinity, we fit a generalized linear model with a binomial error distribution to the count data using the function 'glm' from the 'stats' package in the R statistical computing environment version 3.0.1 (R Development Core Team 2013).

### Effects of salinity on reproduction

Experiments testing the effects of salinity on reproduction were conducted from May to June of 2013 with copepods collected from Rio Vista ( $S = 0.1$ ; Table 1). Within 2 h of collection, copepods were transported to the laboratory and an artificial cohort of juveniles (sensu Kimmerer & McKinnon 1987) was filtered out of the tow contents using 150 and 175  $\mu\text{m}$  mesh sieves. The resulting cohort was composed of late nauplii (mostly NVI) and early copepodites (CI and CII). These copepods were distributed among 10 culture buckets containing GF/F-filtered water from the collection site. Copepods were acclimated gradually from salinity 0.1 to salinities 2, 5, 8, and 12 over 5 d. Duplicate cultures were maintained at each sa-

linity treatment. Salinity in the cultures was increased stepwise, once a day, by adding GF/F-filtered seawater ( $S = 32$ ) until the new target salinity was reached as determined with a handheld meter (YSI Model 30). This rate of acclimation is conservative compared to that used by others in copepod salinity tolerance experiments (Lee & Petersen 2003, Chen et al. 2006). After the final salinity was reached, copepods were left for 3 d while they matured to adults.

Cultures were maintained as in the acute salinity-tolerance experiments except for the live algae species that they were fed (Table 2). Inoculate cultures of algal species were grown in fresh water (WC Medium), salinity 5, and salinity 10 media (WC Medium spiked with filtered and sterilized sea water) to prevent any effect on quality or vitality of algal food because of salinity shock. Copepods at salinities 8 and 12 were fed *Rhodomonas salina* instead of *Cryptomonas ovata* because *C. ovata* could not be successfully acclimated up to salinity 10 culture media and *R. salina* could not be acclimated to grow in fresh water.

Five days after cultures were established, 12–15 ovigerous females were removed from each duplicate culture and placed individually in 15 ml wells in flat-bottom tissue-culture well plates. These females continued to be fed daily with the same diet they received in the culture buckets. Plates were checked twice daily and hatched nauplii were counted. Observations of naupliar condition were recorded, i.e. how many nauplii were actively swimming and how many appeared dead or had failed to hatch with the rest of the clutch. Some females dropped their egg sacs before the eggs were fully developed and no nauplii hatched from those abandoned egg sacs; these clutches (3 of the 125 clutches observed) were not included in the analysis. For each treatment, mean clutch sizes were calculated with 95% confidence intervals.

### Feeding by *P. forbesi* and *L. tetraspina*

Six grazing experiments were conducted with *P. forbesi* in the fall of 2011; 2 experiments also included *L. tetraspina* (Table 1). For the 2 experiments that included *L. tetraspina*, copepods were collected from 2 different locations, one where *P. forbesi* was the dominant species (Antioch) and the other where *L. tetraspina* was the dominant species (Martinez Pier), to facilitate sorting in the laboratory. Tow contents from Martinez Pier were transferred into water from Antioch for 3–4 h before copepods were sorted, to allow for acclimation of copepods to the experi-

mental conditions; both copepod species were incubated in water from Antioch for the experiments (Table 1) so that initial prey concentrations would be the same for both copepod species.

Surface water from the field was gently screened with a 100  $\mu\text{m}$  mesh filter into a clean 20 l bucket to remove larger grazers as previously described. The screened water was then stirred gently to homogenize contents and siphoned into 175 ml incubation bottles. Adult female copepods were sorted into holding vessels containing GF/F-filtered field water (with no food) where they remained for 90–120 min before being added to incubation bottles. Bottles were covered with parafilm and tightly capped to prevent the formation of air bubbles, which can damage fragile protists (Gifford 1993). Once capped, bottles were mounted on a plankton wheel rotating at 1–2 rpm in a temperature-controlled room at 19°C on a 14:10 h light:dark cycle. For each experiment we used 4 initial bottles, 4 controls with no grazers, and 4 experimental bottles for each copepod species.

After 15 min on the plankton wheel, the initial bottles were collected for analysis. This time allows the plankton assemblage to adjust to effects of handling, as most losses due to handling occur immediately (Gifford 1993). Experimental bottles and final controls were incubated for 24 h. From each bottle, a 25–50 ml sample of filtrate was transferred to a 50 ml plastic centrifuge tube and preserved by adding acid Lugol's solution to a final concentration of ~1%. These samples were settled using the Utermöhl method (Sherr & Sherr 1993), and cells were counted at a later date on a Wild M40 inverted microscope at 100 $\times$  magnification. Additional samples were collected for immediate analysis using a FlowCAM (Fluid Imaging Technologies) imaging flow cytometer.

The FlowCAM uses a syringe pump to move fluid through a narrow glass flow chamber at a controlled rate (Sieracki et al. 1998). A section of the flow chamber is oriented in front of a microscope objective and digital camera; as cells pass through the chamber, digital images are captured and stored in a database. In fluorescence-trigger mode, a laser that stimulates chlorophyll *a* autofluorescence is active. When a particle passing through the flow chamber fluoresces, a detector triggers the camera and the contents of the flow chamber are imaged (Sieracki et al. 1998). In auto-image mode, images are captured at consistent, user-defined intervals up to 20 images  $\text{s}^{-1}$ . Visual Spreadsheet software version 9.0 (Fluid Imaging Technologies) automatically measures particle size and other characteristics based on the image and stores these data along with the particle images. In

post-processing, these characteristics can be used to automate sorting and classification of particles.

In Expts 1–3, the fluorescence-trigger mode was used to analyze live samples (Table 1). Bottles were stored in a dark refrigerator at 4°C for a maximum of 2 h to minimize growth and grazer activity during processing (Liu et al. 2005, Ide et al. 2008). Bottles were gently inverted at least 50 times before 1.5 ml subsamples were taken by pipette and processed through the FlowCAM.

In Expts 4–6, the auto-image mode with a frame rate of 20 images  $\text{s}^{-1}$  was used to analyze preserved samples (Table 1). The bottles of filtrate were preserved in 4% glutaraldehyde and stored in a dark refrigerator at 4°C for a maximum of 48 h. Bottles were inverted at least 50 times before 5 ml subsamples were transferred by pipette to be processed through the instrument, which imaged 0.94 ml of the sample.

### Analysis of feeding experiments

Images stored in the FlowCAM database were sorted in the Visual Spreadsheet software and classified into broad taxonomic categories based on visual identification of the cell images. Sample volumes analyzed on the FlowCAM were small ( $\leq 1.5$  ml), so only the most abundant taxa could be counted accurately using this method. Ciliates and dinoflagellates were present at low densities, so these groups were counted only in the larger-volume microscope subsamples. Cells 7–15  $\mu\text{m}$  were counted only in Expts 2 and 3; all other cells counted were  $>15$   $\mu\text{m}$  on their longest axis.

Clearance and ingestion rates were calculated for each taxon using the equations of Marin et al. (1986). Clearance rate was calculated by fitting a generalized linear model with a Poisson error distribution to the cell counts in the final control and treatment bottles using the 'glm' function in the 'stats' package in R. Ingestion rates were calculated by multiplying clearance rates by mean concentrations of cells in initial bottles; 95% confidence intervals of mean ingestion were calculated by accounting for variance of the clearance rates as well as variance of the mean of the initial cell concentrations. Carbon content of cells was estimated by converting average cell dimensions to biovolume using the conversion factors in Hillebrand et al. (1999), then converting biovolume to carbon using the relationships in Menden-Deuer & Lessard (2000) (Table A1 in Appendix 1). Average cell dimensions were calculated using automated

measurements of length and width. At least 30 cell images were selected for each cell type to calculate average measurements. For centric diatoms, side-view images were selected so that the height of the cells could be measured. Ciliate and dinoflagellate dimensions were measured using a calibrated ocular micrometer on the inverted microscope at 400× magnification.

Population clearance rates ( $d^{-1}$ ), the daily fraction of the prey populations consumed by the entire predator population (Uye 1986), were calculated by extrapolating clearance rates of adult females to mean summer–autumn populations of each copepod species (as in Slaughter et al. 2016) using abundance data from the IEP zooplankton monitoring program. Abundance of each gross life stage (nauplius, copepodite, and adult) was averaged from June–October of 2000–2014. *P. forbesi* abundance was averaged across the freshwater stations ( $S \leq 0.5$ ), and *Limnithona* abundance was averaged across stations in the LSZ ( $S = 0.5–5$ ). Prey selection was assumed to be the same across life stages but scaled with carbon mass of the copepods. Prey selection of the *P. forbesi* congener *P. marinus* in the SFE is similar between nauplii and adults (Vogt et al. 2013). The same is true for adults and nauplii of *Oithona davisae* in the SFE (Vogt et al. 2013), a copepod in the same family as *L. tetraspina* and of similar size and predatory mode (Bouley & Kimmerer 2006).

To determine population biomass, we multiplied mean abundance by a representative carbon value for each gross life stage and summed the 3 products. Carbon biomass of adult and copepodite stages was measured in our laboratory (Gould & Kimmerer 2010, W. Kimmerer unpubl.). Carbon biomass of nauplii was calculated by fitting an exponential regression from egg carbon (Kimmerer et al. 2014b) to CI carbon (Gould & Kimmerer 2010) assuming that eggs, NI, and NII have the same carbon biomass. We cross-checked this calculation using a published scaling ratio for *P. marinus* (Vogt et al. 2013) and got a similar result (<3% difference). Clearance rates of adult females were divided by carbon mass to obtain carbon-specific rates which were multiplied by the estimated carbon biomass of each gross stage in the population to calculate a population clearance rate on each prey taxon (Slaughter et al. 2016).

### Predation

Predation on *P. forbesi* nauplii by adult female *L. tetraspina* was tested in an experiment in November

2012. Copepods used in this experiment came from laboratory cultures. The day before the predation experiment, *P. forbesi* cultures were filtered through a 250  $\mu\text{m}$  mesh sieve which retained only adults and late copepodites. Copepods were then washed into a PVC cylinder equipped with a 200  $\mu\text{m}$  mesh screen and suspended in a 4 l bucket containing 3 l of culture media. The day of the experiment, the sieve holding adult *P. forbesi* was removed and the remaining water, containing the nauplii that had hatched overnight, was concentrated on a 53  $\mu\text{m}$  mesh. Nauplii retained on the mesh were resuspended in GF/F-filtered culture media and sorted under a dissecting microscope into 10 groups of 20 individuals, each a mixture of NII and NIII stages. Groups of nauplii were transferred by pipette into glass vials with 15 ml of GF/F-filtered culture media. Meanwhile, *L. tetraspina* cultures were concentrated on a 125  $\mu\text{m}$  mesh sieve and resuspended in the GF/F-filtered culture media. Adult female *L. tetraspina* were sorted under a dissecting microscope into groups of 5 and transferred to 7 of the vials; the remaining 3 vials containing only *P. forbesi* nauplii were left as recovery controls. Vials were maintained in the temperature-controlled room at 19°C for 28 h (Table 1). At the end of this time, copepods were stained, preserved, and counted as in the acute salinity tolerance experiments. Clearance rate and population clearance rate were calculated as described for the other feeding experiments.

## RESULTS

### Distribution

During 1989–1992, abundance of *Pseudodiaptomus forbesi* in the long-term monitoring data exceeded 100, 25, and 10% of the annual freshwater median abundance at salinities up to 9, 15, and 17 respectively (Fig. 1a, and see Table S1 in the Supplement). During 1993–2016, the population contracted up-estuary so that the corresponding salinity values were 3, 5, and 7, respectively. The annual values (means  $\pm$  95% CIs) did not overlap any of those in 1989–1992 (Fig. 1a). Calculations using the full data set or other reductions of the data gave similar results (Fig. S1, Table S1). Over the same time period and in the same sample set, chlorophyll concentration in freshwater exceeded that in the LSZ by  $1.3 \pm 0.5 \mu\text{g l}^{-1}$  with no temporal trend, and annual mean chlorophyll values in each zone varied interannually but also with no trend (Fig. 1b).

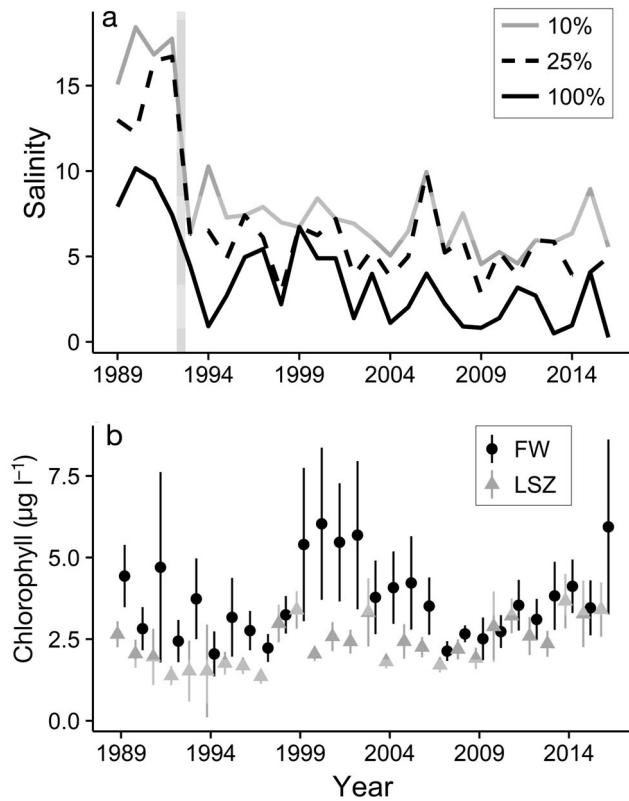


Fig. 1. Temporal variability of distribution in salinity space of *Pseudodiaptomus forbesi* adults, and chlorophyll concentrations in the upper San Francisco Estuary, June–October 1989–2016. (a) Maximum salinity at which abundance exceeded 10% (grey line), 25% (dashed line), or 100% (black line) of the annual median for all freshwater samples (salinity,  $S < 0.5$ ). Mean values of the 10, 25, and 100% values were  $17.0 \pm 2.3$  (95% CI),  $14.6 \pm 3.7$ , and  $8.8 \pm 2.1$ , respectively, for 1989–1992 and  $6.8 \pm 0.6$ ,  $5.4 \pm 0.7$ , and  $2.8 \pm 0.8$ , respectively, for 1993–2014. (b) Annual means with 95% CIs of chlorophyll concentration at stations in fresh water (FW,  $S < 0.5$ ) and in the low-salinity zone (LSZ,  $S = 0.5–5$ )

### Salinity effects

Mean survival of adults in the 48 h salinity-shock experiment was high (above 93%) at all salinity levels from 0 through 8 (Fig. 2a). Survival declined above salinity 8 (as shown by the declining confidence intervals from the generalized linear model), and mean survival in the salinity 16 treatment was 61%. Nauplii were less susceptible to salinity shock than adults over the range of salinities tested. The generalized linear model fit to the data for nauplii (Fig. 2b) predicted survival above 95% at all treatment levels tested.

The clutches produced in the low salinities 0.1 and 2 were smaller than at higher salinities of 5, 8, and 12 (Table 3). An analysis of variance (ANOVA) yielded

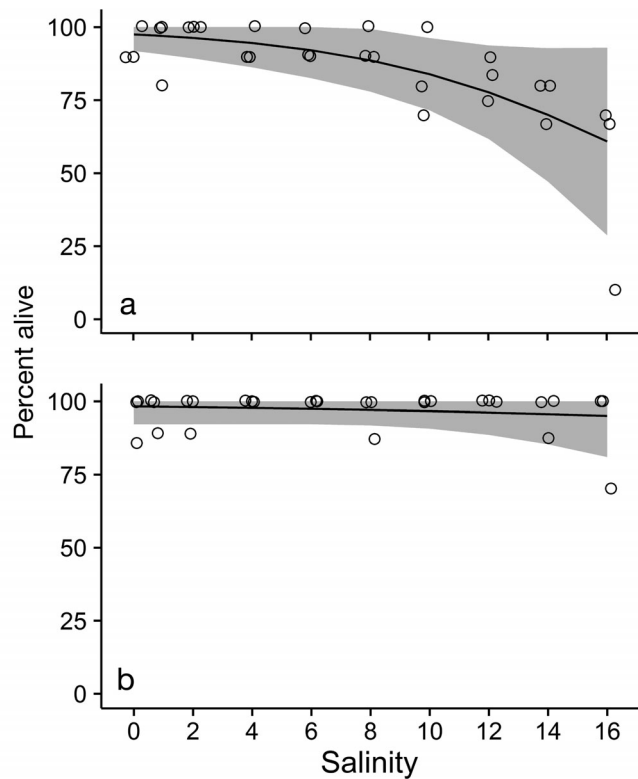


Fig. 2. Percent of *Pseudodiaptomus forbesi* (a) adults and (b) nauplii alive after 48 h salinity shock treatment. Circles show data from each replicate with points moved slightly on the x-axis to reveal overlapping data points. Lines are predicted means from a generalized linear model with a binomial error distribution. Shading shows the 95% confidence interval for the model

significant variation among treatments ( $F_{4,117} = 14.0$ ,  $p < 0.01$ ). A Tukey HSD post hoc test showed significant differences between clutch sizes at salinity 0.1 and all other treatments ( $p < 0.03$ ), and between salinities 5 and 2 (mean difference  $5.8 \pm 4.4$  [95% CI],  $p < 0.01$ ). Differences between clutch sizes at the higher salinity treatments were not statistically significant. The largest average clutch size and highest hatching success were in the salinity 5 treatment (Table 3). Overall hatching success was high and there was no trend.

### Feeding and predation

Clearance rates of *P. forbesi* were higher on centric diatoms than on other cell types (Fig. 3), especially in Expts 1 and 3 when initial concentrations of diatoms were much higher than in the other experiments (Table 4). Clearance rates on cells in the 7–15  $\mu\text{m}$  size class were consistently low, despite the order of mag-



Table 3. Total clutch size and hatching success of *Pseudodiaptomus forbesi* in 5 salinity treatments. Mean number of eggs clutch<sup>-1</sup>, mean number of dead or unhatched nauplii with 95% confidence intervals (CI; N = 22–28 ovigerous females treatment<sup>-1</sup>). Mean hatching success as percent. ANOVA yielded significant variation among treatments ( $F_{4,117}=14.0$ ,  $p < 0.01$ ). A Tukey HSD test showed significant differences between clutch sizes at salinities 0.1 and 2 ( $p < 0.03$ ), and salinities 2 and 5 ( $p < 0.01$ ); differences between the higher salinity treatments were not statistically significant

Treatment salinity	N	Mean $\pm$ CI eggs clutch <sup>-1</sup>	Mean $\pm$ CI dead or unhatched nauplii	Hatching success (%)
0.1	28	16.8 $\pm$ 2.5	1.3 $\pm$ 1.2	92.3
2	22	21.5 $\pm$ 2.9	2.1 $\pm$ 1.3	90.1
5	24	27.4 $\pm$ 1.8	0.7 $\pm$ 0.9	97.4
8	24	23.6 $\pm$ 1.6	1.8 $\pm$ 1.1	92.6
12	24	24.6 $\pm$ 2.2	1.7 $\pm$ 1.2	93.0

nitude higher abundance of these cells than those in the large size class (Table 4). Overall, *P. forbesi* appeared to consume a wide variety of cell types but ingested the most carbon in the form of centric diatoms, flagellates, and ciliates (Fig. 4). Population clearance rates of *P. forbesi* were ~12–27% d<sup>-1</sup> on

centric diatoms, ~4–10% d<sup>-1</sup> on flagellates, and ~6–16% d<sup>-1</sup> on ciliates (Table 5).

Calculated clearance rates of *Limnoithona tetraspina* were positive on flagellates >15  $\mu$ m and ciliates, and negative for all other cell types (Fig. 3). A potential interpretation of negative clearance rates is proposed below. Despite slightly lower clearance rates on flagellates compared to ciliates, most of the carbon ingested by *L. tetraspina* came from flagellates (Fig. 4) because they were much more abundant (Table 4). Population clearance rates were ~8% d<sup>-1</sup> on ciliates and 4–7% d<sup>-1</sup> on flagellates (Table 5).

Prey disappearance in the predation experiment was very low. Nineteen of the 20 *P. forbesi* nauplii originally stocked were recovered from 2 of the experimental vials, and all 20 nauplii were recovered from the remaining 5 experimental vials and the 3 recovery control vials. The small change in numbers of nauplii could have been due to error in counting the nauplii or transferring the small nauplii into or out of the vials rather than to predation. Under the assumption that the missing nauplii were removed by predation, we calculated a mean clearance rate of 0.04 ml d<sup>-1</sup> (*L. tetraspina*)<sup>-1</sup>. The resulting population clearance rate at mean autumn densities of *L. tetraspina* is negligible at only 0.07% d<sup>-1</sup>.

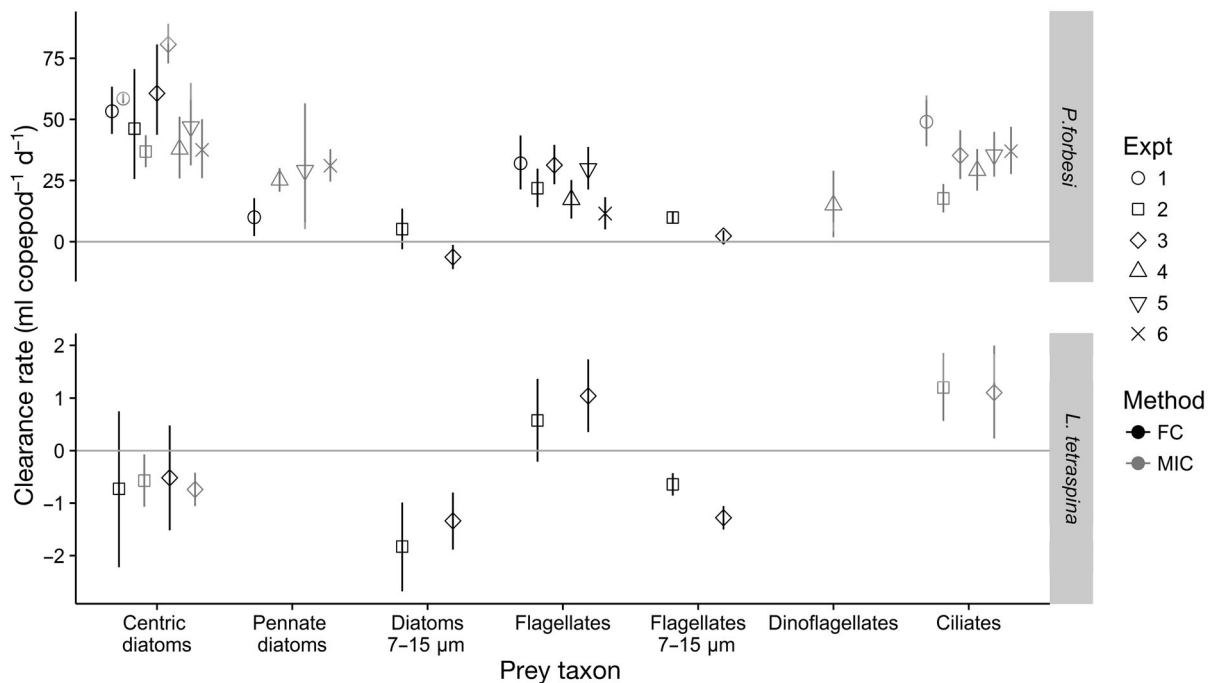


Fig. 3. Clearance rates of *Pseudodiaptomus forbesi* (top) and *Limnoithona tetraspina* (bottom) on different prey taxa in 6 experiments. Symbols represent the experiment; black denotes cells counted with the FlowCAM (FC) and grey shows cells counted on an inverted microscope (MIC). Points are means calculated by fitting a generalized linear model with Poisson error distribution to the experimental data; bars show the 95% confidence intervals. For taxa other than 'Diatoms 7–15  $\mu$ m' and 'Flagellates 7–15  $\mu$ m,' cells in each category are >15  $\mu$ m on their longest axis

Table 4. Abundance of microplankton taxa (cells ml<sup>-1</sup>; mean ± 95% CI) in initial, control, and treatment bottles from all feeding experiments. Shaded counts from FlowCAM, unshaded counts from microscopy. Blank fields indicate taxa not present or not counted

Expt	Type	Centric diatoms		Pennate diatoms	Diatoms 7–15 µm	Flagellates	Flagellates 7–15 µm	Dino-flagellates	Ciliates
1	Initial	19.5 ± 2.4	23.1 ± 6.4	28.4 ± 5.3		34.5 ± 6.7			1.0 ± 0.3
	Control	33.5 ± 5.9	43.1 ± 3.2	22.1 ± 3.2		18.2 ± 5.9			1.0 ± 1.2
	<i>Pseudodiaptomus forbesi</i>	4.5 ± 4.6	6.9 ± 4.6	15.7 ± 6.0		6.1 ± 2.2			0.2 ± 0.1
2	Initial	2.3 ± 0.7	9.1 ± 8.1		15.0 ± 8.4	112 ± 26.5	261 ± 55.1		1.5 ± 0.5
	Control	3.5 ± 0.6	6.6 ± 2.2		17.7 ± 3.2	27.9 ± 13.3	316 ± 56.5		2.8 ± 0.4
	<i>P. forbesi</i>	1.0 ± 0.9	1.4 ± 1.5		14.9 ± 2.9	13.2 ± 4.2	225 ± 54.5		1.5 ± 0.7
	<i>Limnoithona tetraspina</i>	4.1 ± 0.7	8.1 ± 2.3		29.9 ± 9.3	23.6 ± 6.0	379 ± 111		2.0 ± 0.6
3	Initial	4.7 ± 1.3	6.9 ± 3.2		19.3 ± 4.3	79.7 ± 18.0	648 ± 54.8		1.1 ± 0.6
	Control	8.4 ± 0.6	14.9 ± 5.0		54.3 ± 6.8	44.8 ± 38.1	321 ± 84.8		1.4 ± 0.4
	<i>P. forbesi</i>	0.5 ± 0.2	1.9 ± 1.6		67.3 ± 12.6	15.3 ± 5.1	296 ± 146		0.4 ± 0.1
	<i>L. tetraspina</i>	10.4 ± 1.2	17.2 ± 4.4		79.6 ± 8.2	33.3 ± 9.3	463 ± 56.7		1.1 ± 0.6
4	Initial	1.7 ± 0.1		6.4 ± 1.1		61.3 ± 19.5		0.6 ± 0.1	1.1 ± 0.3
	Control	1.2 ± 0.5		5.9 ± 0.9		39.8 ± 9.4		0.6 ± 0.1	2.1 ± 0.3
	<i>P. forbesi</i>	0.3 ± 0.2		2.5 ± 0.8		22.0 ± 2.2		0.3 ± 0.1	0.8 ± 0.5
5	Initial	0.6 ± 0.3		0.7 ± 0.3		65.1 ± 11.2			1.4 ± 0.3
	Control	0.7 ± 0.3		0.2 ± 0.03		44.1 ± 21.7			1.7 ± 0.3
	<i>P. forbesi</i>	0.1 ± 0.1		0.1 ± 0.1		15.9 ± 5.8			0.5 ± 0.2
6	Initial	1.1 ± 0.4		1.9 ± 0.9		66.7 ± 24.7			0.9 ± 0.4
	Control	1.1 ± 0.2		2.9 ± 1.3		50.3 ± 3.8			1.6 ± 0.8
	<i>P. forbesi</i>	0.3 ± 0.1		1.0 ± 0.8		33.9 ± 7.1			0.5 ± 0.3

## DISCUSSION

The persistence of organisms in any habitat depends on their responses to environmental conditions and interspecific interactions. Salinity is the classical driving force of estuarine species' distributions, but physical factors such as temperature, turbidity, and habitat structure often covary with salinity, as do biological factors such as food availability and the distribution of predators and competitors. All of these factors have been invoked to explain distribution patterns of zooplankton (Lakkis 1994, Lee et al. 2013). What caused the shift in distribution of *Pseudodiaptomus forbesi* after 1993, and why is it now confined largely to fresh water? We explore 3 potential explanations: change in salinity tolerance, bottom-up effects through competition with introduced species, and top-down effects through predation pressure from introduced species.

### Abiotic control: salinity effects

The effects of salinity on the mortality or reproduction of this species have not previously been investigated in any system, and the only relevant information on salinity effects is on distribution (Orsi & Walter 1991, Cordell et al. 2008, Bollens et al. 2012).

Our laboratory results are consistent with field observations of the distribution of the species before but not after the introductions in 1993 (Fig. 1a) and reported distributions in other estuaries. In the Columbia River Estuary, *P. forbesi* is found from the brack-

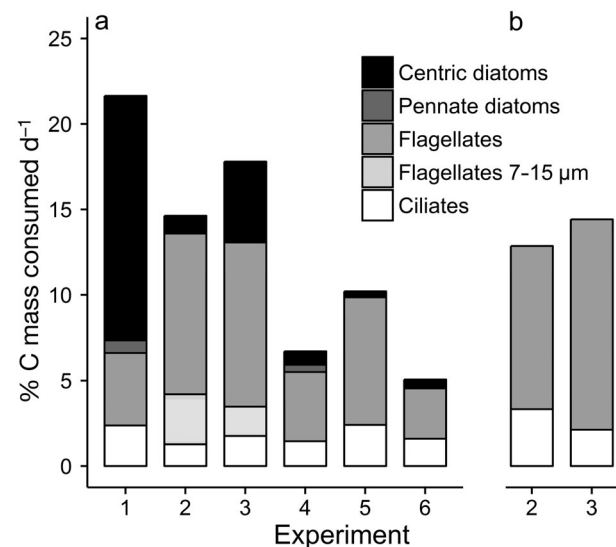


Fig. 4. Ingestion expressed as daily fraction for (a) *Pseudodiaptomus forbesi* and (b) *Limnoithona tetraspina* on different taxa. Proportion of carbon ingested d<sup>-1</sup> to biomass of the copepods: adult female *P. forbesi* 4.26 µg C (Kimmerer unpubl.), and adult female *L. tetraspina* 0.11 µg C (Gould & Kimmerer 2010). Ingestion rates <0.2% d<sup>-1</sup> are not plotted

Table 5. Population clearance (%) of water volume cleared  $d^{-1}$  by each copepod species on each prey taxon (mean  $\pm$  95 % CI). Shaded counts from FlowCAM, unshaded counts from microscopy. Blank fields indicate taxa not present or not counted. Neg: experiment–taxon combinations where calculated clearance rates were negative and therefore were not used in further calculations

Species	Expt	Centric diatoms	Pennate diatoms	Diatoms 7–15 $\mu$ m	Flagellates	Flagellates 7–15 $\mu$ m	Dino-flagellates	Ciliates
<i>Pseudodiaptomus forbesi</i>	1	19.3 $\pm$ 0.7	17.6 $\pm$ 3.3	3.3 $\pm$ 2.6		10.6 $\pm$ 3.8		16.2 $\pm$ 3.5
	2	12.1 $\pm$ 2.2	15.2 $\pm$ 8.0		1.7 $\pm$ 2.7	7.2 $\pm$ 2.6	3.3 $\pm$ 0.7	5.8 $\pm$ 1.9
	3	26.6 $\pm$ 2.8	20.0 $\pm$ 6.6		Neg	10.3 $\pm$ 2.7	0.8 $\pm$ 0.7	11.6 $\pm$ 3.4
	4	12.5 $\pm$ 4.4		8.3 $\pm$ 1.6		5.7 $\pm$ 2.6		9.6 $\pm$ 2.9
	5	15.5 $\pm$ 5.9		9.6 $\pm$ 9.0		9.8 $\pm$ 2.9		11.7 $\pm$ 3.1
	6	12.4 $\pm$ 4.1		10.2 $\pm$ 2.2		3.8 $\pm$ 2.2		12.2 $\pm$ 3.3
<i>Limnoithona tetraspina</i>	2	Neg	Neg		Neg	4.1 $\pm$ 5.6	Neg	8.6 $\pm$ 4.6
	3	Neg	Neg		Neg	7.4 $\pm$ 4.9	Neg	7.9 $\pm$ 6.3

ish lower estuary up into the Snake River tributary (Cordell et al. 2008), and high abundances of *P. forbesi* have been recorded at salinities from 0–12 (Bollens et al. 2012). In its native range in the Yangtze River and estuary in China, *P. forbesi* is found in salinities up to at least 8 (Orsi & Walter 1991).

We found no evidence that physiological tolerance to salinity restricts *P. forbesi* to fresh water in the upper SFE. In fact, our data suggest the opposite: *P. forbesi* seemed physiologically better adapted to live in brackish water than in fresh. Survival of adult females was high up to salinity 8; although we did not test survival of males, the proportion of males in samples from salinity 0.5, 2, and 5 in 2006–2007 averaged  $\sim$ 0.6 and did not vary by salinity (based on data from Kimmerer et al. 2014b), implying similar survival of both sexes. Naupliar survival was near 100% at least up to salinity 14 (Fig. 2). Reproduction was significantly higher at salinity 5 than at salinities of 0.1 or 2 (Tukey's HSD,  $p < 0.01$ ), with a higher mean than in any other treatments (Table 3). Both clutch size and hatching success were numerically highest in the salinity 5 treatment. These results are similar to findings for *Eurytemora affinis* populations that have invaded freshwater habitats (Lee et al. 2013). Chlorophyll concentrations were elevated by  $\sim$ 1.3  $\mu$ g  $l^{-1}$  in fresh water over values in the LSZ, suggesting additional capacity for *P. forbesi* to overcome salinity stress in fresh water (Lee et al. 2013). However, the lack of trend in the chlorophyll data fails to support the hypothesis that a change in food supply was instrumental in the spatial shift of *P. forbesi*. We do not know whether salinity tolerance of *P. forbesi* has changed since its introduction.

Estuarine copepod populations typically are most abundant within a species-specific range of salinity (Miller 1983). Although this pattern is often attributed to salinity tolerance, the observed distributions

are frequently inconsistent with the copepods' physiological responses to salinity (Rippingale & Hodgkin 1975). For example, members of the global species complex *E. affinis* are most often found in freshwater to low-salinity regions of estuaries (Lee 2000), but experiments have invariably shown good survival, reproduction, or growth across a wide range of salinity (Roddie et al. 1984, Nagaraj 1992, Kimmel & Bradley 2001, Devreker et al. 2012). Furthermore, a favorable feeding environment can enable an animal to acclimate to salinity outside its optimum range (Lee et al. 2013). Therefore, the distribution of a copepod species in salinity space likely arises through a combination of salinity tolerance with behaviorally mediated retention mechanisms (Hough & Naylor 1991, Simons et al. 2006, Kimmerer et al. 2014a) and spatially variable growth and mortality (Barlow 1955, Stalder & Marcus 1997, Tiselius et al. 2008) that may offset effects of unfavorable salinity (Lee et al. 2013, Hammock et al. 2016) or restrict distributions to only part of the tolerated range (Rippingale & Hodgkin 1975).

### Biotic control: food availability

Since open-water zooplankton are too dilute to be limited by space, the main resource axis on which they compete is food. Evidence for competition in zooplankton is difficult to find, especially in estuaries. In most cases, competition between zooplankton species is inferred from variation in body size, seasonal occurrence, or spatial distributions with and without a potential competitor (Tranter & Abraham 1971, Vanni 1986, Urabe 1990). Often, competition is inferred from spatial overlap of species and assumptions that they have overlapping diets (Rothhaupt 1990, Lakkis 1994, Bollens et al. 2012). However,

overlaps are insufficient evidence to demonstrate competition. Grazing by a superior competitor must limit the abundance of food available to the lesser competitor in order for competition to be invoked as a major influence on the latter's population dynamics. There are few examples in the literature where this effect has been demonstrated. Larger cladocerans dominate in lakes without fish because they consume so much phytoplankton that the smaller cladocerans are deprived of food. This was attributed to competition because experimental nutrient additions, by increasing the growth of phytoplankton, led to increases in the abundance of the smaller cladoceran species (Vanni 1986).

In the LSZ of the SFE, the major consumers of large phytoplankton and microzooplankton are copepods and the invasive Asian overbite clam *Potamocorbula amurensis* (Greene et al. 2011, Kimmerer & Thompson 2014, York et al. 2014). The invasive clam is implicated in substantially drawing down phytoplankton biomass in the LSZ since its introduction in 1987 (Alpine & Cloern 1992). Grazing by clams, copepods, and microzooplankton on phytoplankton in the LSZ usually outpaces production (Kimmerer & Thompson 2014). The clam *P. amurensis* may consume 50 to 90% of ciliates  $d^{-1}$  (Greene et al. 2011) which can exceed maximum population growth rates of the microzooplankton. Ciliate growth rates in this study, calculated from the log ratio of abundance in the 24 h controls to initial abundance (Table 4), were 0–60%  $d^{-1}$  in the absence of copepods. The invasive clam is thus a primary competitor of planktivores in the SFE, with the potential to both directly and indirectly cause declines in zooplankton (Kimmerer et al. 1994, Kimmerer & Orsi 1996, Kimmerer 2006, Kimmerer & Lougee 2015).

Overall *P. forbesi* appears to be a generalist, consuming whatever cells are abundant (York et al. 2014). *Limnoithona tetraspina* specializes on motile prey (Bouley & Kimmerer 2006, Gifford et al. 2007, York et al. 2014), so any competition with *P. forbesi* is limited to those prey types; in this study, these were flagellates and ciliates. Although both copepods consumed motile prey, *P. forbesi* also consumed considerable amounts of centric diatoms when abundant (Fig. 4). In the Columbia River Estuary, *P. forbesi* also ingests most of its carbon from diatoms and ciliates, at rates comparable to those found in this study (Bowen et al. 2015). Prey selection and clearance rates of *L. tetraspina* reported here are comparable to rates from previous studies in the SFE (Bouley & Kimmerer 2006, York et al. 2014), despite differences in prey density (Bouley &

Kimmerer 2006). Predation on ciliates by both copepod species likely triggered a trophic cascade (York et al. 2014), depressing calculated clearance rates on cells that would have been consumed by ciliates. For *L. tetraspina* this is particularly evident, as experiments consistently yielded negative clearance rates on diatoms and on flagellates 7–15  $\mu m$ . We must assume then that clearance rates for *P. forbesi* on those cell types are underestimates.

The mean population clearance rates of *L. tetraspina* on flagellates >15  $\mu m$  and on ciliates were about 4–8%  $d^{-1}$  (Table 5). During 2006–2007, the population clearance of phytoplankton biomass >5  $\mu m$  by clams, microzooplankton, and copepods during summer in the LSZ was estimated at 26, 14, and 4%  $d^{-1}$ , respectively (data from Kimmerer & Thompson 2014). Thus, the daily consumption of phytoplankton and microzooplankton by *L. tetraspina* is a small part of the overall consumption of each. Although low food availability may limit growth and reproductive rates of both *P. forbesi* (Kimmerer et al. 2014b) and *L. tetraspina* (Gould & Kimmerer 2010), and lack of suitable food can exclude copepods from areas that are otherwise suitable (Modéran et al. 2012), the competitive effect of *L. tetraspina* on *P. forbesi* appears negligible. Moreover, growth rates of *P. forbesi* were similar among salinities of 0.5, 2, and 5 in 2006–2007 (Kimmerer et al. 2014b), suggesting similar trophic conditions across the salinity gradient.

Higher food concentrations in fresh water have facilitated invasions of *E. affinis* into freshwater habitats (Lee et al. 2013) by providing the invader with the energy needed for osmoregulation and ion uptake (Lee et al. 2012). With sufficient food, brackish-adapted species tend to grow faster and have higher fecundity than freshwater species (Peterson 2001), giving them an advantage over freshwater species and allowing them to successfully invade freshwater habitats. However, the elevation of chlorophyll concentration in fresh water over that in the LSZ was rather small and inconsistent among years (Fig. 1b).

### **Biotic control: predation**

Selective predation can exert powerful top-down effects that shape pelagic communities (Brett & Goldman 1997, Cloern & Dufford 2005), mediated through both direct and indirect effects on the distribution of prey species. Predators may remove a species from a potential niche directly through consumption (Ripplingale & Hodgkin 1975, Kimmerer & McKinnon 1989, Kimmerer 1991), or may induce migration or

other predator-avoidance behaviors that change the distribution of prey species (Fancett & Kimmerer 1985, Bollens et al. 1992).

A conservative estimate of the mortality rate that *P. forbesi* nauplii could withstand while maintaining a stable population, based on growth rate and specific egg production, is  $\sim 25\% \text{ d}^{-1}$ . We calculated this estimate (see Kimmerer & Lougee 2015) using the development times of nauplii ( $D_n$ ) and copepodites ( $D_c$ ) (Kimmerer & Gould 2010), the egg production rate (EPR) of the adult females (Kimmerer et al. 2014b), and an estimate of the mortality rate of adults ( $M_a$ ).  $M_a$  was calculated by dividing the ratio of copepodites ( $N_c$ ) to adults ( $N_a$ ) in the population by the development time of the copepodites ( $D_c$ ),

$$M_a = \frac{N_c}{N_a D_c} \quad (1)$$

This assumes that there is no mortality of copepodites, an assumption that yields the maximum mortality of nauplii, and that the population is at steady state. The ratio of adults to copepodites was calculated from the same monitoring data used for estimating population clearance rates.

To maintain the population at steady state, the rate of production of nauplii (EPR) multiplied by the rate of survival to adults must be enough to equal the number of adults that die each day, calculated as the population of adults ( $N_a$ ) times the mortality rate of the adults ( $M_a$ ) (Hirst & Kiørboe 2002). Again, we assumed no mortality of copepodites, and estimated the maximum mortality of nauplii that would allow for steady state,

$$M_a = -\ln\left(\frac{M_a}{0.5 \cdot \text{EPR}}\right) \frac{1}{D_n} \quad (2)$$

In reality there is some mortality of copepodites, which would require a downward adjustment in the estimated maximum mortality of nauplii. But there are also subsidies of nauplii and ovigerous females by dispersion from fresh water, which would allow for higher mortality than estimated. Since the relative magnitude of these adjustments is unknown, we used the  $\sim 25\% \text{ d}^{-1}$  estimate.

The major predators of *P. forbesi* in the LSZ are the clam *P. amurensis* (Kimmerer & Lougee 2015) and the predatory copepod *Acartiella sinensis* (Slaughter et al. 2016). Feeding rates of fish on *P. forbesi* have not been published, but abundance of planktivorous fishes is low in the upper SFE (Sommer et al. 2007) so their predatory impact is probably also low. Small oithonids such as *Oithona nana* are capable of eating calanoid nauplii (Lampitt 1978, Lampitt & Gamble

1982). However, consumption of calanoid nauplii by *L. tetraspina* is negligible, as shown with *P. forbesi* in this study, and *E. affinis* previously (Bouley & Kimmerer 2006).

Benthic grazing likely exerts considerable control on the abundance of *P. forbesi* where the copepod and the clam *P. amurensis* co-occur. Estimated long-term mean population clearance of *P. amurensis* on *E. affinis* nauplii varied seasonally from 4 to 23%  $\text{d}^{-1}$ , with a minimum in spring and a maximum in autumn; during July–October when *P. forbesi* is highly abundant, the mean clearance rate was estimated at  $\sim 14\% \text{ d}^{-1}$  (Kimmerer et al. 1994, Kimmerer & Lougee 2015). Nauplii of *E. affinis* are similar in size and behavior to nauplii of *P. forbesi*, and have nearly identical escape rates from artificial clam siphons (Kimmerer & Lougee 2015), so clearance rates of clams on the 2 copepod species are likely to be similar. Thus, before the introduction of *A. sinensis*, consumption by clams was probably the largest source of predation mortality on *P. forbesi* nauplii in the LSZ, and consumption rates were about half of the maximum mortality the population could withstand without a subsidy. Once *A. sinensis* was introduced to the estuary, it became the other major predator on *P. forbesi* nauplii in the LSZ. Extrapolation of the feeding rates of this predatory copepod to mean long-term summer–autumn population densities indicate that it consumes  $\sim 12\%$  of *P. forbesi* nauplii  $\text{d}^{-1}$  (Slaughter et al. 2016).

The combined predatory impact of *P. amurensis* and *A. sinensis* exceeds the maximum calculated mortality on nauplii that the population can sustain. Therefore *P. forbesi* can co-occur with these predators only by subsidy from fresh water. The demographic structure of the *P. forbesi* population in the LSZ supports the subsidy hypothesis. During July–August 2006–2007 the median proportions of nauplii, copepodites, and adults in the LSZ were 51, 13, and 36%, respectively (based on data in Kimmerer et al. 2014b). The low proportion of copepodites together with very low reproductive rates in the LSZ (Kimmerer et al. 2014b) suggest that the population is unsustainable in isolation, and that copepods are being transported from freshwater habitats into the LSZ.

## Implications

Invasions can provide insight into adaptive responses, as native populations may undergo strong selection when interacting with introduced competitors and predators (Yoshida et al. 2007). The invasive clam *P. amurensis* reduced food availability to cope-

pod in the LSZ and increased mortality through consumption of nauplii (Kimmerer et al. 1994), thus acting as both a competitor and a predator. This effectively eliminated the previously dominant (by numerical abundance and biomass) *E. affinis*, which is now abundant only in winter–spring (Bollens et al. 2011, Kimmerer & Lougee 2015). *P. forbesi* was introduced a year after the clam became abundant; although the timing of its initial introduction was no doubt coincidental, the spread of *P. forbesi* may have been facilitated by the elimination of *E. affinis*. *P. forbesi* has a freshwater refuge from clam predation where it can persist during summers when clams are abundant, while *E. affinis* has been unable to occupy this refuge to any great extent during summer.

The *A. sinensis* population is supported principally by consumption of *L. tetraspina* (Slaughter et al. 2016); thus, the presence of this abundant prey may have facilitated a relatively high abundance of *A. sinensis* and therefore high population consumption rate of this predator on other prey such as *P. forbesi*. Facilitation, once ignored, is now considered a common and structurally important interaction in ecosystems (Bruno et al. 2003). Examples of facilitation are frequently reported in ecosystems where organisms produce physical structure, such as terrestrial plant communities and coral reefs (Bruno et al. 2003). Diffuse facilitation has also been reported in analogous situations to that reported here, e.g. brown tree snakes in Guam which survive by feeding at low levels on lizards but have decimated bird populations (Fritts & Rodda 1998), and garter snakes in California supported by feeding on introduced trout but also preying on and reducing the abundance of amphibians (Pope et al. 2008). These 2 cases and that of *A. sinensis* are instances of hyperpredation (Smith & Quin 1996), by which an abundant prey supports the predator population, which then also preys on the less abundant alternative prey, resulting in population declines.

The concurrent introductions of *A. sinensis* and *L. tetraspina* in 1993 seem to have further intensified the pressures on *P. forbesi* in the LSZ through reduced food availability and increased mortality from predation. These combined pressures may have shifted the seaward limit of the *P. forbesi* distribution to lower salinity (Fig. 1a). According to the calculations presented here along with other literature on the system (Greene et al. 2011, Kimmerer & Thompson 2014, Slaughter et al. 2016), the additional mortality of nauplii due to consumption by *A. sinensis* apparently is the principal cause of this shift. Direct competition with *L. tetraspina* appears to have a minor im-

act on food supply, and the effects of the clam *P. amurensis* were present before the shift.

A consequence of these changes for the food web of the upper SFE was a reduction in prey abundance for the endangered delta smelt, whose principal prey is *P. forbesi* (Slater & Baxter 2014). This species and several other fishes of the region have been in a state of continual decline since ~2002, at least partly as a result of the declining zooplankton abundance in the LSZ (Sommer et al. 2007). The configuration of the food web has changed little since 1993, suggesting that the decline in food available to delta smelt in the open waters of the estuary is unlikely to be reversed without management intervention. Various proposed remedial actions may help to reverse this decline, such as wetland and floodplain restoration designed to enhance pelagic productivity (Herbold et al. 2014), and research is underway to investigate the efficacy of such actions.

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## Appendix 1

Table A1. Parameters used to calculate the carbon cell<sup>-1</sup> for each taxon. Count refers to how many measured cell dimensions were averaged to calculate diameter (*a*, μm) and height (*b*, μm) of the cells. Volumes (*V*, μm<sup>3</sup>) were then calculated based on assignment of geometric shapes (Hillebrand et al. 1999). Calculations of cell carbon (*C*, pg C cell<sup>-1</sup>) from volume were based on conversion equations for various taxa (Menden-Deuer & Lessard 2000). Taxa with a (\*) were measured using an ocular micrometer on an inverted microscope at 400× magnification; all other taxa were measured with the FlowCAM at 100× magnification

Taxon	Count	<i>a</i>	<i>b</i>	Shape	Volume equation	<i>V</i>	Conversion type	Conversion equation	<i>C</i>
Centric diatoms	30	29.8	20.3	Cylinder	$V = (\pi/4)a^2b$	14 176	Diatoms >3000 μm	$C = 0.1167V^{0.881}$	530
Pennate diatoms	137	10.2	28.0	Prolate spheroid	$V = (\pi/6)a^2b$	1525	Diatoms	$C = 0.2877V^{0.811}$	110
Diatoms 7–15 μm	185	6.4	12.8	Cylinder	$V = (\pi/4)a^2b$	412	Diatoms	$C = 0.2877V^{0.811}$	38
Flagellates	252	13.3	19.2	Prolate spheroid	$V = (\pi/6)a^2b$	1775	Protists <3000 μm	$C = 0.2612V^{0.860}$	163
Flagellates 7–15 μm	662	8.3	11.9	Prolate spheroid	$V = (\pi/6)a^2b$	430	Protists <3000 μm	$C = 0.2612V^{0.860}$	48
Dinoflagellates*	30	20.3	20.3	Sphere	$V = (\pi/8)a^2b$	3285	Dinoflagellates	$C = 0.4436V^{0.864}$	484
Ciliates*	40	25.7	29.4	Prolate spheroid	$V = (\pi/6)a^2b$	10 189	Aloricate ciliates	$C = 0.2296V^{0.984}$	2018