

Diets of two congeneric species of crayfish worm (Annelida: Clitellata: Branchiobdellidae) from western Canada

K.S.P. Gale and H.C. Proctor

Abstract: Crayfish worms (Branchiobdellidae) are obligatory ectosymbionts of crustaceans, primarily crayfish. Little is known about their diets or ecology. A single crayfish can host several species and age classes of branchiobdellidans at one time, creating the potential for intra- and inter-specific competition. Coexistence of competitors can be promoted through dietary differences; therefore, we examined gut contents of *Cambarincola chirocephalus* Ellis, 1919 and *Cambarincola vitreus* Ellis, 1919, two species of branchiobdellidan occasionally found on the same host (the crayfish *Orconectes virilis* (Hagen, 1870) in Western Canada) to assess evidence of dietary niche partitioning. We also compared gut contents of conspecific adults and juveniles to determine whether diets differed with age. We found a range of diatoms and invertebrates, particularly rotifers, in the guts of both *Cambarincola* species. The species appear to have different diets, in that guts of *C. chirocephalus* contained more invertebrate material than did those of *C. vitreus*. There was no statistical difference in composition of gut contents between adult and juvenile conspecifics. We found very limited evidence of dietary niche expansion in *C. vitreus* in the absence of *C. chirocephalus*. The observed dietary differences between species may promote their continued coexistence in areas where their ranges overlap.

Résumé : Les branchiobdellidés (Branchiobdellidae) sont des ectosymbiontes obligés des crustacés, particulièrement des écrevisses. On connaît peu leur régime alimentaire et leur écologie. Une même écrevisse peut au même moment porter plusieurs espèces et classes d'âge de branchiobdellidés, ce qui crée potentiellement de la compétition intra- et inter-spécifique. La coexistence des compétiteurs peut être facilitée par des différences alimentaires; nous avons donc analysé les contenus stomacaux de *Cambarincola chirocephalus* Ellis, 1919 et de *Cambarincola vitreus* Ellis, 1919, deux espèces de branchiobdellidés trouvées à l'occasion sur le même hôte (l'écrevisse *Orconectes virilis* (Hagen, 1870) dans l'Ouest canadien) afin de vérifier l'existence d'une partition des niches. Nous avons aussi examiné les contenus des tubes digestifs des adultes et des jeunes de la même espèce afin de voir si le régime alimentaire varie avec l'âge. Nous avons trouvé une gamme de diatomées et d'invertébrés, particulièrement des rotifères, dans les tubes digestifs des deux espèces de *Cambarincola*. Les espèces semblent avoir des régimes différents dans la mesure où les tubes digestifs de *C. chirocephalus* contiennent plus de matériel d'invertébrés que ceux de *C. vitreus*. Il n'y a pas de différence statistique de composition des contenus stomacaux entre les adultes et les jeunes de même espèce. Nous trouvons peu d'indications d'une expansion de niche alimentaire chez *C. vitreus* en l'absence de *C. chirocephalus*. Les différences alimentaires observées entre les espèces peuvent favoriser leur coexistence prolongée dans les régions où leurs répartitions géographiques se recoupent.

[Traduit par la Rédaction]

Introduction

In host–symbiont relationships, host organisms can be viewed as a “landscape” on and in which communities of symbionts interact. General principles relevant to community ecology can also be applied to these systems, including competition and niche theory (Poulin 2007). Although competition is often assumed to act most strongly among closely related taxa, this is not invariably supported when diet or behaviour are closely examined (Dayan and Simberloff 2005). Here we explore whether two congeneric species of crayfish worms that share the same host display evidence of dietary

niche differentiation. We also compare diets between juvenile and adult worms of the same species to test for evidence of age-specific feeding niches (Polis 1984).

Crayfish worms (Annelida: Clitellata: Branchiobdellidae) are obligate ectosymbionts of crustaceans, primarily crayfish (Gelder 1999). There are relatively few ecological studies of branchiobdellidans, with most previous work having focused on systematics, taxonomy, and distribution. Early ecological work investigated general life-history characteristics (McManus 1960; Young 1966; Bishop 1968), but more recent studies have focused on the relationship of crayfish worms with their hosts, exploring whether the worms are parasitic,

Received 10 August 2010. Accepted 11 January 2011. Published at www.nrcresearchpress.com/cjz on 12 April 2011.

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mutualistic, or commensal (Keller 1982; Brown et al. 2002; Lee et al. 2009). Ecological relationships within and among branchiobdellidan species have not received much attention.

Depending on its species and geographic location, an individual crayfish can host hundreds of branchiobdellidans comprising up to eight species from five different genera (Young 1966; Gelder 1996). Because reproduction and growth of the symbionts occurs on the host, both adult and juvenile crayfish worms are frequently present together (Young 1966). Co-occurring branchiobdellidans could be in competition if a common resource such as space or food is limiting. Coexistence of competitors, including symbionts, is generally promoted by some degree of niche partitioning involving differential exploitation of resources (Polis 1984; Friggens and Brown 2005; Nieto-Castañeda and Jiménez-Jiménez 2009). For crayfish worms, this could most likely be accomplished by occupying different microhabitats on a host or by exploiting different types of food.

Aside from determining if the worms are consuming host tissue (e.g., Bishop 1968), the diets of branchiobdellidans have not been well studied. General observations indicate that most crayfish worms are opportunistic omnivores, scraping material from the surface of the crayfish and capturing small invertebrates that pass within their reach (Holt 1973; Jennings and Gelder 1979; Govedich et al. 2009). Branchiobdellidans may also ingest pieces of their host's food or of host tissue, especially when an injury to the host occurs (Jennings and Gelder 1979; Govedich et al. 2009). Branchiobdellidan guts have been observed to contain diatoms and other algae, bacteria, protozoans, nematodes, rotifers, copepods, and other small arthropods (Jennings and Gelder 1979; Govedich et al. 2009), but the relative contribution of each of these food types has not been determined for any species.

It has been suggested that different species of crayfish worms may show differences in feeding tendencies (Govedich et al. 2009; Lee et al. 2009), but this has not been well studied. Several characteristics of branchiobdellidans could promote interspecific dietary variation. The worms' sclerotized jaws are used for manipulating food and often differ morphologically among species; this could reflect different adaptations for food collection (e.g., scraping, tearing), or specialization on different types of food items. There are also body size differences among species and age classes that could influence diet. Smaller branchiobdellidans, either juveniles or small heterospecifics, are likely to have smaller mouths and reduced gape size compared with their larger counterparts. Although this may not affect their ability to scrape diatoms or biofilms from their host's surface, it could potentially limit the size or type of invertebrates that they are able to ingest whole (Lima and Moreira 1993).

In the prairie provinces of Canada, two species of branchiobdellidan are found on the northern, or virile, crayfish (*Orconectes virilis* (Hagen, 1870)) (Williams et al. 2009). *Cambarincola vitreus* Ellis, 1919 occurs from central Alberta through southern Saskatchewan and Manitoba, whereas the larger *Cambarincola chirocephalus* Ellis, 1919 has only been found in southern Saskatchewan and Manitoba (Williams et al. 2009). In areas where the ranges of the two species overlap, both can be found on the same individual crayfish (Williams et al. 2009). *Cambarincola chirocephalus* and *C. vitreus* inhabit a variety of microhabitats on their

hosts' bodies and are particularly common around the mouth and subrostral area (Young 1966; Bishop 1968; present study). The two species do not appear to be spatially segregated, so it is possible that the worms are exploiting different food resources in a way that reduces interspecific competition. This is suggested both by differences in jaw morphology (Ellis 1920) and the larger mouth and body size of *C. chirocephalus*.

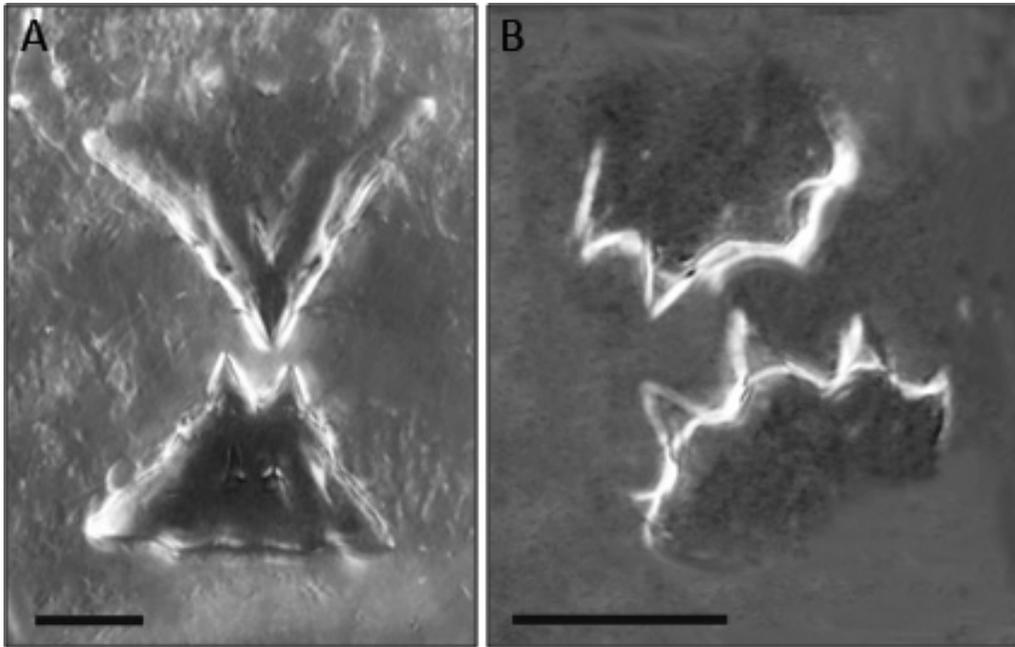
Here we examine intra- and inter-specific variation in diets of these two species from different geographic locations. Particularly strong evidence of niche partitioning would be a broader diet in *C. vitreus* from Alberta, where it is the sole species on *O. virilis*, than in Saskatchewan, where it shares the host with *C. chirocephalus* (i.e., evidence of character displacement) (Bolnick et al. 2010). In addition, to assess if diet is similar through a species' life history, we compared the gut contents of conspecific juvenile and adult *C. chirocephalus* and *C. vitreus* from two sites in Saskatchewan.

Materials and methods

Worm collection

We examined *C. chirocephalus* and *C. vitreus* inhabiting the crayfish *O. virilis* from three localities in Alberta and Saskatchewan. We collected crayfish via hand-held nets in October 2009 from Whitemud Creek off the North Saskatchewan River in Edmonton, Alberta (53°30'9.01"N, 113°33'41.06"W). We also examined ethanol-preserved *C. chirocephalus* and *C. vitreus* from crayfish that had been collected via hand-held nets and minnow traps baited with salmon in July 2008 from the Assiniboine River (51°47'32.45"N, 102°24'28.11"W) and the Qu'Appelle River (50°48'15.81"N, 104°34'50.06"W) in Saskatchewan. Crayfish from Whitemud hosted only *C. vitreus*, but all crayfish from Assiniboine and Qu'Appelle had both *C. chirocephalus* and *C. vitreus* present. The two species were easily differentiated based on overall body shape and jaw structure (Ellis 1920; Figs. 1A, 1B).

Large-bodied ("adult", see below) *C. vitreus* were removed from nine live crayfish from Whitemud under a dissecting scope and preserved immediately in 95% ethanol. Previously preserved adult crayfish worms and small-bodied "juvenile" worms were collected from up to 10 crayfish from each Saskatchewan site (with the exception of juvenile *C. vitreus* from Assiniboine), with no more than 11 worms of each age class and species collected from any individual host. Age classes were differentiated by relative size: within a sample from an individual crayfish, the largest were chosen as adults and the smallest as juveniles. Ideally, age classes would be identified based on presence of a clitellum in adults (Moore 1895), but no clitella could be identified in the specimens we examined. To determine the distinctiveness of these size classes, we measured the body length and mean jaw width of a subsample of mounted worms (7–18 adults and juveniles of each species from each site) using imaging program ImageJ (version 1.43u). Jaw width was measured at the widest part of each jaw, and was averaged between the top and bottom jaws for an individual (see Figs. 1A, 1B). Within a site, adults of both *C. chirocephalus* and *C. vitreus* were significantly longer than conspecific juveniles (Student's *t* test, $p > 0.0001$; JMP version 9.0.0, SAS Institute Inc., Cary, North

Fig. 1. Jaw structures of (A) *Cambarincola chirocephalus* and (B) *Cambarincola vitreus*. Scale bars = 25 μ m.**Table 1.** Comparison of body length and jaw width of adult and juvenile *Cambarincola vitreus* and *Cambarincola chirocephalus*.

	Stage	Body length (mm)			Jaw width (μ m)		
		Mean \pm SE	Range	<i>p</i>	Mean \pm SE	Range	<i>p</i>
Assiniboine							
<i>C. vitreus</i>	Adults (<i>n</i> = 17)	2.128 \pm 0.061	1.805–2.747	<0.0001	31.3 \pm 0.7	28.0–38.0	0.6311
	Juveniles (<i>n</i> = 15)	1.114 \pm 0.028	1.007–1.114		32.0 \pm 1.2	21.5–37.0	
<i>C. chirocephalus</i>	Adults (<i>n</i> = 18)	2.164 \pm 0.076	1.626–2.764	<0.0001	69.5 \pm 3.2	51.5–96.0	<0.0001
	Juveniles (<i>n</i> = 16)	1.360 \pm 0.040	1.139–1.610		43.0 \pm 1.3	33.5–53.5	
Qu'Appelle							
<i>C. chirocephalus</i>	Adults (<i>n</i> = 14)	2.304 \pm 0.126	1.827–3.440	<0.0001	76.6 \pm 2.6	61.5–96.5	<0.0001
	Juveniles (<i>n</i> = 7)	0.811 \pm 0.042	0.645–0.945		35.4 \pm 2.4	25.0–44.5	

Note: The *p* values indicate the results of the Student's *t* test between conspecific adults and juveniles.

Carolina, USA; Table 1). Adult *C. chirocephalus* had wider jaws than juveniles at each site ($p > 0.0001$), but the jaws of adult and juvenile *C. vitreus* were of similar size ($p = 0.6311$). We believe that the significant and nonoverlapping differences in body length are sufficient to differentiate adult and juvenile worms of both species, as little is known about the growth rates of branchiobdellidan jaws in relation to body size.

All crayfish worms were preserved in 95% ethanol, cleared using 85% lactic acid, permanently mounted on slides with PVA (lactic acid, phenol, and polyvinyl alcohol; BioQuip Products, Rancho Dominguez, California, USA), and placed on a slide warmer at 42 $^{\circ}$ C for at least 3 days to cure. In total 593 branchiobdellidans were mounted: 149 adult *C. vitreus* from Whitemud Creek; 106 *C. chirocephalus* (77 adults, 29 juveniles) and 127 *C. vitreus* (96 adults, 31 juveniles) from Assiniboine; and 111 *C. chirocephalus* (96 adults, 15 juveniles) and 100 adult *C. vitreus* from Qu'Appelle (summarized in Table 2).

Gut content analysis

Mounted, cleared worms were inspected using a Leica DMLB compound microscope with differential interference contrast illumination (DIC) at 40 \times magnification. Photographs of gut contents were taken using a Canon Powershot S40 digital camera and imaging program Remote Capture (Canon Utilities). Gut contents were often not readily interpretable in photos taken at a single plane of focus. Therefore, several photos were taken at different levels of focus and combined using auto-blend in Adobe Photoshop CS4 Extended version 11.0.1 (Adobe Systems Inc., San Jose, California, USA).

Because the lactic acid and PVA completely digested some of the gut contents, we mostly observed items that were at least partially resistant to clearing. Worms were first scored for the presence of visible and identifiable gut contents, most of which were rotifers and diatoms. Rotifers were

Table 2. Summary of gut contents of adult and juvenile *Cambarincola chirocephalus* and *Cambarincola vitreus* from the Assiniboine River, Qu'Appelle River, and Whitemud Creek.

	<i>C. chirocephalus</i>						<i>C. vitreus</i>						Total
	Assiniboine			Qu'Appelle			Assiniboine			Qu'Appelle (adult)	Whitemud (adult)		
	Adult	Juvenile	All	Adult	Juvenile	All	Adult	Juvenile	All				
No. of worms examined	77	29	106	96	15	111	96	31	127	100	149	593	
No. of worms with gut contents (%)	71 (92.2)	27 (93.1)	98 (92.5)	38 (39.6)	7 (46.7)	45 (40.5)	59 (61.5)	24 (77.4)	83 (65.4)	14 (14.0)	90 (60.4)	330 (55.6)	
Diatoms (class Bacillariophyceae)	66 (93.0)	24 (88.9)	90 (91.8)	12 (31.6)	5 (71.4)	17 (37.8)	57 (96.6)	24 (100)	81 (97.6)	11 (78.6)	82 (91.1)	281 (85.2)	
<i>Amphora</i> Ehrenberg ex. Kützing	9 (12.7)	3 (11.1)	12 (12.2)	—	—	—	2 (3.4)	—	2 (2.4)	—	7 (7.8)	21 (6.4)	
<i>Cocconeis</i> Ehrenberg	47 (66.2)	13 (48.1)	60 (61.2)	—	—	—	31 (52.5)	7 (29.2)	38 (45.8)	—	4 (4.4)	102 (30.9)	
<i>Cymbella</i> C.A. Agardh	25 (35.2)	8 (29.6)	33 (33.7)	—	—	—	17 (28.8)	5 (20.8)	22 (26.5)	—	3 (3.3)	58 (17.6)	
<i>Diatoma</i> Bory	22 (31.0)	12 (44.4)	34 (34.7)	—	1 (14.3)	1 (2.2)	16 (27.1)	1 (4.2)	17 (20.5)	—	2 (2.2)	54 (16.4)	
<i>Gyrosigma</i> Hass.	—	1 (3.7)	1 (1.0)	—	—	—	1 (1.7)	—	1 (1.2)	—	35 (38.9)	37 (11.2)	
<i>Navicula</i> Bory	45 (63.4)	20 (74.1)	65 (66.3)	8 (21.1)	3 (42.9)	11 (24.4)	32 (54.2)	18 (75.0)	50 (60.2)	5 (35.7)	64 (71.1)	195 (59.1)	
<i>Neidium</i> Pfitzer	3 (4.2)	—	3 (3.1)	—	—	—	1 (1.7)	1 (4.2)	2 (2.4)	—	1 (1.1)	6 (1.8)	
<i>Nitzschia</i> Hass.	13 (18.3)	5 (18.5)	18 (18.4)	2 (5.3)	—	2 (4.4)	10 (16.9)	3 (12.5)	13 (15.7)	3 (21.4)	21 (23.3)	57 (17.3)	
<i>Rhicosphenia</i> Grunow	16 (22.5)	4 (14.8)	20 (20.4)	1 (2.6)	—	1 (2.2)	9 (15.3)	3 (12.5)	12 (14.5)	—	1 (1.1)	34 (10.3)	
<i>Stephanodiscus</i> Ehrenberg	12 (16.9)	1 (3.7)	13 (13.3)	6 (15.8)	—	6 (13.3)	1 (1.7)	1 (4.2)	2 (2.4)	4 (28.6)	10 (11.1)	35 (10.6)	
<i>Synedra</i> Ehrenberg	5 (7.0)	—	5 (5.1)	—	1 (14.3)	1 (2.2)	4 (6.8)	3 (12.5)	7 (8.4)	1 (7.1)	4 (4.4)	18 (5.5)	
<i>Tryblionella</i> W. Sm.	1 (1.4)	—	1 (1.0)	—	—	—	—	—	—	—	1 (1.1)	2 (0.6)	
Invertebrates	44 (62.0)	20 (74.1)	64 (65.3)	29 (76.3)	5 (71.4)	34 (75.6)	19 (32.2)	7 (29.2)	26 (31.3)	6 (42.9)	33 (36.7)	163 (49.4)	
Phylum Rotifera	42 (59.2)	18 (66.7)	60 (61.2)	26 (68.4)	5 (71.4)	31 (68.9)	18 (30.5)	7 (29.2)	25 (30.1)	6 (42.9)	27 (30.0)	149 (45.2)	
Order Bdelloidea	14 (19.7)	4 (14.8)	18 (18.4)	8 (21.1)	—	8 (17.8)	2 (3.4)	2 (8.3)	4 (4.8)	1 (7.1)	10 (11.1)	41 (12.4)	
Order Monogononta													
Suborder Ploima													
Family Notommatidae	15 (21.1)	8 (29.6)	23 (23.5)	13 (34.2)	5 (71.4)	18 (40.0)	9 (15.3)	2 (8.3)	11 (13.3)	4 (28.6)	7 (7.8)	63 (19.1)	
Family Dicranophoridae	6 (8.5)	1 (3.7)	7 (7.1)	1 (2.6)	—	1 (2.2)	2 (3.4)	—	2 (2.4)	—	2 (2.2)	12 (3.6)	
Family Lecanidae	3 (4.2)	4 (14.8)	7 (7.1)	—	—	—	—	1 (4.2)	1 (1.2)	—	—	8 (2.4)	
Family Lepadellidae	10 (14.1)	—	10 (10.2)	—	—	—	2 (3.4)	—	2 (2.4)	—	1 (1.1)	13 (3.9)	
“Loricata”	6 (8.5)	3 (11.1)	9 (9.2)	2 (5.3)	—	2 (4.4)	—	—	—	1 (7.1)	2 (2.2)	14 (4.2)	
“Illoricate”	5 (7.0)	4 (14.8)	9 (9.2)	5 (13.2)	—	5 (11.1)	4 (6.8)	1 (4.2)	5 (6.0)	—	1 (1.1)	20 (6.1)	
Suborder Flosculariacea	20 (28.2)	7 (25.9)	27 (27.6)	5 (13.2)	—	5 (11.1)	2 (3.4)	1 (4.2)	3 (3.6)	—	6 (6.7)	41 (12.4)	
Subclass Oligochaeta	1 (1.4)	1 (3.7)	2 (2.0)	—	—	—	—	—	—	—	11 (12.2)	13 (3.9)	
Family Chironomidae	3 (4.2)	2 (7.4)	5 (5.1)	2 (5.3)	—	2 (4.4)	1 (1.7)	—	1 (1.2)	—	—	8 (2.4)	
Order Branchiobdellida	—	—	—	2 (5.3)	—	2 (4.4)	—	—	—	—	—	2 (0.6)	

Note: Data for gut contents are numbers of worms with each item present; numbers in parentheses are the percentage of those with each item of those that have identifiable gut contents (i.e., prevalence). “Loricata” rotifers belong to either family Lepadellidae or family Mytilinidae. “Illoricate” rotifers belong to either family Epiphanidae or family Proalidae.

identified using trophi (mastax) structure to family or higher taxon using Donner (1956) and Wallace and Snell (2009). Most diatoms were identified to genus by Marianne Douglas (Department of Earth and Atmospheric Sciences, University of Alberta); some groups were identified using Wolle (1894) and Round et al. (1990).

We use “prevalence” to refer to the number of individual crayfish worms in which certain categories of gut items (see definition below) were found, and we use “proportion” and “composition” to refer to the relative amount of certain gut items in individual branchiobdellidans. Counts of each gut-item type (genus or higher taxon) were converted into a proportion of each individual worm’s diet. No biomass estimates were attempted. Prevalence, proportion, and composition values were calculated from worms with identifiable gut contents. After excluding worms that had no identifiable gut contents, a Bray–Curtis similarity matrix of individuals based on gut contents was created using PATN version 3.11 (Blattant Fabrications Pty Ltd., Carlton, Tasmania, Australia). Analysis of similarity (ANOSIM), a multivariate analogue of an ANOVA, was used for statistical comparison of gut content composition between different groups of worms (species, age classes, geographic locations). Intrinsic variables for the analyses included proportions of each gut-item type, diatom and invertebrate richness (number of taxa of each), and raw diatom and invertebrate counts. Gut items that most strongly contributed to differences in diets among groups were determined using SIMPER (PRIMER version 5.2.8; PRIMER-E Ltd., Ivybridge, United Kingdom). Prevalence (see definition below) of invertebrates among groups was compared with a χ^2 and post hoc Marascuilo test (http://amchang.net/StatTools/Multiprop_Pgm.php; accessed 21 July 2010).

Results

Gut-item prevalence

Of the 593 branchiobdellidans that were examined, 330 (55.6%) had gut contents that were both visible and identifiable, which we refer to as “gut items” (Table 2). These gut items may be food items (i.e., they may have provided some form of nutrition), but this distinction was beyond the scope of the present study. Nonetheless, we consider these gut items to be at least partially representative (see Discussion) of the diets of the worms examined. Many of the branchiobdellidans had amorphous, unidentifiable material in their guts. Identifiable gut items were more commonly observed in worms from Assiniboine than in those from Qu’Appelle, and in *C. chirocephalus* compared with *C. vitreus*. Examples of gut items found in both species of branchiobdellidan are shown in Figs. 2A–2D.

Diatoms, with twelve genera represented, were the most prevalent gut item, found in 281/330 (85.2%) of branchiobdellidans with identifiable gut contents (Table 2). Diatom richness was the same between worm species within a site, and was higher in those from Assiniboine and Whitemud (12 genera present) than in Qu’Appelle (6 genera). The genus *Gyrosigma* was common in Whitemud (35/90 guts), rare in Assiniboine (2/181), and absent in Qu’Appelle (0/59). The genus *Cocconeis* was common in Assiniboine (98/181 guts), rare in Whitemud (4/90), and absent in Qu’Appelle (0/59).

Invertebrate material was present in 163/330 (49.4%) of

branchiobdellidans that had identifiable gut contents, with rotifers (eight taxa) being the most common group of invertebrates (149/330). Other invertebrate material present included oligochaete setae (13/330), head capsules of chironomid larvae (8/330), and branchiobdellidan jaws (2/330: one each of *C. vitreus* and *C. chirocephalus*, both in the guts of *C. chirocephalus*). Invertebrate material was less prevalent in *C. vitreus* from Assiniboine and Whitemud than in *C. chirocephalus* from Assiniboine and Qu’Appelle (Fig. 3; Marascuilo procedure, $p < 0.002$). Within a location, invertebrate richness was higher in the guts of *C. chirocephalus* than in *C. vitreus* (Table 2; Assiniboine: 10 taxa in *C. chirocephalus* compared with 8 taxa in *C. vitreus*; Qu’Appelle: 8 compared with 3). Oligochaete setae were most common in *C. vitreus* in Whitemud (11/90 guts), rare in *C. chirocephalus* in Assiniboine (2/98), and absent in all other groups. Lepadellid rotifers were found in branchiobdellidans from Assiniboine and Whitemud but not Qu’Appelle, and lecanid rotifers were only found in Assiniboine.

Diet composition

Analysis of similarity (ANOSIM) was used to test for differences in diet composition among different categories of crayfish worms and different subsets of diet. There was no difference between diet composition of adult and juvenile conspecifics at any site (*C. vitreus* from Assiniboine, *C. chirocephalus* from Assiniboine, *C. chirocephalus* from Qu’Appelle: ANOSIM $p > 0.4$ for each comparison). Therefore for subsequent analyses, age categories were combined. Overall diet composition differed between *C. vitreus* and *C. chirocephalus* at both of the sites where they co-occurred: Assiniboine ($p < 0.0001$) and Qu’Appelle ($p = 0.011$) (Figs. 4A, 4B). An overall pairwise comparison among all five species and location categories found significant differences in diet composition among groups ($p < 0.05$ for all), except between *C. vitreus* in Qu’Appelle vs. in Whitemud ($p = 0.35$). The composition of diatoms in gut contents differed among all groups ($p < 0.05$), except between *C. chirocephalus* and *C. vitreus* from Qu’Appelle ($p = 0.77$). Composition of invertebrate material differed between the two species in Assiniboine ($p = 0.005$), but not in Qu’Appelle ($p = 0.23$). Invertebrate composition differed between Assiniboine and Qu’Appelle for *C. chirocephalus* ($p = 0.003$), as well as between Assiniboine and Whitemud for *C. vitreus* ($p < 0.0001$); that of *C. vitreus* in Qu’Appelle did not differ from that of any other group ($p > 0.05$ for all). Results of the SIMPER analysis indicated that 80% of the difference in diets among branchiobdellidan groups could be explained by 10 gut items: the diatoms *Cocconeis*, *Cymbella*, *Diatoma*, *Gyrosigma*, *Navicula*, *Nitzschia*, *Stephandiscus*; notommatid and bdelloid rotifers; and oligochaetes.

Discussion

We observed some evidence of differentiation in diet between *C. chirocephalus* and *C. vitreus*, with the former having a higher proportion of invertebrate material in their guts; however, there was very little indication of this being due to competitive character displacement. *Cambarincola vitreus* in the absence of *C. chirocephalus* (i.e., in the Whitemud population) had almost the same dietary composition as

Fig. 2. Examples of gut items found in *Cambarincola chirocephalus* and *Cambarincola vitreus*: (A) *Cocconeis* sp. (class Bacillariophyceae); (B) *Diatoma* sp. (Bacillariophyceae); (C) trophi of Notommatidae (phylum Rotifera); (D) trophi of Bdelloidea (Rotifera). Scale bars = 10 μ m.

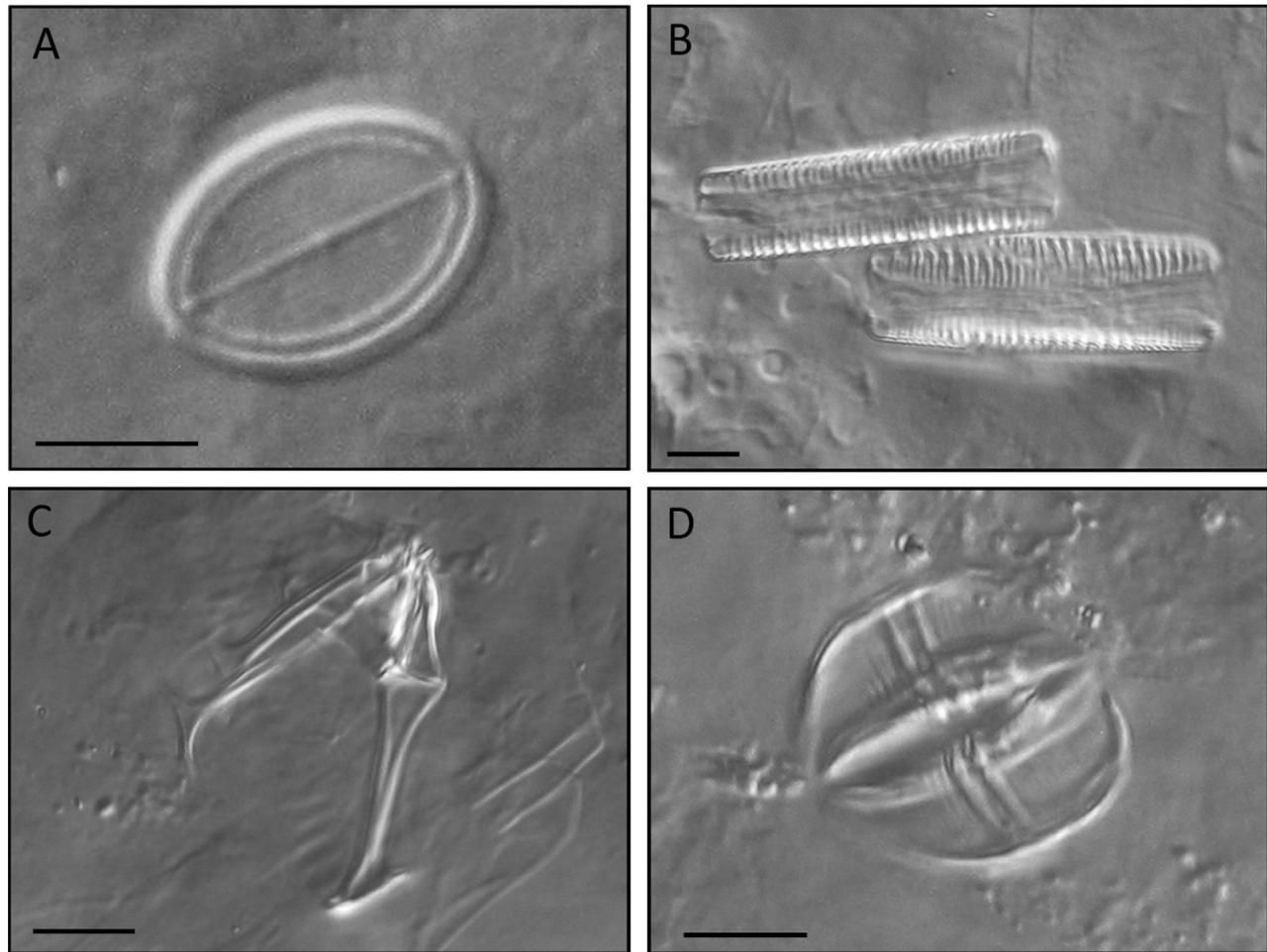
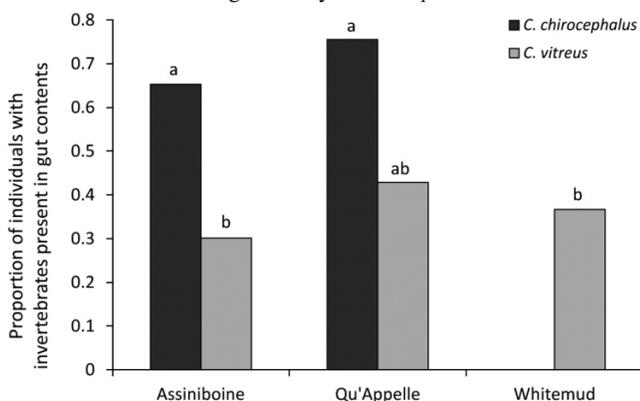


Fig. 3. Proportion of individuals of *Cambarincola chirocephalus* and *Cambarincola vitreus* having at least one invertebrate item present in their guts. Worms lacking identifiable gut contents were excluded. Small letters indicate Marascuilo test results; bars that have the same letter do not significantly differ at $p < 0.05$.

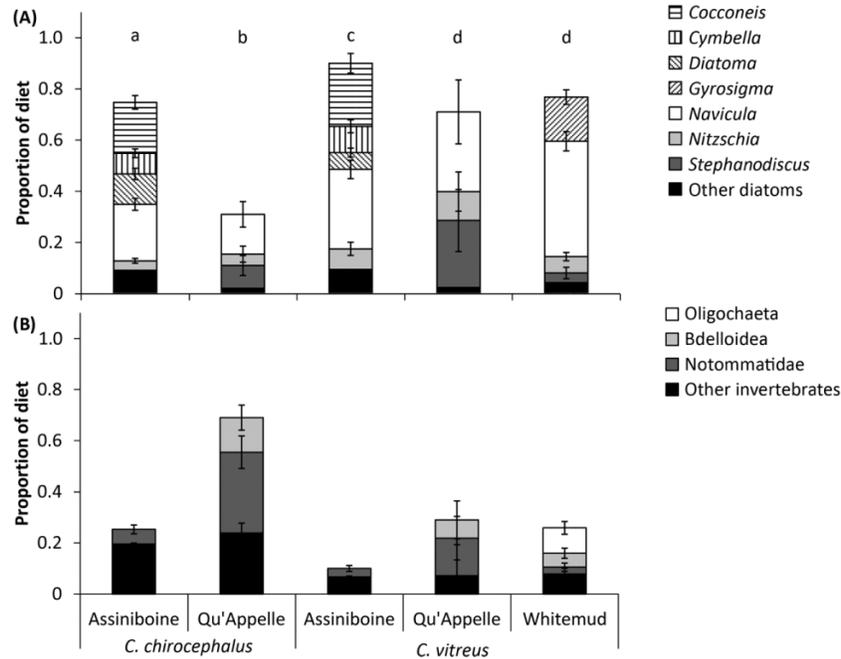


when co-occurring with *C. chirocephalus* at the two Saskatchewan sites (Figs. 4A, 4B). The unusual prevalence of oligochaetes in the diets of *C. vitreus* from Whitemud seems to be the only indication of possible niche expansion. Oligochaetes were never found in *C. vitreus* from either Saskatch-

ewan site, but were present in a small proportion (2.0%) of the guts of *C. chirocephalus* from Assiniboine. The presence of oligochaetes in the diets of *C. vitreus* from Whitemud could indicate an increase in dietary breadth in the absence of the more predatory *C. chirocephalus*, or it could be a simple reflection of availability (i.e., that there were simply more oligochaetes at Whitemud). We found no evidence of diet differences in juveniles and adults of the same species.

Our results support previous work indicating that most branchiobdellidans are omnivorous, ingesting both algae and invertebrates. The greater number of invertebrates in guts of *C. chirocephalus* could be related to differences in morphology between the two branchiobdellidan species. The two jaws of *C. chirocephalus* differ in size and shape, with the upper jaw having one major tooth that occludes with the smaller teeth on the lower jaw, whereas the two jaws of *C. vitreus* are both multitoothed and similarly sized (Figs. 1A, 1B; Ellis 1920). *Cambarincola chirocephalus* also has a large head with lobate lips that can be extended into tentacles, whereas the lips of *C. vitreus* do not form such lobes (Ellis 1920). It is possible that the mouthparts of *C. chirocephalus* are adaptations for capturing and manipulating invertebrate prey; however, assessment of food-handling mechanisms would require observations of live crayfish worms of different species. Juvenile branchiobdellidans likely have smaller mouths and

Fig. 4. Composition of (A) diatoms and (B) invertebrates in the guts of *Cambarincola chirocephalus* ($n_{\text{Assiniboine}} = 98$; $n_{\text{Qu'Appelle}} = 45$) and *Cambarincola vitreus* ($n_{\text{Assiniboine}} = 83$; $n_{\text{Qu'Appelle}} = 14$; $n_{\text{Whitemud}} = 90$). Each section of a bar represents the proportion of those worms' diets composed of each gut item, averaged within groups. Error bars are SE. Worms without identifiable gut items are excluded. Only diet items that contributed to more than 5% of the difference in diets among groups (as per a SIMPER analysis) are shown; the remaining items are pooled in either "other diatoms" or "other invertebrates". Lowercase letters indicate the analysis of similarity (ANOSIM) results for overall diet composition; bars that have the same letter do not significantly differ at $p < 0.05$.



are presumably more gape-limited than adults (although their jaw structures may be similarly sized; see Materials and methods); however, we observed that conspecific adult and juvenile worms ingest similar amounts of invertebrate material (Table 2). Because our methods did not allow us to determine original body size of most consumed invertebrates, we cannot exclude the possibility of some dietary differentiation based on item size.

Our methods of cleaning and mounting revealed more invertebrate material in the guts of *C. chirocephalus* than in those of *C. vitreus* (Fig. 3); however, branchiobdellidans can also pierce holes in large prey and suck out tissues (Govedich et al. 2009), and the resultant soft material would not have been detected in this study. It is therefore possible that invertebrates are more common in the diets of either species of crayfish worm than we observed. Such feeding behaviours could potentially account for the low proportion of identifiable gut contents present in both species from Qu'Appelle (40.5% of *C. chirocephalus*, 14% of *C. vitreus*), as well as the frequent presence of amorphous material in the guts of worms from all sites. Similar unidentifiable material in branchiobdellidan guts has been interpreted as host hemolymph from gill tissue (Moore 1895; Holt 1973; Govedich et al. 2009), but this seems unlikely for the two species we studied. Bishop (1968) did not find *C. chirocephalus* in the gill chambers of its hosts in Ontario, and found no evidence of ingestion of host tissue using serological techniques. *Cambarincola vitreus* has not been observed on the gills of *O. virilis* from the North Saskatchewan River in Edmonton (J. Gagnon, unpublished data (undergraduate thesis, University of Alberta)). As suggested by Jennings and Gelder

(1979), we think it most likely that the amorphous material in the guts of *C. chirocephalus* and *C. vitreus* represents a mixture of detrital material, pieces of the crayfish's food, and possibly the soft tissues of invertebrates.

In conclusion, we found that these two congeneric species of crayfish worms have different diets, with individuals of *C. chirocephalus* having a greater proportion of invertebrates in their guts than did individuals of *C. vitreus*. Given the lack of evidence of intraspecific dietary differentiation between small juvenile and large adult worms, we feel that this intraspecific variation is not solely due to differences in body size but could be related to mouthpart morphology. There was very limited evidence of niche expansion in *C. vitreus* that occurred outside the range of *C. chirocephalus*, but we do not find this convincing enough to imply that competition strongly influences diet choice when the species do coexist. Instead, intrinsic dietary differences between species may promote their continued coexistence in areas where they share hosts. Past competition may have selected for these differences, but this cannot be easily tested (see Connell 1980). It is also possible that food is not an important limiting factor for these branchiobdellidans, e.g., if population size on a crayfish is maintained below carrying capacity by parasitism, predation, disease, or accidents (Strong 1982). There was a great deal of site-associated variation in observed diet within each species, suggesting that these worms are mainly opportunistic feeders. It would be interesting to compare the diversity of potential food items on the carapace of the host crayfish to the gut contents of the branchiobdellidans to determine if the worms graze randomly or if they select a subset of available items. Observations of interactions among

live crayfish worms and manipulative experiments will help to resolve the nature of relationships among these and other species of coexisting branchiobdellidans.

Acknowledgements

We are indebted to Bronwyn Williams (Department of Biological Sciences, University of Alberta) for having collected and identified the Saskatchewan branchiobdellidans, and for providing literature and helpful advice. We also thank Marianne Douglas (Department of Earth and Atmospheric Sciences, University of Alberta) for identifying the diatoms in this study, as well as two reviewers for helpful comments on an earlier version of the manuscript. This research was supported by a Natural Sciences and Engineering Research Council of Canada Discovery Grant to H.C.P.

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