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Physiological properties of the gut lumen of terrestrial isopods (Isopoda: Oniscidea): adaptive to digesting lignocellulose?

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Abstract Since any given trait of an organism is considered to represent either an adaptation to the environment or a phylogenetic constraint, most physiological gut characteristics should be adaptive in terms of optimizing digestion and utilization of the respective food source. Among the Crustacea, the taxon Oniscidea (Isopoda) is the only suborder that includes, and essentially consists of, species inhabiting terrestrial environments, feeding on food sources different from those of most other Crustacea (i.e., terrestrial leaf litter). Microelectrodes were used to assay physiological characteristics of the gut lumen from representatives of four families of terrestrial isopods: *Trichoniscus pusillus* (Trichoniscidae), *Oniscus asellus* (Oniscidae), *Porcellio scaber* (Porcellionidae), and *Trachelipus rathkii* (Trachelipodidae). Microsensor measurements of oxygen pressure (Clark-type oxygen microelectrodes) revealed that O₂-consuming processes inside the gut lumen created steep radial oxygen gradients. Although all guts were oxic in the periphery, the radial center of the posterior hindgut was micro-oxic or even anoxic in the

adults of the larger species. The entire gut lumen of all examined species was strongly oxidizing (Pt microelectrodes; apparent redox potential, E_h : +600–700 mV). Such conditions would allow for the coexistence of aerobic and anaerobic microorganisms, with both oxidative and fermentative activities contributing to digestion. Although bacterial O₂ consumption was also observed in the midgut glands (hepatopancreas), they remained entirely oxic, probably owing to their large surface-to-volume ratio and high oxygen fluxes across the hepatopancreatic epithelium into the gland lumen. Measurements with pH microelectrodes (LIX-type) showed a slight pH gradient from acidic conditions in the anterior hindgut to neutral conditions in the posterior hindgut of *O. asellus*, *P. scaber* and *T. rathkii*. By contrast, the pH in the hindgut lumen of *T. pusillus* was almost constant. We discuss to what extent these physiological characteristics may be adaptive to the digestion of terrestrial food sources that are rich in lignocellulose.

Keywords Gut microbiota · Gut pH · Microelectrodes · Oxygen consumption · Redox potential

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Introduction

During the last few decades, the significance of gut microbiota in phytophagous, xylophagous and saprophagous animals has received increasing attention (e.g., Martin 1984; Slaytor 1992; Breznak and Brune 1994; Douglas 1995). In terrestrial isopods and other saprophagous soil animals, microorganisms ingested along with the food support digestive processes (for discussion, see Zimmer 2002). Microbial activity partly determines physiological gut conditions such as pH level (Zimmer and Topp 1997, in the woodlouse *Porcellio scaber*), redox potential (Bignell 1984; Kappler and Brune 2002), or oxygen concentration (Bignell 1984; Brune et al. 1995a, b), generating physiological gradients

between gut sections that differ with respect to microbial density. On the other hand, the environmental conditions inside the gut lumen not only affect digestion (e.g., Appel 1993, for phytophagous insects), but also determine microbial metabolism (Brune and Friedrich 2000); the oxygen status, for instance, determines whether anaerobic fermentation or aerobic oxidation of nutrients prevails.

The hindgut of terrestrial isopods (Isopoda: Oniscidea), where digestive processes mainly take place, is a straight tube that can be morphologically subdivided into different sections (Hassall and Jennings 1975; Hames and Hopkin 1989). Despite the lack of structural compartmentalization, differences in pH levels (Nicholls 1931; Zimmer and Topp 1997) and enzymatic activities (Hassall and Jennings 1975; Zimmer and Topp 1998a, b; Zimmer 1999) of different sections of the hindgut have been described. Since terrestrial isopods comprise the only crustaceans that became completely independent of an aquatic habitat, we consider this taxon an excellent model for studying the adaptiveness of physiological gut characteristics to terrestrial food sources, but so far detailed data on conditions inside the gut lumen are scarce (see Zimmer 2002).

Based on the predominance of facultatively anaerobic bacteria in the hindgut, it has been suggested that the intestinal tract of isopods is partly anoxic (Reyes and Tiedje 1976); a concept that – to our knowledge – is presently not supported by any experimental evidence. Anoxic hindgut conditions would favor microbial fermentations, and the resulting products would be expected to affect the pH level of the hindgut lumen (cf. Kane 1997). However, the pH in the hindgut of isopods is maintained at slightly acidic levels (Nicholls 1931; *Ligia oceanica*; Hartenstein 1964; *Oniscus asellus*; Wood and Griffiths 1988; *O. asellus*, *P. scaber*; Zimmer and Topp 1997; *P. scaber*), indicating the existence of pH homeostasis (Zimmer and Topp 1997). So far, pH measurements have only been performed in homogenates (Nicholls 1931; Wood and Griffiths 1988; Zimmer and Topp 1997) or by feeding pH- and redox-sensitive dyes (Hartenstein 1964); fine-scale in situ measurements are lacking. In the latter study, the author concluded that the hindgut of *P. scaber* possesses a positive redox potential, i.e., oxidizing conditions (Hartenstein 1964), but again, no experimental confirmation has been presented.

The present study aims at the analysis of fine-scale physiological characteristics influenced by, and influencing, the intestinal microbiota. Comparing four species of terrestrial isopods, representing a gradient of adaptations to fully terrestrial environmental conditions, we focussed on the following hypotheses: (1) Since digestive processes inside the lumen of the anterior hindgut require oxygen, the lumen of this gut section is largely oxic and exhibits positive redox conditions. (2) Due to oxygen consumption by dense microbial populations in both the midgut glands and the posterior hindgut, the lumina of these gut sections are largely

anoxic. (3) According to previous findings (Zimmer and Topp 1997), the pH inside the gut lumen follows a gradient from slightly acidic conditions in the anterior hindgut, favoring digestive processes, to neutral conditions in the posterior hindgut, favoring microbial proliferation.

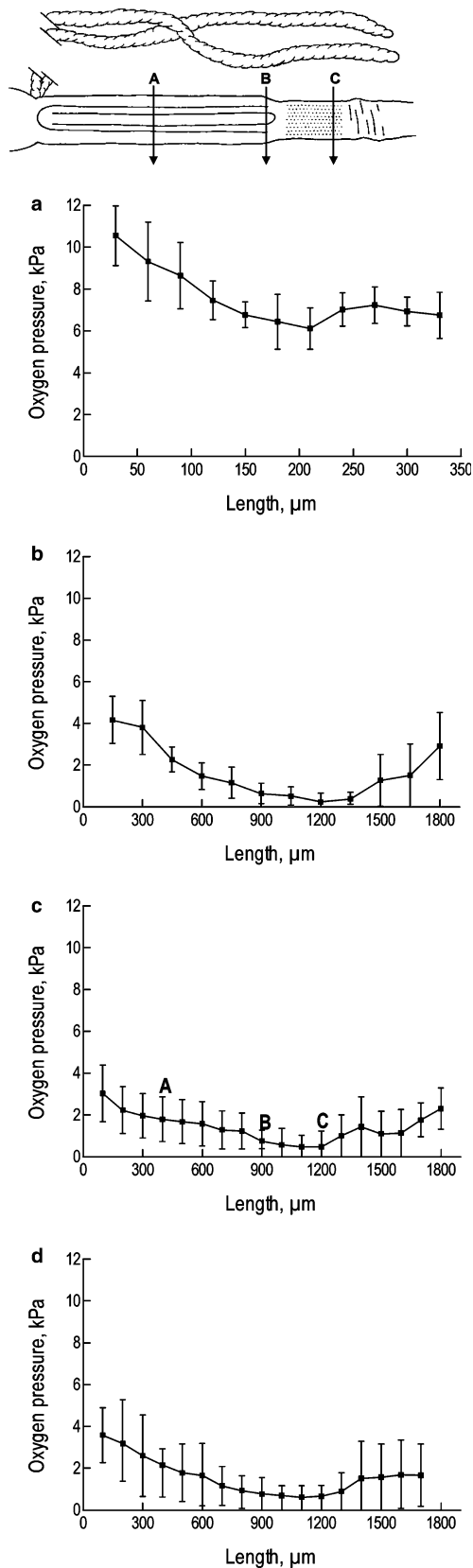
Material and methods

Adults of both sexes of *Trichoniscus pusillus* Brandt 1833 (Trichoniscidae), *Oniscus asellus* Linnaeus 1761 (Oniscidae), *Porcellio scaber* Latreille 1804 (Porcellionidae), and *Trachelipus (Porcellio) rathkii* (Brandt 1833) (Trachelipodidae) were collected in the vicinity of Cologne, Germany. In the laboratory, they were maintained on moist soil and fed with a mixture of poplar litter and decomposing willow wood for up to 2 weeks before they were used for measurements.

Immediately before measurement, the isopods were decapitated, and the hindgut—site of digestion of leaf litter (Hassall and Jennings 1975; Hames and Hopkin 1989) and microbial cells (Reyes and Tiedje 1976; Coughtrey et al. 1980; Zimmer and Topp 1998a) in the anterior part, site of microbial proliferation and home to dense populations of microorganisms (Gunnarsson and Tunlid 1986; Zimmer and Topp, 1998a) in the posterior part—and midgut glands—home to dense populations of symbiotic bacteria (*O. asellus*: Wood and Griffiths 1988; *P. scaber*: Wood and Griffiths 1988; Zimmer and Topp 1998a, b; Wang et al. 2004a, b; *Ligia pallasii*: Zimmer et al. 2001, 2002)—were pulled carefully from the body cavity by the last pleonal segment. After dissection, the guts were embedded in a vertical microchamber (10 mm high, 25 mm wide, and 4 mm deep) in a gel made up of 1% agarose in Ringer's solution (300 mM Cl⁻, 250 mM Na⁺, 15 mM Ca²⁺, 10 mM Mg²⁺, and 5 mM K⁺), which was adjusted according to the ionic composition of the hemolymph of woodlice (cf. Lagarrigue 1969; Wright et al. 1997; Ziegler and Scholz 1997). A fresh gut was placed flat and fully extended onto a 2–3 mm thick agarose layer at the microchamber bottom, and was quickly covered with an identical layer of molten agarose (40°C), which cooled and solidified immediately. Microelectrodes were positioned with a manual micromanipulator (MM33, Märzhäuser, Wetzlar, Germany). All measurements were carried out at room temperature. Each set of measurements was conducted independently with five specimens.

In measurements of axial gradients (oxygen, pH, redox potential, potassium), data were ascertained every 30 µm (*T. pusillus*) or every 150 µm (*O. asellus*, *P. scaber*, *T. rathkii*); every 100 µm for oxygen gradients in the latter two species).

For in vivo measurements of radial oxygen gradients in the hindgut of *P. scaber*, isopods were fixed upside-down in a wax bed. The oxygen microelectrode was inserted from the ventral side at three different sections of



the hindgut (a, b, c, in Fig. 1). Measurements were done in 100 μm steps, after allowance for stabilization of readings.



Fig. 1 Axial oxygen profile along the center of the hindgut (anterior: left, posterior: right) in four species of terrestrial isopods: *T. pusillus* (a), *O. asellus* (b), *P. scaber* (c) and *T. rathkii* (d). Data represent median \pm median absolute deviation of five specimens. A, B, C, in (c) indicate the localization of radial profiles in Fig. 2

Clark-type O_2 microelectrodes with guard cathodes (Revsbech 1989) had tip diameters of 10–15 μm and 90% response times of < 5 s, and were constructed and calibrated as described by Brune et al. (1995a). The experimental setup used for microelectrode measurements in isopod guts was essentially the same as that described previously for termite guts (Ebert and Brune 1997).

Electrically shielded platinum redox microelectrodes with response times of 5–10 s had tip diameter of 15–30 μm (Ebert and Brune 1997). The working electrode was connected to a high-impedance electrometer amplifier ($R_i > 10^{14} \Omega$) via a low-noise coaxial cable, and electrode potentials were measured against a saturated calomel electrode (Ref 401, Radiometer, Copenhagen, Denmark), which was in contact with the agarose-filled microchamber via a KCl-filled agar bridge. Reference and casing were grounded. Redox electrodes were tested and calibrated with saturated quinhydrone solutions made up with commercial pH calibration standards (pH 4.0–7.0).

Ion-selective pH and potassium microelectrodes (LIX-type) were constructed using the design described by Revsbech and Jørgensen (1986) and were equipped with an external casing filled with 1 M KCl to minimize electrical noise (Jensen et al. 1993). The microelectrodes had tip diameters of 10–30 μm and 90% response times of < 10 s, and had a log-linear response when calibrated with commercial pH standard solutions (pH 5, 6, and 7) or KCl standards before and after each set of measurements, observing the necessary temperature corrections. The electrical circuit was identical to that of the redox potential measurements (see above).

Most of our data showed significant deviation from normal distribution. Thus, results are presented as median \pm median absolute deviation, and non-parametric statistics were used for statistical comparison between species. Groups of data were analyzed through Kruskal–Wallis H tests, and pair-wise comparison was performed using Mann–Whitney U tests (Bonferroni-corrected, where applicable). Gradients of measured parameters along radial, dorsal-ventral, or longitudinal axes were checked for significance either through direct comparison of selected sections (U test) or through Cox–Stuart trend tests.

Results

Oxygen conditions in the gut lumen

In every tested isopod, the lumen of the dissected anterior hindgut was largely oxic with oxygen pressures ranging from 1 kPa to 2 kPa. The oxygen content of the central

region of the hindgut decreased continuously from the anterior to the posterior region. *T. pusillus* (Fig. 1a) differed from *O. asellus* (Fig. 1b), *P. scaber* (Fig. 1c) and *T. rathkii* (Fig. 1d) in exhibiting oxic conditions in the center of the posterior hindgut, while in the latter species this hindgut region was largely micro-oxic to anoxic. Even in *T. pusillus*, however, the posterior hindgut was characterized by significantly lower oxygen concentrations than the anterior region ($P < 0.05$). In all examined species, the midgut glands (hepatopancreas) showed higher oxygen contents than the entire hindgut ($P < 0.01$). In these glands (Table 1), no changes or trends in oxygen concentration were obvious in *T. pusillus*, *O. asellus* or *P. scaber*, but in *T. rathkii*, the distal region of the hepatopancreas contained significantly ($P < 0.05$) less oxygen than the proximal part.

The pattern of low oxygen concentrations in the hindgut center was confirmed by in vivo measurements in *P. scaber*. Measurements of radial oxygen gradients revealed a steep decrease in oxygen content from the ventral cuticle to the center of the hindgut. The dorsal hemolymph was characterized by an oxygen saturation of 65–85%. The anterior hindgut (Fig. 2a), the posterior hindgut (Fig. 2c), and the transition zone between anterior and posterior hindgut (Fig. 2b), were oxic in their radial peripheries, but anoxic in their radial centers. The dorsal increase in oxygen pressure was less steep in the posterior section of the body cavity than in the other sections. The contrary results obtained from axial measurements in dissected guts and from radial measurements in vivo are most likely due to higher oxygen consumption in vivo and lower microbial activity in the dissected guts, or to limited oxygen diffusion into the hindgut in vivo. If our in vivo results of *P. scaber* also apply to *O. asellus* and *T. rathkii*, we will have to consider the anterior hindgut oxic only in the periphery but anoxic in the center. Due to the oxygen gradient in the lumen of the anterior hindgut being less steep (Fig. 2), however, a larger volume will be oxic in this region compared to the posterior hindgut.

Redox conditions in the gut lumen

Coinciding with the described oxic conditions, the redox potential indicated strongly oxidizing conditions of +600–+700 mV in all examined species (Table 2). Both hepatopancreas and hindgut lumen were characterized by relatively constant redox conditions. Only in *P. scaber* were these gut sections different from each other ($P < 0.05$), while in the other species the entire gut exhibited virtually constant conditions where redox potential was concerned.

pH conditions in the gut lumen

In *T. pusillus*, the pH level of the anterior hindgut level ranged between 6.5 and 6.8, and slowly decreased to

Table 1 Oxygen partial pressure (kPa) and pH level in the central region of the hepatopancreas (embedded in agarose) of different isopod species. Data are given as median \pm median absolute deviation of five specimens with 15 measurements, each ($n = 75$)

| | O ₂ pressure | pH |
|--------------------|-------------------------|------------------------------|
| <i>T. pusillus</i> | 12 \pm 1 ^a | 6.5 \pm 0.2 ^a |
| <i>O. asellus</i> | 8 \pm 3 ^b | 6.1 \pm 0.3 ^{b,c} |
| <i>P. scaber</i> | 12 \pm 2 ^a | 6.1 \pm 0.2 ^b |
| <i>T. rathkii</i> | 6 \pm 2 ^b | 6.5 \pm 0.3 ^{a,c} |

Superscript letters indicate significant differences ($\alpha = 0.05$)

slightly more acidic values of pH 6.3–6.4 in the posterior hindgut (Fig. 3a). Altogether, the hepatopancreas (Table 1) did not differ from the hindgut with respect to pH conditions. However, there was a weak pH gradient with the proximal region slightly less acidic than the distal region ($P < 0.05$; data not shown). In contrast to *T. pusillus*, the anterior hindgut lumen of the other isopod species was clearly acidic, ranging from pH 5.1 to 6.1, depending on the examined species (Fig. 3b–d). The axial pH profile revealed continuously increasing pH levels in all species, with a relatively sharp transition between the anterior and the posterior hindgut in *P. scaber* (Fig. 3c) and *T. rathkii* (Fig. 3d). In these species, the pH of the most posterior hindgut region ranged between 5.6 and 6.6. In *O. asellus* (Fig. 3b), the axial dynamics in gut pH were larger, but resulted in similar conditions in the posterior hindgut (pH 5.8–6.8). The pH of the hepatopancreas of *O. asellus* (Table 1) was rather constant (pH 5.7–6.3). While the hepatopancreatic pH was significantly higher than that of the anterior hindgut lumen ($P < 0.05$), it did not differ from that of the posterior hindgut lumen. Similar values were obtained for the distal region of the hepatopancreas of *P. scaber*, but the proximal region was slightly more acidic in this species (pH 5.8–6.1; $P < 0.05$; data not shown). In *T. rathkii*, the hepatopancreas was significantly less acidic (pH 6.1–6.8) than the entire hindgut ($P < 0.01$).

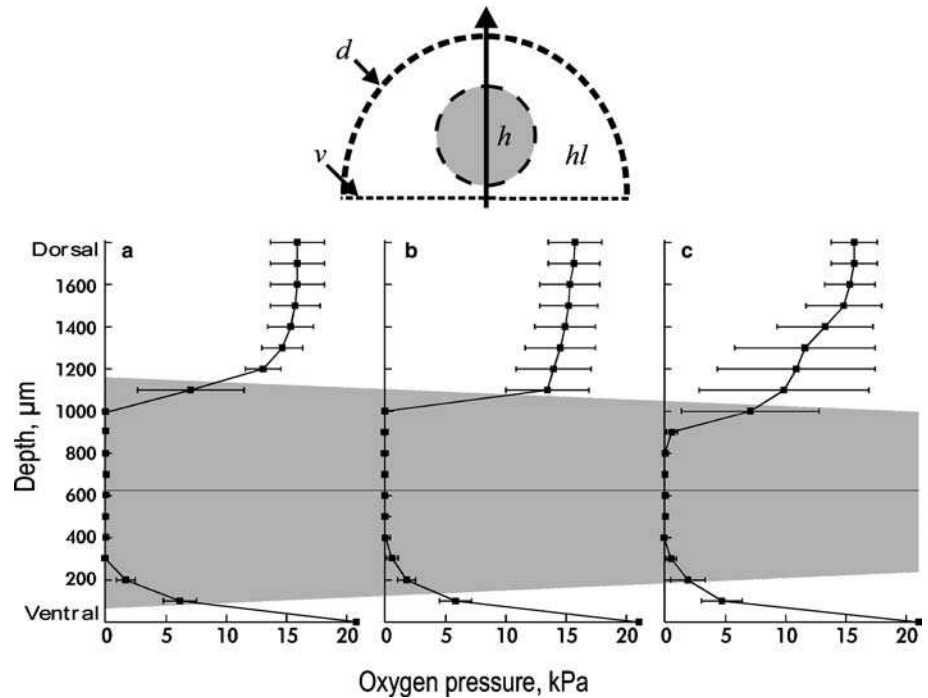
Since *P. scaber* has been described as responding homeostatically to changes in food pH (Zimmer and Topp 1997), we compared the pH profiles in the hindgut pH of this species to the corresponding potassium concentrations in the gut lumen (Fig. 4). The axial K⁺ profile strikingly resembled the pH profile of the hindgut. Potassium concentrations were low (0.4–0.9 mM) in the anterior hindgut and increased about fivefold at the transition between anterior and posterior hindgut. In the posterior most region, the potassium concentration again decreased to average values of 1.3–1.9 mM.

Discussion

Oxygen conditions in the gut lumen

The gut lumen of the examined isopod species exhibited steep oxygen gradients, spanning from a constantly oxic

Fig. 2 Schematic cross section of an isopod (*upper part*), and radial oxygen profiles (*lower part*) in the anterior (**a**), medial (**b**), and posterior (**c**) body region of *P. scaber*. A, B, C: cf. Fig. 1. Data represent median \pm median absolute deviation of five specimens. *d* strong dorsal cuticle; *h* hemolymph; *hg* hindgut; *v* soft ventral cuticle. Arrow indicates the direction in which the profile was measured



periphery to a mostly anoxic center. While Coughtrey et al. (1980) suspected air in the gut lumen of *Oniscus asellus*, and Hames and Hopkin (1989) observed air bubbles in the anterior gut of juvenile *O. asellus* and *Porcellio scaber*, our findings confirmed the hypothesis already proposed by Reyes and Tiedje (1976) that microbial oxygen consumption leads to anoxic gut conditions. The axial gradient of oxygen concentrations from anterior to posterior hindgut and positive redox potentials are in keeping with the hypothesis that an efficient digestion of lignocellulose requires oxygen (Breznak and Brune 1994; Brune 1998). Taking the anoxic hindgut center into account, we may expect aerobic digestive processes to take place mainly in the periphery of the hindgut, whereas anaerobic fermentation processes would be restricted to the center of the hindgut lumen (for consequences with respect to gut pH see next section).

Despite the high density of symbiotic bacteria in the midgut glands (see Zimmer 2002), the lumen of these glands is clearly oxidic. This is most likely a conse-

quence of the enormous surface-to-volume ratio of the thin hepatopancreatic lobes, which allow for large oxygen fluxes across the epithelium of the midgut glands; the other possibility: a lack of oxygen consumption due to non-oxidative metabolism of the bacteria colonizing the hepatopancreas is less likely given their phylogenetic affiliation with the mostly aerobic α -Proteobacteria and Mycoplasmatales (Wang et al. 2004a, b).

pH conditions in the gut lumen

The pH conditions in the gut lumen of the examined species provide optimal conditions for digestive processes in the anterior hindgut (cf. Zimmer and Topp 1997) and for microbial proliferation in the posterior hindgut (cf. Zimmer and Topp 1998a).

In *P. scaber*, the pH levels of the different regions of the gut correlate with the potassium concentration of the same region. In lepidopteran larvae, Dow (1984, 1992) demonstrated that potassium transport via a K^+ -ATPase is responsible for the generation of an alkaline pH in the midgut. The gut pH in terrestrial isopods is slightly acidic, but pH homeostasis (Zimmer and Topp 1997) may be maintained by a similar mechanism, counter-balancing respiratory CO_2 production by microorganisms proliferating in the posterior hindgut (Zimmer and Topp 1998a), and the formation of acidic fermentation products by (facultatively) anaerobic bacteria (Kane 1997). K^+ -ATPases have been described in the hindgut epithelium of terrestrial isopods (Warburg and Rosenburg 1989), but detailed studies are needed to substantiate these speculations.

Table 2 Redox potential (mV) in the central region of the hepatopancreas and the hindgut (embedded in agarose) of different isopod species. Data are given as median \pm median absolute deviation of five specimens with 15 measurements, each ($n = 75$)

| | hepatopancreas | hindgut |
|--------------------|---------------------------|---------------------------|
| <i>T. pusillus</i> | 609 \pm 5 ^a | 612 \pm 5 ^a |
| <i>O. asellus</i> | 659 \pm 3 ^b | 651 \pm 7 ^b |
| <i>P. scaber</i> | 699 \pm 20 ^c | 631 \pm 21 ^b |
| <i>T. rathkii</i> | 613 \pm 5 ^a | 611 \pm 10 ^a |

Superscript letters indicate significant differences ($\alpha = 0.05$)

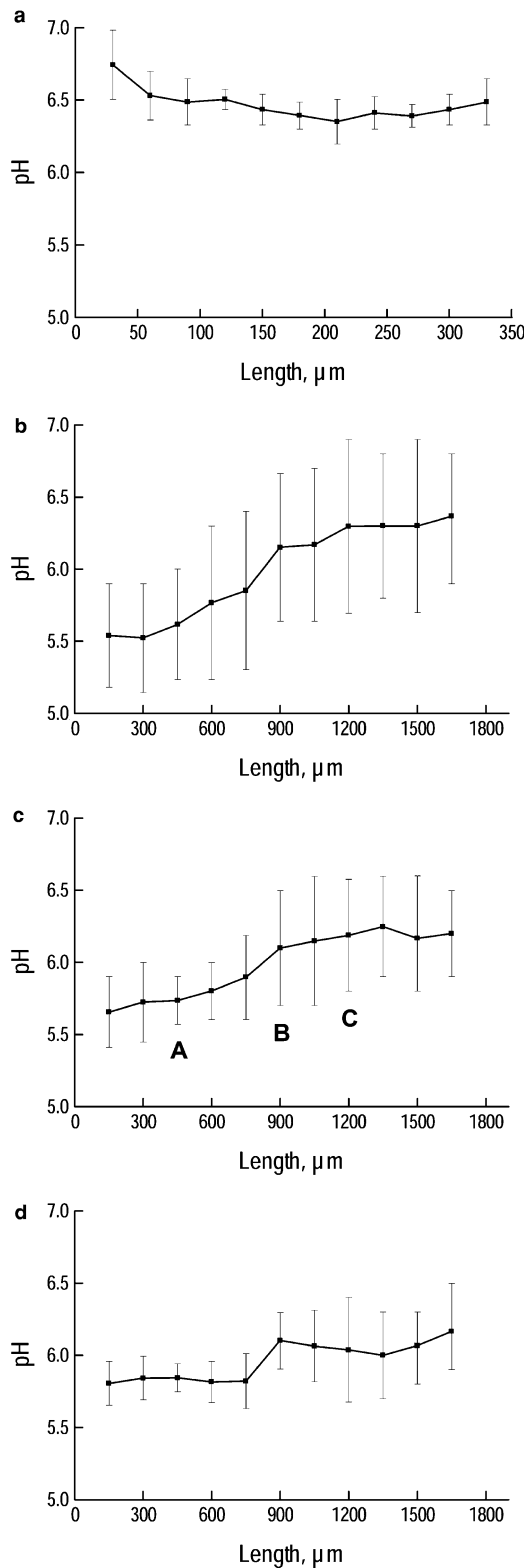


Fig. 3 Axial pH profile along the center of the hindgut (anterior: left, posterior: right) in four species of terrestrial isopods: *T. pusillus* (a), *O. asellus* (b), *P. scaber* (c) and *T. rathkii* (d). Data represent median \pm median absolute deviation of five specimens. A, B, C, in (c) indicate the localization of radial profiles in Fig. 2

Gut characteristics adaptive to the digestion of terrestrial food sources

According to Garland and Adolph (1994), comparative studies of two (or, as here, four) species do not suffice to infer adaptations to particular environmental conditions; such comparisons may only hint at characteristics that appear to be adaptive. Clearly, detailed phylogenetic studies, including the examination of an outgroup, and appropriate quantitative analyses of both environmental differences between tested species and effects of the trait on animal performance (e.g., in terms of growth, reproduction, or mortality) are required before any conclusions on physiological adaptations of isopods to their terrestrial life style can be deduced. Thus, we would need to know (a) the individual variability of the studied traits, (b) its heritability, and (c) the relationship between the trait in its various expressions and the individual's fitness. Further, detailed knowledge of the phylogeny of the studied taxon would be necessary.

The exact phylogenetic relationships within the taxon Oniscidea are still debated. With respect to morphological adaptations and ecological preferences, however, the four isopods species examined here clearly represent a gradient of terrestriality. One of these species, the small and extremely hygrophilic (cf. Beyer 1964) soil-dwelling (endogeic) *Trichoniscus pusillus* (Trichoniscidae) belongs to the taxon Synocheta. The litter-dwelling (epigeic) species, *O. asellus* (Oniscidae), *P. scaber* (Porcellionidae), and *Trachelipus rathkii* (Trachelipodidae), are members of the Crinocheta, thought to be best adapted to the terrestrial lifestyle. The colonization of terrestrial habitats required digestive adaptations to terrestrial food sources, and both symbiotic and litter-colonizing microbiota that are ingested along with the food may have been significant for the colonization of land through facilitating the utilization of terrestrial food sources (for discussion, see Zimmer 2002). Thus,

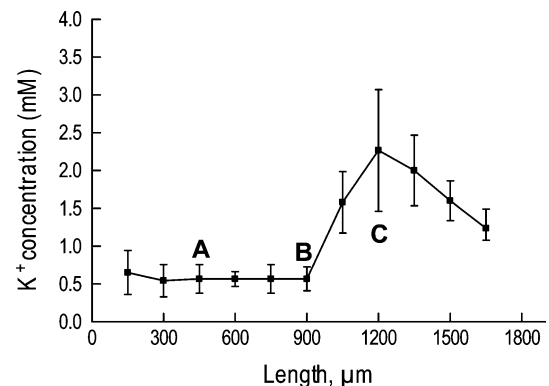


Fig. 4 Axial profile of potassium concentration along the center of the hindgut of *P. scaber* (anterior: left, posterior: right). Data represent median \pm median absolute deviation of five specimens. A, B, C, indicate the localization of radial profiles in Fig. 2

we expected the gut of terrestrial isopods to exhibit physiological characteristics that (1) make possible the digestion of terrestrial leaf litter inside the lumen of the anterior hindgut which requires oxygen and positive redox conditions, (2) reflect oxygen consumption by dense microbial populations in the midgut glands and the posterior hindgut, and (3) ensure optimal pH conditions for digestive processes in the anterior hindgut and for microbial proliferation in the posterior hindgut. Further, differences in gut physiology might be expected between species of different feeding strategies, e.g., microbivory *versus* detritivory.

Cellulose, the most prominent carbon source for saprophagous woodlice (Kozlovskaja and Striganova 1977), is intimately associated with lignin (Ljungdahl and Eriksson 1985; Breznak and Brune 1994). Since lignin can only be degraded oxidatively (Breznak and Brune 1994; Brune 1998), the digestion of lignocellulose requires oxidizing conditions. Also microbial phenol-oxidizing enzymes, which have been suggested to be produced by hepatopancreatic bacteria and may be responsible for the degradation of phenolic components of leaf litter in *P. scaber* (Zimmer and Topp 1998b; Zimmer 1999), require molecular oxygen. Thus, an oxic status of the gut lumen appears to be adaptive to feeding on terrestrial food sources. Oxidizing conditions were observed in all examined species, and at least the periphery of the anterior hindgut was always oxic. Oxygen-consuming digestive processes mainly take place in the anterior hindgut (summarized in Zimmer 2002), and result in micro-oxic or even anoxic conditions at the center of the anterior hindgut. Since the gut periphery experiences a continuous influx of oxygen from the surrounding hemolymph via the gut epithelium, oxygen-dependent processes are thought to occur in this region. In the anoxic center, we expect fermentation to prevail that, in turn, would effect the pH regime of the central hindgut lumen (see below).

Compared with other saprophagous or xylophagous invertebrates, the redox potential in terrestrial isopods is very high, ranging from +600 mV to +700 mV. In hindguts of different termite species, negative (−300 mV) to weakly positive (+200 mV) redox potentials were observed (Ebert and Brune 1997; Kappler and Brune 2002); slightly higher redox potentials (up to +300 mV) have been demonstrated in millipedes (Bignell 1984). Not only does the adaptive significance of redox potentials, as high as those we measured in isopod guts, remain unclear, but such conditions may even have deleterious effects on consumers, since ingested phenolic compounds may cause serious physiological impact under oxidizing conditions (Appel 1993). However, the risk of being adversely affected by phenolic compounds and their oxidation products is counteracted by permeability characteristics of the hindgut cuticle and surfactants in the hindgut fluids (in *P. scaber*: Zimmer 1997, 1999). Comparing the examined isopod species, the synochete *T. pusillus* and the crinochete *O. asellus* and *P. scaber* differed significantly with respect to

gut lumen redox potentials. However, the epigeic litter-feeder *T. rathkii* did not differ from the endogeic microbivore *T. pusillus*. The adaptive significance of these extremely high redox potentials and the lack of an obvious pattern between isopod species warrant detailed studies on redox conditions in isopod guts.

In vivo, the hemolymph of the body cavity is characterized by high oxygen concentrations in *P. scaber*. When the hindgut was not surrounded by oxygen-consuming tissue and hemolymph, but was embedded in iso-osmotic agarose, the lumen of the anterior hindgut was largely oxic in every examined species. Following the pathway of the food bolus, the oxygen concentration of the gut content clearly decreased to anoxic conditions in the posterior hindgut which is most likely due to the activity of proliferating oxygen-consuming hindgut microbiota that serve as an oxygen sink (cf. Brune et al. 1995a; Ebert and Brune 1997; Schmitt-Wagner and Brune 1999).

The hindguts of the similarly-sized species *O. asellus* and *T. rathkii* exhibited oxygen concentration patterns similar to those observed in *P. scaber*, indicating that anoxic conditions in the central hindgut lumen are created by the same mechanism of microbial oxygen consumption. Adults of these species have large hindguts with diameters of 1,200–1,400 μm in the anterior region, and 800–1,000 μm in the posterior region. In contrast, the hindgut of the small *T. pusillus* showed different oxygen profiles. In this species, the hindgut has a diameter of 500–600 μm in its anterior region and 300–400 μm in the posterior region. Although Drobne (1995) did not find any bacteria attached to the hindgut cuticle of the closely related *Hyloniscus riparius* (Trichoniscidae), there are, presumably, abundant microorganisms in the lumen of the posterior hindgut of *T. pusillus*, as has been demonstrated in other isopod species (e.g., Griffiths and Wood 1985; Ullrich et al. 1991; Zimmer and Topp 1998a), resulting in decreased oxygen levels in this gut section. However, the smaller hindgut dimensions decrease the diffusion barrier and facilitates diffusive oxygen transport in the smaller isopod species, resulting in oxic conditions throughout the gut. Whether this difference, however, reflects an evolutionary trend or simply the interspecific differences in size has yet to be clarified.

Agarose-embedded hepatopancreas exhibited oxygen partial pressure of 6–12 kPa in different species. In vivo, when in contact with the hindgut and surrounded by hemolymph, rather than agarose gel, oxygen-consumption of the surrounding tissues and body fluids might reduce the oxygen pressure inside the hepatopancreas lumen, though anoxic conditions are unlikely. However, in our experimental design, oxygen flux through the agarose gel was sufficient to support oxic conditions in the hepatopancreas. As discussed for the hindgut of the small *T. pusillus*, diffusion through the hepatopancreatic epithelium is obviously sufficient to render the lumen of the glands oxic, providing favorable conditions for aerobic bacterial metabolism and their (potential) contributions to the host's nutrition.

Fermentation products of (facultatively) anaerobic microbiota tend to decrease the surrounding pH level (Kane 1997). Thus, it is quite possible that the slightly acidic pH levels in the anterior hindgut lumen of the examined isopod species of Crinocheta are kept constant through fermentation, rather than by homeostasis through the release of microbial cell contents (for discussion, see Zimmer 2002). As for oxidizing conditions, the probability of being affected by phenolic compounds increases with decreasing pH values in the gut lumen (Berenbaum 1980; Martin and Martin 1983; Schultz and Lechowicz 1986). Consequently, in many phytophagous and saprophagous insects extremely high pH values have been described (Martin and Martin 1983; Dow 1984, 1992; Brune and Kühl 1997). However, acidic pH conditions appear to be adaptive with respect to the digestion of terrestrial food sources in the anterior hindgut (Zimmer and Topp 1997). Proliferation of those ingested microbiota that survived digestive processes in the anterior hindgut is promoted by neutral pH conditions in the posterior hindgut (Zimmer and Topp 1998a). Microbial proliferation in the posterior hindgut ensures high microbial densities on isopod feces that may serve as an important food source in terrestrial isopods (discussed by Kautz et al. 2002). The synochete *T. pusillus* also exhibits optimal pH conditions for microbial proliferation in the posterior hindgut, but even higher pH levels in the anterior hindgut. This physiological difference may reflect an ecological difference. In contrast to the other species, *T. pusillus* is not a detritivore sensu strictu in that it consumes detritus as such, but rather it acts as a microbivore, grazing on leaf-litter-colonizing microorganisms (cf. Kautz et al. 2000). We hypothesize that, as in crinochete isopods, the pH in the anterior hindgut of *T. pusillus* is optimal for the activity of digestive enzymes corresponding with their nutritional ecology, and thus, is also adaptive to their respective food source.

Conclusions

The present study is a first approach towards a better understanding of the characteristics of the isopod gut lumen as influenced by, and influencing, gut microbiota and digestive processes. Clearly, these digestive processes reflect the terrestrial food source that is high in cellulose and phenolic content. Both aerobic digestive processes and fermentation are possible in the anterior hindgut. Homeostatic responses of the isopod and/or microbial fermentation products render the gut pH at levels optimal for digestive processes. In the posterior hindgut, microbial proliferation is promoted to ensure dense colonization of isopod feces that, in turn, serve as a high-quality supplementary food source to leaf litter. The resulting high microbial activity renders the lumen of the posterior hindgut anoxic. A comparison of marine isopods and intertidal species of oniscid isopods, considered prototypal models of isopod evolution, would

reveal further information on physiological gut characteristics that are adaptive to the digestion of terrestrial food sources.

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