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Hepatopancreatic endosymbionts in coastal isopods (Crustacea: Isopoda), and their contribution to digestion

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Abstract Three isopod species (Crustacea: Isopoda), commonly found in the intertidal and supratidal zones of the North American Pacific coast, were studied with respect to symbiotic microbiota in their midgut glands (hepatopancreas). *Ligia pallasii* (Oniscidea: Ligiidae) contained high numbers of microbial symbionts in its hepatopancreatic caeca. Numbers of endosymbionts were strongly reduced by ingestion of antibiotics. By contrast, the hepatopancreas of *Idotea wosnesenskii* (Valvifera: Idoteidae) and *Gnorimosphaeroma oregonense* (Sphaeromatidea: Sphaeromatidae) did not contain any microbiota. Results of feeding experiments suggest that microbial endosymbionts contribute to digestive processes in *L. pallasii*, the most terrestrial of the three isopods that we studied. The acquisition of digestion-enhancing endosymbionts may have been an important evolutionary step allowing isopods to colonize terrestrial habitats where relatively indigestible leaf litter is the primary food source. By contrast, the ability to digest phenolic compounds was most developed in one of the more marine species, suggesting that this trait may have evolved independently in isopod species that consume a phenolic-rich diet, whether in marine habitats or on land.

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Introduction

Endosymbionts are well known to play a key role in the digestive processes of many terrestrial species (summarized in Martin 1983; Slaytor 1992; Breznak and Brune 1994); however, their role in marine invertebrate species is poorly understood. While studies have shown that gut microbiota exist in some marine invertebrates, knowledge of their nutritional role is scanty (cf. Vitalis et al. 1988). At present it is unclear if the limited information on endosymbionts of marine species reflects the unimportance of endosymbionts in these systems, or simply a lack of attention.

The order Isopoda (Peracarida: Crustacea) originated in marine habitats, but has radiated to include many freshwater and terrestrial species. While many marine species feed on algae, terrestrial isopods (Isopoda: Oniscidea Latreille, 1829) mostly consume leaf litter. Thus, with terrestrialization, isopod diets changed from macroalgae, with comparatively high nitrogen and low lignin and cellulose contents (Kennish and Williams 1997; Olivier et al. 1996), to leaf litter with the opposite features. In particular, feeding on leaf litter requires that isopods are capable of both digesting cellulose and tolerating various phenolic compounds present in leaf litter. In some species of terrestrial isopods, the digestive midgut glands (hepatopancreas) contain abundant symbiotic bacteria (Donadey and Besse 1972; Hopkin and Martin 1982; Wood and Griffiths 1988; Hames and Hopkin 1989) that appear to contribute to the digestion of lignocellulose and phenolic compounds of the food (Zimmer and Topp 1998a, b; Zimmer 1999), and thus, may have facilitated the colonization of terrestrial habitats by isopods (cf. Zimmer and Topp 1998a) whose

ancestors inhabited marine habitats. Comparing marine and terrestrial isopods could therefore be very valuable in shedding light both on the importance of endosymbionts in digestion, and on the colonization of land by marine arthropods.

Whether marine isopods contain midgut symbionts is not known, although the prototypal land colonizers *Ligia* spp. are known to host numerous bacteria (Carefoot 1993) and symbiotic fungi (Lichtwardt 1986) in their hindguts that have as yet unknown roles in digestion (cf. Carefoot 1993). To address this gap in our knowledge, we studied three isopod species from the northwest coast of North America, with partly overlapping small-scale distribution.

According to recent studies on the evolution of terrestrial isopods, the isopodan suborders Sphaeromatidea Wägele, 1989 (''Flabellifera'' Sars, 1882) and Valvifera Sars, 1882 are probably linked to terrestrial isopods as parts of a monophyletic sister group sharing a common marine ancestor with the monophyletic Oniscidea (Schmalfuss 1989; Wägele 1989; Brusca and Wilson 1991). Hence, representatives of Valvifera and Sphaeromatidea may serve as valuable models in comparing the eco-physiological capabilities of terrestrial isopods with those of their marine relatives. We studied a single species from each of these three isopodan suborders.

The genus *Ligia* (Oniscidea: Ligiidae Brandt, 1833) represents a prototypal terrestrial isopod (Schmalfuss 1978, 1989; Carefoot 1993; Carefoot and Taylor 1995) with respect to eco-physiological, morphological and behavioral adaptations to terrestrial habitats. These semi-terrestrial isopods inhabit a narrow zone just above the tide line. By feeding primarily on the litter of kelp and other seaweeds (Pennings et al. 2000), *Ligia* species resemble their marine ancestors with respect to nutrition, while more terrestrial isopod species have shifted evolutionarily towards feeding on the litter of terrestrial plants. The eco-physiology of *Ligia pallasii* Brandt, 1833, the oniscidean species used in the present study, has been investigated thoroughly by Carefoot (e.g. 1973a, b, 1984a, b, 1987; Carefoot et al. 2000).

The family Idoteidae (Isopoda: Valvifera) includes marine-intertidal species living on macroalgae. *Idotea* (*Pentidotea*) *wosnesenskii* (Brandt, 1851) is a valviferan that inhabits the rocky intertidal zone along the North American Pacific coast (Morris et al. 1980). It lives on various intertidal macroalgae, such as *Fucus* spp., and grazes on their surfaces (Morris et al. 1980). The color of individual isopods often matches the color of the algae upon which they are found (cf. Kozloff 1996). At our study site, we found *I. wosnesenskii* mainly in *Fucus* forests, where it clung to seaweed blades or hid under rocks during low tide (authors' observations). Little is known about the ecology of *I. wosnesenskii*.

The third study species, the sphaeromatid *Gnorimosphaeroma oregonense* (Dana, 1855), is a phytophagous high-level inhabitant of the marine intertidal zone in the Pacific northwest, favoring under-rock habitats in quiet-water regions. Little information is available on its feeding ecology (cf. Brooks 1994).

The present study focused on a microbiological comparison of the guts of these three phylogenetically related isopod species as a first approach to understanding nutritional aspects associated with the successful colonization of land by isopod crustaceans.

Materials and methods

Collection and care of animals

All field and most laboratory work was conducted at the Bamfield Marine Station (BMS) (Vancouver Island, B.C., Canada), or on nearby beaches and small islands, in July 1998. The determination of C:N ratios of algae and isopod feces was performed at the University of British Columbia (Vancouver, B.C., Canada).

Ligia pallasii was collected by hand from a cobble beach on Seppings Island. Adults of 21–25 mm body length (132 ± 21 mg dry mass) were chosen for the experiments. *Idotea wosnesenskii* was collected from *Fucus* sp. growing on rocky intertidal shores of Wizard Island. Large-sized adults of 16–19 mm length (78 ± 12 mg dry mass) proved to be solely females. Thus, we did not compare males and females of this species. *Gnorimosphaeroma oregonense* (Isopoda: Sphaeromatidea) were collected from under large stones at Grappler Inlet. Mature, non-gravid females and immature males (cf. Brooks et al. 1994) with a size of 5–7 mm (7.9 ± 0.8 mg dry mass) were used for experiments, due to their relatively high consumption rates (Brooks 1994).

In all feeding trials isopods were kept individually (*L. pallasii*), or in groups of five individuals (*I. wosnesenskii*) in circular 200 ml plastic containers with screw-fitted lids, or in groups of five individuals in small Petri dishes (*G. oregonense*). Each assay was performed with nine replicates. Due to their physiological requirements (W. Pope, unpublished BMS report), we moved individuals of *I. wosnesenskii* and *G. oregonense* back and forth between seawater-filled containers and dry containers every 6 h to simulate tidal conditions.

Microbial counts

To determine numbers of hepatopancreatic microbiota, we dissected the hepatopancreas out of individual isopods, homogenized it in 1 ml of pepton (0.1% in H_2O), and stained the material with 1 ml of an aqueous phenol-alinine solution (Parkinson et al. 1971). After a 1 h incubation, the extract was filtered (0.1 μm), and microorganisms were counted under a microscope.

To compare the numbers of microbiota in the hepatopancreas of animals feeding on their natural diet in the field with animals feeding on an artificial diet in the laboratory, we maintained some isopods on seaweed and some on an artificial diet (Zimmer 1999, modified after Carefoot 1984a, b) mixed with seawater to meet the ionic requirements of marine isopods, for 5 days. To determine the effects of antibiotics on endosymbionts, a 1% (dry mass) antibiotics mixture (equal parts of penicillin, streptomycin and amphotericin) was added to the artificial diet or to the fresh seaweed by soaking the plants in a 1% solution for 24 h. These antibiotics have previously been used to reduce the number of hepatopancreatic bacteria in the terrestrial isopod *Porcellio scaber* (Zimmer 1999; Zimmer and Topp 1998a, b). A similar mixture reduced the number of platable hindgut bacteria in *L. pallasii* to about 0.1% (Carefoot, unpublished results). As has been demonstrated in *P. scaber*, feeding on artificial diet resulted in strongly reduced microbial counts in the hindgut, while bacteria in the hepatopancreas were not affected by this food source (Zimmer and Topp 1998a; Zimmer 1999). Thus, we did not examine microbiota inside the hindgut, although these microorganisms may be important with respect to

nutrition (e.g. Hassall and Jennings 1975; Kaplan and Hartenstein 1978; Carefoot 1984a, b, 1993; Zimmer and Topp 1997, 1998a; Zimmer 1999).

Consumption and digestibility

We measured consumption and digestibility (relative consumption rate, RCR, and approximate digestibility, AD, after Waldbauer 1968) of different diets in a series of feeding experiments. We conducted experiments using a high-preference food for each species, and using a single common food to facilitate comparisons. Based on our knowledge of food preferences of each isopod, we fed *L. pallasii* with *Nereocystis luetkeana* (henceforth *Nereocystis*) (cf. Carefoot 1973a; Pennings et al. 2000), *G. oregonense* with *Ulva lactuca* (henceforth *Ulva*) (Brooks 1994), and *I. wosnesenskii* with *Fucus* cf. *gardneri* (henceforth *Fucus*) (authors' observations). Due to Schaffelke et al. (1995) and our own observations on feeding behavior of *I. wosnesenskii*, only apical parts of the blades of *Fucus* were offered in our experiments. As a common food, we offered *Macrocystis integrifolia* (henceforth *Macrocystis*) to all three species.

To test for the contribution of endosymbionts, isopods were pretreated with either artificial diet or artificial diet containing antibiotics. Isopods were fed pretreatment diets for a period that lasted 2 days after the first appearance of feces derived solely from artificial diet. By this, we made sure that the guts of isopods did not contain remnants of natural food. Feces samples were collected by hand in the case of *L. pallasii*, and via filtering the seawater from the experimental containers through glass microfiber filters (Whatman 934-AH) for *I. wosnesenskii* and *G. oregonense*. We compensated the dry weights of filtered feces for the salt loads of the filtered volume of seawater.

Oven-dried (60 °C, 24 h) feces and food remnants were used for the calculation of consumption indices. To calculate the dry weight of fresh diet at the beginning of the trials, we determined a fresh mass:dry mass regression ($n = 15$) for each food source. In controls without isopods to estimate the autogenic change in dry weight of the food sources, pre- and post-experimental samples did not differ significantly with respect to their weight. Thus, we considered autogenic weight changes to be negligible.

Chemical analyses

Seaweed and isopod feces were characterized by the carbon content and the nitrogen content in order to obtain information on the C:N ratio, which has been used in many nutritional studies to estimate food quality and chemical changes in the food material during digestion (cf. White 1993). Further, the phenolic content was determined, since phenolics are known to affect nutrition in many marine herbivores (Valiela and Rietsma 1984; Rietsma et al. 1988; Bärlocher and Newell 1994).

Carbon and nitrogen content were measured in a CALRO ERBA NA-1500 C-N-analyzer. Phenol content of seaweed and feces was determined by using a modified Folin–Denis assay: samples were lyophilized, weighed (dry mass) and immediately extracted in 10 ml of 80% methanol (cf. van Alstyne 1995). After centrifuging the extract, the supernatant was stored frozen (−20 °C) until the phenol content was measured. Folin–Denis reagent was obtained by mixing and refluxing 100 g sodium tungstate ($Na_2WO_4 \cdot 2H_2O$), 20 g phosphomolybdic acid and 50 ml H_3PO_4 in 1000 ml double-distilled water. A supersaturated solution of sodium carbonate was made by dissolving 35 g anhydrous Na_2CO_3 in 100 ml double-distilled water (70–80 °C), and adding a crystal of $Na_2CO_3 \cdot 10H_2O$ after cooling.

Aliquots of 1 ml of tissue extract were mixed with 7.5 ml double-distilled water, 0.5 ml Folin–Denis reagent and 1 ml saturated sodium carbonate solution. After an incubation of 30 min in the dark and mixing the samples, absorbance at $\lambda = 725$ nm (cf. Swain and Hillis 1959) was determined. Phloroglucinol (Sigma, St. Louis, Mo.) served as a standard.

Statistics

Most of our data were not normally distributed. Consequently, data are presented as median ± median absolute deviation (M ± MAD) or as range (minimum–maximum), and we predominantly used non-parametric statistics for comparison of data sets. Multiple comparison tests were performed with Kruskal–Wallis *H*-tests. Subsequently, significant differences were localized by using Mann–Whitney *U*-tests. Prior to parametric statistics (ANOVA), data were transformed to normality and approximate homoscedasticity (cf. Levy 1980).

Results

In the midgut glands of *Ligia pallasii* microbiota were present in numbers of 2.5×10^6–3.5×10^6 cells per animal. Taking the animals' dry weight into account, *L. pallasii* contained $(3 \pm 1)\ 10^5$ cells mg^{-1} (Fig. 1). By contrast, *Idotea wosnesenskii* and *Gnorimosphaeroma oregonense* did not appear to have microbial symbionts inside the hepatopancreas. In 30 specimens of *I. wosnesenskii* from three different sites (Grappler Inlet, Pachena Bay, Wizard Island), and 20 specimens of *G. oregonense* from Grappler Inlet, we could not detect any hepatopancreatic microbiota.

Microbiotal counts in male and female field-collected *L. pallasii* did not differ ($P > 0.3$). Therefore, males and females were pooled for all subsequent analyses. Further, we did not detect differences between freshly collected animals (data not shown) and those that had been feeding on seaweed for 5 days in the laboratory ($P > 0.4$), nor did changing the food source from seaweed to an artificial diet affect the number of microbial endosymbionts ($P > 0.5$). By contrast, antibiotics significantly reduced the number of hepatopancreatic microbiota in *L. pallasii* to 6% that of control animals ($P < 0.001$; Fig. 1).

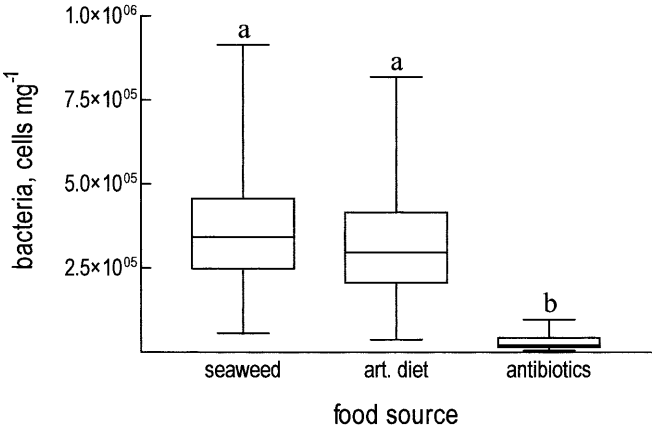

Fig. 1 Density of endosymbiotic bacteria in midgut glands (hepatopancreas) of *Ligia pallasii* feeding on different food sources, seaweed and artificial diet, with or without antibiotics. Data are given as median and first and third quartile, and minimum and maximum ($n = 18$); *letters* indicate significant differences ($\alpha = 0.05$) between treatments

Relative consumption rates (RCR) depended on the algal diet (species) in *I. wosnesenskii* and *G. oregonense* (Fig. 2; Table 1), but not in *L. pallasii* (Fig. 2; Table 1). Previous treatment with antibiotics did not show any effect on RCR for any isopod feeding on any seaweed (Fig. 2, $P > 0.4$). Approximate digestibility (AD) strongly depended on both the algal diet and the antibiotic treatment in *L. pallasii* (Fig. 3; Table 1), but only on the food source in *G. oregonense* (Fig. 3; Table 1). In *I. wosnesenskii*, neither parameter affected the digestibility of the food (Fig. 3; Table 1).

Changes in the C: N ratio, when comparing seaweed and feces (Table 2), were most pronounced in *L. pallasii*, where the content of carbon (Fig. 4) slightly increased during digestion of *Nereocystis* ($P < 0.05$) and *Macrocystis* ($P < 0.05$), while the nitrogen content (Fig. 5) of the feces was significantly lower than that of *Nereocystis* ($P < 0.001$) or *Macrocystis* ($P < 0.01$). Ingesting antibiotics did not affect the changes in carbon or nitrogen content during digestion in *L. pallasii* ($P > 0.2$).

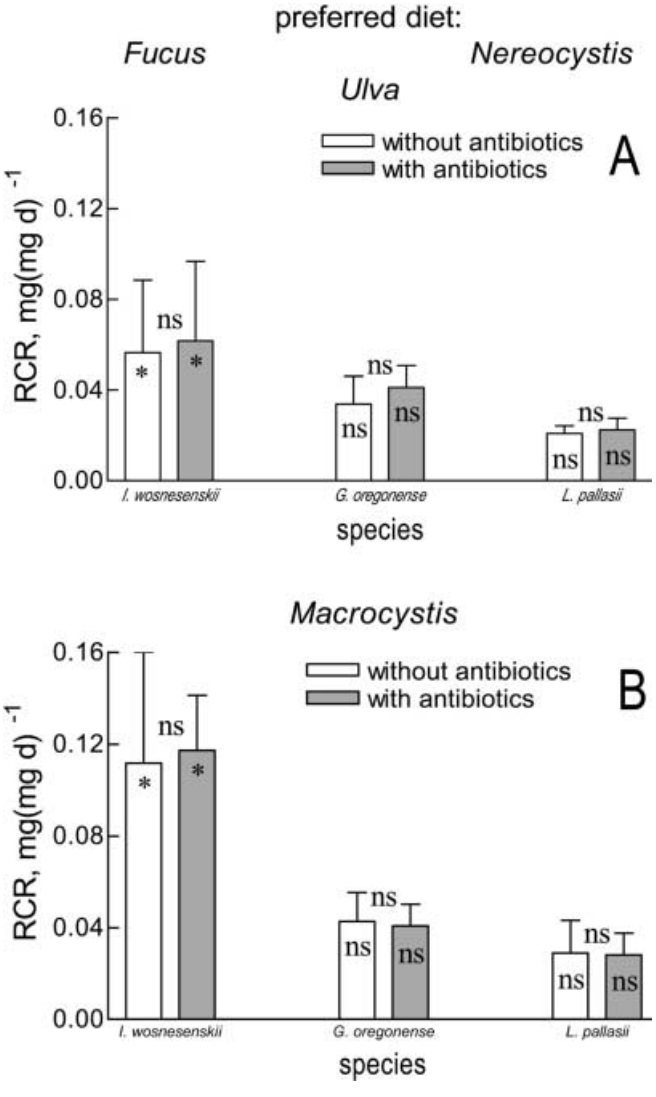

Fig. 2 Consumption (relative consumption rate, *RCR*) of seaweed (**A**: high-preference diet; **B** *Macrocystis*) by the coastal isopods *Idotea wosnesenskii*, *Gnorimosphaeroma oregonense* and *Ligia pallasii*. Data are given as median ± median absolute deviation ($n = 9$); *asterisks* indicate significant differences ($\alpha = 0.05$) between diets and between treatments (without and with antibiotics) (*ns* not significant)

Table 1 ANOVA of food source and antibiotic treatment on consumption (*RCR*) and digestion (*AD*) of seaweed by coastal isopods. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, *n.s.* not significant

	df	RCR		AD	
		F	r^2	F	r^2
Idotea wosnesenskii					
Model	2	5.2*	0.4	$0.7^{n.s.}$	–
Food source	1	10.4**	0.4	$0.1^{n.s.}$	–
Antibiotics	1	$0.1^{n.s.}$	–	$1.3^{n.s.}$	–
Gnorimosphaeroma oregonense					
Model	2	5.9**	0.2	8.9**	0.5
Food source	1	10.9**	0.2	16.9***	0.5
Antibiotics	1	$0.9^{n.s.}$	–	$1.0^{n.s.}$	–
Ligia pallasii					
Model	2	$0.5^{n.s.}$	–	33.6***	0.8
Food source	1	$0.4^{n.s.}$	–	28.1***	0.3
Antibiotics	1	$0.6^{n.s.}$	–	39.2***	0.5

In the case of *I. wosnesenkii*, the carbon content significantly decreased when animals fed on *Fucus* ($P < 0.01$), but no changes in the carbon content occurred when this isopod species fed on *Macrocystis* ($P > 0.3$). Significantly lower nitrogen content in the feces than in *Macrocystis* ($P < 0.01$) indicated strong nitrogen digestion when consuming this food source (Fig. 5), but no changes were observed when *I. wosnesenskii* fed on *Fucus* ($P > 0.5$). Consequently, the C:N ratio of feces was significantly higher than that of *Macrocystis* ($P < 0.01$), but significantly lower than that of *Fucus* ($P < 0.01$). Antibiotics had no quantitative effects on carbon or nitrogen digestion in *I. wosnesenskii* ($P > 0.4$).

In *G. oregonense*, digestion of *Ulva* ($P < 0.01$) and *Macrocystis* ($P < 0.01$) led to lower C:N ratios, indicating a relative increase of nitrogen, but the nitrogen content of the feces did not differ from that of the food ($P > 0.5$). The carbon content dropped during digestion of *Macrocystis* ($P < 0.05$), but not when fed the natural food source, *Ulva* ($P > 0.2$). When feeding on *Macrocystis* after previous antibiotic treatment, no changes in carbon content were observed ($P > 0.6$), and the changes in the C: N ratio were not significant ($P > 0.2$). When feeding on *Ulva* after previous antibiotic treatment, the carbon content in the feces was lower than in the algal tissue ($P < 0.001$), and the C: N ratio of the feces was changed to lower values ($P < 0.05$).

The phenol content of the feces of *I. wosnesenskii* was reduced by factors of 3–15 from that of its food (Table 3, $P < 0.05$ to $P < 0.001$). The phenol content of the feces of *L. pallasii* was slightly higher than that of its food (Table 3, $P < 0.05$ to $P > 0.3$). In *G. oregonense*, phenol content did not vary between feces and food (Table 3, $P > 0.4$).

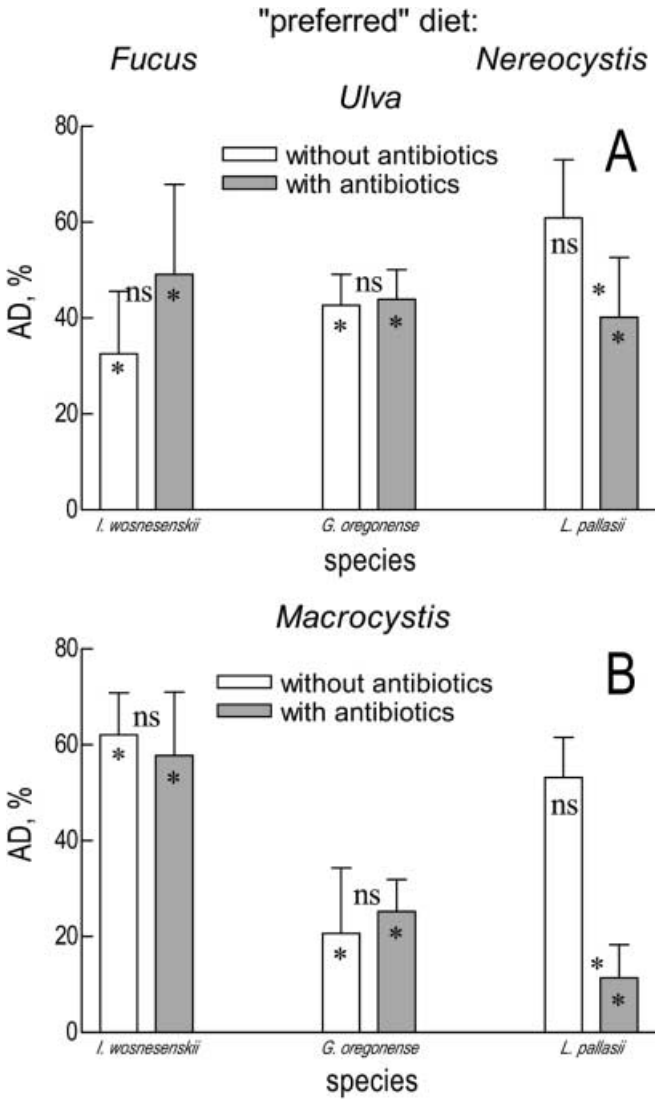

Fig. 3 Digestion (approximate digestibility, *AD*) of seaweed (**A** high-preference diet; **B** *Macrocystis*) by the coastal isopods *Idotea wosnesenskii*, *Gnorimosphaeroma oregonense* and *Ligia pallasii*. Data are given as median ± median absolute deviation ($n = 9$). *Asterisks* indicate significant differences ($\alpha = 0.05$) between food sources (preferred diet vs. *Macrocystis*) and between treatments (without and with antibiotics) (*ns* not significant)

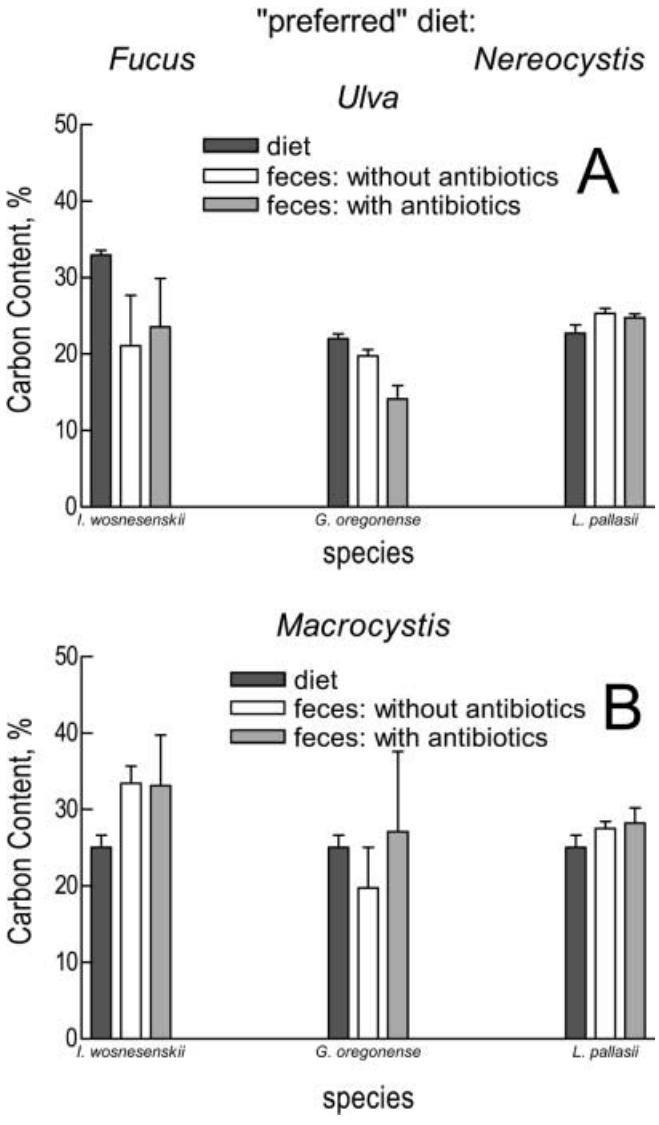

Fig. 4 Carbon content of seaweed (**A** high-preference diet; **B** *Macrocystis*) and feces of the coastal isopods *Idotea wosnesenskii*, *Gnorimosphaeroma oregonense* and *Ligia pallasii*, having fed on these food sources in treatments without and with antibiotics. Data are given as median ± median absolute deviation ($n = 9$)

Discussion

Hepatopancreatic bacteria have previously been described only in three terrestrial isopods: *Porcellio dilatatus* (Oniscidea: Porcellionidae) (Donadey and Besse 1972), *Porcellio scaber* (Oniscidea: Porcellionidae) (Hopkin and Martin 1982; Wood and Griffiths 1988; Zimmer and Topp 1998a, b) and *Oniscus asellus* (Oniscidea: Oniscidae) (Wood and Griffiths 1988; Ullrich et al. 1991). In *P. scaber*, their density is correlated with

Table 2 Changes in C:N ratio during digestion of seaweed by coastal isopods as a function of the food source and antibiotic treatment (−, without antibiotics; +, with antibiotics)

	C:N ratio		
	Seaweed	Feces (−)	Feces (+)
Idotea wosnesenskii on *Fucus*	24 ± 1	14 ± 2	16 ± 2
I. wosnesenskii on *Macrocystis*	13.6 ± 0.9	23 ± 1	22 ± 1
Gnorimosphaeroma oregonense on *Ulva*	13.1 ± 0.8	10 ± 2	11 ± 2
G. oregonense on *Macrocystis*	13.6 ± 0.9	10 ± 2	12 ± 2
Ligia pallasii on *Nereocystis*	15.2 ± 0.5	27 ± 2	31 ± 1
L. pallasii on *Macrocystis*	13.6 ± 0.9	20 ± 3	22 ± 1

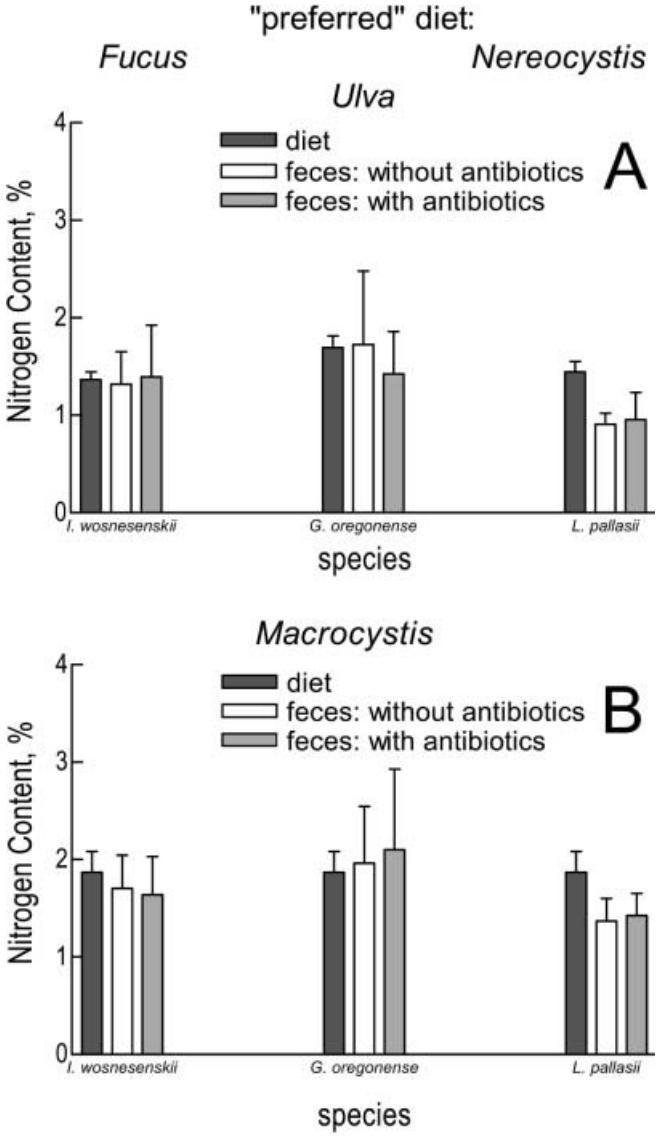

Fig. 5 Nitrogen content of seaweed (**A** high-preference diet; **B** *Macrocystis*) and feces of the coastal isopods *Idotea wosnesenskii*, *Gnorimosphaeroma oregonense* and *Ligia pallasii*, having fed on these food sources in treatments without and with antibiotics. Data are given as median ± median absolute deviation ($n = 9$)

digestive processes (Zimmer and Topp 1998a,b). The present study demonstrates that the prototypal semi-terrestrial isopod *Ligia pallasii* (Oniscidea: Ligiidae) also contains microbial symbionts in its midgut glands. However, compared to the terrestrial *P. scaber* (5×10^7 cells mg^{-1}: Zimmer and Topp 1998a), the densities of symbionts found in *L. pallasii* (3×10^5 cells mg^{-1}) are relatively low. The types of bacteria also differed between the terrestrial and supralittoral species. In *L. pallasii*, there were only very few morphotypes of small coccoid bacteria. In *P. scaber*, coccoid hepatopancreatic bacteria were found (Ullrich et al. 1991; Zimmer 1998) as well as rod-shaped cells (Wood and Griffiths 1988), but the most abundant bacteria appeared to be filamentous (Zimmer 1998; cf. Ullrich et al. 1991). No microbial symbionts were found in the hepatopancreas of *Idotea wosnesenskii* and *Gnorimosphaeroma oregonense*. In their ultrastructural studies on the hepatopancreas of *I. baltica* (Pallas, 1772), Guarino et al. (1994) did not present any hint of the presence of hepatopancreatic bacteria, and in *Limnoria tripunctata* Menzies, 1951, belonging to the same suborder as *G. oregonense* (''Flabellifera''), the midgut glands were shown to be void of microbiota (Sleeter et al. 1978).

In contrast to the findings of Wood and Griffiths (1988) in *Oniscus asellus*, but coinciding with Zimmer and Topp (1998a) and Zimmer (1999) in *Porcellio scaber*, every checked individual of the observed populations of *L. pallasii* contained hepatopancreatic bacteria. Given that hepatopancreatic bacteria contribute to the digestion of food (Zimmer and Topp 1998a, b; present study), a lack of endosymbionts in some isopods would be disadvantageous for these individuals. Similar conclusions can be drawn from our results with respect to the digestibility of ingested food in *L. pallasii*. In this species, previously ingesting antibiotics resulted in reduced counts of hepatopancreatic endosymbionts (Fig. 1) and a significant decrease in AD (Fig. 3; Table 1). Since antibiotics did not affect consumption rates in either of the examined species and had no influence on AD in *G. oregonense* and *I. wosnesenskii*, we propose that the reduction of AD in *L. pallasii* is due solely to the antibiotics' effect on hepatopancreatic symbionts. Thus, in agreement with the terrestrial *P. scaber*, *L. pallasii* appears to profit from the activity of their microbial endosymbionts with respect to digestive processes. Although further evidence is certainly needed, this observation may be interesting from an evolutionary point of view, because it may reflect one of the adaptations acquired in the course of terrestrialization of isopods (cf. Zimmer and Topp

Table 3 Changes in phenol content during digestion of seaweed by coastal isopods as a function of the food source and antibiotic treatment (–, without antibiotics; +, with antibiotics)

	Phenol content (%)		
	Seaweed	Feces (–)	Feces (+)
Idotea wosnesenskii on *Fucus*	6.1 ± 0.8	0.5 ± 0.2	0.4 ± 0.1
I. wosnesenskii on *Macrocystis*	0.9 ± 0.3	0.3 ± 0.1	0.3 ± 0.2
Gnorimosphaeroma oregonense on *Ulva*	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.2
G. oregonense on *Macrocystis*	0.9 ± 0.3	0.8 ± 0.5	1.3 ± 0.6
Ligia pallasii on *Nereocystis*	0.5 ± 0.1	1.3 ± 0.6	0.9 ± 0.3
L. pallasii on *Macrocystis*	0.9 ± 0.3	1.1 ± 0.4	1.9 ± 0.9

1998a). Endosymbionts were lacking in *I. wosnesenskii* and *G. oregonense*, and thus clearly played no role in digestive processes in these more marine species.

The high mean values of digestibility described here (Fig. 3) are comparable to those found in other studies of isopods. Strong and Daborn (1979) calculated assimilation efficiencies of 45–65% in *I. baltica*. In *G. oregonense*, Brooks (1994) observed AD of up to 70%, and comparative values for "food energy absorption" in *L. pallasii* were even higher (Carefoot 1973a).

Differences in the digestion of carbonic and nitrogenous compounds were observed depending on the food source. These differences may be due to phenolics that appear to be deterrent to many marine invertebrates feeding on macroalgae (Hay and Fenical 1988; Steinberg 1988, 1992; Denton and Chapman 1991). Although there surely exist individual (Steinberg 1989), seasonal (Ragan and Jensen 1978; Steinberg 1995), geographical (Steinberg 1989; Targett et al. 1992) and intraspecific differences (Tuomi et al. 1989) in the phenolic contents of seaweeds, *Nereocystis luetkeana* can unambiguously be classified as a phenol-poor diet (Targett et al. 1992; cf. Table 3). *Macrocystis integrifolia* contained about twice as much phenolics (Table 3), but generally the genus *Macrocystis* is characterized by < 15 mg phenolics g^{-1} (dry mass; cf. Targett et al. 1992), which is still a relatively low value. *Ulva lactuca* contains only trace amounts of phenolic compounds (Table 3). By contrast, seaweeds of the genus *Fucus* frequently contain > 50 mg g^{-1} (dry mass) phenolics (Denton and Chapman 1991; Targett et al. 1992), and the phenol content of *F.* cf. *gardneri* was significantly higher ($P < 0.001$) than that of the other species studied here (Table 3). In a roughly coinciding order (Table 2), *Ulva*, *Macrocystis* and *Nereocystis* were characterized by relatively low C:N ratios. The significantly higher C:N ratio in *Fucus* ($P < 0.05$) is probably partly due to the high phenol content in this seaweed.

Feces of *I. wosnesenskii* fed *Fucus* contained an order of magnitude less phenol than the seaweed, suggesting a striking ability to digest phenolic compounds (Table 3). The relative accumulation of phenolics in the feces of *L. pallasii* compared to its food suggests only weak digestion of phenolic seaweed compounds. *L. pallasii* is very reluctant to eat *Fucus* (Pennings et al. 2000), perhaps because it cannot process high levels of phenolics in its diet. In *G. oregonense*, minor reductions in the phenolic content egested versus ingested matter suggest at best weak digestion of phenolics. In none of the examined species were changes in phenolic content influenced by antibiotics, suggesting that endosymbionts, if present, do not play a role in digestion of phenolics in these species. Since terrestrial isopods must deal with the high phenolic content found in the litter of terrestrial plants, a simple model of the colonization of land by isopods would predict that more terrestrial species would have a greater ability to digest phenolics than would more marine species. The weak ability of *L. pallasii* to digest phenolics compared with *I. wosnesenskii* is inconsistent with such an overly simplistic model. Most likely, the striking ability of *I. wosnesenskii* to digest phenolics evolved in association with its specialization upon seaweeds containing extremely high levels of phenolics. If so, the ability to digest phenolics may have independently evolved several times within isopod lineages, both in species that specialized on high-phenolic seaweeds, and in species that colonized land. If so, the weak ability to digest phenolics observed in *L. pallasii* and *G. oregonense* may simply represent the ancestral state for this trait. Evaluation of this hypothesis will require a survey of the phenolic-digestion ability of a wide range of isopod taxa.

Our conclusion of high rates of phenol digestion in *I. wosnesenskii* is supported by the decreased C:N ratio of feces versus diet of *I. wosnesenskii* fed *Fucus*, suggesting high levels of digestion of carbonic compounds (likely phenolics), while nitrogenous compounds were digested less effectively (cf. Figs. 4, 5). By contrast, mainly nitrogenous seaweed compounds were digested when *I. wosnesenskii* fed on *Macrocystis*, while the carbon content did not change. This dependence of digestive processes on the food source is probably due to qualitative differences in a wide variety of seaweed compounds, including phenolics and proteins. In *G. oregonense*, the nitrogen content of the ingested plant material did not change during digestion (Fig. 5), while the decrease of carbon content when feeding *Macrocystis* (Fig. 4) suggests the digestion of carbonic compounds. When feeding on the latter food source, the unchanged carbon and nitrogen contents suggest similarly effective digestion of carbonic and nitrogenous compounds. The decrease in carbon content when feeding *Ulva* after having ingested antibiotics might be explained by decreased digestion of nitrogenous compounds due to the antibiotic treatment, but with the present results, this question has to remain open. The contents of carbon and nitrogen in the food and the feces of *L. pallasii* clearly indicate extensive digestion of nitrogenous compounds, while carbonic seaweed compounds are assimilated less effectively. This conclusion is in agreement with the lack of phenol breakdown. Considering these results, digestion of ingested food is disproportionately due to the digestion of nitrogenous compounds in *L. pallasii*. Thus, the comparison of two intertidal species and a supratidal species reveals a more effective utilization of food nitrogen in the latter. Limited availability of nitrogen in the food has been proposed to be the major restriction to animal performance (White 1993).

We can only speculate on reasons for interspecific differences in carbon and nitrogen utilization during digestion of the experimental food sources. If *L. pallasii* is similar to the terrestrial *P. scaber* in containing surfactants in the hindgut lumen (Zimmer 1997), these surfactants could prevent protein precipitation by ingested seaweed phlorotannins under acidic (Nicholls 1931, in *L. oceanica*) gut conditions, allowing for the assimilation of nitrogenous compounds (cf. Fig. 5)

and resulting in phenolics passing through the gut unchanged, and, thus, being contained in the feces in higher concentration than in the food (cf. Table 3). Yet, we do not know whether there are such surfactants in the gut fluids of *L. pallasii*. Tugwell and Branch (1992) demonstrated the presence of surfactants in gut fluids of the marine isopods *Paridotea reticulata* Barnard, 1914 and *P. rubra* Barnard, 1914 (both Valvifera), feeding on phenol-rich and phenol-free seaweed species, respectively, that prevented protein-precipitation and digestibility-reduction by phenols in vitro. These authors suggest that surfactants are ubiquitous in the gut fluids of invertebrate animals (cf. Martin and Martin 1984), since they may be formed during digestion (of lipids), or may originate from resident or ingested microbiota (Tugwell and Branch 1992). Thus, their concentration and composition clearly depend on the nutritional history of the animal (cf. Zimmer 1997), and, in turn, determine the efficacy of preventing protein-precipitation by phenols (Goldstein and Swain 1965).

By contrast, digestive breakdown of phenolics may be expected in *I. wosnesenskii* feeding on *Fucus* (cf. Table 3) and *G. oregonense* feeding on *Macrocystis*. Qualitative differences in the phenolics of *Fucus*, the natural food source of *I. wosnesenskii*, and *Macrocystis* may explain the less extensive digestion of carbonic compounds by *I. wosnesenskii* feeding on *Macrocystis*, resulting in increased nitrogen assimilation due to the lack of protein-precipitating phenolic degradation products (cf. Appel 1993). Reasons for the lack of nitrogen assimilation in *G. oregonense* (cf. Fig. 5) feeding on the phenol-poor *Ulva* (Table 3), or the poor digestion of carbonic compounds of *Macrocystis* (Fig. 4), remain unclear.

In conclusion, the present study strongly indicates that hepatopancreatic symbionts are important in digestion in *L. pallasii*. Their significance does, however, not appear to be related to the selective digestion of carbonic, phenolic, or nitrogenous compounds in particular. Microbial endosymbionts were absent from *I. wosnesenskii* and *G. oregonense*, indicating that they do not contribute to digestive processes in these species. The ability of these three species to digest phenolics was correlated with diet, but not with increasing terrestrialization. The great variability observed between these isopod species and the potential importance of various digestive adaptations in allowing the colonization of land warrants further investigations.

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