Feeding dynamics of the invasive calanoid copepod *Pseudodiaptomus inopinus* in two northeast Pacific estuaries

Jade Jacobs[1],*, Gretchen Rollwagen-Bollens[1], Stephen M. Bollens[1,2]

[1]School of the Environment, Washington State University, 14204 NE Salmon Creek Avenue, Vancouver, WA 98686-9600, USA
[2]School of Biological Sciences, Washington State University, 14204 NE Salmon Creek Avenue, Vancouver, WA 98683-9600, USA

ABSTRACT: The Asian calanoid copepod *Pseudodiaptomus inopinus*, first observed in the Columbia River Estuary in the early 1990s, has since become the dominant copepod species in many estuaries along the US Pacific coast, but its feeding behavior has not been previously studied. In October 2019 and 2020, when *P. inopinus* was at peak seasonal abundance, we conducted incubation experiments with this species feeding on natural microplankton prey assemblages sampled from 2 invaded estuaries: the Chehalis River estuary, Washington, and the Yaquina River estuary, Oregon. In both estuaries, diatoms were the most numerically abundant prey group, with 11–15 µm *Chaetoceros* sp. and 21–25 µm *Cyclotella* sp. dominating the Chehalis and Yaquina estuaries, respectively. Diatom and ciliate biomass were highest in both estuaries, with all prey cells in the Yaquina estuary typically larger than those in the Chehalis estuary. *P. inopinus* fed omnivorously on microplankton prey, with a preference for prey >20 µm and occasional avoidance of cyanobacteria and cells <10 µm. Ingestion rates were highest on ciliates and diatoms. The omnivory of *P. inopinus* may contribute to its success as an invader in northeast Pacific estuaries.

KEY WORDS: Copepod · Feeding ecology · Trophic ecology · Estuaries · Invasive species

1. INTRODUCTION

Aquatic invasive species can have large-scale ecological impacts on plankton community composition and food web dynamics in aquatic ecosystems (Bollens et al. 2002, Strayer 2010, Havel et al. 2015, Dexter & Bollens 2020, Dexter et al. 2020a). For example, the spread of zebra and quagga mussels throughout North America has resulted in major community shifts and structural alterations of freshwater environments (Vanderploeg et al. 2002, Cuhel & Aguilar 2013). Ecological impacts such as these are likely to worsen, as some models predict a substantial increase in the distributional range and/or abundance of aquatic invasive invertebrates in response to climate change (Bellard et al. 2013, Dexter et al. 2020b).

Estuaries are often the locations where non-indigenous aquatic taxa are initially introduced, due largely to the global transport of plankton via ballast water (Carlton & Geller 1993, Ruiz et al. 2000) and because in these areas they can reach high enough abundances or otherwise disrupt native communities so as to become invasive. This is especially true for estuaries of the northeast Pacific Ocean, which have experienced numerous aquatic invasions in recent decades (Bollens et al. 2002, Cordell et al. 2008, Dexter et al. 2015). Notably, these estuaries have been invaded by at least 9 species of planktonic copepods native to Asia, most of which first appeared in the San Francisco Estuary (SFE) in California, USA (Bollens et al. 2002, Cordell et al. 2010), as well as the bosminid cladoceran *Bosmina coregoni* (Smits et al.

2013) and planktonic juveniles of the Asian clam *Corbicula fluminea* (Counts 1986, Hassett et al. 2017).

One such invasion is that of the Asian copepod *Pseudodiaptomus inopinus*, introduced to the Columbia River Estuary (CRE), Washington, USA, in the early 1990s (Cordell et al. 1992). *P. inopinus* is native to Japan and South Korea, where it has been recently redescribed as distinct from other closely related species (Eyun et al. 2007, Ueda & Sakaguchi 2019). Since its first observation in the CRE, *P. inopinus* is now present as the dominant copepod species in at least 11 other estuaries along the west coast of North America (Cordell & Morrison 1996, Dexter et al. 2018, 2020c).

The distribution of *P. inopinus* in northeast Pacific estuaries is strongly related to temperature, salinity, and water column stratification (Cordell et al. 2010), and the estuaries where it has successfully invaded fall within a narrowly defined geographic range of 43–47° N (Dexter et al. 2020c). This latitudinal distribution has been static for more than 20 yr and thus may represent the maximum extent of its range, even though other estuaries of the northeast Pacific have been shown to contain potentially suitable habitat (Cordell et al. 2010, Dexter et al. 2020b). These invaded estuaries are characterized by bottom salinities between 0 and 6, mean autumn water temperature of 19.3 ± 1.5°C, and estuarine transition zones extending for at least 1 km upstream (Cordell & Morrison 1996, Bollens et al. 2002, Cordell et al. 2007). These conditions are consistent with those observed in the native range of *P. inopinus*, where its abundance is believed to be controlled primarily by salinity and chlorophyll *a* concentration (Park et al. 2013). In every northeast Pacific estuary where it is observed as well as in its native range (Cordell & Morrison 1996), *P. inopinus* is highly abundant (~10^3 m^{-3}) (Dexter et al. 2020c) and is the dominant copepod in late summer and early autumn, with juveniles peaking in abundance between August and mid-September followed by peak adult abundance from October through mid-December (Cordell et al. 2007).

Despite its numerical dominance in many north Pacific estuaries, very little is known about the trophic dynamics of *P. inopinus*. Of the few such studies, all have focused on *P. inopinus* as a prey source for larger aquatic invertebrates and larval fish. For instance, in their native range in Japan, laboratory experiments indicated that *P. inopinus* plays an important ecological role in coastal ecosystems by contributing the fatty acid docosahexaenoic acid (DHA) in polar lipids to larval red bream fish *Pagrus major* (Matsui et al. 2021). In the Chehalis River, Washington (where *P. inopinus* is invasive and highly numerically abundant), native vertebrate and invertebrate predators—especially the bentho-pelagic invertebrates *Neomysis mercedis* and *Crangon franciscorum*—readily consume, but otherwise do not show preference for, *P. inopinus* over native copepods (Bollens et al. 2002). However, nothing (to our knowledge) has ever been published on the feeding dynamics of *P. inopinus* in either its native or invaded range.

Addressing this knowledge gap is necessary to understand the role of this aquatic invader in general and is of particular interest in relation to estuaries of the northeast Pacific since information about the trophic behavior of *P. inopinus* could provide insights as to why it has now become rare or absent in the CRE, an estuary where its congener species *P. forbesi* has since invaded and become established (Sytsma et al. 2004, Cordell et al. 2008, Dexter et al. 2020c). Indeed, the CRE and SFE are the only 2 northeast Pacific estuaries that do not contain populations of *P. inopinus* but do contain *P. forbesi* (Dexter et al. 2020c). The feeding dynamics of *P. forbesi* have been studied previously in both the CRE (Bowen et al. 2015) and SFE (Kayfetz & Kimmerer 2017). However, thorough investigation of the feeding dynamics of *P. inopinus* is a prerequisite to any future investigations into whether these species are able to coexist or if they may compete for prey resources.

Therefore, we conducted incubation experiments with *P. inopinus* feeding on the natural prey assemblages from 2 northeast Pacific estuaries where this species has become dominant during the autumn months (Dexter et al. 2020c). We had 2 specific objectives: first, to quantify the composition of the potential prey community as well as the feeding rates and prey selectivity of *P. inopinus* in the Chehalis River and Yaquina River estuaries in October of 2 consecutive years (2019 and 2020); second, to compare how *P. inopinus* feeding behaviors may differ between these 2 estuaries and between these 2 years.

2. MATERIALS AND METHODS

2.1. Study sites

Experiments were conducted using copepods and the ambient prey assemblage from 2 estuaries—the Chehalis River estuary and the Yaquina River estuary—each located within 200 km north and south, respectively, along the Pacific coast from the CRE,

where *Pseudodiaptomus inopinus* was first introduced (Fig. 1). These estuaries were selected because both had high abundances of *P. inopinus* and each was within a 2 h drive from our laboratory, which minimized the time between collection of live specimens and the start of the feeding incubation experiments. The Chehalis River estuary is located 100 km north of the mouth of the Columbia River in southwest Washington, USA (Fig. 1). The river runs 200 km from its headwaters in the Willapa Hills to its outlet in Greys Harbor, with discharge being lowest in August and highest in January (Gendaszek 2011). Our sample site on the Chehalis River was near Cosmopolis (46° 57′ 27.8″ N, 123° 46′ 16.9″ W), approximately 15 river km from the outlet to Greys Harbor, where the tidal range is 5 m and salinity ranges tidally from 0 to 12.

The Yaquina River estuary is located 170 km south of the mouth of the Columbia River along the central coast of Oregon, USA (Fig. 1). The river runs 95 km from its headwaters in the Oregon Coast Range to the Pacific Ocean, with discharge annually averaging 6.9 m^3 s^{-1} (Sigleo & Frick 2007). Our sampling site in the Yaquina River was near Toledo (44° 36′ 13.8″ N,
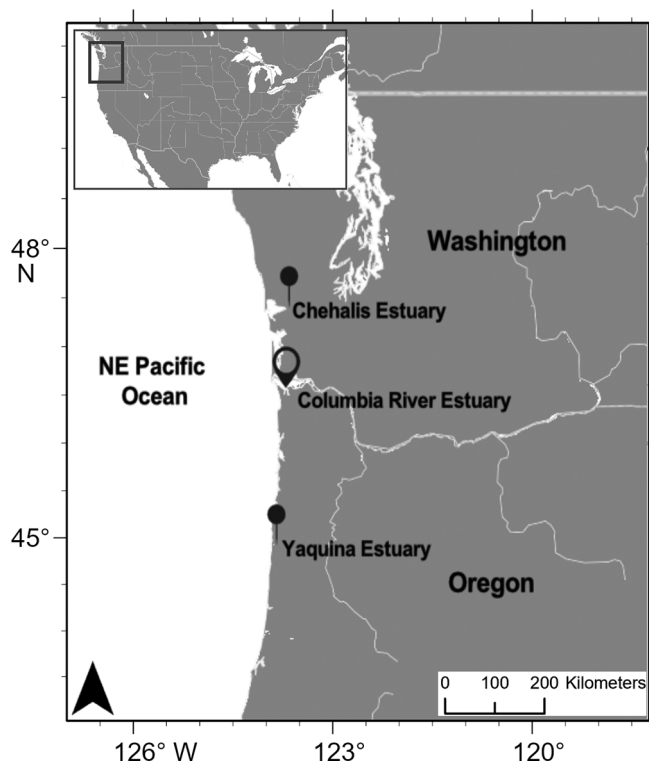

Fig. 1. Study sites (Chehalis and Yaquina estuaries) where *Pseudodiaptomus inopinus*, microplanktonic prey, and environmental conditions were sampled in October 2019 and 2020, as well as the Columbia River Estuary, the site of several related historical studies

123° 54′ 8.8″ W), approximately 30 river km upstream from the Pacific Ocean, where the tidal range is 1.9 m (Brown & Ozretich 2009) and the salinity ranges tidally from 0 to 15.

2.2. Field sampling

Water and copepods for incubation experiments were collected at one site from each estuary during October 2019 and again in October 2020. At each site in each estuary, sampling occurred within 1 h of high slack tide at a location near the center of the channel, where both the surface and bottom salinities were between 2–5 as measured with a YSI portable salinity–temperature meter. Water depth, surface and bottom temperature, and surface and bottom salinity were recorded immediately before and after plankton sampling. Oblique net tows from near bottom to the surface were conducted from a small (4 m) boat using a 0.5 m diameter, 75 µm mesh plankton net; net contents were immediately rinsed from the net and transferred to a clean bucket. Oblique tows were used to ensure that *P. inopinus* were collected regardless of their vertical position in the water column, which can fluctuate on a diel basis (Bollens et al. 2002). Surface water for the laboratory incubation experiments was then gently filtered into carboys through a 300 µm mesh sieve to remove large grazers. In addition, three 200 ml samples of unfiltered surface water were collected and preserved in 5% Lugol's solution for later microscopical analysis. All samples were transported to the lab within 6 h of collection and stored in conditions consistent with those recorded during sampling (12 h light:12 h dark cycles and temperature of 16°C).

2.3. Incubation experiments

Following the methods of Rollwagen-Bollens et al. (2013) and Bowen et al. (2015), 40 non-ovigerous adult female *P. inopinus* for each experiment were hand-picked from the plankton net tow samples and transferred into each of four 500 ml incubation bottles containing filtered surface water from the collection site. Additionally, 4 replicate 500 ml bottles were filled with filtered surface water to serve as initial controls and another 4 replicate bottles were filled with filtered surface water as final controls. All replicate bottles were covered with parafilm and sealed to prevent bubbles. All treatment and final control bottles were then incubated for 12 h, overnight, on a ro-

tating (0.5–1 rpm) plankton wheel in the dark and kept at ambient temperature to mimic field conditions.

After incubation, copepods were removed from the treatment bottles by filtering the incubation water over a 300 µm mesh sieve and then preserved in 5% buffered formalin solution in 20 ml glass vials. Initial control bottles were sub-sampled immediately prior to the start of the incubations, and final control and treatment bottles were sub-sampled at the end of the incubations as follows: 200 ml sub-samples were taken from each bottle then preserved in 5% Lugol's solution for microscopical analysis.

2.4. Microplankton sample processing and analysis

Taxonomic composition, abundance, biomass, and cell size of microplankton prey in each incubation bottle were assessed by settling aliquots of 24.5 ml from each sample bottle overnight in Utermöhl chambers and then observing the contents using an inverted microscope (Leica DMI4000B) at 400× magnification. For each sample (aliquot), cells up to 200 µm in their longest dimension were counted and sized in microscope fields along transects of the settling chamber until at least 300 cells >10 µm were observed (Kirchman 1993). Every cell counted was further identified to the lowest practical taxonomic level using Patterson & Hedley (1992) and Wehr et al. (2015). The cells were then grouped into 1 of 6 major prey taxonomic categories: diatoms, dinoflagellates, flagellates, ciliates, chlorophytes, and cyanobacteria, as well as 4 major size categories based on their longest dimension: 1–10, 11–20, 21–30, and >30 µm. Carbon biomass was calculated for each individual using biovolume calculated from geometric shape (Hillebrand et al. 1999) and estimated from the algorithms of Menden-Deuer & Lessard (2000). Any one of our experiments was considered to have resulted in a feeding effect if there was a significant ($p < 0.05$) reduction in abundance and/or biomass of any individual prey group between final control and treatment bottles as measured using Student's t-tests (Rollwagen-Bollens et al. 2013, Bowen et al. 2015).

Copepod clearance rates (ml copepod^{-1} h^{-1}) and ingestion rates (µg C copepod^{-1} h^{-1}) were calculated from the changes in abundance (cells ml^{-1}) or biomass (µg C ml^{-1}) of each prey taxonomic category and each size category over the course of the incubation, following the approach of Marin et al. (1986). Feeding selectivity and preference were then estimated using 2 approaches. First, significant differences in clearance rates among prey taxa and size categories were assessed within each experiment using Kruskal-Wallis ANOVA by ranks, since these data did not meet the assumptions of a parametric ANOVA. Second, an electivity index ($E^\star$; Vanderploeg & Scavia 1979) was calculated for each prey taxonomic and size category in each experiment, and each mean $E^\star$ value was tested for being significantly different from zero using 1-sample t-tests. $E^\star$ values range from +1 to −1; significantly positive values of $E^\star$ were interpreted to indicate a preference for that particular prey category whereas significantly negative $E^\star$ values were indicative of avoidance of that particular prey category.

Body mass of each *P. inopinus* specimen was also determined from both incubation experiments from each estuary using length–weight–carbon biomass conversions measured by Ara (2001). Daily weight-specific ingestion rates ([µg C prey] [µg C copepod]$^{-1}$ d^{-1}) and daily ration (% body carbon consumed d^{-1}) were then calculated for each experiment to more accurately draw comparisons to feeding rates of other copepods taxa reported in the literature.

3. RESULTS

3.1. Microplankton prey assemblages

In October 2019 and 2020, microplankton abundance in the Chehalis estuary was primarily composed of diatoms (59 and 63%, respectively) and flagellates (31 and 29%, respectively) (Fig. 2A). However, while ciliates only comprised 8% of the microplankton abundance in 2019, these cells contributed 60% of microplankton biomass, with diatoms sub-dominant in terms of biomass (20%). In 2020, this pattern was reversed: diatoms were dominant with a relative biomass of 46% and ciliates sub-dominant, contributing 30% of relative biomass (Fig. 2B). In both years, chlorophytes, cyanobacteria, and dinoflagellates were particularly scarce, each comprising less than 5% of both total abundance and total biomass of microplankton. The dominant prey size category in the Chehalis estuary in 2019 and 2020 was 11–20 µm in the longest dimension, comprising 70.0 and 61.2% of available prey, respectively. This size category primarily contained diatoms, dinoflagellates, flagellates, and ciliates. Sub-dominant to this size category were cells 1–10 µm in their longest dimension (21.3% in 2019 and 20.3% in 2020), primarily comprising flagellates, chlorophytes, and cyanobacteria (Table 1).
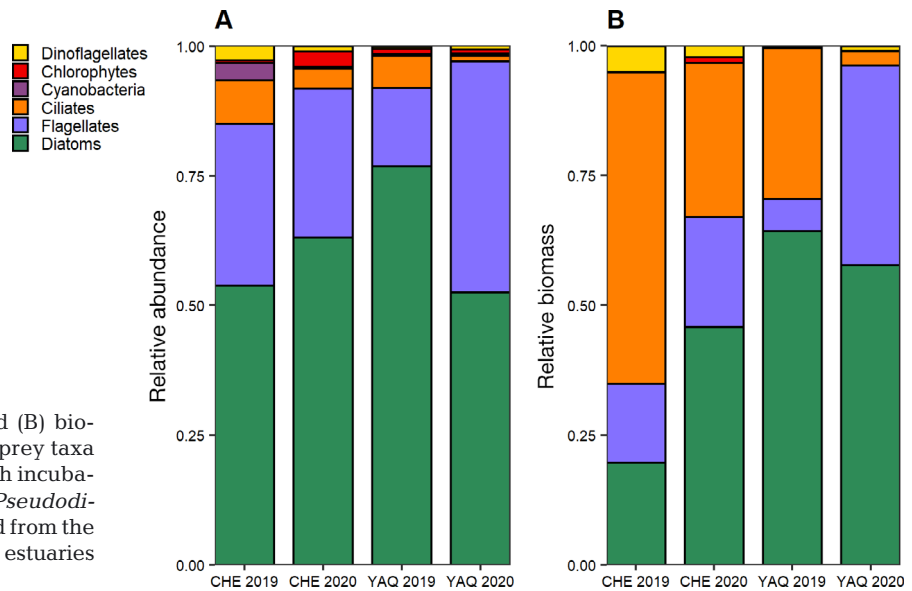
Fig. 2. Relative (A) abundance and (B) biomass of each major microplankton prey taxa category available at the start of each incubation experiment conducted with *Pseudodiaptomus inopinus* and prey collected from the Chehalis (CHE) and Yaquina (YAQ) estuaries in 2019 and 2020

In the Yaquina estuary, diatoms were the most abundant microplankton prey taxon in 2019 (77%) followed by flagellates (15%); in 2020, diatoms and flagellates were roughly equal in relative abundance (53 and 45%, respectively) (Fig. 2A). With respect to microplankton biomass, diatoms were consistently dominant in 2019 and 2020 (64 and 58%, respectively), although in 2019, ciliates, which only accounted for 6% of the total prey abundance, contributed 30% of the total prey biomass (Fig. 2B). In both years, chlorophytes, cyanobacteria, and dinoflagellates each comprised less than 5% of both total abundance and total biomass of microplankton. The dominant prey size category in the Yaquina estuary in 2019 and 2020 was that of cells 21–30 µm in their longest dimension, comprising 42.9 and 62.5% of available prey, respectively. This size category primarily contained diatoms, flagellates, and ciliates. Sub-dominant to this size category were cells 11–20 µm in their longest dimension (30.2% in 2019 and 31.0% in 2020), primarily containing dinoflagellates, flagellates, and ciliates (Table 1).

3.2. *P. inopinus* prey preferences

In the Chehalis estuary, *P. inopinus* clearance rates on prey taxa cate-

Table 1. Microplankton taxa observed in the Chehalis and Yaquina estuaries during October 2019 and 2020. Asterisks indicate the presence of the listed taxon in the estuary

Group	Size (µm)	Genus sp.	Chehalis	Yaquina
Diatoms		Unknown	*	*
	1–10	*Melosira*	*	*
		Skeletonema	*	*
		Tabellaria	*	*
	11–20	*Chaetoceros*	*	*
		Navicula	*	*
		Nitzschia	*	*
	21–30	*Amphora*	*	*
		Cocconeis	*	*
		Cyclotella	*	*
	30+	*Asterionella*		*
		Gomphonema	*	
		Gyrosigma	*	*
		Synedra	*	*
		Unknown	*	*
Dinoflagellates	11–20	*Gynmodinium*	*	*
		Peridinium	*	
		Unknown athecate	*	*
		Unknown thecate	*	*
Flagellates	1–30	Cryptomonad	*	*
		Unknown	*	*
Ciliates	11–20	*Mesodinium rubrum*	*	*
		Unknown loricate	*	*
	21–30	*Strombidium*	*	*
Chlorophytes	1–10	*Coelastrum*	*	*
		Crucigenia	*	*
		Tetrastrum	*	
		Scenedesmus	*	*
		Spirulina		*
	11–20	*Ankistrodesmus*		*
		Staurastrum	*	*
		Unknown	*	*
Cyanobacteria	1–10	*Anabaena*	*	*
		Microcystis		*
		Unknown	*	*

gories ranged from −0.25 to 1.23 ml copepod^{-1} h^{-1} in 2019 and −2.67 to 1.24 ml copepod^{-1} h^{-1} in 2020 (Fig. 3). Clearance rates on prey size categories ranged from −0.04 to 0.34 ml copepod^{-1} h^{-1} in 2019 and −0.17 to 1.96 ml copepod^{-1} h^{-1} in 2020 (Table 2). Statistical analyses indicated that there were no significant differences ($\chi^2_5 = 9.12$, p > 0.05) in *P. inopinus* clearance rates among prey taxonomic categories in 2019. However, there was a significant difference ($\chi^2_5 = 11.014$, p < 0.05) in *P. inopinus*

Table 2. Mean (±SE) clearance rates and electivity (E^*) values of *Pseudodiaptomus inopinus* on prey size categories in the Chehalis and Yaquina estuaries in October 2019 and 2020. E^* values significantly different from zero shown in **bold**

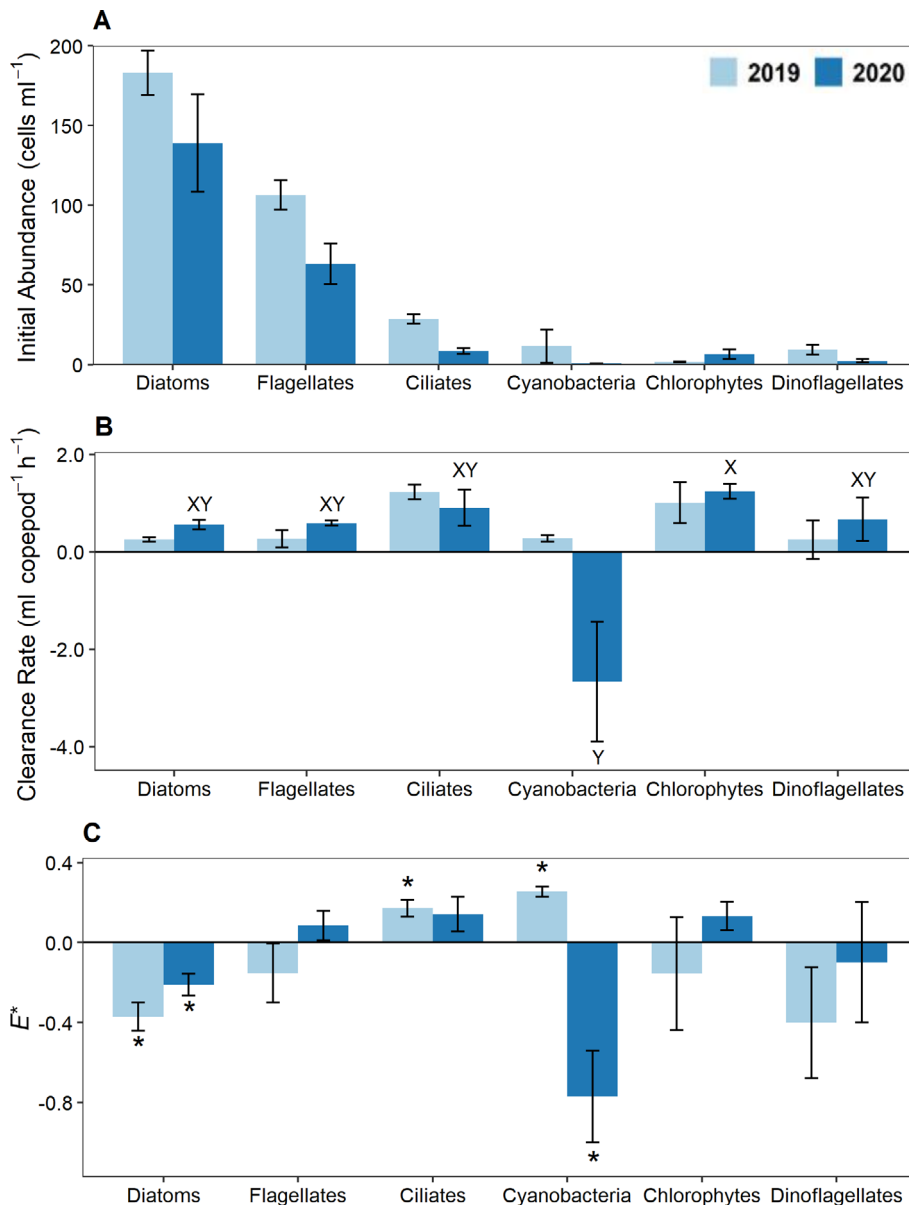| Size (μm)	Clearance rate	E^*	Clearance rate	E^*
	Chehalis 2019		Yaquina 2019	
1–10	−0.037 (±0.058)	−0.064 (±0.086)	1.094 (±0.629)	−0.032 (±0.331)
11–20	0.382 (±0.069)	0.136 (±0.126)	0.097 (±0.108)	**−0.501 (±0.148)**
21–30	0.340 (±0.174)	0.064 (±0.107)	0.027 (±0.118)	−0.412 (±0.248)
30+	0.192 (±0.616)	−0.688 (±0.312)	0.708 (±0.303)	0.120 (±0.115)
	Chehalis 2020		Yaquina 2020	
1–10	−0.169 (±0.302)	−0.636 (±0.211)	0.017 (±0.126)	**−1.0 (±0)**
11–20	0.704 (±0.351)	−0.267 (±0.237)	0.280 (±0.096)	−0.515 (±0.182)
21–30	1.959 (±0.362)	**0.306 (±0.043)**	1.403 (±0.109)	**0.248 (±0.018)**
30+	0.806 (±0.157)	−0.147 (±0.293)	4.168 (±1.952)	**0.321 (±0.028)**

Fig. 3. Mean (±SE) (A) initial prey abundance, (B) clearance rates, and (C) electivity (E^*) values for each prey category during the 12 h incubation experiments conducted with *Pseudodiaptomus inopinus* and microplankton prey from the Chehalis estuary. Clearance rates for prey taxa categories that share letters are not significantly different from each other (p > 0.05). E^* values significantly different from zero (p < 0.05) are marked with an asterisk

clearance rates among prey categories in 2020; a post hoc Dunn's test of the 2020 data revealed that *P. inopinus* cleared chlorophytes at a higher rate than cyanobacteria. With respect to prey size, there were no significant differences in clearance rates among prey size categories in 2019, but in 2020, *P. inopinus* cleared prey in the 21–30 µm size category at a significantly higher rate than prey cells 1–10 µm in size (Table 3).

E^* values for prey taxa categories in the Chehalis estuary ranged from −0.37 to 0.25 in 2019 and from −0.77 to 0.14 in 2020 (Fig. 3). Among prey size categories in the Chehalis estuary experiments, E^* ranged from −0.69 to 0.14 in 2019 and from −0.64 to 0.31 in 2020 (Table 2). E^* values calculated from the 2019 experiment were significantly (p < 0.05) positive for cyanobacteria (t_6 = −10.28, p = 0.00005) and ciliates (t_6 = −4.06, p = 0.007) and negative for diatoms (t_6 = 5.25, p = 0.002) (Fig. 3), and there were no E^* values significantly different from zero among prey size categories (Table 2). Conversely, in the 2020 Chehalis estuary experiment, E^* values were significantly negative for both cyanobacteria (t_6 = 3.35, p = 0.015) and diatoms (t_6 = 3.26, p = 0.017) (Fig. 3) and were significantly positive (t_6 = −7.12, p = 0.006) for prey cells 21–30 µm in size (Table 2).

Among prey taxa categories in experiments from the Yaquina estuary, clearance rates ranged from −0.12 to 1.31 ml copepod^{-1} h^{-1} in 2019 and from 0.94 to 2.21 ml copepod^{-1} h^{-1} in 2020, and E^* values ranged from −0.66 to 0.34 in 2019 and from −0.47 to 0.20 in 2020 (Fig. 4). Among prey size categories, clearance rates ranged from 0.03 to 1.09 ml copepod^{-1} h^{-1} in 2019 and from 0.02 to 4.17 ml copepod^{-1} h^{-1} in 2020, and E^* values ranged from −0.57 to 0.20 in 2019 and from −1 to 0.32 in 2020 (Table 2). In the Yaquina estuary, no significant differences in *P. inopinus* clearance rates were observed among prey taxonomic or size categories in the 2019 experiment (Table 4); however, *P. inopinus* exhibited significantly positive E^* for chlorophytes (t_6 = −5.93, p = 0.001) and significantly negative E^* for both flagellates (t_6 = 2.79, p = 0.032) and prey cells 11–20 µm in size (t_6 = 3.84, p = 0.031) in 2019. In 2020 in the Yaquina estuary, *P. inopinus* similarly did not exhibit significantly different clearance rates for any prey taxon but did clear prey >30 µm at a significantly higher rate than prey cells in the 1–10 and 11–20 µm size categories (Table 4). *P. inopinus* did not show significant E^* for any prey taxa but did show significantly positive E^* for prey cells 21–30 µm (t_6 = −14.02, p = 0.001) and 30+ µm in size (t_6 = −11.42, p = 0.001) in the 2020 Yaquina experiment (Table 2).

Table 3. Statistical comparison of *Pseudodiaptomus inopinus* clearance rates on microplankton prey taxa and size categories in experiments conducted from the Chehalis River estuary in 2019 and 2020. Significant (p < 0.05) values shown in **bold**. Chloro: chlorophytes; Cil: ciliates; Cyano: cyanobacteria; Diat: diatoms; Dino: dinoflagellates; Flag: flagellates

Chehalis 2019, taxa; Kruskal-Wallis			Chehalis 2020, taxa; Kruskal-Wallis					
df	χ^2	p		df	χ^2	p		
Prey 5	9.12	0.10	Prey	5	11.01	**0.05**		
				Cil	Cyano	Diat	Dino	Flag
			Chloro	1	**0.02**	0.66	1	1
			Cil		0.77	1	1	1
			Cyano			1	0.56	1
			Diat				1	1
			Dino					1

Chehalis 2019, size; Kruskal-Wallis			Chehalis 2020, size; ANOVA					
df	χ^2	p		df	SS	MS	*F*	p
Size 3	5.38	0.15	Size	3	9.15	3.06	4.03	**0.03**
			Residual	12	9.08	0.76		
				11–20	**21–30**	**30+**		
			1–10	0.51	**0.01**	0.42		
			11–20		0.23	0.10		
			21–30			0.29		

3.3. *P. inopinus* ingestion rates

In the Chehalis estuary, average total ingestion rates by *P. inopinus* were 0.021 ± 0.007 and 0.015 ± 0.0008 µg C copepod^{-1} h^{-1} in 2019 and 2020, respectively (Fig. 5). There were significant differences in *P. inopinus* ingestion rates among prey categories in both 2019 and 2020 (Fig. 5). In October 2019, ciliate biomass was ingested at the highest rate (0.031 µg C copepod^{-1} h^{-1}), and a post hoc Dunn's test revealed that the biomass of this group was ingested at a significantly higher rate than the biomass of chlorophytes (z_3 = 3.03, p = 0.035) and cyanobacteria (z_3 = −3.18, p = 0.022) (Table 5). In October 2020 in the Chehalis estuary, ciliate, diatom, and flagellate
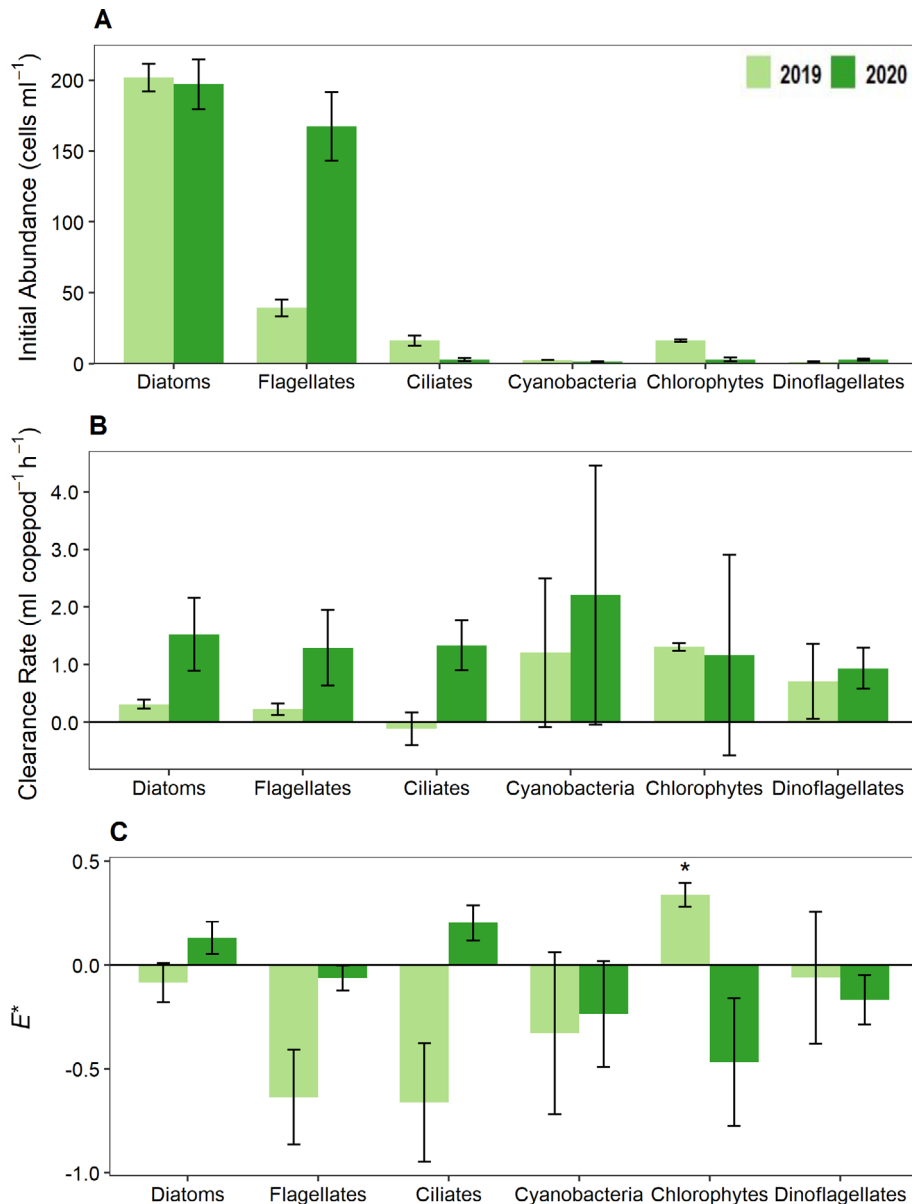
Fig. 4. Same as Fig. 3, but for the Yaquina estuary

biomass was ingested at rates between 0.0031 and 0.0062 µg C copepod^{-1} h^{-1}, and a post hoc Dunn's test revealed that both ciliate ($z_3 = -3.40$, p = 0.010) and diatom ($z_3 = 3.32$, p = 0.013) biomass were ingested at significantly higher rates than that of cyanobacteria (Table 5).

In the Yaquina estuary, average total ingestion rates by *P. inopinus* were 0.007 ± 0.011 and 0.053 ± 0.001 µg C copepod^{-1} h^{-1} in 2019 and 2020, respectively (Fig. 6). Similar to the Chehalis estuary, significant differences in *P. inopinus* ingestion rates on different prey taxa were observed in both years (Fig. 6). In October 2019, diatom biomass was ingested at the highest rate (0.014 µg C copepod^{-1} h^{-1}) and a post hoc Dunn's test revealed that this taxon was ingested

at a significantly higher rate than cyanobacteria biomass ($z_3 = 3.71$, p = 0.003) (Table 6). Similarly, in October 2020 in the Yaquina estuary, diatom biomass was again ingested at the highest rate (0.038 µg C copepod^{-1} h^{-1}), and a post hoc Dunn's test showed that diatoms were ingested at a significantly higher rate than the biomass of chlorophytes ($z_3 = 3.29$, p = 0.014); both diatom ($z_3 = 3.75$, p = 0.003) and dinoflagellate ($z_3 = 2.94$, p = 0.043) biomass was ingested at a significantly higher rate than cyanobacteria (Table 6).

The average individual biomass of copepods in the Chehalis estuary was higher than in the Yaquina estuary (3.36 and 2.86 µg C copepod^{-1}, respectively). Weight-specific ingestion rates of *P. inopinus* on the total microplankton community in the Chehalis estu-

Table 4. Statistical comparison of *Pseudodiaptomus inopinus* clearance rates on microplankton prey taxa and size categories in experiments conducted from the Yaquina River estuary in 2019 and 2020. Significant (p < 0.05) values shown in **bold**

Yaquina 2019, taxa; Kruskal-Wallis			Yaquina 2020, taxa; Kruskal-Wallis		
df	χ^2	p	df	χ^2	p
Prey 5	7.36	0.20	Prey 5	3.20	0.67

Yaquina 2019, size; Kruskal-Wallis			Yaquina 2020, size; Kruskal-Wallis		
df	χ^2	p	df	χ^2	p
Size 3	4.02	0.26	Size 3	13.26	**0.004**

	11-20	21-30	30+
1–10	0.48	0.13	**0.005**
11–20		0.48	**0.047**
21–30			0.48

ary in 2019 and 2020 were 0.147 ± 0.619 and 0.104 ± 0.250 (µg C prey) (µg C copepod)$^{-1}$ d^{-1} (respectively), representing a daily biomass ration of 14.8 ± 4.9 % in 2019 and 10.3 ± 0.6 % in 2020. In the Yaquina estuary, *P. inopinus* weight-specific ingestion rates in 2019 and 2020 were 0.055 ± 0.495 and 0.441 ± 1.160 (µg C prey) (µg C copepod)$^{-1}$ d^{-1}, respectively, which resulted in daily rations of 10.3 ± 6.2 % in 2019 and 44.0 ± 0.9 % in 2020.

4. DISCUSSION

Pseudodiaptomus inopinus has invaded and is now highly abundant in at least 11 northeast Pacific estuaries (Dexter et al. 2020c), including the Chehalis and Yaquina estuaries in Washington and Oregon states, respectively, yet this is the first investigation of *P. inopinus* feeding behavior in either its native or invasive range. We found that *P. inopinus* consumed prey omnivorously with a preference for ciliates, diatoms, and prey 21–30 µm. Its ingestion rate was highest on prey that comprised greater relative biomass rather than greater relative abundance. We also provide the first report of abundance and composition of microplankton in the Chehalis estuary, as well as a more comprehensive report of the microplankton assemblage in the Yaquina estuary.

4.1. Microplankton assemblage structure

During October 2019 and 2020, the microplankton prey assemblages in the Chehalis and Yaquina estu-aries were dominated by diatoms and flagellates, specifically *Chaetoceros* sp. in the 11–15 µm (Chehalis) and *Cyclotella* sp. in the 21–25 µm (Yaquina) size ranges. In both estuaries, ciliates ranged in size from 10 to 25 µm and flagellates ranged from 6 to 25 µm.

Our findings from the Yaquina estuary are consistent with previous studies of this estuary which reported that diatoms dominated in the spring–autumn and dinoflagellates and cyanobacteria increased in abundance in the summer (Karentz & McIntire 1977, Gilbert et al. 2010). Blooms of the nontoxic, red-tide-forming ciliate *Myrionecta rubra* also recur in the Yaquina estuary during spring in the vicinity of our sampling location (Brown & Nelson 2010). To our knowledge, our study is the first to report abundance and composition of the microplankton assemblage in the Chehalis estuary.

Moreover, the microplankton assemblage patterns observed in our study are consistent with many other temperate estuaries. *Cyclotella* and *Chaetoceros* dominated the upper eutrophic and middle transitional sections, respectively, of the Urdaibai estuary of northern Spain (Trigueros & Orive 2001, Revilla et al. 2002), and the autumn assemblage of the Guadiana estuary on the Iberian Peninsula was marked by high diatom abundance and biomass (Domingues & Galvão 2007). In the northeast Pacific, autumn microplankton assemblages in the SFE are dominated by *Chaetoceros* sp. and pennate diatoms (Rollwagen-Bollens & Penry 2003, Rollwagen-Bollens et al. 2006, Bouley & Kimmerer 2006); however, the CRE (located between the Chehalis and Yaquina estuaries along the US Pacific coast) is low in diatom and flagellate abundance in autumn but has occasional blooms of ciliates and consistently high abundance of cyanobacteria at this time of year (Bowen et al. 2015, Breckenridge et al. 2015, Rollwagen-Bollens et al. 2020).

4.2. *P. inopinus* prey selection

Despite the dominance of diatoms, flagellates, and ciliates in the Chehalis and Yaquina River estuaries, *P. inopinus* cleared most prey types at similar rates in both estuaries and both years while showing a slight preference for chlorophytes, ciliates, and cells >20 µm. These results generally align with the
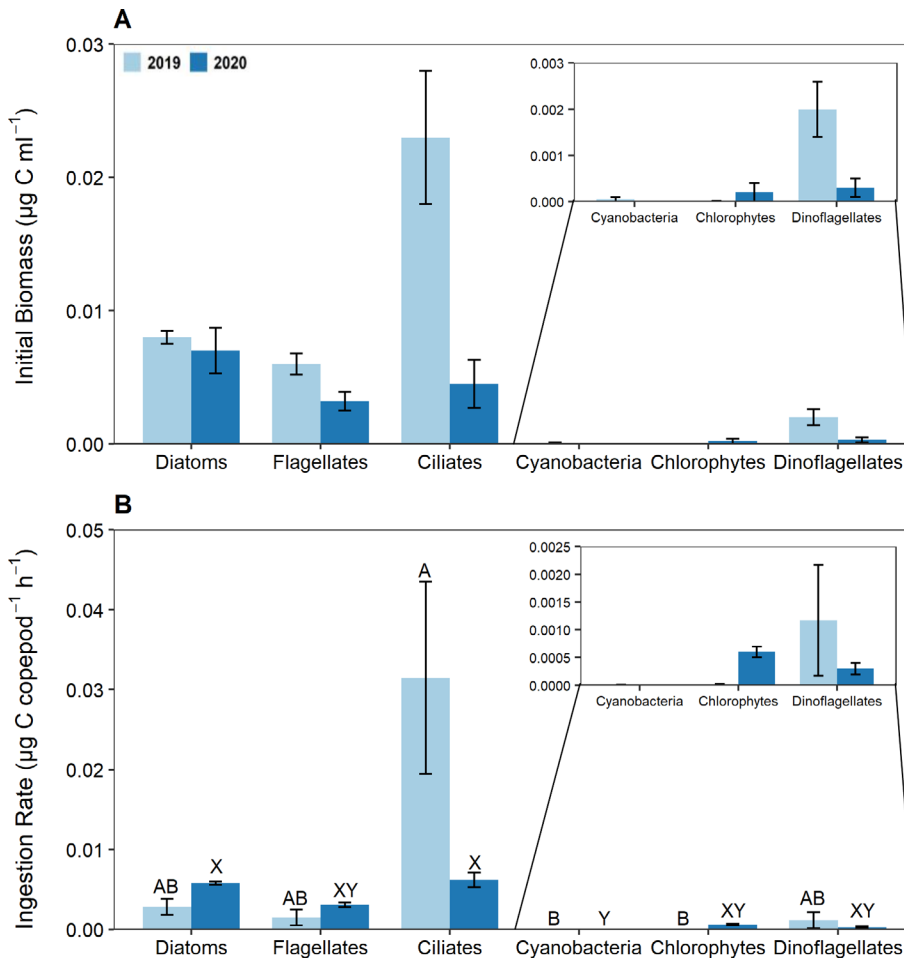
Fig. 5. Mean (±SE) (A) initial prey biomass and (B) ingestion rates for each prey taxa category during the 12 h incubation experiments conducted with *Pseudodiaptomus inopinus* and microplankton prey from the Chehalis estuary. Ingestion rates for prey taxa categories that share letters are not significantly different from each other (p > 0.05)

Table 5. Statistical comparison of *Pseudodiaptomus inopinus* ingestion rates on multiple categories of microplankton prey taxa in experiments conducted from the Chehalis River estuary. Significant (p < 0.05) values shown in **bold**. Abbreviations as in Table 3

	Chehalis 2019; Kruskal-Wallis					Chehalis 2020; Kruskal-Wallis				
	df	χ^2	p				df	χ^2	p	
Prey	5	12.7970	0.0254			Prey	5	18.141	**0.003**	
	Cil	Cyano	Diat	Dino	Flag		Cil	Cyano	Diat	Dino	Flag
Chloro	**0.035**	1	1	1	1	Chloro	0.45	1	0.45	1	1
Cil		**0.022**	1	0.30	0.46	Cil		**0.010**	1	0.45	1
Cyano			1	1	1	Cyano			**0.013**	1	0.45
Diat				1	1	Diat				0.45	1
Dino					1	Dino					1

The pattern of prey preference for ciliates is not surprising, as ciliates commonly comprise large portions of calanoid copepod diets, particularly when phytoplankton biomass is low (Rollwagen-Bollens & Penry 2003, Calbet & Saiz 2005, Gifford et al. 2007), and ciliates may be more carbon-rich than diatoms of similar volumes (Menden-Deuer & Lessard 2000). Size selection by calanoid copepods was also observed in a tropical lagoon (Cote d'Ivoire), where Pagano et al. (2003) observed *P. hessei* to generally prefer particles up to 39 µm. In the Bay of Biscay, bordering Spain and France, *Temora longicornis* selected for prey >40 µm (Vincent & Hartmann 2001). In the SFE, *Acartia* spp. preferred prey >15 µm and often targeted prey >25 µm (Rollwagen-Bollens & Penry 2003). Kayfetz & Kim-

patterns observed in the native range of *Pseudodiaptomus* spp., such as was found by Chen et al. (2018) using a stable isotope approach in Guangyang Bay, Korea, where *P. marinus* and *P. koreanus* as well as other brackish copepod species were observed to feed omnivorously and across a broad size spectrum.
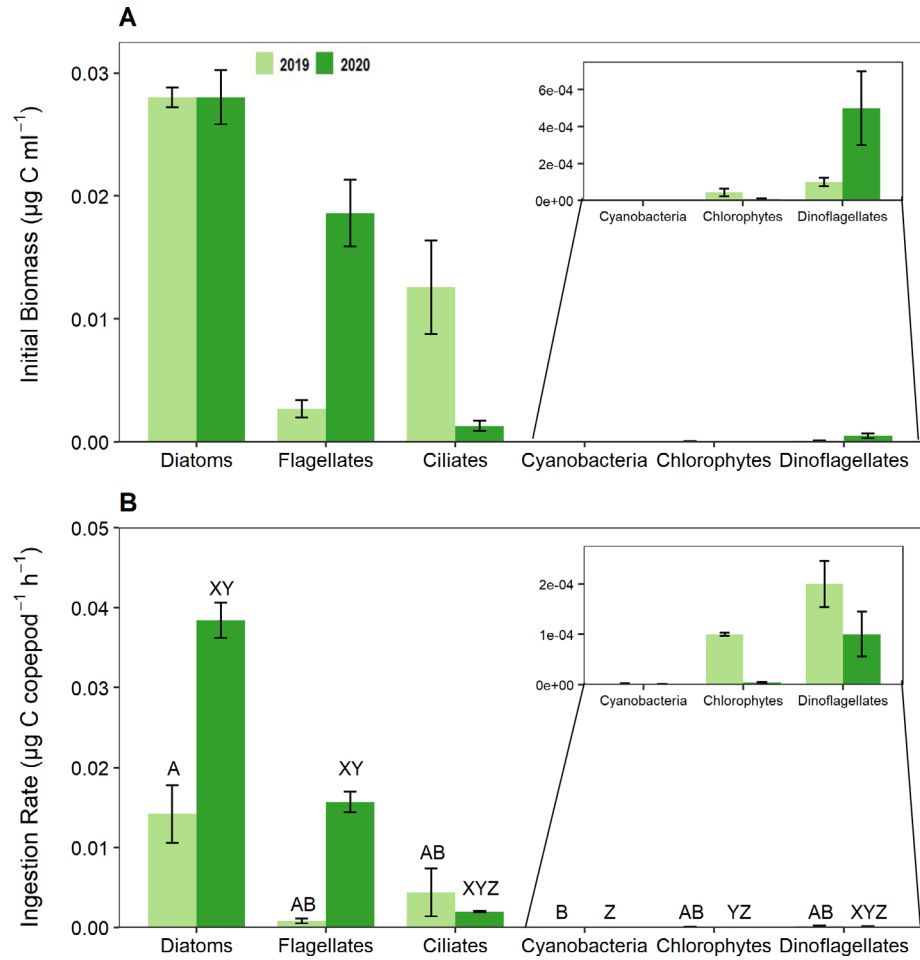
Fig. 6. Same as Fig. 5, but for the Yaquina estuary

Table 6. Statistical comparison of *Pseudodiaptomus inopinus* ingestion rates on multiple categories of microplankton prey taxa conducted from the Yaquina River estuary. Significant (p < 0.05) values shown in **bold**. Abbreviations as in Table 3

	\multicolumn{4}{c}{Yaquina 2019; Kruskal-Wallis}				\multicolumn{4}{c}{Yaquina 2020; Kruskal-Wallis}		
	df	χ^2		p			df	χ^2		p	
Prey	5	12.51		**0.028**		Prey	5	20.695		**0.0009**	
	Cil	Cyano	Diat	Dino	Flag		Cil	Cyano	Diat	Dino	Flag
Chloro	1	1	0.18	1	1	Chloro	0.76	1	**0.014**	1	0.14
Cil		0.83	0.48	1	1	Cil		0.33	0.76	1	1
Cyano			**0.003**	0.67	0.57	Cyano			**0.003**	1	**0.043**
Diat				0.66	0.75	Diat				0.08	1
Dino					1	Dino					0.55

merer (2017) found that *P. forbesi* showed low clearance rates on prey 7–15 µm, and *Limnoithona tetraspina* showed preference for flagellates >15 µm in the SFE. Additionally, the brackish environment of estuaries may impact prey preference of *P. inopinus*, as Galloway & Winder (2015) found that the long-chain essential fatty acid content of chlorophytes cor-

related positively with salinity, was variable but highest at intermediate salinities for diatoms, and was relatively low for cyanobacteria regardless of salinity. Thus, under brackish conditions, chlorophytes and diatoms could be more nutritious than cyanobacteria (Galloway & Winder 2015).

Although cyanobacteria were scarce in both of our estuaries in October of both years and are relatively low in nutritional value, *P. inopinus* demonstrated a slight preference for this taxon in the Chehalis estuary in October 2019. Conversely, *P. inopinus* showed a distinct avoidance of cyanobacteria in the Chehalis estuary in October 2020. This is of note, as previous studies have shown estuarine calanoid copepods are able to feed on cyanobacteria and overcome the nutritional deficiencies of this prey

taxon to sustain growth. For instance, in the Baltic Sea, all field-collected zooplankton taxa (copepods, cladocerans, and rotifers) as well as laboratory-fed specimens of the copepod *Acartia tonsa* showed the presence of picocyanobacteria DNA in their gut contents, even when alternative food was plentiful, suggesting direct consumption of cyanobacteria (Motwani & Gorokhova 2013). *P. hessei* (Kâ et al. 2012) and *P. forbesi* (Bowen et al. 2015, Owens et al. 2019) have been shown to ingest cyanobacteria found in natural prey assemblages, and nauplii of *P. marinus* were reported to feed on the cyanobacterium *Synechococcus* sp. under cultured laboratory conditions (Vogt et al. 2013).

4.3. Patterns of *P. inopinus* prey consumption

In both estuaries, *P. inopinus* most often exhibited the highest ingestion rates on prey categories that were high in biomass rather than abundance. This may be due to the high abundance of large *Cyclotella* diatoms in the Yaquina estuary, which could have provided adequate carbon to support the copepods′ diet and lessened the need to ingest heterotrophic prey such as ciliates—which tend to have a slightly lower carbon:nitrogen ratio than autotrophic prey (Broglio et al. 2003), even though they can be a more efficient source of proteins and amino acids (Kiørboe et al. 1985).

The omnivorous diet of *P. inopinus* is not surprising, as copepod omnivory is common, particularly in systems where phytoplankton biomass is low and nanophytoplankton abundance is high (Gifford & Dagg 1988, Rollwagen-Bollens & Penry 2003, Bouley & Kimmerer 2006). The ingestion of heterotrophic taxa (e.g. ciliates and some dinoflagellates) by copepods has been observed in other temperate estuaries and may have cascading impacts on phytoplankton. For instance, in the SFE, the invasive copepods *L. tetraspina*, *P. forbesi*, and *Acartiella sinensis* are well established and broadly distributed (Bollens et al. 2011, 2014), and in the lower salinity zone exhibited grazing impacts on ciliates, which at times released phytoplankton growth from grazing pressure by the microzooplankton (Kratina et al. 2014, York et al. 2014). *P. inopinus* may have a similar impact in its invaded range, particularly in the Chehalis estuary, where we observed this species to selectively consume ciliates. Seasonal monitoring of both *P. inopinus* and the microplankton assemblage of this estuary would provide further insight into the potential role of *P. inopinus* to exert top-down control of the

microplankton assemblage. We also note that the significant consumption of ciliate biomass by *P. inopinus* in our incubations could have resulted in higher growth of small phytoplankton in those experiments through cascading effects (e.g. Nejstgaard et al. 2001, York et al. 2014) and might have artificially reduced the estimation of *P. inopinus* ingestion rates upon these smaller prey taxa. However, the ambient abundance of such small-sized phytoplankton groups was already quite low in both the Chehalis and Yaquina estuaries; therefore, we consider such cascading effects, if present, to be quite limited in our incubations.

More broadly, the potential trophic implications of omnivory are 2-fold: first, there is the possibility that *P. inopinus* could exert direct top-down control on both the phytoplankton and microzooplankton assemblages; and second, there are potential bottom-up impacts on higher level consumers, depending on whether *P. inopinus* is acting as a primary consumer (e.g. ingesting diatoms) or a secondary consumer (e.g. ingesting ciliates). In a laboratory setting with linear 3- and 4-trophic-level food chains, Malzahn et al. (2010) found that when autotrophic quality was low, copepods reared as secondary consumers grew faster than those reared as primary consumers. The authors concluded that if intermediary consumers are in high enough abundance they may, counterintuitively, increase higher trophic level production.

The weight-specific ingestion rates *of P. inopinus* measured in our study (6–44% body carbon consumed d^{-1}) fell well within the ranges reported for other *Pseudodiaptomus* species and were especially comparable with rates measured for *P. forbesi* in its invasive range. Bowen et al. (2015) found total weight-specific ingestion rate of *P. forbesi* in the CRE to be 0.163 (µg C prey) (µg C copepod)$^{-1}$ d^{-1}, or 16.3% body carbon d^{-1}, and in the SFE, Kayfetz & Kimmerer (2017) measured the average daily ration of this copepod as ranging from 5 to 22%, primarily on centric diatoms. Similarly, several studies have reported weight-specific ingestion rates for *P. hessei* that align with those for *P. inopinus* and *P. forbesi*, although with a much wider range of daily ration. Specifically, *P. hessei* in a northern African tropical lagoon (Cote d′Ivoire) exhibited daily rations between 13 and 100% based on incubations with the natural assemblage of microplankton (Pagano et al. 2003), and from 5 to 150% based on the gut fluorescence technique (Kouassi et al. 2001); and in a southern African estuary, Froneman (2004) measured daily biomass rations of *P. hessei* ranging from 4 to 64%.

Our measurements of *P. inopinus* ingestion rates in the Chehalis and Yaquina estuaries also align generally with rates reported for marine and estuarine copepods more broadly, although the weight-specific ingestion rates for the genus *Pseudodiaptomus* appear to be on the low end of the range based on reviews by Saiz & Calbet (2007, 2011) as well as 24 additional studies of calanoid and small cyclopoid copepod feeding rates published between 1992 and 2017 using a range of experimental approaches. For instance, daily biomass rations of mixed copepod assemblages estimated using the gut fluorescence technique (sensu Mackas & Bohrer 1976) have been reported to range from as low as 4 to >1000% (and in some cases, biologically impossible rates of >7000%) (e.g. Hansen & van Boekel 1991, Debes et al. 2008, Calliari & Tiselius 2009). When provided with cultured algae as prey in the laboratory, calanoid and small cyclopoid copepods have been observed to consume prey biomass from 0.5 to 250% of body carbon d^{-1} (e.g. Durbin & Durbin 1992, Koski et al. 1998, Dam & Lopes 2003, Garrido et al. 2013, Zamora-Terol & Saiz 2013, Saiz et al. 2014, van Someren Gréve et al. 2017). In feeding experiments using wild-caught copepods incubated with natural prey assemblages (such as in our study), daily rations have ranged from 1 to 150% (Pagano et al. 2003, Zamora-Terol et al. 2014, Bowen et al. 2015, Kayfetz & Kimmerer 2017). There are numerous biological explanations for such large differences in weight-specific ingestion rates by copepods, including past feeding history, availability of preferred prey taxa, temperature, season, latitude, etc. However, the variation in these rates also highlights the differences between experimental approaches used to assess feeding dynamics and the assumptions upon which they are based.

4.4. Co-occurrence and potential competition with congeners

P. inopinus is now the dominant zooplankton taxon in many northeast Pacific estuaries (Dexter et al. 2020c), yet this species has not been observed in the CRE (where it first arrived in the northeast Pacific) since 2002 (Cordell et al. 2008, Dexter et al. 2020c). Its congener species, *P. forbesi*, however, is now the second-most dominant zooplankton taxon along the northeast Pacific coast (Dexter et al. 2020c) and since 2002 has been present in very high abundance during August and September in the CRE (Cordell et al. 2008, Dexter et al. 2020c). In the CRE, *P. forbesi* is omnivorous, ingesting ciliates, algae, and cyanobac-

teria, and it consumes these prey taxa non-selectively (Bowen et al. 2015). Similarly, in the upper SFE, *P. forbesi* has been reported to ingest diatoms, ciliates, and flagellates at the highest rates (Kayfetz & Kimmerer 2017). Although *P. forbesi* is found to co-occur with other estuarine calanoid copepods in the CRE, such as *Eurytemora affinis* (Bollens et al. 2012, Bowen et al. 2015), it has not been observed to co-occur with *P. inopinus* (Bouley & Kimmerer 2006, Dexter et al. 2020c). *P. inopinus*, however, has been found to co-occur with another congener, *P. poplesia*, in the Mankyung River estuary, South Korea, where it is native (Park et al. 2013).

Both *P. inopinus* and *P. forbesi* are at peak abundance during the autumn wherever they are found. In the lower CRE, cyanobacteria become the most highly abundant taxon during this time, while other prey taxa decline in abundance (Bowen et al. 2015, Rollwagen-Bollens et al. 2020). Our current results from the Chehalis and Yaquina estuaries show that *P. inopinus* occasionally avoids cyanobacteria and shows preference for ciliates and diatoms — prey taxa that are not highly abundant in the lower CRE during autumn. Thus, the lower CRE may not be conducive to supporting a *P. inopinus* population due to prey limitation, a hypothesis that merits testing. It is also possible, given the overlap in prey preferences between *P. inopinus* and *P. forbesi* as well as the greater tolerance of broader environmental conditions by *P. forbesi* (Cordell et al. 2010, Dexter et al. 2015), that the subsequent introduction of *P. forbesi* to the CRE may have contributed to the disappearance of *P. inopinus* from this estuary, as suggested by Dexter et al. (2015). Indeed, the issue of potential congeneric displacement in zooplankton is not novel to *P. inopinus* and *P. forbesi*. In the Gulf of Finland, Baltic Sea, the native *E. affinis* and its invasive congener *E. carolleeae* currently co-exist, but multiple biotic factors — in particular, similarities in population dynamics and differences in body size — point to the potential for *E. carolleeae* to displace native *E. affinis* (Sukhikh et al. 2019). Further studies to address the possibility of resource competition or competitive exclusion between *P. inopinus* and *P. forbesi* would provide insight into the current and future invasions of this type.

5. CONCLUSIONS

This study is the first, to our knowledge, to determine the prey preferences and feeding rates of the Asian calanoid copepod *Pseudodiaptomus inopinus*

on natural prey assemblages, in either its native or invasive range. We found that *P. inopinus* is omnivorous in these estuaries, primarily consuming ciliates and diatoms and selecting for prey in the 21–30 μm size range. Its ingestion rates were highest on prey taxa that comprised the largest proportion of the available biomass in these estuaries, and when compared to other congeneric species, *P. inopinus* had a weight-specific ingestion rate similar to that of *P. forbesi*. This study expands our understanding of the trophic role of *P. inopinus* in 2 invaded northeast Pacific estuaries where it has become dominant by furthering our knowledge of the feeding rates, prey selection, and potential impacts of *P. inopinus* in these invaded systems.

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