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Ecology of a highly abundant, introduced cyclopoid copepod in a temperate estuary

P. Bouley*, W. J. Kimmerer

Romberg Tiburon Center, San Francisco State University, 3152 Paradise Drive, Tiburon, California 94920, USA

ABSTRACT: The cyclopoid copepod *Limnoithona tetraspina* (Oithonidae) was introduced into the San Francisco Estuary (SFE) in 1993 and within a year became the most abundant copepod in the low-salinity zone. *L. tetraspina* makes up ~95% (median) of the total adult copepods in the low-salinity zone, and the biomass of adults is similar to that of 2 larger co-occurring calanoids, *Pseudodiaptomus forbesi* and *Eurytemora affinis*. The main goal of our research was to understand which food resources *L. tetraspina* uses in the low-salinity region of the SFE. Incubation experiments using natural water revealed feeding by *L. tetraspina* on mixotrophic and heterotrophic aloricate ciliates, but rarely on loricate tintinnids or diatoms. The co-occurring calanoids consumed similar prey, but also readily consumed diatoms. Capture and consumption of *Strombidium* spp. by *L. tetraspina* was confirmed visually, and experiments using cultured prey also showed that these copepods fed on motile phytoplankton but not on diatoms. Estimated grazing rates were low (median 2.3, range 0.6 to 8.3% body weight d^{-1}); although these rates may be underestimates because of high concentrations of copepods in experimental containers, they are consistent with low specific egg production of females (0.3 ± 0.2% body weight d^{-1}). Low selectivity of one fish species for *L. tetraspina* suggests that this copepod may not be an important food resource for visually-selective fishes in the SFE. The low abundance of filter-feeding predators in this region of the estuary may be responsible for the high abundance of this cyclopoid copepod, despite its low potential population growth rate.

KEY WORDS: Estuary · Cyclopoid · Foodweb · Introduced species · Ciliates

INTRODUCTION

Small copepods in the family Oithonidae are among the most abundant and productive groups of zooplankton to occur in marine and estuarine waters across the globe (Turner 2004). Ecologically they may function as important prey sources for the larval stages of some key fishery species (Viñás & Ramirez 1996), or conversely as energetic sinks in the metazoan foodweb (Atkinson & Snÿder 1997). They directly influence the downward flux of calanoid fecal-pellet material in pelagic zones (González & Smetacek 1994), contribute to the regeneration of nutrients supporting primary production (Hiromi 1995), and facilitate complex trophic interactions between the protozoan and metazoan foodwebs (Nakamura & Turner 1997). Oithonids differ from the better-studied suspension-feeding calanoid copepods in being primarily raptorial predators that use hydromechanical signals to detect and capture motile prey (Svensen & Kiørboe 2000).

Limnoithona spp. (Oithonidae, length 0.5 mm) were first described from freshwater and brackish regions of the Yangtze River, China. The genus has been introduced to estuaries of the Eastern Pacific, most likely in the ballast water of trade ships originating from estuaries on the Asian continent (Orsi & Ohtsuka 1999). *Limnoithona* spp. have been recorded in the northernmost low-salinity areas of the San Francisco Estuary (SFE) since 1979 (Orsi & Ohtsuka 1999): *L. sinensis* was first detected in 1979, and *L. tetraspina* in 1993. Within a year of its introduction, *L. tetraspina* became the most abundant copepod to inhabit the low-salinity regions of the SFE and this pattern persists to date.

*Email: paola@tirn.net

The SFE is the second-largest estuary in the USA and may be the most heavily invaded estuary in the world (Cohen & Carlton 1998), particularly by copepods in the low-salinity region, where the copepod fauna now resembles that of East Asia (Orsi & Ohtsuka 1999). The trophic ecology of copepods in this estuary is not well studied; only 2 studies to date have addressed the energy sources used by copepods (Orsi 1995, Rollwagen Bollens & Penry 2003). The main purpose of this study was to investigate the feeding ecology of *Limnoithona tetraspina* by studying its grazing behavior on naturally-occurring prey assemblages in Suisun Bay, a shallow embayment of the SFE with notably suppressed phytoplankton production (Alpine & Cloern 1992, Kimmerer 2004). We also present egg production rates in relation to chlorophyll *a* (chl *a*) for *L. tetraspina*, as well as population biomass trends in relation to temperature, salinity, and the abundance and biomass of 2 co-occurring calanoids, *Eurytemora affinis* and *Pseudodiamptomus forbesi*. Additionally, we present evidence that *L. tetraspina* is rarely utilized by juvenile fishes in the SFE and likely represents an energetic dead-end in the metazoan foodweb.

MATERIALS AND METHODS

Suisun Bay is a brackish, river-dominated, shallow embayment (mean depth = 3 meters), that makes up ~5% of the total volume of the San Francisco Estuary (volume = 0.3×10^9 m^{-3}, Kimmerer 2004) (Fig. 1). This embayment is a nutrient-rich, turbid, and net heterotrophic system with low phytoplankton production (Alpine & Cloern 1992), that receives seasonally-variable flows from the Sacramento–San Joaquin River Delta and is also strongly influenced by tides.

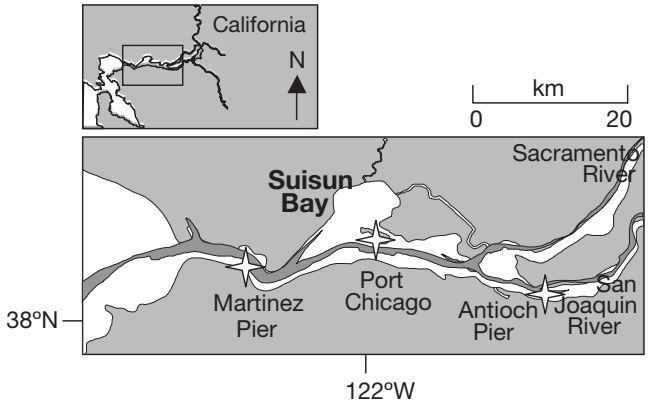

Fig. 1. Study sites for natural-water grazing experiments in northern San Francisco Estuary (SFE). Copepods and water samples were collected from piers along Suisun Bay, except for the May experiment when samples were collected offshore (from Port Chicago) using a boat

We obtained chl *a*, water temperature, and copepod abundance data from the California Interagency Ecological Program (IEP; www.iep.water.ca.gov) zooplankton monitoring survey for 3 species of copepods occurring in low-salinity areas (0.5 to 10 psu) of the northern SFE: *Limnoithona tetraspina* (Cyclopoida), *Eurytemora affinis,* and *Pseudodiaptomus forbesi* (Calanoida). Carbon and nitrogen content data for adult *L. tetraspina* were obtained from previously published relationships to length for related *Oithona* species (Uye 1982). Carbon and nitrogen data for *E. affinis* and *P. forbesi* were obtained by collecting 3 separate replicates of 15 female and male copepods each from Suisun Bay, drying each replicate on acid-washed septa at 60°C for 15 to 20 min, and then transferring them to foil cups for an extended drying period of 12 h at 60°C. Replicate samples, including blanks, were analyzed on a PE II 2400 CHN Analyzer at the University of California at Davis Stable Isotope Laboratory (Table 1).

Five natural prey-field grazing incubations were carried out between October 2003 and May 2004 using the adult stages of *Limnoithona tetraspina*. In April and May 2004, grazing incubations were also carried out using *Eurytemora affinis* and *Pseudodiaptomus forbesi*. Copepods, natural water samples for feeding incubations, and temperature and salinity data were collected from 3 different sites in Suisun Bay (Fig. 1). Field collection and experimental methods were adapted from previously published protocols (Båmstedt et al. 2000, Rollwagen Bollens & Penry 2003). Copepods were collected from Suisun Bay with a 100 µm mesh net using a gentle subsurface tow. Water samples used for experimental incubations were collected from the surface using a clean bucket and immediately back-siphoned gently into an insulated carboy through a submerged 200 µm mesh to remove all large grazers. Water samples were not filtered after this initial screening to prevent damage to fragile cells. Water samples were also collected to determine ambi-

Table 1. *Limnoithona tetraspina*, *Eurytemora affinis* and *Pseudodiaptomus forbesi*. Mean (±95% CI) carbon and nitrogen content of adult copepods (µg copepod^{-1}). Calanoid samples were collected from Suisun Bay in April 2003. Data for *L. tetraspina* from published length–weight relationships for *Oithona* (Uye 1982)

Species	Carbon	Nitrogen
L. tetraspina	0.27	0.06
P. forbesi females	3.53 (0.83)	0.67 (0.23)
P. forbesi males	1.55 (4.70)	0.28 (0.95)
E. affinis females	2.00 (1.30)	0.35 (0.23)
E. affinis males	1.49 (1.10)	0.31 (0.21)

ent chl *a* concentrations at each study site, except for the October experiment (for which we have no chl *a* data), and the February experiment when we used chl *a* data collected by a USGS research vessel on the same day from stations adjacent to the Martinez pier (USGS Stns 7 to 10, unpubl. data, sfbay.wr.usgs.gov/access/wqdata/). Water samples were collected from the surface in clean Nalgene bottles and immediately stored in the dark on ice. In the laboratory 3 replicate 50 ml water samples were filtered onto either GF/F filters or 5 µm polycarbonate filters, and both sets of filters were extracted in 90% acetone for 24 h. Blank filters were simultaneously extracted. Standard fluorometric techniques using acidification were used to measure *in vitro* chl *a* concentration (Holm-Hansen et al. 1965) on a calibrated Turner 10-AU fluorometer. Chl *a* concentrations were <3 µg l^{-1} for all experiments, peaking slightly during the May experiment at 2.5 µg l^{-1}. The fraction of ambient chl *a* >5.0 µm ranged from 76% in March 2004 to 68% in May 2004 (Table 2).

For the grazing experiments, copepods and natural water samples were returned to the lab within 2 h of collection and stored in a constant-temperature room at *in situ* field temperatures. Copepods were sorted while detritus-rich water collected from Suisun Bay for experimental incubation purposes was settled out in a carboy for approximately 3 h. The top 80% of the contents of the carboy was then gently siphoned into a clean bucket, mixed thoroughly, and allocated to the respective treatment bottles. Treatments included 3 or 4 replicate initial, control, and experimental bottles (Table 3). At the start of each experiment, 62 to 74 copepods were added to the 175 ml experimental bottles and all treatment bottles were filled with Suisun Bay water, sealed with parafilm, and capped to prevent the formation of air bubbles. All treatments were incubated on a plankton wheel at 1 revolution min^{-1} for 24 h at field temperatures on a 12:12 h light:dark cycle, except the initial treatments which were removed from the plankton wheel after 1 h. All treatments were preserved immediately after removal from the wheel in a 10% acid Lugol's solution.

Using the Utermöhl method, samples from preserved incubation treatments were settled through 27 ml columns into settling chambers, and the entire area of the chamber was counted for ciliates and diatoms using an inverted microscope at 200× magnification. Counts of cells for all taxa combined typically exceeded 200 per chamber. Since the large quantity of detrital clumps prevented accurate counts of cells smaller than 10 µm using these methods, we excluded small flagellates from our analyses. Counts for all experiments were completed within 45 d of the experiment, with the exception of the October 2003 experiment, which was analyzed within 90 d. All taxa grazed were measured and assigned a geometric shape for volume estimates. Carbon biomass was calculated for aloricate ciliates using a carbon-to-volume ratio of 0.22 pg carbon μm^{-3} (Stoecker et al. 1994), and for loricate cells using previously-published relationships to biovolume (Menden-Deuer & Lessard 2000) assuming a lorica:cell volume of 0.35 (Rollwagen Bollens & Penry 2003). To determine significant decreases in prey resulting from grazing by copepods, control and experimental cell concentrations were compared (*t*-test, equal variance) for all taxonomic groups that were more abundant than 10 cells per 27 ml. Clearance and ingestion rates with 95% confidence limits were calculated for all prey taxonomic categories using the equations of Marin et al. (1986), and daily ration was calculated according to Båmstedt et al. (2000).

The concentration of copepods in experimental containers (~70 per 175 ml) was selected to ensure that any feeding could be detected. In terms of biomass this concentration (~110 µg C l^{-1}) is similar to that used in other feeding experiments (e.g. Rollwagen Bollens & Penry 2003, ~125 µg C l^{-1} at 2.5 µg C copepod^{-1},

Table 2. Surface salinity (psu), temperature (°C), and chl *a* (µg l^{-1}) at copepod and natural-water sampling locations along Suisun Bay. nd: no data

Date	Sample site	Salinity	Temp.	Chl *a*	
				GF/F	>5 µm
27 Oct 2003	Antioch Pier	1.2	21.1	nd	nd
10 Feb 2004	Martinez Pier	4.5	10	2.2	nd
30 Mar 2004	Martinez Pier	9.5	15	2.1	1.6
24 Apr 2004	Martinez Pier	3.5	15	0.9	0.7
18 May 2004	Port Chicago	1.8	18	2.5	1.7

Table 3. *Limnoithona tetraspina*, *Eurytemora affinis* and *Pseudodiaptomus forbesi*. Number of copepods and incubator volumes (ml) used to measure copepod grazing on Suisun Bay plankton assemblages

Experiment	No. of replicates	Species	Gender	No. of copepods	Incubator volume
27 Oct 2003	4	*L. tetraspina*	♀	68–74	175
10 Feb 2004	4	*L. tetraspina*	♀	62–77	175
30 Mar 2004	4	*L. tetraspina*	♀	65–78	175
24 Apr 2004	4	*L. tetraspina*	♀	72–75	175
24 Apr 2004	3	*E.affinis*	(5 ♀, 5 ♂)	10	600
18 May 2004	4	*L. tetraspina*	♀	68–77	175
18 May 2004	4	*P. forbesi*	♀	10	600

weight from W. J. Kimmerer unpubl.). Nevertheless, the large calculated reductions in food concentration in some experiments (see 'Results') suggest that calculated grazing rates might be underestimated in some experiments. To further study selection by *Limnoithona tetraspina* of motile or non-motile prey, we also ran separate grazing experiments using cultured phytoplankton. *L. tetraspina* were starved for 1 h, after which sets of 5 to 10 copepods were incubated separately in cultures of either *Rhodomonas salina*, *Thallasiosira weissflogii, or Skeletonema costatum*. After allowing copepods to graze on separate groups of phytoplankton for up to 1 h, live copepods were observed under blue (450 to 490 nm) and green (510 to 560 nm) light with a Nikon Eclipse E400 epifluorescence microscope at 200 to 400× magnification and visually analyzed for gut fluorescence. In addition, *L. tetraspina* were collected from surface waters offshore of Port Chicago in Suisun Bay on May 17, 2004 and analyzed for chl *a* gut fluorescence. Calculations to determine chl *a* gut content of copepods were made according to Båmstedt et al. (2000).

We also determined the egg production rate of *Limnoithona tetraspina* using samples collected from Suisun Bay on 17 occasions between 1999 and 2001 (Kimmerer et al. 2005). Copepod samples were taken approximately monthly using a 50 µm mesh, 50 cm diameter net towed vertically through the water column at a single station in Suisun Bay. Measurements were also made of temperature and salinity using a Seabird SBE-19 CTD, and chlorophyll concentration by extracted fluorescence as described above (Kimmerer et al. 2005). Copepods were identified and the number of egg sacs was determined. A subsample of 20 egg sacs from 11 samples had an average of 6 eggs per sac, and these were used to calculate eggs per female. Loose eggs in some samples were also counted and assigned to *L. tetraspina* in proportion to its abundance relative to total Oithonids. A total of 48 to 243 females (median = 127) were counted and 0 to 138 (median = 24) eggs estimated for *L. tetraspina*. Egg production rate and weight-specific rate were estimated using temperature-dependent egg development times and egg carbon content (7.4 ng C) for *Oithona davisae* (Uye & Sano 1995).

RESULTS

Limnoithona tetraspina occupied higher salinities and also occurred at densities an order of magnitude higher than its congener *L. sinensis* (Fig. 2). Of the *L. tetraspina* population, 80% occurred within the 0.5 to 10 psu salinity range, and the population was most abundant (>20 000 m^{-3}) during the late summer and fall, coincident with elevated water temperatures and periods of low phytoplankton biomass (i.e. chl *a* concentrations <5 µg l^{-1}; Fig. 3). Abundance of the adult *L. tetraspina* population was 1 to 2 orders of magnitude greater than those of the co-occurring calanoids *Eurytemora affinis and Pseudodiaptomus forbesi*, whereas the carbon and nitrogen biomass of adult *L. tetraspina* was roughly equivalent to that of adults of the larger calanoids (Fig. 4).

Both adult and immature stages of *Limnoithona tetraspina* behave as ambush predators that remain

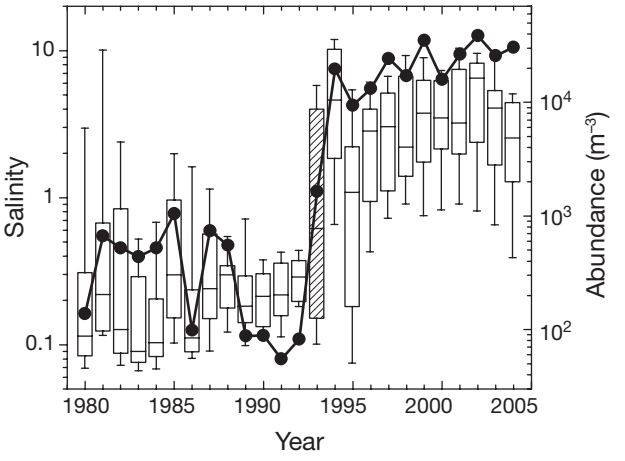

Fig. 2. *Limnoithona sinensis* and *Limnoithona tetraspina*. Abundance from monitoring data (species were not distinguished routinely). Line shows mean abundance in summer (July to October) for adult copepods. Box plot distribution of *Limnoithona* spp. in terms of salinity; boxes give medians with 90th, 75th, 25th and 10th percentiles. Hatched box indicates year (1993) in which *L. tetraspina* was first detected in the SFE, during and after which *L. sinensis* was no longer detected in Interagency Ecological Program zooplankton samples (IEP monitoring data)

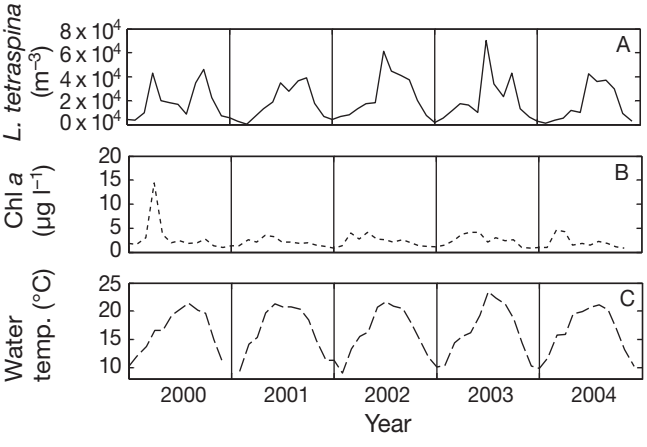

Fig. 3. *Limnoithona tetraspina*. (A) Mean monthly adult copepod abundance, (B) mean chl *a*, and (C) mean temperature in the 0.5 to 10 psu salinity range of the northern SFE (IEP monitoring data)

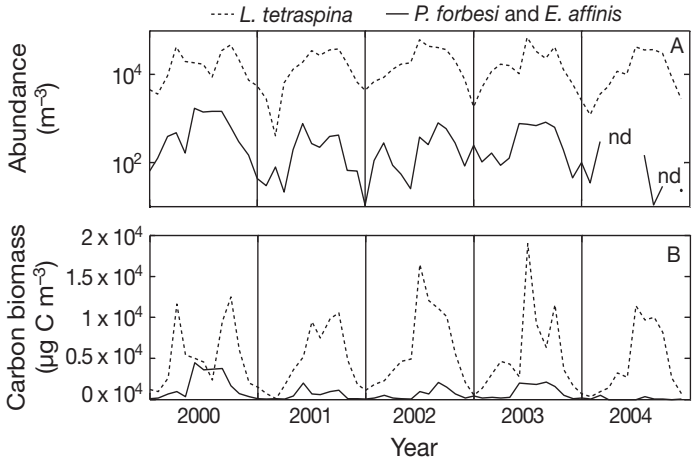

Fig. 4. *Limnoithona tetraspina*, *Eurytemora affinis*, and *Pseudodiaptomus forbesi*. (A) Mean adult abundance and (B) mean carbon biomass (nd: no data) in the northern SFE. All data are for salinity ranges ≥0.5 and <10 psu; data for calanoids are combined for comparison with *L. tetraspina*. (IEP monitoring data)

relatively motionless in the water column before striking out towards motile prey; to date we have not observed a feeding current. Live capture and consumption of ciliates by adult copepods was directly observed under light microscopy. While capture of prey was not always successful, once capture occurred ciliates were ingested whole.

In situ gut pigment analyses of *Limnoithona tetraspina* adults collected from Suisun Bay showed that chl *a* pigments were an order of magnitude higher in copepods collected directly from the field 1.2×10^{-3} µg chl *a* copepod^{-1}) than for copepods starved for 24 h in GF/F water (0.4×10^{-3} µg chl *a* copepod^{-1}). Grazing on cultured phytoplankton sources, as detected by gut fluorescence, was observed only for copepods incubated with the motile phytoflagellate *Rhodomonas salina* (length = 7 µm, width = 5 µm). Guts of copepods that had been incubated with *R. salina* fluoresced brightly under both blue and green excitation. We saw a single diatom cell, *Thalassiosira weissflogii* (length = 15 µm, width = 7.5 µm), in the gut of a single copepod; otherwise there was no evidence of feeding on this diatom or on *Skeletonema costatum* (length = 7 µm, width = 5 µm).

Abundance of heterotrophic aloricate ciliates in Suisun Bay was greatest during the spring (April and May) experiments (Table 4), while mixotrophic aloricates (i.e. *Mesodinium* sp.) and heterotrophic loricates usually occurred at low relative abundances. Diatoms were most abundant in March when *Skeletonema costatum* dominated the prey-field, and during the October experiment when *Chaeotoceros* sp. and pennate diatoms were dominant in the plankton. In the April 2004 experiment no diatoms were detected, and dinoflagellates were detected only in May 2004. Total prey abundances were highest in February as a result of the *S. costatum* bloom.

During the October and March experiments no grazing by *Limnoithona tetraspina* was detected on diatoms although they dominated the prey field (Table 5). Grazing on diatoms was detected in only 1 of 5 experiments. Grazing was detected on ciliates in all experiments, but grazing rates depended on the ciliate taxon. For instance, a significant grazing effect was detected on heterotrophic aloricate ciliates in all 5 experiments, but grazing on heterotrophic loricate ciliates was detected only 1 of 5 times. Two size-morphs of *Mesodinium rubrum* (length = 18 µm and length = 28 µm) were observed during each experiment, but grazing was detected only on the smaller-sized form. Daily rations of *L. tetraspina* feeding on ciliates, measured as the percentage of copepod carbon consumed as ciliates d^{-1}, ranged from 0.6 to 8% d^{-1}.

Significant grazing on heterotrophic aloricate and loricate ciliates was observed for both *Eurytemora affinis* (mean ingestion rate = 5 ± 3 ciliates copepod^{-1} h^{-1} (95% CI), and daily ration = 34 ± 7%), and *Pseudodiaptomus forbesi* (average ingestion rate = 8 ± 4, and daily ration = 40 ± 22%). Unlike *Limnoithona tetraspina*, *P. forbesi* also grazed on diatoms and dinoflagellates that were present. No diatoms were present in plankton samples at the time of the *Eurytemora affinis* grazing experiment, but we have previously maintained cultures of this copepod on diatoms (W. J. Kimmerer unpubl.). These experiments demonstrated partial overlap in the diets of *L. tetraspina* and the larger calanoids, although clearance rates for *E. affinis* (mean clearance rates = 32 to 33 ml copepod^{-1} d^{-1}) and *P. forbesi* (mean clearance rate = 28 to 50 ml copepod^{-1} d^{-1}) were an order of magnitude higher than those of the smaller cyclopoids.

Table 4. Initial concentrations for all potential prey taxa (>10 µm) present in water samples collected for Suisun Bay copepod grazing incubations (cells ml^{-1}; mean ± 95% CI). np: not present

Experiment	Heterotrophic aloricate	Mixotrophic aloricate	Heterotrophic loricate	Diatoms	Dinoflagellates
Oct 2003	3.2 ± 0.9	1.5 ± 0.4	0.9 ± 0.4	6.5 ± 1.3	np
Feb 2004	1.1 ± 0.2	1.2 ± 0.1	1.5 ± 0.3	0.4 ± 0.1	np
Mar 2004	1.7 ± 0.3	np	0.9 ± 0.2	61.2 ± 6.1	np
Apr 2004	10.7 ± 0.5	np	1.2 ± 0.1	np	np
May 2004	7.9 ± 0.7	0.8 ± 0.3	1.0 ± 0.2	1.4 ± 0.3	0.8 ± 0.3

Table 5. *Limnoithona tetraspina*. Prey abundance for all incubations (cells ml^{-1}; mean ± 95% CI), and *t*-test comparison of prey abundance for control versus grazed incubators at t = 24 h. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$; ns = non-significant ($p > 0.05$)

Expt	Prey field		Cells ml^{-1} (95% CI)		
			Initial	Control t_{24}	Copepods t_{24}
October 2003		n =	3	4	4
	Strombidium sp. A		2.8 ± 0.8	4.9 ± 0.8	2.3 ± 1.1**
	Strombidium sp. B		0.4 ± 0.1	0.6 ± 0.2	0.0 ± 0.0**
	Mesodinium sp. (large)		0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.0^{ns}
	Mesodinium sp. (small)		1.3 ± 0.3	2.0 ± 0.2	0.5 ± 0.3***
	Total Aloricate Ciliates		4.7 ± 1.3	7.7 ± 0.8	2.9 ± 1.2***
	Codonellopsis sp. A		0.5 ± 0.3	1.6 ± 0.5	1.8 ± 0.6^{ns}
	Tintinnid sp. A		0.4 ± 0.2	0.7 ± 0.3	0.4 ± 0.1^{ns}
	Total Loricate Ciliates		0.9 ± 0.4	2.3 ± 0.5	2.2 ± 0.7^{ns}
	Amphiprora sp.		1.1 ± 0.3	1.0 ± 0.3	0.7 ± 0.6^{ns}
	Chaetoceros sp.		4.7 ± 1.2	7.0 ± 0.5	4.7 ± 3.0^{ns}
	Navicula sp.		0.1 ± 0.2	0.2 ± 0.0	0.1 ± 0.1^{ns}
	Pleurosigma sp.		0.3 ± 0.1	0.1 ± 0.0	0.2 ± 0.1^{ns}
	Other centric diatoms		0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1^{ns}
	Total Diatoms		6.5 ± 1.3	8.4 ± 0.7	5.9 ± 2.8^{ns}
February 2004		n =	4	5	4
	Strombidium sp. C		1.1 ± 0.15	1.3 ± 0.1	0.7 ± 0.3**
	Mesodinium sp. (large)		0.8 ± 0.0	0.7 ± 0.1	0.5 ± 0.2^{ns}
	Mesodinium sp. (small)		0.4 ± 0.1	0.5 ± 0.0	0.2 ± 0.1***
	Total Aloricate Ciliates		2.2 ± 0.2	2.4 ± 0.2	1.4 ± 0.4**
	Total Loricate Ciliates — Tintinnid sp. B		1.5 ± 0.3	1.3 ± 0.2	1.1 ± 0.1^{ns}
	Total Diatoms — *Amphiprora* sp.		0.4 ± 0.1	0.4 ± 0.1	0.3 ± 0.1*
March 2004		n =	5	5	4
	Strombidium sp. D		1.7 ± 0.3	3.1 ± 0.3	1.3 ± 0.3***
	Tintinnid sp. C		0.9 ± 0.2	1.7 ± 0.3	0.9 ± 0.3**
	Total Ciliates		2.6 ± 0.4	4.8 ± 0.4	2.2 ± 0.5***
	Skeletonema costatum		61 ± 49	66 ± 17	75 ± 11^{ns}
	Amphiprora sp.		0.7 ± 0.2	0.6 ± 0.2	0.7 ± 0.2^{ns}
	Total Diatoms		61 ± 49	66 ± 16	76 ± 11^{ns}
April 2004		n =	4	4	4
	Strombidium sp. E		5.8 ± 0.5	9.9 ± 1.8	6.4 ± 1.2**
	Unidentified sp.		4.9 ± 0.3	4.7 ± 0.5	3.5 ± 0.5**
	Total Aloricate Ciliates		11 ± 0.5	15 ± 2.0	10 ± 1.4**
	Total Loricate Ciliates — Tintinnid sp. D		1.2 ± 0.1	1.2 ± 0.3	1.2 ± 0.1^{ns}
May 2004		n =	4	4	4
	Strombidium sp. *F*		7.0 ± 0.6	10 ± 1.0	8.2 ± 1.4*
	Unidentified ciliate A		0.8 ± 0.1	0.5 ± 0.1	0.5 ± 0.2^{ns}
	Mesodinium sp. (large)		0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1^{ns}
	Mesodinium sp. (small)		0.2 ± 0.1	0.3 ± 0.3	0.1 ± 0.1^{ns}
	Total Aloricate Ciliates		8.6 ± 0.9	11 ± 1.4	8.9 ± 1.5
	Total Loricate Ciliates — Tintinnid sp. E		1.0 ± 0.2	0.9 ± 0.5	0.5 ± 0.2^{ns}
	Ciliates		18.9 ± 1.5	12.3 ± 1.8	9.5 ± 1.5*
	Total Dinoflagellates — Unidentified sp.		0.8 ± 0.3	0.6 ± 0.1	0.5 ± 0.1^{ns}
	Total Diatoms		1.4 ± 0.3	1.2 ± 0.4	0.9 ± 0.1^{ns}

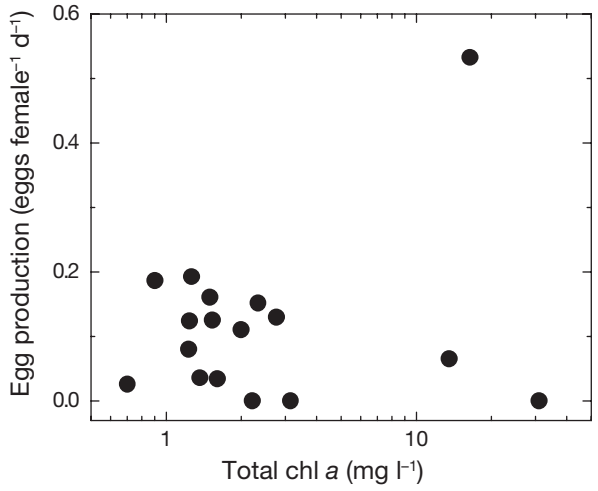

Fig. 5. *Limnoithona tetraspina*. Monthly egg production rate as a function of chl *a* in Suisun Bay during 17 mo between November 1999 and September 2001

Egg production rates of *Limnoithona tetraspina* averaged 0.2 ± 0.1 eggs female^{-1} d^{-1} and were unrelated to chlorophyll concentration in Suisun Bay (Fig. 5). The mean egg production rate in weight-specific terms was 0.3% d^{-1}.

DISCUSSION

Across a range of experimental conditions, and a wide geographic range, *Oithona* species are predominantly raptorial omnivores (Turner 2004) preying opportunistically on ciliates, autotrophic and heterotrophic flagellates, dinoflagellates, and copepod nauplii (Lampitt & Gamble 1982, Turner 1986, Tsuda & Nemoto 1988, Lonsdale et al. 2000). Feeding by oithonids on non-motile prey such as diatoms has been detected (Tsuda & Nemoto 1988), but overall there is greater selection for motile taxa (Atkinson 1996) across a wide range of particle sizes (2 to >20 µm, Lampitt & Gamble 1982, Tsuda & Nemoto 1988, Nakamura & Turner 1997, Calbet et al. 2000). Additionally, Castellani et al. (2005) showed that oithonid metabolic demands are approximately 8 times lower than that of a calanoid copepod of equivalent body weight, which may help to explain the relatively high population abundances of oithonids across a wide range of habitats.

Our results show that, like *Oithona* spp., *Limnoithona tetraspina* is an omnivorous, raptorial copepod that consumes motile mixotrophic and heterotrophic ciliates with prey carbon content ranging between 560 and 5900 pg C cell^{-1}, and phytoflagellates, but not diatoms (Table 5). Our analyses of gut-pigment showed that *L. tetraspina* consumed chl *a* in Suisun Bay. Since small-sized mixotrophic protozoans (*Mesodinium* sp.) were

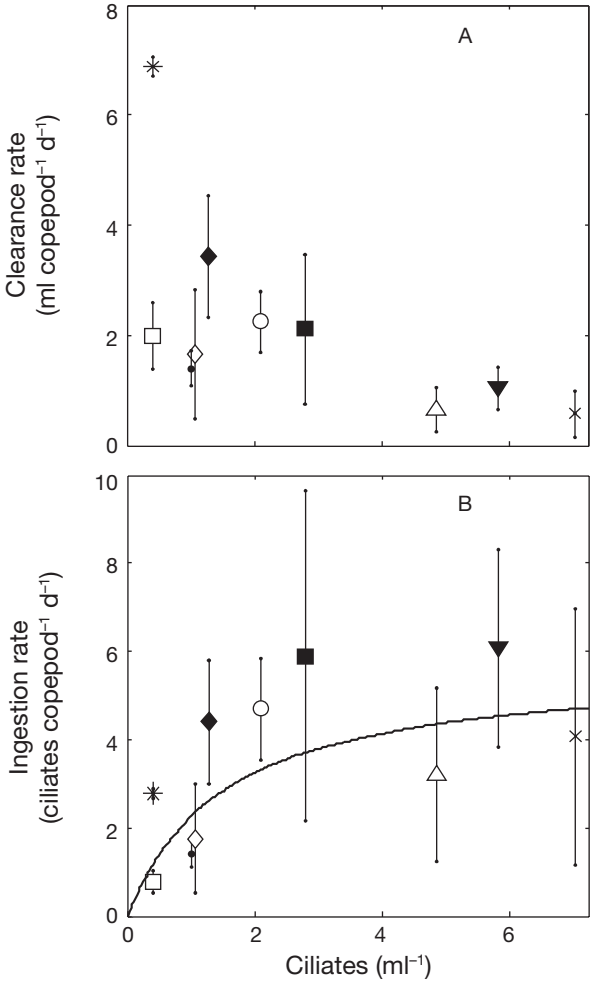

Fig. 6. *Limnoithona tetraspina*. (A) Clearance rates and (B) ingestion rates on naturally-occurring ciliates as a function of initial ciliate density. Bars show 95% confidence intervals. The curved line in (B) is a rectangular hyperbola fitted to the data, (5.7 × Initial Prey Conc.) / (1.5 + Initial Prey Conc.). *Strombidium* A (■), *Strombidium* B (✳), *Strombidium* C (□), *Strombidium* D (○), *Strombidium* E (▼), *Strombidium* F (×), *Mesodinium* A (◆), *Mesodinium* B (◇), *Tintinnid* B (•), Unidentified ciliate (△)

present in these Suisun Bay samples, and concurrent incubation experiments confirmed predation on these taxa, it is likely that the gut chl *a* signal reflected predation on *Mesodinium*. The chl *a* signal may also have resulted from copepods grazing on naturally occurring phytoflagellates, which we were unable to count in our grazing experiments. While phytoflagellates may represent a potentially significant food source for *L. tetraspina* (as our gut-content experiments with monocultures of *R. salina* indicated), this would be surprising as *L. tetraspina* biomass peaks in the SFE during periods of very low phytoplankton biomass (chl *a* < 5 µg l^{-1}) and egg production rates of *Limnoithona* in the SFE were unrelated to phytoplankton biomass (Fig. 5).

Grazing rates determined in the incubation experiments may underestimate grazing under natural conditions, particularly in experiments in which proportional decreases in cell concentrations were large (e.g. March 2004 for *Strombidium* D). However, weight-specific egg production rates determined during 1999 to 2001 were very low, and with a gross growth efficiency of ~30% imply weight-specific ingestion rates of ~0 to 5% d^{-1} (median 1% d^{-1}), consistent with the range of rates we observed (0.6 to 8% d^{-1}). Our results for *Limnoithona tetraspina* ingestion rates are also consistent with the generally low metabolic rates characteristic of Oithonids (Castellini et al. 2005).

Mean clearance rates for all taxa across all experiments were highest for heterotrophic and mixotrophic aloricate ciliates reflecting selection for these motile taxa, consistent with the raptorial feeding behaviour of this copepod species (Fig. 6). Ingestion rates on ciliates increased with ciliate density, and a rectangular hyperbola provided a reasonably good fit to the ingestion rate data (Fig. 6).

Clearance rates for *Limnoithona tetraspina* (0.01 to 0.38 ml copepod^{-1} h^{-1}) feeding on ciliates are similar to those reported for *Oithona similis* (0.03 to 0.38 ml copepod^{-1} h^{-1}) in Buzzards Bay, Massachusetts (Nakamura & Turner 1997). However, our ingestion rates for *L. tetraspina* were an order of magnitude lower (average 3.1 ± 2.6 cells copepod^{-1} d^{-1}) than those found for *O. similis* in Buzzards Bay primarily as a result of low ciliate concentrations. The low ingestion rates and the saturated feeding on ciliates at the higher range of observed ciliate concentrations (Fig. 6) suggests that the population is food limited much of the time. This is consistent with observations from higher (Kimmerer et al. 2005) and lower (Mueller-Solger et al. 2002) salinity regions, where zooplankton were chronically food-limited. It is also consistent with the persistently low level of phytoplankton biomass in the low-salinity region of the estuary since 1987 (Alpine & Cloern 1992, Kimmerer 2004).

Nonetheless, we cannot rule out the possibility that other foods supplement ciliates in the diet of this copepod. Alternative food sources in Suisun Bay may include copepod nauplii, other pelagic larvae, nano- and micro-flagellates, and detritus. *Limnoithona* spp., like *Oithona* sp. (Gonzáles & Smetacek 1994), could be utilizing sinking particulate matter as a food source. In Suisun Bay this particulate material is mostly associated with bacteria (Hollibaugh & Wong 1999), and bacterial aggregation with larger particles may permit picoplankton-sized bacterial food sources to be more readily available to copepod grazers. As for feeding on copepod nauplii, preliminary investigations of *Limnoithona* feeding on Nauplius stages 1 and 2 of *Eurytemora affinis* incubation methods showed no predatory impact in that zero change in abundance of nauplii occurred in the experimental containers (P. Bouley unpubl.). Further experiments are needed to test these results. Naturally occurring phytoflagellates may be a significant food source, and further study on *Limnoithona* spp. feeding habits using methods developed for studying field populations of nanoflagellates should yield further insight.

Omnivory by copepods is prevalent in, but not restricted to, environments with low phytoplankton biomass, e.g. oligotrophic oceanic systems, or regions with high suspended particulate matter or nanophytoplankton (Gifford & Dagg 1988, Ohman & Runge 1994, Rollwagen Bollens & Penry 2003). Phytoplankton biomass in the northern SFE is low compared to other estuarine systems (Alpine & Cloern 1992, Kimmerer 2004). Bacterial production was estimated to exceed phytoplankton production by 5-fold during 1988 to 1989 (Hollibaugh & Wong 1996) and a significant portion of phytoplankton carbon occurs in cells less than 10 µm (Sobczak et al. 2002). Light limitation caused by high suspended particulate matter and benthic grazing are considered the primary reasons for low phytoplankton productivity in this turbid region (Alpine & Cloern 1992). The invasion of the SFE by *Corbula amurensis* in 1986 (as *Potamocorbula amurensis*, Nichols et al. 1990) eliminated the summer high-phytoplankton biomass in Suisun Bay previously associated with periods of low outflow and high residence time (Alpine & Cloern 1992). Chl *a* concentrations decreased by 69% between pre- and post-*C. amurensis* years (Kimmerer et al. 1994), concurrent with decreases in the proportion of cells in larger size classes, diatom abundance, and dissolved silica uptake in Suisun Bay and the lower Sacramento–San Joaquin Delta (Kimmerer 2004, 2005). The consequences of the above conditions in Suisun Bay included notable declines in abundance of some copepod and mysid shrimp species as a result of the reduction in food supply, as well as predation on nauplii by clams, and a reduction in abundance of several common fish species including northern anchovy (Kimmerer 2006, this volume).

In bays and estuaries where the dominant mode of predation is visual or benthic, predation can cause significant mortality of larger copepod prey favoring smaller, less visible copepods (Kimmerer 1991). *Limnoithona tetraspina* may be able to escape predation by predators using sight or hydro-mechanical signals because of 3 factors: (1) small copepod body size (500 µm total length, 0.27 µg carbon copepod^{-1}), (2) the relatively motionless behavior of all life stages, and (3) the ability of the copepods to detect and escape from predators.

The reduction of predation pressure on small pelagic copepods due to the decline of the northern anchovy,

previously the biomass dominant among planktivores in Suisun Bay, may have facilitated the large increase in the *Limnoithona* population (Kimmerer 2006). We do not have gut composition for SFE anchovies, but significant predation by larval and juvenile anchovies on *Oithona davisae*, a similarly small marine oithonid, has been documented (Mitani 1988). Additionally, nauplii of small *Oithona* spp. are important prey for larval anchovy (Viñás & Ramírez 1996). In addition, although *L. tetraspina* comprised as much as 80% of the SFE plankton during the summer and fall months between 1993 and 1996, it rarely made up more than 10% of the diet of juvenile delta smelt *Hypomesus transpacificus*, a visual predator, over that same period (Fig. 7).

In other systems dominated by small oithonids, small copepods are consumed primarily by non-visual, gelatinous predators. In Tokyo Bay, a eutrophic system dominated by *Oithona davisae*, jellyfish have been described as 'enormously successful' (Uye 1994). In Fukuyama Harbor, Japan, *O. davisae* typically dominated copepod abundances in the warm summer and fall months (Uye & Liang 1998) but suffered declines due to cannibalism and during periods when lobate ctenophores (gelatinous predators) were abundant in the plankton (Uye & Sano 1995). A similar pattern was observed in the Black Sea in the late 1980s as overexploitation led to the collapse of the Black Sea anchovy fishery. The subsequent abundance of mesozooplankton opened up a key trophic niche in the system that facilitated the rapid and successful invasion by the lobate ctenophore *Mnemiopsis leidyi*. The once-abundant, smaller-sized zooplankton suffered severe declines over this period and only began to shown signs of increase as jellyfish populations began to decline (Shiganova 1998). Three species of hydromedusae indigenous to the Black Sea have become established in small harbors of the SFE (Mills & Rees 2000). However, gelatinous zooplankton overall do not occur at significantly high abundances in open waters of the SFE (Orsi & Mecum 1986, W. J. Kimmerer unpubl.) which may contribute to the high abundance of *L. tetraspina*.

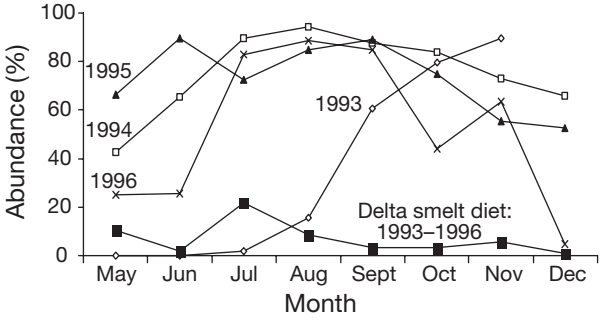

Fig. 7. *Limnoithona tetraspina*. Relative abundance among adult copepods in the plankton (IEP monitoring data for salinity of 0.5 to 10 psu) compared to the percentage of *Limnoithona* in the gut contents of juvenile Delta smelt between May and December, 1993 to 1996 (data from Lott 1998)

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