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Habitat-specific gut microbiota of the marine herbivore *Idotea balthica* (Isopoda)

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ABSTRACT

We hypothesised that the generalist herbivore *Idotea balthica* may harbour intestinal symbiotic bacteria that aid in food utilisation by the host, and that the number and/or identities and thus the contribution of such symbionts may vary between the habitats. We investigated the gut microbiota and its contribution to host use ability of the *I. balthica* originating from two host assemblages, dominated by either the bladderwrack *Fucus vesiculosus* or the seagrass *Zostera marina*. In *I. balthica*, the host use abilities of populations were habitat-specific suggesting divergent evolution for digestion mechanism between different selective environments. We found bacteria both in the hindgut of *I. balthica* and in the digestive midgut glands, demonstrating for the first time the presence of bacterial midgut symbionts in a marine isopod. To study the effects of gut microbes on isopod performance, we reared isopods on two distinct diets, *Fucus* and *Zostera*, and manipulated their gut microbes with antibiotic treatment. The effect of antibiotic treatment on the bacterial counts depended on the isopod origin: antibiotics reduced bacterial counts in isopods originating from the *Fucus* assemblage, but had no effect on isopods originating from the *Zostera* assemblage, supporting the hypothesis that the bacterial communities in the gut differ between the habitats. On the *Fucus* diet, isopods with natural gut bacteria grew less well than those treated with antibiotics, whereas no such difference in growth was found on the much lower quality *Zostera* diet. Symbiotic bacteria, depending on the diet, may thus be harmful or indifferent to their isopod host.

1. Introduction

Generalist herbivores inhabit diverse habitats with varying availabilities of potential host species. The different host species vary in quality, some being low in nutrients or difficult to digest, some inhibiting digestion due to the presence of secondary plant compounds (Karban and Agrawal, 2002). Being polyphagous, i.e. utilizing several host species, challenges the digestive system of herbivores, because overcoming different defence compounds and structural materials may require specific enzymes. Consequently, the same digestive processes are hardly suitable for utilising efficiently a range of qualitatively different host species. Therefore, instead of being completely polyphagous, herbivores could be expected to specialise to exploit only a few host species with a trade-off in the ability to utilise other species (Fox and Morrow, 1981). Furthermore, if the local selection pressures vary, the specialisation process may have population-specific outcomes (Nosil, 2004; Schluter, 2000). Such among-population differentiation in host-use ability has been found in marine crustacean herbivores (Sotka and Hay, 2002; Sotka and Reynolds, 2011; Stachowicz and Hay, 2000; Vesakoski et al., 2009).

Supposedly the most important trait subject to natural selection in this case is the suite of mechanisms by which the food is digested inside the herbivore gut. Many invertebrates possess obligate or facultative bacterial, fungal or viral symbionts in their gut, the effects of which on the host can be parasitic, mutualistic or neutral (commensalistic) and may vary in space and/or time (Bronstein, 1994a, 1994b; De Vries et al., 2004). The herbivores depending on bacterial symbionts include species that live on diets low in nutrients (Bernays and Klein, 2002; Wilkinson and Ishikawa, 2000) or on diets that are difficult to digest (Breznak and Brune, 1994; Brune and Friedrich, 2000). Bacterial symbionts can enhance their hosts' ability to live on qualitatively poor diets by improving conditions inside the gut lumen for digestive processes, by providing essential nutrients that lack from the diet, or by producing digestive enzymes (Brune and Friedrich, 2000; Zimmer et al., 2002).

Angiosperm plants are challenging food for herbivores as their cell walls have high contents of lignocellulose that inhibit digestive processes (Oliver et al., 1996; Zimmer, 2005a). Cellulose hydrolysis has long been considered an ability restricted to bacteria and fungi, but recently an increasing number of invertebrates have been shown to produce endogenous cellulases (e.g. Linton and Greenaway, 2007; Zimmer and Bartholmé, 2003). In addition to cellulases, the oxidative breakdown

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of lignins (Breznak and Brune, 1994) is contributed by peroxidases and phenol oxidases (s.l.). The activity of phenol oxidases in the gut environment is poorly understood. In addition to oxidative breakdown of lignins, they may degrade non-hydrolysable phenolic compounds such as condensed tannins in angiosperm tissue and phlorotannins in brown algae although this ability has remained somewhat controversial since their major function is phenol-polymerization rather than breakdown. Furthermore, by oxidising phenolics these enzymes may generate oxidative stress. Thus, the consequences of phenol oxidase activity in the gut cannot be predicted and they may well vary depending on the diet.

In several isopod (Crustacea) species, bacterial symbionts have been shown to inhabit the digestive midgut glands (hereafter hepatopancreas) (e.g. Eberl, 2010; Fraune and Zimmer, 2008; Wang et al., 2007; Zimmer and Bartholmé, 2003), and have been suggested to contribute to the utilisation of low-quality diets (Fraune and Zimmer, 2008; Zimmer, 2002; Zimmer and Bartholmé, 2003; Zimmer et al., 2002). By contrast, the few studies on marine isopods did not find bacteria inside their hepatopancreas (Boyle and Mitchell, 1978; Daniel et al., 1991; Guarino et al., 1994; Wang et al., 2007; Zimmer et al., 2001). The differences among isopod species may indicate adaptations to different selective environments (Zimmer et al., 2002). However, when a generalist herbivore inhabits habitats with qualitatively different host species the contribution of the gut microbiota to digestion may differ also among populations. Earlier studies have shown, that in isopod *Idotea balthica* local populations differ in their ability to utilise different host species (Bell and Sotka, 2012; Vesakoski et al., 2009). However, it is not known to what extent this is caused by varying contributions of microbial communities inside the herbivores' digestive system. Therefore, *I. balthica* is a fruitful species to study the impact of gut microbes.

Here we studied the occurrence of intestinal bacteria and their contribution to digestion in *I. balthica* (Pallas, 1772) populations from the northern Baltic Sea. The isopods inhabit both the shallow soft-bottom seagrass habitats with the eelgrass *Zostera marina* mixed with other vascular plants such as *Potamogeton spp.* and hard-bottom communities dominated by the bladderwrack *Fucus vesiculosus*. The brown alga *F. vesiculosus* and the angiosperms are qualitatively very dissimilar hosts; consuming *F. vesiculosus* may for example require the ability to cope with phlorotannins, while the efficient utilisation of angiosperms may require the ability to degrade cellulose. Thus the same digestive processes are hardly suitable for exploiting efficiently both the diet species and a cost of specialisation in one or the other host can be expected in this system.

In this study, we tested the hypothesis that the abundance and the role of bacterial and fungal intestinal symbionts vary among isopod populations originating from angiosperm and *Fucus*-habitats. First we collected a sample of isopods from both selective environments (*Fucus*- and *Zostera* assemblages) to characterize their gut microbiota. In a laboratory experiment, we fed isopods originating from the two habitat types (three replicate habitats) with either *F. vesiculosus* or the angiosperms *Z. marina* and *Potamogeton pectinatus* and manipulated their intestinal microbes with antibiotics in order to reveal their role in the ability to utilise these food sources as hosts. We further hypothesised that the occurrence phenol oxidation and cellulolytic activities in the gut fluid may differ between populations from the two habitat types or between diets composed of either *Fucus* or angiosperms.

2. Material and methods

The gut of *I. balthica* can be divided into three sections: foregut, hepatopancreas, and hindgut (method and picture in Zimmer, 1999). The hepatopancreas of *I. balthica* consists of three pairs of tubular midgut glands (caeca), which are the key site for the secretion of digestive fluids and for the absorption of nutrient (Zimmer, 2002, and references therein). Fragments of food pass through the foregut into the hindgut where digestion takes place with the aid of digestive fluids secreted by the hepatopancreas (Zimmer, 2002, and references therein; Zimmer and Bartholmé, 2003).

2.1. Sampling

We sampled isopods from six populations (referred hereafter by the collection sites: Björkö, Höglandet, Säppi, Kolaviken, Ryssholmen and Sandön) separated by 5 to 170 km. The first three of the sites represent rocky littoral zones dominated by the bladderwrack *F. vesiculosus*, and the rest are seagrass meadows dominated by *Z. marina* (for a description of habitats and a map, see Vesakoski et al., 2009). For the performance experiments (see below), we collected 10 gravid females from each population in June 2006, totalling 60 females. For studying the natural variation in gut microbes and enzymatic activities in the gut fluid, we collected immature males and females from 17th Oct to 15th Nov, 2006. For this, we sampled 20 males and 20 females from each population, in total 240 individuals.

2.2. Performance experiment

To study the effects of the gut microbiota on isopod performance (growth rates), we set up a factorial experiment where we reared isopods on two distinct diets and manipulated their gut microbes. We collected gravid females from three *F. vesiculosus* assemblages and three *Z. marina* meadows, and placed them individually in 2.7 l aquaria. We used laboratory-born juveniles in order to remove any direct environmental effects; thus the differences are likely to represent genetic differences among populations. However, our experimental design cannot exclude totally maternal effects on juvenile performance, e.g. the diet or the size of the mother. Juveniles were collected after they left the mother's brood pouch and placed individually in one-dl jars. From each female we aimed to get a total of 12 juveniles, i.e. three juveniles to each of the four treatment combinations (see below); due to mortality the actual number varied between 2 and 12 juveniles per female. In total 418 juveniles were used. Prior to the actual performance experiment, we fed all the juveniles with an assortment of easily consumed filamentous algae, *F. vesiculosus*, *Z. marina* and *P. pectinatus*. We initiated the experiment gradually in September–October when the juveniles were big enough ($\geq$10 mm) to start feeding on *Fucus* and the angiosperms. At the beginning of the experiment the isopods were weighted. Through the experiment, isopods were kept in one-dl jars with aeration.

During the experiment, juveniles were provided with either *F. vesiculosus* diet (hereafter *Fucus* diet) or a diet consisting of *Z. marina* and *P. pectinatus* (hereafter angiosperm diet) as we knew from earlier that the isopods would not grow with *Zostera* diet only (Vesakoski et al., 2009). Half of the isopods from both diet groups received a multi-spectrum antibiotic treatment (0.1% concentration of penicillin G, streptomycin sulfate and amphotericin B in water; Sigma A5955) to reduce their gut microbes, and the other half remained as a control without antibiotic treatment. Antibiotic treatment continued throughout the experiment and the antibiotic–water mixture was replaced in at 7-day intervals. In the control group, water was changed at similar intervals. Experiment lasted for one intermoult period (ca. 3 weeks). Occurrence of intermoult was observed daily. For the moulted isopods the experiment was over and they were re-weighted.

We measured growth rate in terms of the fresh weight increment during the duration of one intermoult (mg/day). We also determined the number of gut microbes and enzymatic activities in the gut from a random subsample. We randomly selected two mothers per population, and took randomly four of their offspring for further analyses; one from each treatment. In total 43 of the hepatopancreas and 40 of the hindgut were measured.

2.3. Microbes, phenol oxidation and cellulose hydrolysis

Before dissection, the isopods were surface dried, wet-weighed and surface sterilized. Surface sterilization was done by dipping into 70% ethanol. We dissected either the complete intestinal tract including the hepatopancreas (field-collected isopods) or the hepatopancreas and hindgut separately (isopods from the performance experiment) in sterile conditions and homogenized and preserved them in 1 ml PBS buffer.

We determined the total number of bacteria from a subsample of the homogenates that was filtered on 0.2 μm filter, stained with 4:6-diamidino-2-phenyl-indole (DAPI) and counted under a fluorescence microscope. From the samples of natural populations we also counted the colony forming bacteria and fungi (hereafter CFUs), respectively, by diluting gut homogenates to nutrient and potato dextrose agars (as described in Treves and Martin, 1994).

The phenol oxidation capacity of the gut fluid was determined from the homogenates using the kinetic spectrophotometric method described in Zimmer (2005b). The determination was done separately for two different substrates, phloroglucinol and catechol. We determined the cellulase activity of the gut homogenates as the capability of gut homogenate to degrade α-cellulose to glucose following the method described in Zimmer (2005a), except we used a Glucose Assay Kit (Sigma product code GAHK-20) for quantifying glucose. We conducted these determinations either for the gut as a whole or separately for hepatopancreas and the rest of the gut (see above).

2.4. Statistical analyses

2.4.1. Natural among-population variation

The distributions of bacterial counts and numbers of CFUs were non-normal; we therefore analysed them using generalized linear models (PROC GLIMMIX in SAS 9.2, SAS Institute Inc., 2008). We used negative binomial (total bacterial count, bacterial CFUs) and Poisson (fungal CFUs) error distribution and a logarithmic link function. We checked the goodness of fit of the model by close-to-one deviance/df value (Dobson, 2002). We included habitat, sex and their interaction as fixed factors and population (nested within habitat) and population-by-sex interaction as random factors into the model. We used the initial weight of the animal as a covariate. We then simplified the model by removing population-by-sex interaction with the aid of AIC-values.

Phenol oxidation and cellulolytic activities were normally distributed and we analysed them using a mixed model ANOVA (PROC MIXED in SAS 9.2) using a similar model as described above. Adjusted degrees of freedom were determined with the Kenward–Rogers method. The homogeneity of variances was checked through visual inspection of residuals and by Levene's test. In the analysis of phenol oxidation with catechol substrate, the levels of sex and population had unequal variances and we therefore included the heterogeneity of residual variation into the statistical model. Based on the Akaike Information Criterion the model fit was improved by doing so (Littell et al., 2006). We evaluated the significance of the random factors in all analyses (GLIMMIX and MIXED) by calculating the likelihood ratio test and using χ^2 distribution (as described in Littell et al., 2006).

2.4.2. Performance experiments

We analysed growth rate with mixed model ANOVAs (PROC MIXED in SAS 9.2). Growth rate was explained by habitat, diet, antibiotic treatment and their interactions as fixed factors. Isopod population (nested under habitat) and its interactions with the fixed factors were treated as random factors. We used the AIC values to select the best-fit model. The significant fixed factor interactions were further analysed with contrasts. Statistical significances for random factors were obtained the assumptions of ANOVAs checked as described above.

As the distributions of bacterial counts were non-normal we used generalized linear models (PROC GLIMMIX in SAS 9.2, SAS Institute Inc., 2008) to analyse them. We included habitat, diet, antibiotic treatment, gut section and their interactions as fixed factors and population (nested within habitat) as random factors into the model.

Enzyme activities were measured from each individual both from the hepatopancreas and hindgut. We included both the measurements to the same model and treated individuals as repeated subjects in mixed model repeated ANOVA (PROC MIXED in SAS 9.2). Enzyme activities were explained with the fixed factors habitat, diet, antibiotic treatment, gut section and their interactions as fixed factors. Isopod population (nested under habitat) and its interactions with the fixed factors were treated as random factors. We further explored possible covariation of growth rate and enzyme activities by analysing growth rate as described above but adding enzyme activity into the model as a continuous covariate. We did these analyses separately for enzyme activity from the hepatopancreas and hindgut and separately for phenol oxidase activity with the two different substrates as well as for cellulolytic activity. Of these six analyses, enzyme activity was significant in only one that we report in the results.

3. Results

3.1. Natural variation in gut microbes and enzymatic activities

The numbers of both bacterial and fungal CFUs differed among populations (Fig. 1; Table 1) but were similar between the habitats and sexes.

Bacterial counts were higher in males than in females (Fig. 2 A; Table 1). There were differences neither between bacterial counts in isopods originating from the *Fucus* and *Zostera* assemblages nor among populations within the habitat.

Gut homogenates effectively oxidized phenolic substrates. When using phloroglucinol as substrate, phenol oxidation was similar in

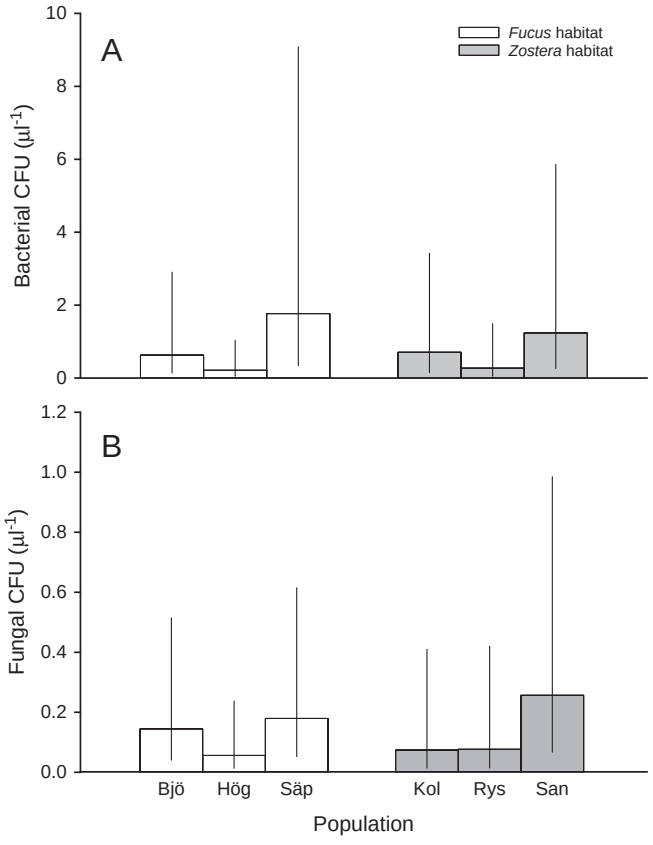

Fig. 1. Bacterial CFU (A) and fungal CFU (B) sampled from the six populations in the field. Mean ± 95% confidence limits are shown.

Table 1
Results of generalized mixed linear models for testing the effects of habitat, sex and isopod population on total bacterial counts and the bacterial and fungal CFUs from the isopods were sampled from two habitat types, either the *Fucus* or *Zostera* assemblage, and three random populations were sampled from both the habitat types. The initial weight of an animal was used as a covariate in all models.

	Total bacterial counts			Bacterial CFU			Fungal CFU		
Source of variation	df	F	P	df	F	P	df	F	P
Fixed factors									
Habitat	1, 4	1.58	0.2774	1, 4	2.32	0.2028	1, 4	3.27	0.1450
Sex	1, 231	5.18	0.0237	1, 207	0.07	0.7902	1, 226	1.71	0.1921
Habitat × sex	1, 231	1.59	0.2082	1, 207	0.30	0.5830	1, 226	0.81	0.3687
	df	χ^2	P	df	χ^2	P	df	χ^2	P
Random factors									
Isopod population (habitat)	1	0	1.0	1	10.02	0.0008	1	5.16	0.0115
	Chi-Square/DF: 1.00			Chi-Square/DF: 0.97			Chi-Square/DF: 1.13		

both habitats and sexes (data not shown), but isopod populations differed in their phenol oxidation activity ($\chi^2 = 13.06$, df = 1, P =0.0001). Also when using catechol as substrate, there were no differences between the habitats ($F_{1, 3.8} = 0.54$, P = 0.5; habitat-by-sex: $F_{1, 121} = 0.49$, P = 0.49), but populations varied in their phenol oxidation ability ($\chi^2 = 4.4$, df = 1, P < 0.05). Here, males had higher phenol oxidation activities than females (Fig. 2 B; sex: $F_{1, 121} = 8.58$; P = 0.0041).

Gut fluid possessed cellulolytic activity as the mean activity was significantly higher than zero (27 μg glucose/ml, n = 240, 95% confidence limits 23–31). However, cellulolytic activity did not differ between the habitat, populations or sex, nor were there interactions of these (data not shown).

3.2. Microbial contribution to performance

Isopods grew considerably better on *Fucus* (mean ± SE: 0.11 ± 0.009 mg d^{-1}) than on angiosperms (0.013 ± 0.009 mg d^{-1}) diet. This however depended on the antibiotics treatment (Fig. 3; diet × antibiotics, $F_{1, 403} = 3.94$, P = 0.048). On the *Fucus* diet, antibiotic-treated isopods had better performance than individuals of the control group (contrast: $F_{1, 403} = 6.37$, P = 0.01). By contrast, there were no differences between the antibiotic treatment and the control group on the angiosperm diet (contrast: $F_{1, 403} = 0.16$, P = 0.7). Growth rates also varied among the isopod populations depending on the diet species ($\chi^2 = 3.5$, P = 0.031; for similar results in the analysis of weight gain, see Vesakoski et al., 2009).

Bacteria occurred in both the hepatopancreas and hindgut as revealed by direct counts. Interestingly, numbers of bacteria did not differ between the gut sections. The effect of antibiotic treatment on the total bacterial counts depended on the habitat of origin (Fig. 4, Table 2): the addition of the antibiotic reduced bacterial counts in isopods originating from the *Fucus* assemblage ($F_{1, 36} = 8.91$, P < 0.01), whereas the antibiotic treatment did not have any effect on the total numbers of bacteria in isopods originating from the *Zostera* assemblage ($F_{1, 36} = 0.50$, P = 0.48).

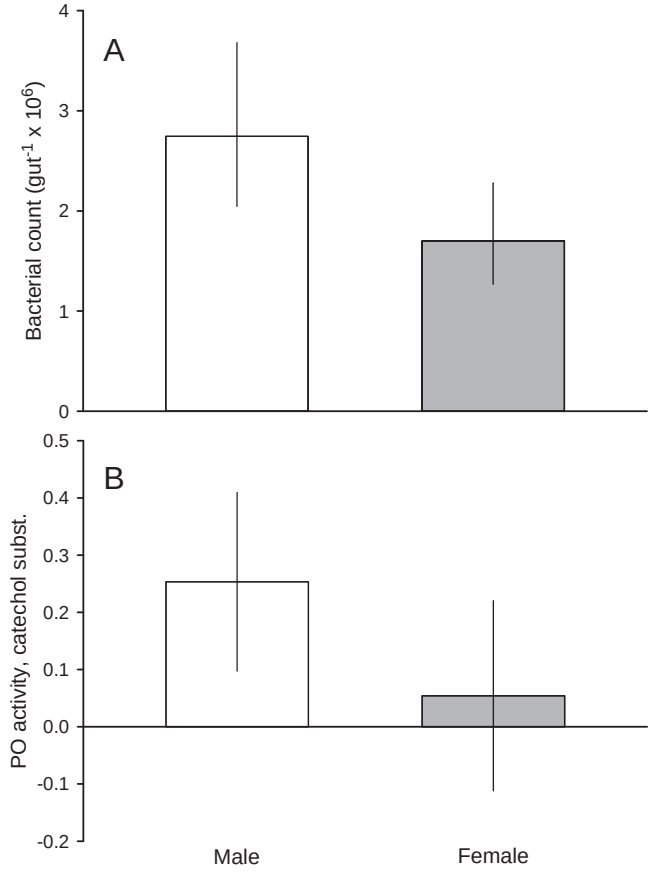

Fig. 2. Total bacterial count per gut (mean ± 95% confidence limits) (A) and phenol oxidation activity (Δ Absorbance d^{-1}; mean ± 95% confidence limits) of gut homogenates, measured using catechol as a substrate (B), separately for males and females sampled from the six populations in the field.

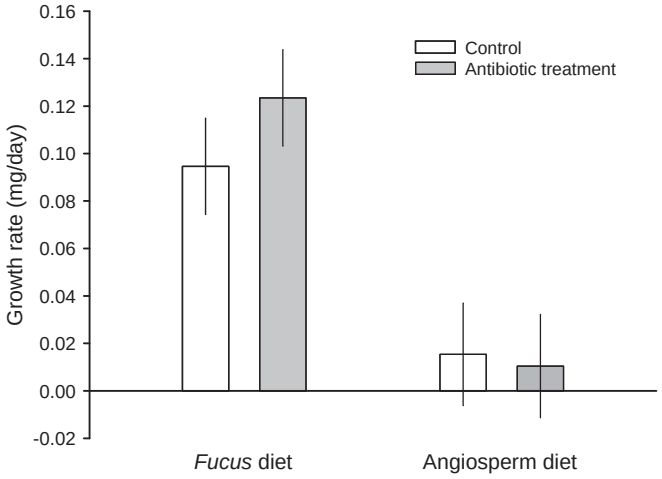

Fig. 3. Growth rate of *Idotea balthica* during one intermoult (in weight increment per day; mean ± 95% confidence limits) in the control and antibiotic treatment levels on the two diets.

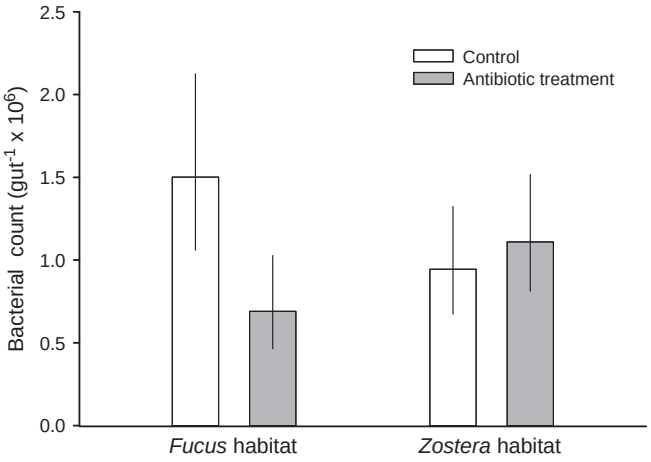

Fig. 4. Total bacterial count per gut (mean ± 95% confidence limits) in the control and antibiotic treatment levels for isopods originating from *Fucus* and *Zostera* assemblages.

When using phloroglucinol as a substrate, phenol oxidation activity was higher in the hindgut than in the hepatopancreas (Fig. 5 A; Table 2). Habitat, antibiotic treatment, diet or their interaction did not affect phenol oxidation activity (Table 2). When using catechol as substrate, oxidation activity did not differ between habitat, antibiotic treatment, gut section or diet, nor were there interactions of these (data not shown).

In the hepatopancreas, an analysis of covariance of growth rate with phenol oxidation activity showed differences between diets (phenol oxidation activity-by-diet interaction: $F_{1, 30} = 5.57$, $P = 0.025$), when using phloroglucinol as substrate. On the *Fucus* diet, phenol oxidation activity covaried positively with growth rate (0.09 ± 0.065), whereas on the angiosperm diet the covariation was negative (-0.10 ± 0.069). When using catechol as substrate, growth rate and oxidation activity did not correlate.

Cellulolytic activity occurred in both hindgut (mean ± SE: 13 ± 4 μg glucose/ml) and hepatopancreas (10 ± 4 μg glucose/ml), but the activity did not differ between gut sections. Cellulolytic activity in the gut fluid was higher on *Fucus* than on angiosperm diet (Fig. 5 B; Table 2). There were no other significant effects. Growth rate did not covary with cellulolytic activity.

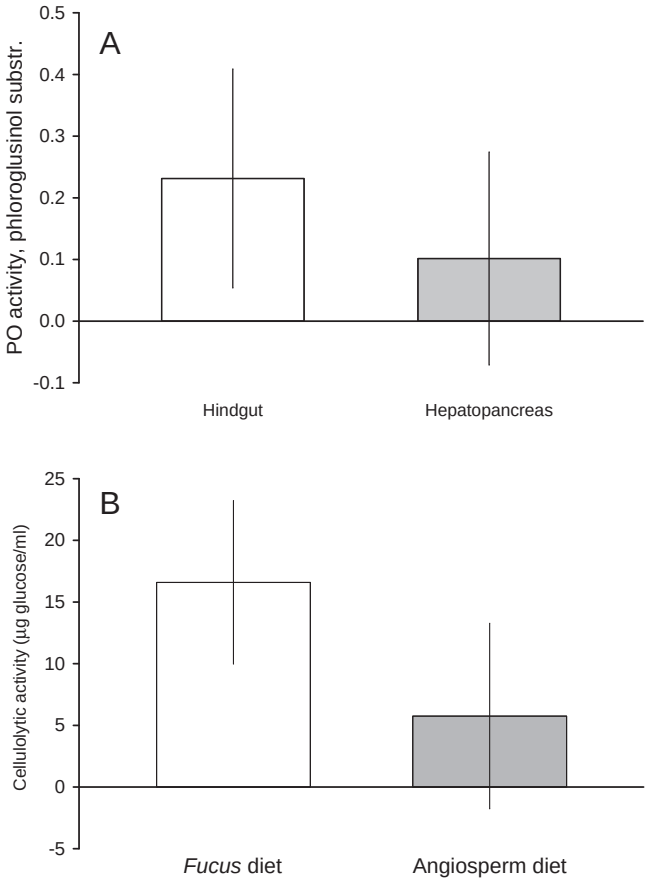

Fig. 5. Phenol oxidation activity (Δ Absorbance d^{-1}) of different gut sections, measured using phloroglucinol as a substrate (A). Cellulolytic activity (in μg glucose produced ml gut homogenate) on the two diets (B). Mean ± 95% confidence limits are shown.

Table 2
Results of generalized mixed linear models for testing the effects of habitat type (*Fucus* or *Zostera* assemblage), antibiotic treatment (antibiotics either added or not), diet (*F. vesiculosus* or angiosperms), gut section (hindgut or hepatopancreas), mother and isopod population (total of six populations nested under the habitat type) on bacterial counts and enzymatic activities in the performance experiment.

Source of variation	Bacterial counts			Cellolytic activity				PO activity, phloroglucinol substr.			
	df	F	P	df	F	P		df	F		P
Fixed factors											
Habitat	1, 35.0	0	0.9754	1, 4	1.05	0.3644		1, 4	1.81		0.2495
Antibiotic treatment	1, 35.7	3.17	0.0836	1, 56	0.45	0.5029		1, 61	0.77		0.3851
Diet	1, 35.8	1.16	0.2887	1, 56	5.65	0.0209		1, 61	1.36		0.2478
Gut section	1, 37.4	0.43	0.5172	1, 56	0.38	0.5427		1, 61	5.26		0.0253
Habitat × diet	1, 35.8	1.84	0.1832	1, 56	0.75	0.3898		1, 61	2.97		0.0899
Antibiotic treatment × diet	1, 36.4	0.39	0.5346	1, 56	0.04	0.8333		1, 61	0.99		0.3231
Diet × gut sect.	1, 39.3	0.16	0.6917	1, 56	1.21	0.2768		1, 61	0.30		0.5876
Habitat × gut sect.	1, 37.4	1.17	0.2863					1, 61	2.68		0.1066
Antibiotic treatment × gut sec.	1, 39.6	0.19	0.6685					1, 61	0.92		0.3410
Habitat × antibiotic treatment	1, 35.7	7.36	0.0102					1, 61	0.16		0.6890
Habitat × antibiotic treatment × diet	1, 36.4	1.94	0.1724					1, 61	1.61		0.2094
Habitat × antibiotic treatment × gut sec.	1, 39.6	0.08	0.7816								
Habitat × diet × gut sec.	1, 39.3	0.66	0.4211								
Diet × antibiotic t. × gut sec.	1, 41.5	0.94	0.3388								
				s^2	SE	χ^2	P	s^2	SE	χ^2	P
Random factors											
Mother				0	–	–	–	0.000013	0.000025	1.1	0.1471
Isopod population (habitat)				0.000012	0.000027	0.2	0.3274	0.001427	0.004865	0.1	0.3759
Residual				0.000350	0.000094	–	–	0.05668	0.02890	–	–

4. Discussion

4.1. Occurrence of microbial symbionts in I. balthica

We found evidence for the presence of bacteria in both the hindgut and hepatopancreas in *I. balthica*. Many terrestrial and semiterrestrial isopods species carry symbiotic bacteria in their hepatopancreas (e.g. Fraune and Zimmer, 2008; Wang et al., 2007; Zimmer, 2002, and references therein), but such symbionts have not been found in marine isopod species (Wang et al., 2007; Zimmer et al., 2001). Thus, our findings demonstrate for the first time the presence of bacterial symbionts in the hepatopancreas of a marine isopod species. In addition, we found fungi that may be either symbiotic or trespassing transient microbes in the intestinal tract of *I. balthica*.

Our finding that a marine isopod species carries intestinal symbionts is of great interest, because intestinal symbionts have been suggested to be uncommon in marine invertebrates (Plante et al., 1990). Plante et al. (1990) reasoned that the conditions within marine invertebrates' guts differ less from the external microbial environment than that in terrestrial species. Chemical conditions, for example ionic and osmotic levels, in the guts of marine invertebrates are almost alike with the external environment. Thus, for marine bacteria it is not necessarily advantageous to colonize the gut of an invertebrate instead of living in seawater. It is worth to note that in the previous studies marine isopods were collected from fully marine or at least clearly more marine environments in the Western Baltic Sea (Wang et al., 2007; Zimmer et al., 2001) than in the present study. Instead, our study area in the northern Baltic Sea is low-salinity brackish-water environment that is characterized by a number of angiosperms originating from fresh-water habitats. Therefore, the selective environment for evolution of intestinal symbiosis may resemble more the one in freshwater habitats.

Bacterial and fungal CFUs were found in all field populations, and their numbers varied among populations. Endogenous bacteria grow in highly distinctive conditions of the gut and most likely only few bacterial or fungal taxa will form colonies on the selective growing media. Therefore, variation in the amount of CFUs most likely indicates variation in microbial community composition among populations. This variation may represent variation in transient microbes due to spatial variation in availability and composition of natural microbial communities, or, it may indicate that local populations are differentiated in their intestinal characteristics, thus allowing the development of dissimilar microbial communities.

The total number of bacteria in the whole intestinal tract differed between the sexes being higher in males than females. This was not explained by the size difference of the sexes. We suggest that the dissimilar host exploitation patterns between the sexes may contribute to the difference in the bacterial numbers: Males favour apical parts of *Fucus*, which contain less phenolic compounds (phlorotannins) than the basal parts, while females do not show such a preference (Jormalainen et al., 2001). However, phenolics disturb the growth of bacteria (Boettcher and Targett, 1993) and thus, it is possible that males either get more bacteria than females via food or the male diet allow better conditions for bacterial growth in the hindgut. Bacteria may oxidise phenolics (Zimmer, 2002, and references therein) and that may generate oxidative stress and reduce the availability of important nutrients in the gut by binding many types of molecules (Appel, 1993; Barbehenn et al., 2003). Thus, the sexual difference in the bacterial abundance may reflect the sex-specific ability to utilise phenolic-rich food.

Antibiotics reduced bacterial counts in isopods originating from the *Fucus* assemblages, but did not have any effect on the isopods originating from the *Zostera* assemblages. As antibiotics are typically selectively efficient against different bacteria, the above finding most likely indicates differences in intestinal bacterial community composition between the assemblages. We hypothesise that the differential assortment of diet species, the digestion of which necessitates distinct enzymatic pathways, has acted as a selective agent for the evolution of symbiotic relationship with gut microbiota and may drive differentiation in the gut characteristics relevant for housing symbiotic bacterial taxa. Our data does provide some evidence for this hypothesis: The differences between the habitats of origin in the bacterial response to antibiotics were detected in isopods born and reared in the laboratory. Thus all of them had equal opportunities to gain bacteria with the ingested food. Therefore, the difference among populations originating from different assemblage may indicate that the populations are genetically differentiated in their gut characteristics relevant for gaining symbiotic gut bacteria.

Numerous studies have shown that invertebrates usually suffer from elimination of symbionts (e.g. Douglas, 1998, and references therein; Zimmer, 2002, and references therein). However, on *Fucus* diet, the isopods with abundant gut bacteria grew less than the isopods treated with antibiotics. By contrast, antibiotic treatment had no effect on growth rate on the angiosperm diet. Thus, the effects of gut bacteria on performance were diet-dependent; on low-quality angiosperm diet they had no effect whereas gut bacteria incurred a cost on the higher-quality *Fucus* diet. Possibly higher growth rates on *Fucus* diet without bacteria can be explained by bacterial activity increasing the amount of toxic oxidation products in the gut (Zimmer et al., 2002). If bacterial activity increases oxidative stress, it seems likely that the lower abundance of bacteria in females may allow them to better grow on phenolic-rich food.

4.2. Enzymatic activity

Phenol oxidation activity occurred in both the hepatopancreas and hindgut of *I. balthica* hinting at this species' ability to oxidatively degrade phenolics. The enzyme activity was higher in the hindgut than in hepatopancreas which may indicate contribution of transient bacteria to phenol oxidase production. Bacterial contribution is further supported by the finding that males exhibited both the higher abundance of bacteria and higher phenol oxidation activity than females. Alternatively, the phenol oxidases can be produced by the animal itself, supported by the finding that the antibiotic treatment affected neither the phenol oxidation activity nor the covariation of enzymatic activity and growth rate. Symbiotic bacteria participate in the degradation of phenolics in some terrestrial and aquatic isopods species (Zimmer, 2002, and references therein; Zimmer and Bartholmé, 2003). In the semi-terrestrial *Ligia pallasii*, however, antibiotic treatment reduced hepatopancreatic bacteria, but did not affect phenol oxidation (Zimmer et al., 2002). The authors thus assumed that phenol oxidases are likely produced endogenously by the isopods.

Interestingly, the covariation of phenol oxidation activity and growth rate varied between the diets implying that efficiency in the ability to oxidize phenolics was beneficial on the *Fucus* diet whereas it was harmful on the angiosperm diet. Thus, phenol oxidation activity in hepatopancreas, originating from either endogenous or bacterial source, provides a mechanism leading to differential ability to utilise different diet species and trade-offs among hosts. However, it remains unclear by which process phenol oxidation activity is more beneficial when feeding on *Fucus* than on angiosperm diet.

Cellulolytic enzymes can be produced endogenously by some invertebrates, for example *Gammarus pulex* (Zimmer and Bartholmé, 2003), land crabs (Linton and Greenaway, 2007) and wood-boring isopods (King et al., 2010). On the other hand, it has been suggested, that many terrestrial and semi-terrestrial isopod species are able to utilise cellulose-rich plant material and digest cellulose with the aid of symbiotic bacteria in the hepatopancreas (Zimmer, 2002, and references therein; Zimmer et al., 2002). However, some marine isopods species, *Idotea wosnesenskii* and *Gnorimosphaeroma oregonense*, digest cellulose even though their hepatopancreas does not contain bacteria (Zimmer et al., 2001), which suggest either endogenous production of cellulases or contribution of hindgut bacteria to digestion. In *I. balthica*, cellulolytic

activity that was found in both the hepatopancreas and hindgut may result from activity of enzymes secreted by isopods, from the activity of symbiotic bacteria or from transient gut passengers. The finding that antibiotic treatment did not affect cellulolytic activity may imply rather endogenous production than contribution by hepatopancreatic and hindgut bacteria, but as antibiotics did not remove all the bacteria the possibility of the latter cannot be excluded.

We found that cellulolytic activity was present in the gut independently of the occurrence of cellulose in the food and that enzyme activity was higher on *Fucus* than on angiosperm diet. This implies that the cellulolytic enzymes were not induced by the presence of the substrate but present continuously. Because the amount and presence of the substrate do not affect enzyme activity by depleting the enzyme there may be something in the angiosperm hosts dampening the action of the enzymes. This may be for example phenolics; seagrasses are particularly rich in phenolic acids, condensed tannins and lignins (Arnold and Targett, 2002).

In conclusion, our results suggest that the marine *I. balthica* harbour symbiotic bacteria in their gut and hepatopancreas. Bacterial counts and numbers of colony forming units varied among isopod populations indicating that both the bacterial abundance and their community composition vary among populations due to either differential availability of microbes in the local environment or population differentiation in gut characteristics. Furthermore the effects of antibiotics on bacterial numbers of laboratory-reared populations depended on the habitat of origin of the these populations, supporting the hypothesis that *I. balthica* populations from distinct host plant assemblages harbour different the intestinal bacterial communities due to habitat-specific (i.e. genetic) differences in some (unknown) gut characteristics. Bacteria are either harmful or indifferent to their isopod hosts depending on the diet and may contribute to digestive processes by affecting the ability to oxidize phenolics and digest cellulose. This suggests that bacterial symbionts likely played a significant role in the host range evolution and among-population differentiation in host use ability in *I. balthica*.

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