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SHORT COMMUNICATION

Feeding of the invasive copepod *Pseudodiaptomus forbesi* on natural microplankton assemblages within the lower Columbia River

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The Asian copepod *Pseudodiaptomus forbesi* has conspicuously invaded the Columbia River, but its feeding behavior is poorly understood. We conducted feeding experiments with *P. forbesi* and natural assemblages of microplankton collected from three sites in the Columbia River. *Pseudodiaptomus forbesi* primarily consumed diatoms, ciliates, flagellates and dinoflagellates, exhibiting a general preference for diatoms and ciliates, and an avoidance of chlorophytes and cyanobacteria. These results suggest potential competition with native copepods and other food web impacts.

KEYWORDS: *Pseudodiaptomus forbesi*; Columbia River; invasive zooplankton; copepod

Invasive zooplankton can significantly alter plankton community composition and ecosystem dynamics (Bollens *et al.*, 2002; Roohi *et al.*, 2008; Strecker and Arnott, 2008). The Columbia River has experienced numerous zooplankton invasions in recent years (Cordell *et al.*, 2008; Bollens *et al.*, 2012; Dexter *et al.*, 2015). Most strikingly, the Asian copepod *Pseudodiaptomus forbesi*, first discovered

in 2002 (Cordell *et al.*, 2008), now dominates the zooplankton community during late summer and early autumn throughout the lower Columbia River (LCR) (Bollens *et al.*, 2012; Dexter *et al.*, 2015; Emerson *et al.*, 2015). With its broad distribution and high abundance, there is potential for *P. forbesi* to compete with native zooplankton, and to impact ecosystem dynamics in the LCR (Bollens *et al.*, 2012).

Additionally, an association between *P. forbesi* abundance and warm water temperatures has been observed, suggesting a relationship between zooplankton invasion success and expected climate change (Dexter *et al.*, 2015; Emerson *et al.*, 2015).

Little is known about the prey distribution and feeding dynamics of *P. forbesi* and the native copepods that it may be competing with in the LCR (Bollens *et al.*, 2012). Thus, our objective was to investigate the feeding dynamics of *P. forbesi* on natural microplankton taxa in the LCR, in order to address these research questions:

- (i) How does *P. forbesi* prey availability, diet composition and prey selectivity vary from the lower estuary, to the free-flowing river, to a reservoir in the LCR?
- (ii) What is the potential for *P. forbesi* to compete with native copepods in the LCR?

Water sampling and feeding experiments were conducted with *P. forbesi* incubated in unfiltered water from three locations in the LCR: (i) the LCR estuary (46°11.4'N, 123°49.5'W), 29 river kilometers (rkm) upstream of the estuary mouth, which has relatively high, tidally influenced flows and salinity of 0–12; (ii) a free-flowing river site in Vancouver, WA (45°37.3'N, 122°40.7'W), 171 rkm upstream, with some tidal fluctuation but entirely fresh-water and (iii) the reservoir above Bonneville Dam (45°41.6'N, 121°52.6'W), 241 rkm upstream with moderate flows.

Feeding experiments were conducted in October 2010 with copepods and water collected at all three locations, and in November 2012 at the river and reservoir locations only, due to insufficient abundance of *P. forbesi* in the estuary, for a total of five experiments in the LCR. At all locations, zooplankton were collected via horizontal tows of a 250- μ m mesh plankton net; net contents were transferred into a clean bucket with 10- μ m filtered surface water, and transported to the laboratory within 6 h of collection. Unfiltered surface water was also collected with a clean bucket for the grazing experiments, and for microscopical analysis.

For each experiment, 40 adult female *P. forbesi* were transferred within 24 h of collection into each of four 500-mL incubation bottles containing unfiltered water from the collection site. This copepod density, while high, has been observed in the lower Columbia River estuary (Bollens *et al.*, 2012) and on average resulted in a reduction of 47.5% of prey biomass in the incubations, which was sufficient to dampen the effects of any ambient grazers. In addition, four replicate 500-mL bottles were filled with unfiltered water to serve as initial controls, and another four bottles were filled with unfiltered

water as final controls. All copepod treatment and final control bottles were incubated for 12 h, overnight, on a rotating (0.5–1 rpm) plankton wheel in the dark, at ambient temperatures (13–14°C) to best mimic the physical condition of the field sites.

All subsampling of experimental bottles, plankton identification, enumeration and biomass estimations, grazing rate and electivity calculations were performed following the methods described in Rollwagen-Bollens *et al.* (Rollwagen-Bollens *et al.*, 2013) and Rollwagen-Bollens and Penry (Rollwagen-Bollens and Penry, 2003). Briefly, 200 mL from each incubation bottle was preserved in 5% Lugol's for microscopical analysis using the Utermöhl's method to identify and enumerate all microplankton (individuals ~5–200 μ m in size, plus cyanobacteria cells). Cyanobacteria were enumerated, since colony sizes were consistently >5 μ m in length (size range ~15–150 μ m) and were at times significantly consumed by *P. forbesi*. Microplankton were sorted into six taxonomic categories: diatoms, dinoflagellates, ciliates, flagellates, chlorophytes and cyanobacteria.

Copepod clearance rates ($\text{mL copepod}^{-1} \text{h}^{-1}$) and biomass ingestion rates ($\mu\text{gC copepod}^{-1} \text{h}^{-1}$) were calculated from the changes in abundance or biomass of each prey category over the incubations. We estimated copepod feeding selectivity via two approaches: a comparison of clearance rates on each prey type and a comparison of electivity index. The differences in ingestion rates and clearance rates across all three sites in 2010 and the reservoir and river sites in 2012 were analyzed with two-way general linear model ANOVA, and were interpreted in conjunction with the calculated electivity values.

In October 2010, microplankton prey abundance and biomass were lowest in the estuary and highest in the upstream sites, with flagellates and cyanobacteria dominant in abundance (Fig. 1a and c), but ciliates and diatoms dominant in biomass (Fig. 1b and d). In November 2012, when experiments were only conducted for the river and reservoir sites, microplankton prey abundance was comparable with that of 2010 at the river site, but twice as high in the reservoir due to high cyanobacteria abundance (Fig. 1b). The pattern was similar for biomass; however, ciliate biomass was substantially lower in 2012 at both upstream sites (Fig. 1d). The taxonomic composition of microplankton prey assemblages did not vary significantly from our downstream to upstream locations. Our results are in agreement with other studies in the LCR, although these have been limited to the lower estuary (Breckenridge *et al.*, 2015 and references therein).

During the 2010 feeding experiments, there were significant differences in *P. forbesi* clearance rates between prey categories and between the three LCR sites, as well

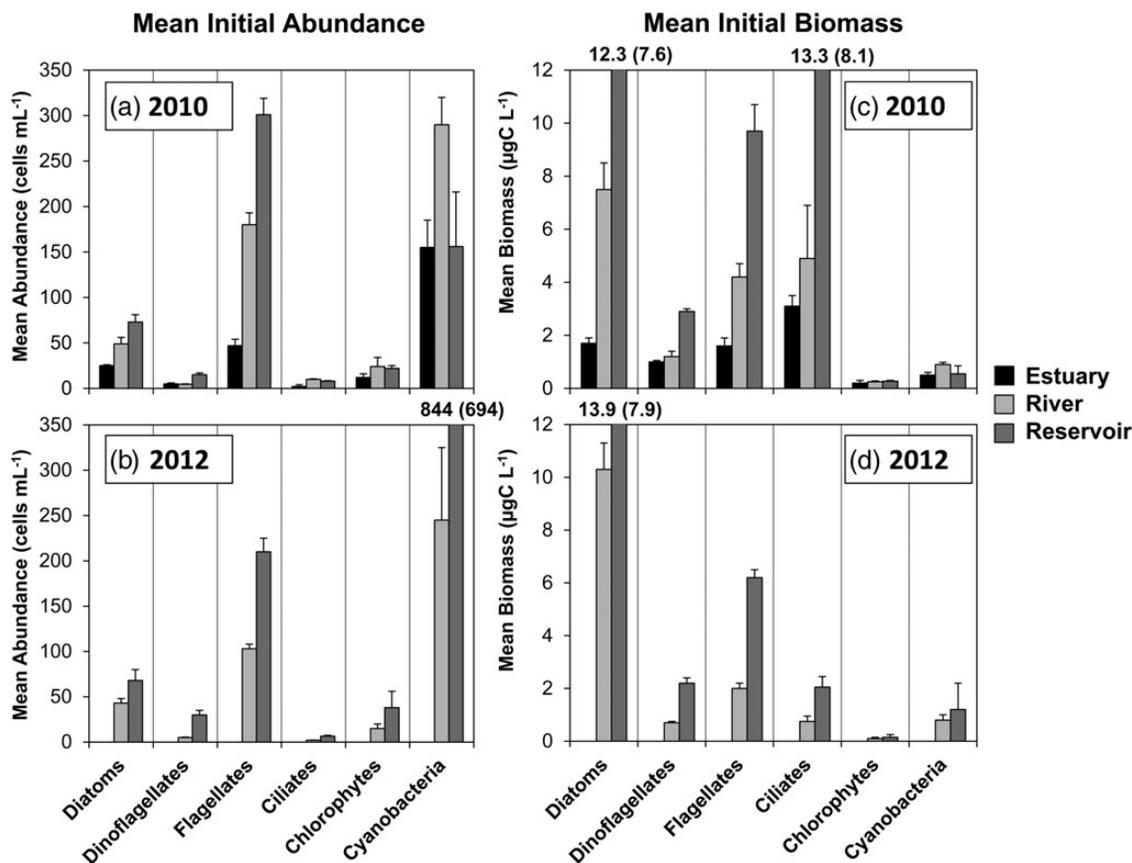


Fig. 1. Mean (\pm SE) initial abundance (**a** and **b**) and biomass (**c** and **d**) of microplankton taxa collected in the LCR across years, sites and prey groups. Where a value exceeds the y-axis range, it is provided above the bar with \pm SE in parentheses.

as a significant interaction among sites and prey category, suggesting that in 2010 *P. forbesi* preferred different prey in different parts of the river (Fig. 2a and b, Table I). Clearance rates were higher in the upstream (river and reservoir) sites than that in the estuary, and a *post hoc* Tukey test revealed that diatoms were cleared at higher rates than flagellates and chlorophytes in 2010 (Table I). Positive electivity values support a preference by *P. forbesi* for diatoms in 2010 particularly in the river and reservoir locations, and negative electivity values indicate avoidance of flagellates and chlorophytes (Fig. 2c and d).

For the two 2012 feeding experiments, clearance rates were significantly different among prey categories, but not between sites (Fig. 2b). The Tukey multiple comparison test showed that diatoms, flagellates and ciliates were all cleared at higher rates than chlorophytes and cyanobacteria; and ciliates were preferred over dinoflagellates (Table I). Mean electivity values in 2012 also showed a preference for diatoms and ciliates, and an avoidance of chlorophytes and cyanobacteria (Fig. 2d). However, no distinct trend in preference for flagellates was seen in the electivity indices (Table I). In most aquatic systems,

diatoms and ciliates are typically the largest microplankton individuals, making them energy-efficient food for copepods (Merrell and Stoecker, 1998). The lack of motility of diatoms (Brandl, 1998) and the higher nutritional value and hydrodynamic signaling of ciliates (Calbet and Saiz, 2005) are additional factors favoring the selection of these prey taxa by copepods.

In general, *P. forbesi* from the LCR cleared diatoms and ciliates at the highest rates, and cleared chlorophytes and cyanobacteria at the lowest rates, with these latter prey categories largely avoided. However, in October 2010, *P. forbesi* in the estuary did not show a significantly higher clearance rate for any particular prey category, although the electivity indices demonstrated a preference for cyanobacteria and general avoidance of ciliates. This result in the estuary is counter to what we expected, since many studies have found ciliates to comprise a significant proportion of copepod diets in estuarine environments (see Rollwagen-Bollens and Penry, 2003 and references therein), especially when phytoplankton biomass is low (Calbet and Saiz, 2005; Gifford *et al.*, 2007), as often occurs in autumn and winter. Non-preferential feeding in

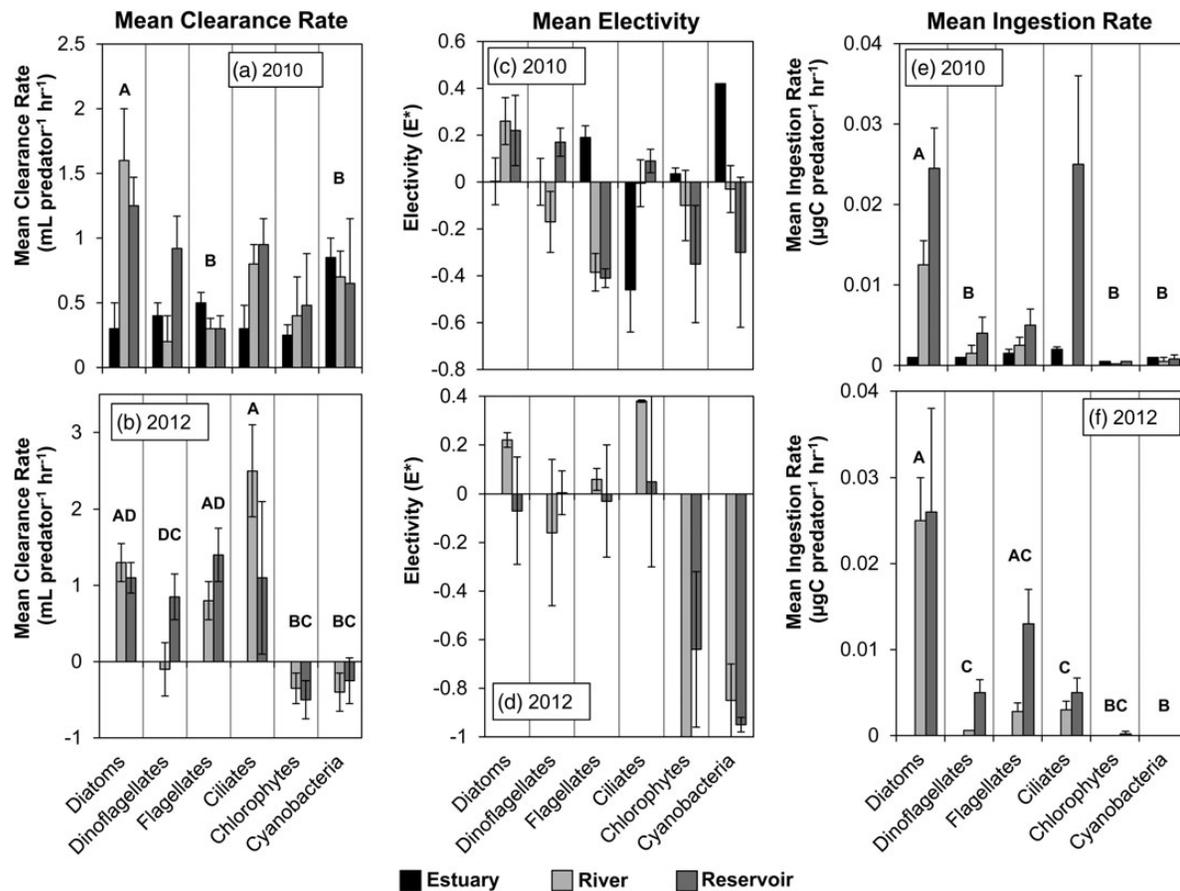


Fig. 2. Mean (\pm SE) clearance rates (a and b), electivity (c and d) and ingestion rates (e and f) of *P. forbesi* feeding on natural microplankton assemblages. Letters represent the results of the *post hoc* Tukey test for the two-way ANOVAs (site vs. prey category) within each year; prey categories that share letters are not significantly different from each other ($\alpha = 0.05$) within experiments conducted during that year.

our estuarine site could have been due to the high relative abundance of cyanobacteria, coupled with the relatively low microplankton abundance and small ciliate cell size in the estuary compared with the reservoir site. High concentrations of cyanobacteria are known to depress copepod clearance rates of higher quality foods (DeMott, 1995; Gifford *et al.*, 2007).

In 2012, we observed negative clearance rates for both chlorophytes and cyanobacteria in the river and reservoir locations, indicating that these two prey groups increased in abundance in the presence of copepod grazers during the incubations. This net growth could be a result of *P. forbesi* selecting for diatoms and ciliates during these experiments, thus releasing the smaller chlorophyte and cyanobacteria cells from microplankton grazing pressure, in a “trophic cascade” [see Rollwagen-Bollens *et al.* (Rollwagen-Bollens *et al.*, 2013) for discussion of cascading effects in feeding experiments]. Based on these results, it is possible that preferential feeding by *P. forbesi* in the freshwater reaches of the LCR could contribute to

conditions favorable for enhanced growth of cyanobacteria taxa, some of which can produce harmful toxins (Lee *et al.*, 2015).

Ingestion rates on microplankton prey biomass during the October 2010 experiments differed significantly between prey categories and sites, with a significant interaction term. *Post hoc* Tukey tests revealed that *P. forbesi* from the reservoir had significantly higher ingestion rates than those from the estuary and the river sites. In particular, diatoms were ingested at a significantly higher rate than dinoflagellates, chlorophytes and cyanobacteria (Table I, Fig. 2e and f). In November 2012, ingestion rates were significantly different among prey categories only; Tukey test results showed significantly higher ingestion of diatoms than ciliates by *P. forbesi* from the upstream, freshwater sites, as well as significantly higher ingestion rates on dinoflagellates, flagellates and ciliates than cyanobacteria (Table I, Fig. 2e and f).

Pseudodiaptomus forbesi reaches its maximum abundance in the LCR in late summer and early autumn, during

Table I: Results of two-way ANOVA of grazing rates calculated from *P. forbesi* incubation experiments conducted in October 2010 and November 2012 in the lower Columbia River

Factor	2010 (three sites)					2012 (two sites)				
	DF	SS	MS	F	P-Value	DF	SS	MS	F	P-Value
Clearance rates										
Site	2	1.074	0.537	3.59	0.038	1	0.004	0.004	0.00	0.946
Prey Category	5	3.327	0.665	4.45	0.003	5	35.13	7.026	8.37	<0.001
Interaction	10	3.815	0.381	2.55	0.019	5	6.685	1.337	1.59	0.188
Error	36	5.381	0.149			35	29.39	0.840		
	Din	Fla	Cil	Cph	Cya	Din	Fla	Cil	Cph	Cya
Dia	0.059	0.005	0.458	0.007	0.572	0.548	1.000	0.715	0.008	0.014
Din		0.918	0.877	0.960	0.790		0.581	0.047	0.337	0.454
Fla			0.313	1.000	0.229			0.685	0.009	0.016
Cil				0.400	1.000				<0.001	<0.001
Cph					0.302					
Ingestion rates										
Site	2	4.889	2.445	9.20	0.001	1	0.286	0.286	1.02	0.320
Prey Category	5	5.761	1.152	4.34	0.003	5	20.75	4.150	14.8	<0.001
Interaction	10	9.758	0.976	3.67	0.002	5	2.437	0.487	1.73	0.152
Error	36	9.579	0.266			35	9.835	0.281		
	Din	Fla	Cil	Cph	Cya	Din	Fla	Cil	Cph	Cya
Dia	0.033	0.120	0.091	0.003	0.005	<0.001	0.060	0.006	<0.001	<0.001
Din		0.996	0.998	0.944	0.980		0.463	0.975	0.947	0.022
Fla			1.000	0.730	0.834			0.911	0.098	<0.001
Cil				0.761	0.859				0.607	0.004
Cph					1.000					0.164

Boldface *P*-values are significant (<0.05).

Dia, diatoms; Din, dinoflagellates; Fla, flagellates; Cil, ciliates; Cph, chlorophytes; Cya, cyanobacteria.

which it co-occurs with the native copepods *Diacyclops thomasi* and *Eurytemora affinis* (Bollens *et al.*, 2012). The native cyclopoid *D. thomasi* is omnivorous, feeding on aloricate ciliates, flagellates and dinoflagellates, and less commonly, juvenile copepods, rotifers and larval fish (Brandl, 1998; Rollwagen-Bollens *et al.*, 2013). Ingestion rates of *D. thomasi* in Vancouver Lake, a tidally influenced freshwater lake in the LCR (and <1 mile from our river site), were determined to be $<0.6 \mu\text{gC predator}^{-1} \text{h}^{-1}$ for all prey groups except cyanobacteria, which were ingested at rates as high as $1.4\text{--}2.0 \mu\text{gC predator}^{-1} \text{h}^{-1}$ during bloom periods (Rollwagen-Bollens *et al.*, 2013). When considering only our river and reservoir sites, we found *P. forbesi* to have ingestion rates comparable with *D. thomasi* for diatoms at both sites, as well as for dinoflagellates, flagellates and ciliates in our reservoir site (Fig. 2). With both *P. forbesi* and *D. thomasi* selecting for ciliates and diatoms, competitive interactions between these species seem likely.

The native euryhaline calanoid *E. affinis*, like *P. forbesi*, is omnivorous, and in estuaries can ingest ciliates, algae, cyanobacteria and detritus (Gasparini and Castel, 1997; Merrell and Stoecker, 1998; Ger *et al.*, 2010). This omnivory, coupled with high ingestion rates, suggests that *E. affinis* may be a strong competitor with *P. forbesi* in our estuary site. Indeed, Bollens *et al.* (Bollens *et al.*, 2012)

found that *E. affinis* and *P. forbesi* overlap considerably in seasonal abundance (June–September) as well as in temperature–salinity space in the LCR.

Finally, the presence of *P. forbesi* in the LCR may have trophic implications for their predators. The sustainability of salmonids, which are planktivorous as juveniles (Keeley and Grant, 2001; Bollens *et al.*, 2010), is of great concern to managers in the Pacific Northwest. Adams *et al.* (Adams *et al.*, unpublished data) found that juveniles of the threatened Chinook salmon (*Oncorhynchus tshawytscha*) showed very strong positive selection for the native cladoceran, *Daphnia retrocurva*, over *P. forbesi*, but neutral selection between native cyclopoid copepods and *P. forbesi*. Thus, the invasive copepod *P. forbesi*, which is highly abundant and broadly distributed within the Columbia River, is likely to have significant but as yet unquantified food web impacts, both as a grazer of microplankton and as prey for planktivores.

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REFERENCES

- Bollens, S. M., Breckenridge, J. K., Cordell, J. R., Rollwagen-Bollens, G. and Kalata, O. (2012) Invasive copepods in the Lower Columbia River Estuary: seasonal abundance, co-occurrence and potential competition with native copepods. *Aquat. Invas.*, **7**, 101–109.
- Bollens, S. M., Cordell, J. R., Avent, S. and Hooff, R. (2002) Zooplankton invasions: a brief review, plus two case studies from the northeast Pacific Ocean. *Hydrobiologia*, **480**, 87–110.
- Bollens, S. M., vanden Hooff, R., Butler, M., Cordell, J. R. and Frost, B. W. (2010) Feeding ecology of juvenile Pacific salmon (*Oncorhynchus* spp.) in a northeast Pacific fjord: diet, availability of zooplankton, selectivity for prey, and potential competition for prey resources. *Fish. Bull.*, **108**, 393–407.
- Brandl, Z. (1998) Feeding strategies of planktonic cyclopoids in lacustrine ecosystems. *J. Mar. Syst.*, **15**, 87–95.
- Breckenridge, J., Bollens, S. M., Rollwagen-Bollens, G. and Roegner, C. (2015) Plankton assemblage variability in a river-dominated temperate estuary during late spring (high-flow) and late summer (low-flow) periods. *Estuar. Coasts*, **38**, 93–103.
- Calbet, A. and Saiz, E. (2005) The ciliate-copepods link in marine ecosystems. *Aquat. Microb. Ecol.*, **38**, 157–167.
- Cordell, J. R., Bollens, S. M., Draheim, R. and Sytsma, M. (2008) Asian copepods on the move: recent invasions in the Columbia-Snake River system, USA. *ICES J. Mar. Sci.*, **65**, 753–758.
- DeMott, W. R. (1995) Optimal foraging by a suspension-feeding copepod: responses to short-term and seasonal variation in food resources. *Oecologia*, **103**, 230–240.
- Dexter, E., Bollens, S. M., Rollwagen-Bollens, G., Emerson, J. and Zimmerman, J. (2015) Persistent versus ephemeral invasions: 8.5 years of zooplankton community dynamics in the Columbia River. *Limnol. Oceanogr.*, **60**, 527–539.
- Emerson, J. E., Bollens, S. M. and Counihan, T. D. (2015) Seasonal dynamics of zooplankton in Columbia-Snake River system reservoirs, with special reference to the invasive copepod *Pseudodiaptomus forbesi*. *Aquat. Invas.*, **10**, 25–40.
- Gasparini, S. and Castel, J. (1997) Autotrophic and heterotrophic nano-plankton in the diet of the marine copepods *Eurytemora affinis* and *Acartia bifilosa*. *J. Plankton Res.*, **19**, 877–890.
- Ger, K. A., Arneson, P., Goldman, C. R. and Teh, S. J. (2010) Species specific differences in the ingestion of *Microcystis* cells by the calanoid copepods *Eurytemora affinis* and *Pseudodiaptomus forbesi*. *J. Plankton Res.*, **32**, 1479–1484.
- Gifford, S. M., Rollwagen-Bollens, G. and Bollens, S. M. (2007) Mesozooplankton omnivory in the upper San Francisco Estuary. *Mar. Ecol. Prog. Ser.*, **348**, 33–46.
- Keeley, E. R. and Grant, J. W. A. (2001) Prey size of salmonid fishes in streams, lakes, and oceans. *Can. J. Fish. Aquat. Sci.*, **58**, 1122–1132.
- Lee, T., Rollwagen-Bollens, G., Bollens, S. and Faber-Hammond, J. (2015) Environmental influences on cyanobacteria abundance and microcystin toxin production in a shallow temperate lake. *Ecotoxicol. Environ. Saf.*, **114**, 318–325.
- Merrell, J. R. and Stoecker, D. K. (1998) Differential grazing on protozoan microplankton by developmental stages of the calanoid copepod *Eurytemora affinis* Poppe. *J. Plankton Res.*, **20**, 289–304.
- Rollwagen-Bollens, G., Bollens, S. M., Gonzalez, A., Zimmerman, J., Lee, T. and Emerson, J. (2013) Feeding dynamics of the copepod *Diacyclops thomasi* before, during and following filamentous cyanobacteria blooms in a large, shallow temperate lake. *Hydrobiologia*, **705**, 101–118.
- Rollwagen-Bollens, G. and Penry, D. L. (2003) Feeding dynamics of *Acartia* spp. copepods in a large, temperate estuary (San Francisco Bay, CA). *Mar. Ecol. Prog. Ser.*, **257**, 139–158.
- Roohi, A., Yasin, Z., Kideys, A. E., Hwai, A. T. S., Khanari, A. G. and Eker-Develi, E. (2008) Impact of a new invasive ctenophore (*Mnemiopsis leidyi*) on the zooplankton community of the Southern Caspian Sea. *Mar. Ecol.*, **29**, 421–434.
- Strecker, A. L. and Arnott, S. E. (2008) Invasive predator, *Bythotrephes*, has varied effects on ecosystem function in freshwater lakes. *Ecosystems*, **11**, 490–503.