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PHYSIOLOGICAL ECOLOGY - ORIGINAL RESEARCH

Sex- and habitat-specific movement of an omnivorous semi-terrestrial crab controls habitat connectivity and subsidies: a multi-parameter approach

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Abstract Distinct habitats are often linked through fluxes of matter and migration of organisms. In particular, intertidal ecotones are prone to being influenced from both the marine and the terrestrial realms, but whether or not small-scale migration for feeding, sheltering or reproducing is detectable may depend on the parameter studied. Within the ecotone of an upper saltmarsh in the United States, we investigated the sex-specific movement of the semi-terrestrial crab *Armases cinereum* using an approach of determining multiple measures of across-ecotone migration. To this end, we determined food preference, digestive abilities (enzyme activities), bacterial hindgut communities (genetic fingerprint), and the trophic position of *Armases* and potential food sources (stable isotopes) of males versus females of different sub-habitats, namely high saltmarsh and coastal forest. Daily observations showed that *Armases* moved frequently between high-intertidal (saltmarsh) and terrestrial (forest) habitats. Males were encountered more often in the forest habitat, whilst gravid females tended to be more abundant in the marsh habitat but moved more frequently. Food preference was driven by both sex and habitat. The needlerush *Juncus* was preferred over three other high-marsh detrital food sources, and the periwinkle *Littoraria* was the preferred prey of male (but not female) crabs from the forest habitats; both male and female crabs from marsh habitat preferred the fiddler crab *Uca* over three other prey items. In the field, the major food sources were clearly vegetal, but males have a higher trophic position than females. In contrast to food preference, isotope data excluded *Uca* and *Littoraria* as major food sources, except for males from the forest, and suggested that *Armases* consumes a mix of C4 and C3 plants along with animal prey. Digestive enzyme activities differed significantly between sexes and habitats and were higher in females and in marsh crabs. The bacterial hindgut community differed significantly between sexes, but habitat effects were greater than sex effects. By combining multiple measures of feeding ecology, we demonstrate that *Armases* exhibits sex-specific habitat choice and food preference. By using both coastal forest and saltmarsh habitats, but feeding predominantly in the latter, they possibly act as a key biotic vector of spatial subsidies across habitat borders. The degree of contributing to fluxes of matter, nutrients and energy, however, depends on their sex, indicating that changes in population structure would likely have profound effects on ecosystem connectivity and functioning.

Keywords Saltmarsh · Coastal forest · Land crab · Sexual dimorphism · Spatial subsidy · Habitat connectivity · Motile link organism

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Introduction

Historically, ecologists considered food webs as static representations of communities, and habitats as closed systems comprising these communities. This approach was useful

for addressing many questions; however, ecosystems and communities are in fact neither closed nor static, and the fluxes of matter and migration of organisms across ecosystem boundaries contribute significantly to community dynamics, food web dynamics, and ecosystem functioning (Polis et al. 1997; Loreau et al. 2002; Knight et al. 2005). Spatial subsidies across habitat boundaries (allochthonous inputs) are ubiquitous and can be mediated by passive physical transport or by active biotic movement. For example, the deposition of macrophyte wrack of marine origin ashore in the marine–terrestrial ecotone is driven by wind or water movement (Orr et al. 2005). In contrast, many organisms actively move between different habitats that provide feeding grounds versus shelter (Polis et al. 1997; Lewis et al. 2007; Garcia et al. 2011) or change habitats during their life cycle (e.g., crustaceans and insects, Knight et al. 2005; Gratton et al. 2008; Gratton and Vander Zanden 2009; Hoekman et al. 2011; Vander Zanden and Gratton 2011) and, hence, act as biotic vectors of spatial subsidies by translocating material (prey, fecal matter, etc.) between habitats. These "motile link organisms" (sensu Lundberg and Moberg 2003) transfer not just nutrients but also ecological processes. For example, a motile consumer can affect prey densities in multiple habitats, potentially propagating interactions from one habitat into the other (Henry and Jefferies 2009), and food web dynamics in the two habitats are linked (McCann 2012).

Tidal saltmarshes are ecologically important (Barbier et al. 2011) habitats situated between subtidal marine and upland habitats. There is an extensive body of research examining the physical and biotic mechanisms driving subsidies between saltmarshes and subtidal systems. Studies of physical subsidies have focused on "outwelling", the idea that a large proportion of the extraordinarily high primary production of saltmarshes is moved by tides into subtidal habitats, where it fuels heterotrophic coastal metabolism (Teal 1962; Valiela and Teal 1974; Peterson and Howarth 1987; Krest et al. 2000; Cai 2011) and may affect community structure (Peterson and Howarth 1987; Currin et al. 1995; Créach et al. 1997; Fantle et al. 1999; Guest et al. 2004). Studies of biotic subsidies have focused on juvenile nekton living in coastal waters next to marshes (Kneib 1997; Jones et al. 2002; Mazumder et al. 2006). In contrast to this extensive body of research, relatively little is known about biotic subsidies between marshes and adjacent upland habitats. It has long been recognized that fish, mammals, alligators and birds move between marshes and upland habitats to feed and rest (Rosenblatt et al. 2013), but few studies have quantified the extent of these linkages (but see Garcia et al. 2011; Brittain et al. 2012; Platt et al. 2013). Along this line, tidal inundation may remove organic matter from the intertidal into coastal waters (c.f. Orr et al. 2005, and references therein), but motile link organisms that move between the intertidal and the supratidal may help sustain nutrient balances in the former.

A relatively unstudied aspect of subsidies driven by motile link organisms is the extent to which these subsidies are sexually dimorphic. Male and female organisms differ in many aspects of their behavior and ecology (Breed et al. 2006; Thaxter et al. 2009; Helfer et al. 2012), and so it is likely that they contribute to subsidies in different ways, or to different extents, but this has rarely been studied. Furthermore, spatial subsidies are mostly considered in one direction, fueling one receptor habitat (sink) from one donor habitat (source), but back-and-forth movements of "motile link organisms" among adjacent habitats may result in bi-directionally subsidizing both habitats, making a distinction between sink and source difficult or even obsolete. Deciding upon source versus sink habitats may depend upon the parameter under investigation: studying only one measure of habitat connectivity through motile links might result in a different picture than using an integrated approach of quantifying multiple parameters.

Here, we focus on a crab, *Armases cinereum* (hereafter *Armases*), that is common in southeastern USA saltmarshes. *Armases* is often described as a "saltmarsh" crab (Pennings et al. 1998; Buck et al. 2003; Zimmer et al. 2004; Ho and Pennings 2008), but it prefers drier and higher areas in the intertidal region, and is usually found under wrack and wood (Seiple 1979) at the terrestrial border of the marsh or in the coastal forest. From there, these crabs range far into either habitat (Pennings et al. 1998), but must return to the water to release their eggs during the reproductive period (c.f. Anger 1995 for terrestrial crabs). *Armases* is broadly omnivorous (Buck et al. 2003; Ho and Pennings 2008; Ewers et al. 2012) and so may act in translocating both organic matter and consumer–prey interactions back and forth between the intertidal and supratidal area. Male and female *Armases* differ morphologically in that males have larger claws and are better able to eat hard-shelled prey (Buck et al. 2003). Hence, sex-specific migration patterns or spatiotemporal changes in population structure (sex ratio) may have profound effects on the ecological role of this motile link organism that connects saltmarshes and coastal forest. The magnitude and nature of this putative cross-boundary linkage depends on how *Armases* uses adjacent habitats, that is, where and what *Armases* crabs feed upon, and how they move between habitats. Using field and laboratory approaches, we tested the hypothesis that individual male and female *Armases* crabs at Sapelo Island (GA, USA) regularly move between the upper saltmarsh and the coastal forest, versus the alternate hypothesis that crabs stay for extended periods in individual habitats, forming stable subpopulations of marsh crabs and forest crabs. For this, we used two complementary approaches to obtain a comprehensive picture of the potential of saltmarsh crabs to act as biotic vectors of spatial subsidies:

(1) observations of crab movements in the field, and (2) laboratory analysis of feeding ecology based on four parameters. First, food preferences: preference tests provide information about potential food preferences, when and if different foods are available (which might or might not be the case in the field). Second, digestive abilities (enzyme activities): the activities of different digestive enzymes inform about recently ingested food sources, assuming that most digestive enzymes are inducible in that their activities correspond with the mixture of food sources inside the digestive tract. Third, bacterial gut communities (genetic fingerprint): the gut microbiota are an indicator of sex, habitat, and food sources (at a range of temporal scales) (Dittmer et al. 2012; Mattila et al. 2014). And fourth, trophic positions (stable isotopes) of crabs found in both habitats: the stable isotope signature of consumers provides a long-term integration of actual food choice in the field.

Previous studies have examined species-specific contributions to connecting adjacent habitats. We asked whether movement and feeding patterns differed among individuals of the same species depending on sex and habitat. If so, this would reveal the potential for intraspecific variation in contributing to spatially subsidizing neighbor habitats through movement and feeding patterns that differ between sexes and among subpopulations from adjacent habitats. Moreover, if predictable intraspecific variation in subsidies exists, it will allow for predicting changes in spatial connectivity of adjacent habitats upon changes in population structure.

Materials and methods

Study site and species

Field work was performed during May–July 2009 in the coastal forest and saltmarsh on western Sapelo Island, GA, USA (31°27′N, 81°15′W). Laboratory work for enzyme measurements, genetic fingerprinting and preparing stable isotope analysis was carried out at the University of Georgia Marine Institute (UGAMI) on Sapelo Island and the Zoological Institute of the Christian-Albrechts-Universtität, Kiel, Germany.

Saltmarshes around Sapelo Island are typical of the south-eastern Atlantic coast of the US (Ho and Pennings 2008) and are dominated by the C4 cordgrass, *Spartina alterniflora* Loisel (Pennings et al. 1998; Zimmer et al. 2004). The most common high marsh plant is the C3 black needlerush, *Juncus roemerianus* Scheele (Parsons and De La Cruz 1980; Pennings et al. 1998). The composite shrub *Borrichia frutescens* (Linnaeus) is common at the terrestrial border (Pennings et al. 1998), and the coastal forest adjacent to the marsh is dominated by live oak, *Quercus virginiana* Miller. Both saltmarsh plants, *Spartina* and *Juncus*, and plants from the terrestrial habitat, especially *Quercus*, produce large amounts of litter that accumulates in the high marsh (Zimmer et al. 2004). Other plants, like *Iva frutescens* Linnaeus and *Sarcocornia* spp. Linnaeus, are also common near (*Sarcocornia*) or at (*Iva*) the terrestrial border of Georgia saltmarshes (Pennings et al. 1998) but were not dominant in the area where the research was conducted. Hence, *Quercus*, *Borrichia*, *Juncus* and *Spartina* were used in feeding experiments, because they were common at the study site in different habitats that crabs might move between, and because *Armases* is known to feed on all these plants (Pennings et al. 1998; Buck et al. 2003; Zimmer et al. 2004).

Four small invertebrates, two gastropods and two crustaceans, were common in the study area, and *Armases* is also known to feed on these small invertebrates living either in the terrestrial habitat or in the saltmarsh (Buck et al. 2003; Ho and Pennings 2008). The periwinkle, *Littoraria irrorata* (Say), is most abundant at lower latitudes, and the coffeebean snail, *Melampus bidentatus* Say, occurs along the entire US east coast (Silliman and Zieman 2001). Both snail species were consumed by *Armases* in previous experiments (Buck et al. 2003), although the amount eaten differed between male and female crabs and depended on crab and snail size. Fiddler crabs, *Uca* spp., are abundant in lower Georgia saltmarshes (Plumley et al. 1980), with an average density of ca 200 crabs m^{-2} (Wolf et al. 1975). *Armases* crabs grow rapidly when fed dead fiddler crabs [*Uca pugnax* (Smith)] (Buck et al. 2003). The common amphipod, *Orchestia grillus* (Bosc), was chosen to represent a high-marsh invertebrate that is known to be readily eaten by both female and male *Armases* (Buck et al. 2003).

Movement in the field

According to the site of capture, crabs were designated as either "marsh crabs" or "forest crabs". We further identified crabs as males, females or gravid females. An area of 20 m width (parallel to the coastline) and stretching from the marsh into the forest (perpendicular to the coastline) over a length of 25 m (Fig. 1) was divided into 5 m × 5 m squares and marked with colored flags for orientation. This area included a 10-m-wide coastal forest area on the inland site (forest), a 5-m-wide transition habitat at the terrestrial border (transition) and a 10-m-wide high marsh habitat dominated by *Juncus* (marsh). These areas will henceforth be referred to as forest, transition, and marsh.

To observe the movement of *Armases* between the habitats, 300 crabs were collected in May 2009 and marked. Of these, 150 crabs were collected in the forest and marked with yellow nail polish with numbers from 1 to 150. The other 150 crabs were collected in the marsh and marked with pink nail polish from 1 to 150. All animals were scored as males, females or gravid females. Carapace width and length were measured with calipers. We compared the

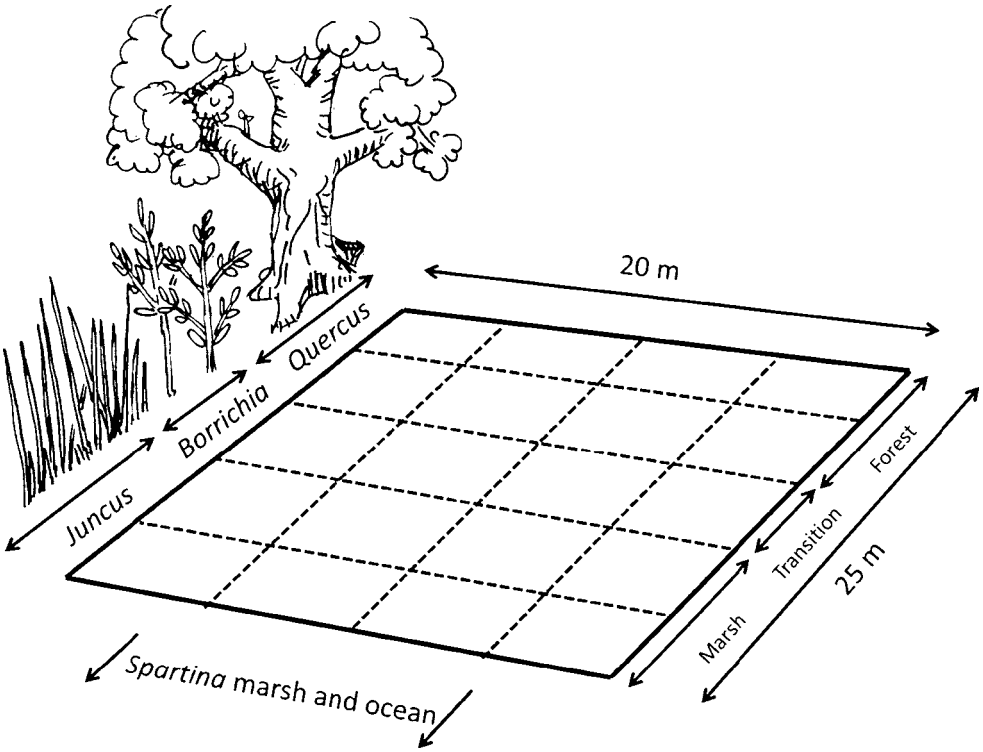

Fig. 1 The sampling setting, ranging from the upper marsh (*Juncus*) into the forest (*Quercus*)

size of males and females, and forest crabs and marsh crabs through pair-wise comparisons ($\alpha = 0.05$) by using Mann–Whitney *U* tests (GraphPad Prism 5).

Crabs were released in the center of the marked areas in which they were captured. Marked *Armases* were observed once daily for about 1 h for 25 days around slack low tide during daytime. In each area of 5 m width and 20 m length, a single investigator searched for marked *Armases* for 15 min while slowly walking the transect. Each numbered crab that was observed was recorded and counted as a "recapture", although crabs were not physically captured during daily monitoring.

To show the ranges of movement of crabs that were captured more than two times during the observation, an individual ellipse of the recapture range was created for each crab. Ellipses show the centroid of ellipsoid range and maximum distance moved by the crabs along (horizontally) and perpendicular to (vertically) the marsh–forest gradient in the marked area. Statistical analyses were performed using the Wilcoxon signed-rank test (SPSS 20) to test for differences in the lengths of the two axes of ellipses (vertical vs. horizontal) between forest crabs and marsh crabs, further separated into males and females.

Food preference

Food preference among leaf litter of the four plant species were separately evaluated for forest crabs versus marsh crabs and females versus males. *Armases* and leaf litter were collected in the area described above and taken to the laboratory. The fresh weight of each crab was determined. Leaf litter was used because it was preferred over fresh leaves in previous experiments (data not shown; Buck et al. 2003), but litter was in an early stage of decomposition.

Crabs ($n = 7$ for each group) were maintained at room temperature individually in the laboratory in Petri dishes (14 cm diameter). A small bowl of water (15 psu) was present to prevent dehydration of the crabs during the experiment. For each feeding trial, detrital leaves were cut into two pieces of approximately same size. One piece was used for the treatment, the other piece was used in a crab-free control to monitor autogenic changes in mass (Peterson and Renaud 1989). Fresh weight (FW) of each half was determined. Each feeding trial contained four leaves per crab, one of each plant species. Based on consumption rates as determined in pilot experiments (not shown), feeding trials ran for 3 days, and crabs were then released at a separate location to ensure that they were only used once in experiments. Leaf remains and leaf pieces from the controls were weighed, oven-dried at 60 °C to constant weight, and weighed again. We estimated the initial dry weight of the leaves using the fresh:dry weight ratio of the control leaves and calculated mass loss of experimental leaves on a dry weight basis upon correction for autogenic mass loss of control leaves. Total amount eaten of all food sources together by one crab was considered 100 % consumption. For each crab, we calculated the proportion of feeding on each of the four food sources. Statistical analyses of

Table 1 Summary of studied enzymes and their relevance for digestive processes

Enzyme group	Digestive function	Indicative of …
Proteases	Proteolytic breakdown (proteolysis) of proteins, poly- and oligo-peptides from various (vegetal, animal, microbal) sources	All trophic groups; more important in carnivores
Cellulase	Hydrolytic breakdown (cellulolysis) of cellulose from vegetal and microbial sources	Herbivores, detritivores
Amylase	Hydrolytic breakdown of starch from vegetal sources	Herbivores, detritivores
Phenol oxidases	Oxidative breakdown of (condensed) tannins, other phenolics, and lignins from vegetal sources	Herbivores, detritivores[a]
Esterases	Hydrolytic cleavage of aliphatic and aromatic esters into an acid and an alcohol; very diverse group with broad spectrum of, mostly not clearly defined, reactions, e.g., breakdown and detoxification of hydrolyzable tannins	All trophic group; more important in herbivores and detritivores[b]

[a] Carnivores also exhibit phenol oxidase activity in the context of immune response, wound-healing and molting (arthropods) but not inside their digestive tract

[b] Carnivores also exhibit multiple esterase activities, but herbivores and detritivores appear to more directly depend on esterases sensu strictu, whereas carnivores are more in need of lipases

feeding preference were performed using the poptools add-on to MS Excel for Resampling and Monte Carlo Analysis with 9999 permutations (Frenkel 2004).

Since *Armases* prefers invertebrate prey over vegetal food sources (Buck et al. 2003), we also investigated food preference among potential prey items (dead *Uca*, *Orchestia*, *Melampus* and *Littoraria*) for forest crabs versus marsh crabs and females versus males. *Armases* and prey were collected as described above. The fresh weight of each crab was determined, and soft body mass of each prey was estimated based on previously determined live mass:soft body mass ratios.

Crabs ($n = 7$ for each group) were offered the four prey items simultaneously for 3 days, and afterwards the remaining soft body mass of prey items was estimated. The total amount eaten of all food sources by each crab was considered 100 % consumption. For each crab, we calculated the proportion of feeding on each of the four food sources. Statistical analyses of food preference were performed using the poptools add-on to MS Excel for Resampling and Monte Carlo Analysis with 9999 permutations (Frenkel 2004).

Digestive capabilities and natural food sources

In order to determine actual food sources of male and female crabs from different habitats through an integrated approach of measuring multiple parameters, 50 crabs (25 forest crabs, 25 marsh crabs) were collected for analysis of enzyme activities, gut microbial communities and stable isotope signatures. By using multiple indicators of diet, we obtained a more comprehensive picture than one could gain from any one of these. These measures have different strengths and weaknesses. The enzyme activities provide an indication of recent diet, but are fairly general measures for broad categories of compounds; the gut microbial communities are potentially quite diverse and so can potentially distinguish among many different types of diets, but the link between particular microbial species and particular dietary items is often obscure; and the stable isotope signature provides an indication of diet integrated over a longer time period, but may have low resolution if multiple food types have similar isotopic signatures.

For each individual, carapace width and length was measured and fresh weight was determined before crabs were anesthetized individually in ice-water for 5 min. Claws were cut off and stored in 3 mL ethanol (~100 %) until processing. We followed the protocol of Pavasovic et al. (2004), using K/Na-phosphate buffer (pH 6.2) for preparation of the digestive tract. The carapace was opened and tissue remains around the stomach and above the mid-gut gland removed. The mid-gut gland (MG) was dissected out and washed in buffer (K/Na-phosphate buffer, pH 6.2) to minimize the risk of contamination with microbial enzymes which may have been present in the gut (Pavasovic et al. 2004). After washing, the MG was stored in 1 mL of the above buffer until use for enzyme measurements. The hindgut was dissected out and stored in 1 mL absolute ethanol without removing gut content remains for analyses of microbial diversity.

Enzyme activities

The activity of gut enzymes provides information on the nature of major food sources (Table 1). For example, species that feed on a high-protein diet possess a wide range of proteolytic enzymes, whereas species that feed on large amounts of carbohydrates exhibit highly active carbohydrases (Johnston and Freeman 2005). Crustaceans are known to possess many of the enzymes required to break

down key nutrients such as proteins and carbohydrates (Pavasovic et al. 2004).

Individual midgut glands were homogenized in 1 mL K/Na-phosphate buffer (pH 6.2), and homogenates were centrifuged for 10 min with 10,000*g* at 4 °C. The supernatant was diluted by adding 1 mL of K/Na-phosphate buffer (pH 6.2) and stored in aliquots at −20 °C until use for individual enzyme measurements.

Total protease activity was measured using azocasein as a substrate following the protocol described by Dìaz-Tenorio et al. (2006) in K/Na-phosphate buffer (pH 6.2). Aliquots of 20 μL of MG extract were added to 480 μL of K/Na phosphate buffer (pH 6.2) and mixed. The reaction was started by adding 500 μL of 1 % azocasein solution to the samples and stopped after 1 h by adding 500 μL of 20 % TCA and maintaining at 0 °C for 10 min. Samples were centrifuged at 10,000*g* at 4 °C for 5 min, and absorbance was recorded at 366 nm with a spectrophotometer. For the blank, 20 % TCA was added before adding MG extracts.

Cellulase activity was measured using α-cellulose as substrate (Zimmer 2005). Following the protocol of Zimmer (2005), 20 mg α-cellulose was added to 200 μL aliquots of MG extract and mixed. The Glucose (HK) Assay Kit (Sigma) was used to quantify the amount of enzymatically released glucose following the manufacturer's protocol. 200 μL citrate phosphate buffer containing 0.05 % NaN_3 was added to the samples that were then incubated on a shaker with 200 rpm for 24 h at room temperature. Samples were centrifuged for 10 min (10,000*g*, 4 °C) after incubation, and the absorbance of supernatants was measured at 340 nm.

Amylase activity was measured as described for cellulase but using starch instead of cellulose as substrate.

Total phenol oxidase activity was measured using pyrocatechol as a substrate, following the protocol described by Zimmer (2005). Aliquots of 100 μL of MG extract were added to 900 μL of 50 mM pyrocatechol solution and mixed. Change in absorbance was recorded at 340 nm and followed at 1-min intervals for 10 min. Relative phenol oxidase activity was determined through linear regression analysis (GraphPad Prism 5).

Total esterase activity was measured using 1-naphthyl acetate as a substrate, modified after He (2003). Aliquots of 100 μL of MG extract were added to 900 μL of 50 μM 1NA solution and mixed. Change in absorbance was recorded at 320 nm and followed at 1 min intervals for 10 min. Relative Esterase Activity (REA) was determined through linear regression analysis (GraphPad Prism 5).

We compared enzyme activities of males, females, forest crabs and marsh crabs using two-way ANOVA, with sex and habitat as factors (SPSS 20).

Gut bacterial community composition

In addition to species-specific intestinal microbes, the gut microbial community reflects the recent actual diet, and intraspecific differences in gut microbial community composition suggest individual differences in food sources (Dittmer et al. 2012). We used denaturating gradient gel electrophoresis (DGGE) to visualize differences in the composition of gut bacterial communities of the crabs collected in different habitats and crabs of different sexes. Hindgut samples ($n = 6$ for each group) stored in ethanol (see above) were centrifuged for 1 min (6000*g*), and the supernatant ethanol was removed. DNA was extracted using the Qiagen DNAesy kit following the bench protocol for animal tissues.

Following the protocol used by Lachnit et al. (2009) based on Muyzer et al. (1993), PCR-amplification of bacterial 16S rRNA genes of bacterial community DNA present in gut samples was performed using PuReTaq Ready-To-Go PCR Beads (GE Healthcare) in a total PCR volume of 25 μL, containing 10 pmol of each bacterial primer, 341F-GC [5′-(CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GC) CTA CGG GAG GCA GCA G-3′] and 534R (5′-ATT ACC GCG GCT GCT GG-3′). After initial denaturation at 94 °C for 2 min, 15 touchdown cycles (annealing at 65 °C for 40 s, incremental reduction by 1 °C per cycle; elongation at 72 °C for 40 s; denaturation at 95 °C for 30 s) were followed by 25 annealing cycles (annealing at 50 °C for 40 s; elongation at 72 °C for 40 s; denaturation at 94 °C for 30 s) and a final annealing (42 °C for 60 s) and elongation (72 °C for 5 min). The correct size of the amplified DNA fragments was verified through electrophoresis of 2.5 μL of the PCR reaction volume in 1.5 % agarose (150 V).

DGGE was performed using double gradient polyacrylamide gels (Lachnit et al. 2009) with a denaturing gradient from 40 to 80 % (100 %: 7 M urea and 10 M formamide) and an acrylamide gradient from 6 to 8 %. Electrophoresis was run at 60 °C for 13.5 h at 80 V in 0.5 × TAE buffer in a CBS Scientific DGGE-2001 system. After electrophoresis, the gel was stained for 45 min in SYBR Gold® (Invitrogen), rinsed for 30 min in 1× TAE buffer, and photographed under UV light. DGGE gels were analyzed through the generation of a presence–absence matrix based on the individual band pattern. All visible bands in the gel lane were taken into account and assigned to OTU's for further calculation using PRIMER v.6.1.9 (Primer-E). We calculated Bray–Curtis values without transformation, and visualized sample similarities (ANOSIM, two-way crossed with replicates) using cluster analysis and non-metric multidimensional scaling (NMDS).

Isotopic signatures

We used stable isotope analysis to identify carbon sources of *Armases*, to compare the trophic position of *Armases* with the trophic positions of potential food sources, and to detect diet differences in crabs from different habitats or of different sexes.

A randomly chosen mix of leaf litter from numerous individuals of each species and animals were collected at the study site where fieldwork was conducted. *Armases* were collected from the forest and marsh; all other species were collected only from their primary habitat. Shells of the snails ($n = 3$ for each species) were removed, guts were discarded, and muscle tissue was used for analysis of isotopic signatures. Amphipods ($n = 3$) were taken as a whole, because *Armases* fed on whole amphipods during previous experiments. For crabs [*Armases* ($n = 5$ for each group) and *Uca* ($n = 3$)], claws were cut off and muscle tissue was taken out after drying. Samples were washed with distilled water and oven-dried at 60 °C to constant weight before being ground for 2 min at 60 % speed with a pebble mill (Retsch, Polzin Laborbedarf) and homogenized. Samples were weighed and encapsulated in tin capsules following the direction for the preparation of C and N solid samples of the UC Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu/), where samples were analyzed. We compared isotope values of males and females, and forest crabs and marsh crabs pair-wise ($\alpha = 0.05$) by using Mann–Whitney *U* tests (GraphPad Prism 5).

In order to estimate the proportional contribution of different food sources to the nutrition of crabs, we calculated mixing models using the R package SIAR. For this, we complemented our dataset with average isotope values for benthic diatoms, a potential food source that we had not taken into account in our experiment, using data from Kasai et al. (2004) (for a temperate estuary: Japan), encompassing data provided by Riera et al. (1996) and Kurata et al. (2001) (for saltmarshes: France and Japan, respectively), Doi et al. (2005) (for a temperate estuary: Japan) and Kanaya et al. (2007) (for a temperate brackish lagoon: Japan), as well as Haines (1976a, b) and Haines and Montague (1979) for benthic micro-algae (diatoms) at Sapelo Island.

Results

Movement in the field

Captured male crabs were on average 43 % heavier (1.0 ± 0.3 vs. 0.7 ± 0.2 g; ANOVA: $p < 0.01$) but not larger (carapace width: 14 ± 3 vs. 14 ± 2 mm; $p = 0.45$) than females. Irrespective of their sex, crabs captured in the forest were 25 % heavier (1.0 ± 0.3 g; $p < 0.01$) and slightly (7 %) larger (15 ± 1 mm; $p < 0.05$) than those captured in the marsh (0.8 ± 0.2 g; 14 ± 0.9 mm).

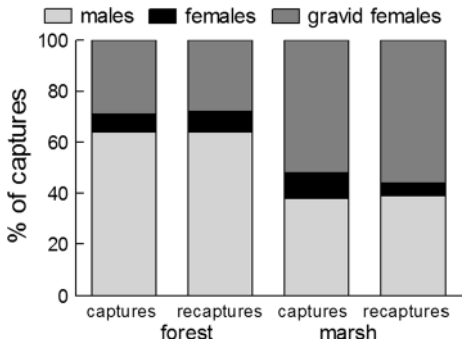

Fig. 2 Proportions of males, females and gravid females of the crab, *Armases cinereum*, in captures and recaptures in the forest and marsh: 100 % captures corresponds to 150 captured and marked crabs in each habitat; 100 % recaptures corresponds to the total number of recaptured crabs in each habitat, irrespective of where they had been originally caught. In total, 78 of the forest crabs and 89 of the marsh crabs were recaptured at least once

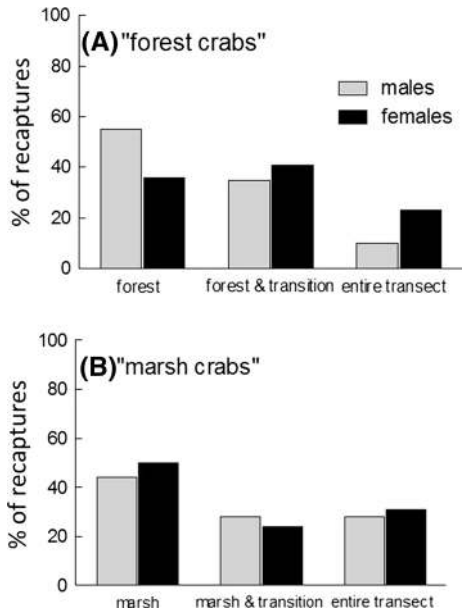

Fig. 3 Proportional distribution of recaptures of forest crabs (**a**) and marsh crabs (**b**) in either the habitat of their capture (forest or marsh, respectively), in their respective habitat and the transition zone (forest and transition or marsh and transition, respectively), or over the entire transect

The *Armases* population in the forest was biased towards males (>60 %), whereas the population in the marsh was biased towards gravid females (>50 %; Fig. 2). Non-gravid females were rare in both habitats. The high frequency of gravid females was consistent with previous reports that females are gravid from mid-April to November (Seiple 1979; Seiple and Salmon 1987).

Most of the forest males were recaptured in the forest (ca 55 %) or transition zone, and only a few (ca 10 %) were

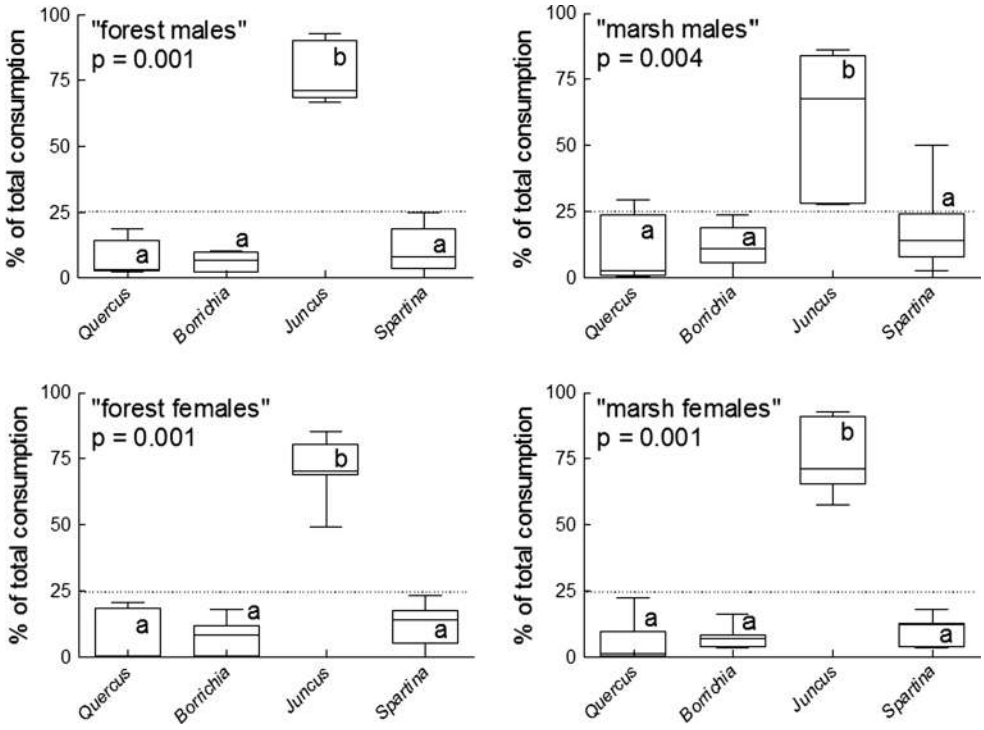

Fig. 4 Proportions of amounts eaten of different vegetal food sources by male and female forest and marsh *Armases* in preference tests. 100 % corresponds to the total amount eaten by each individual crab. *Box plots* represent minimum, first quartile, median, third quartile and maximum ($n = 7$). *Lower case letters* indicate pair-wise differences; the *dotted line* indicates equal consumption (25 %) of each food source

found in the marsh area (Fig. 3a). Forest females (gravid and non-gravid) showed less pronounced habitat choice and were more likely to be recaptured in the transition zone or in all habitats including the marsh (~22 %). By contrast, marsh crabs commonly were observed in all areas including the forest (males ~42 %, females ~46 %) (Fig. 3b). In sum, marsh crabs of both sexes were quite likely to move into the forest, but forest males were more likely to remain in the forest than forest crab females "Appendix 1".

Food preference

Male and female crabs from both habitats strongly preferred *Juncus* litter over the other three detrital food sources ($p < 0.001$; Fig. 4). Among the available prey items, males (both from the forest and the marsh habitat) preferred *Littoraria*, whereas marsh crabs (both females and males) preferred *Uca* (Fig. 5). Hence, marsh males did not exhibit any preference among *Littoraria* and *Uca* but preferred both over *Melampus* and *Orchestia*, and forest females did not show any significant food preference.

Digestive capabilities and natural food sources

Enzyme activities

Enzyme activities differed between female and male crabs, and between forest crabs and marsh crabs (Fig. 6). Except for cellulase activity, all enzyme activity depended on both habitat and sex (protease—habitat: $p < 0.001$, sex: $p = 0.003$, interaction: $p = 0.4$; amylase—habitat: $p < 0.001$, sex: $p = 0.001$, interaction: $p = 0.6$; phenol oxidase—habitat: $p < 0.001$, sex: $p < 0.001$, interaction: $p = 0.003$; esterase—habitat: $p < 0.001$, sex: $p < 0.001$, interaction: $p = 0.4$). Cellulase activity (Fig. 6b) depended on habitat ($p < 0.001$) but not sex ($p = 0.277$).

Gut bacterial community composition

The bacterial community present in the hindgut differed ($p = 0.03$) between habitats and sex, although similarities within habitat ($R = 0.124$) and sex ($R = 0.209$) were low. Six of the female crabs analyzed (two of them being forest crabs) harbored bacterial communities in their hindguts that were clearly distinct from those of male crabs (45 % similarity), whereas the other females (mostly forest crabs) resembled males by >65 % similarity; 9 out of 12 forest crabs clustered together, with only 3 marsh crabs included in this cluster of >60 % similarity (Fig. 7). Accordingly, forest crabs and marsh crabs formed distinct groups in MDS with an overlap of only four (three males and one female) forest crabs included in the marsh cluster and only one (male) marsh crab included in the forest cluster (inset in Fig. 7). Overall, crabs of different sexes were more similar than crabs of different habitats, and sex was a weaker predictor for dissimilarity in the gut microbiota than was habitat.

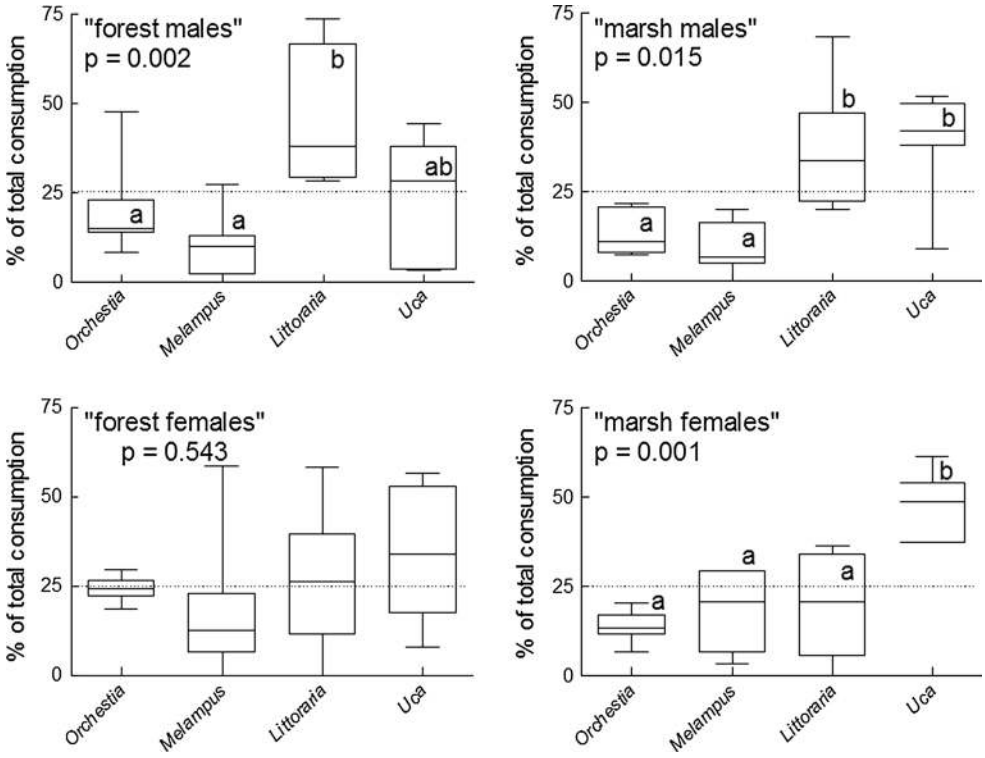

Fig. 5 Proportions of amounts eaten of different animal food sources by male and female forest and marsh *Armases* in preference tests. 100 % corresponds to the total amount eaten by each individual crab. *Box plots* represent minimum, first quartile, median, third quartile and maximum ($n = 7$). *Lower case letters* indicate pair-wise differences; the *dotted line* indicates equal consumption (25 %) of each food source

Isotope signatures

Carbon isotopic values of *Spartina* ($\delta^{13}C = -12.97$ to -13.36 ‰) were different from those of transition and upland plant litter from *Quercus*, *Borrichia* and *Juncus* ($\delta^{13}C = -25.70$ to -31.06 ‰) (Fig. 8). Carbon isotope values of potential gastropod prey were clearly separated from those of the potential crustacean prey. *Melampus* ($\delta^{13}C = -14.91$ to -15.25 ‰) and *Littoraria* ($\delta^{13}C = -14.35$ to -15.57 ‰) both had carbon isotopic values similar to *Spartina*, whereas values of *Orchestia* ($\delta^{13}C = -18.82$ to -20.08 ‰) and *Uca* ($\delta^{13}C = -20.08$ to -22.96 ‰) had values similar to those of *Armases* ($\delta 13C = -16.55$ to -21.64 ‰). The mean carbon isotope value of forest crabs ($\delta^{13}C = -20 \pm 1$ ‰) and the mean value of marsh crabs (-20.9 ± 0.5 ‰) did not differ ($p = 0.1$) and fell between terrestrial and saltmarsh plants.

Nitrogen isotope values of plants ranged from $\delta^{15}N = -0.77$ ‰ in *Quercus* to $\delta^{15}N = 3.04$ ‰ in *Spartina*. *Spartina* receives nutrients from coastal water bodies and is therefore enriched in heavy nitrogen relative to terrestrial plants (Currin et al. 1995). Nitrogen isotope values of *Orchestia* (1.10–1.89 ‰) and *Melampus* (1.41–1.84 ‰) were lower than those of *Armases*, whereas those of *Littoraria* (5.92–6.35 ‰) and *Uca* (5.68–6.20 ‰) were similar to those of *Armases* (Fig. 8). Mean nitrogen isotopic values did not differ significantly ($p = 0.3$) between forest crabs ($\delta^{15}N = 5.7 \pm 0.9$ ‰) and marsh crabs (5.5 ± 0.5 ‰), but males were characterized by significantly ($p = 0.043$) higher values (5.9 ± 0.6 ‰) than females (5.2 ± 0.6 ‰). The larger variation in carbon signature of marsh males than of other crabs suggests a greater variety of food sources among male *Armases* that live in the high marsh.

According to the diet mixing model used herein, forest males ate approximately the same amount (c. 5–15 %, each) of all 9 food sources considered ("Appendix 2"). All other crabs ingested significantly more *Quercus*, and to a lesser degree *Juncus*, *Orchestia* and *Melampus* (forest females) or *Borricchia* (marsh crabs) than of *Littoraria*, *Uca* or benthic diatoms.

Discussion

Movement in the field

Based on our data on spatial distribution and migration of male and female crabs within the marsh–forest ecotone, we propose that habitat choice (marsh vs. forest) is sex-specific. Males prefer the coastal forest habitat, whereas gravid females prefer the marsh habitat. Captures of non-gravid females were low, due to the synchronized breeding period, but they seem to exhibit only weak selectivity for either habitat. Crabs found in the marsh, i.e. mostly gravid females, exhibit a large activity range, including both the marsh and the coastal forest. Male crabs, by contrast, and

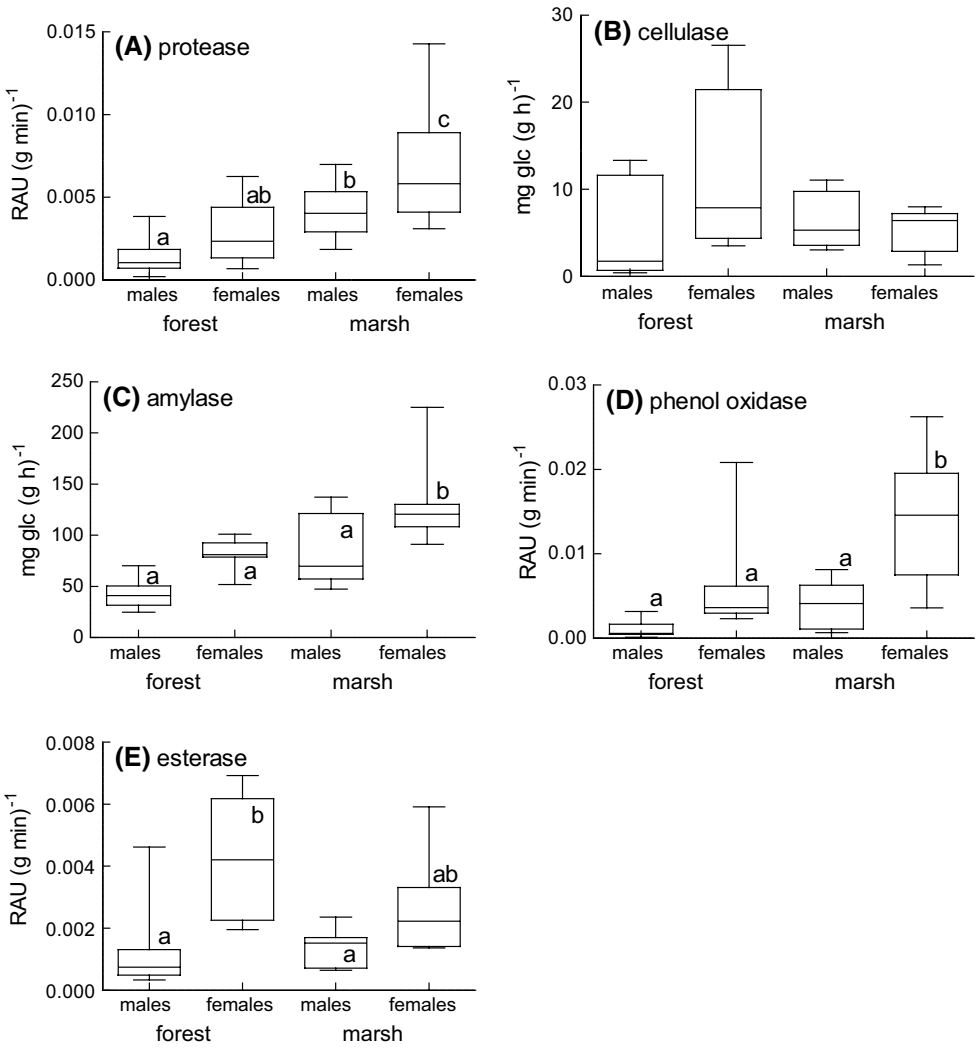

Fig. 6 Digestive enzyme activities in midgut gland extracts of male and female forest and marsh crabs. **a** protease, **b** cellulase, **c** amylase, **d** phenol oxidase, **e** esterase. *Box plots* represent minimum, first quartile, median, third quartile and maximum ($n = 12$). *Lower case letters* indicate pair-wise differences

particularly those caught in the forest, exhibit a much more restricted activity range that largely excludes the marsh ("Appendix 1"). Nonetheless, our data indicate that *Armases* moves regularly between the upper marsh and the forest and does not establish highly specialized subpopulations in the forest versus in the marsh. Hence, *Armases* has the potential to act as a vector for organic matter between the saltmarsh and adjacent coastal forests, but both the type of matter and its spatial fate will depend on the crab's sex.

The choice of the marsh habitat by gravid females could be related to the vicinity to water where they release their larvae after incubation (Lee 1998). Moreover, exposed eggs are likely vulnerable to desiccation in the drier forest habitat. Additionally, gravid female crabs as well as fish and shellfish may use the marsh, because dense plant cover in the marsh protects them from predators (McIvor and Odum 1988). However, both male and female crabs move into the marsh habitat and, according to our findings on digestive capabilities and nutrition, do so for feeding. It thus remains an essentially unanswered question why male crabs remain in the drier and cooler coastal forest with (detrital) food sources of relatively low nutritive value; however, forest males seem to have access to a more diverse diet than their conspecifics that spend more time in the marsh, and this may have advantages that were not captured in this study.

Integrated feeding ecology

According to food preference, enzyme activities and stable isotope signatures, diet choice by *Armases* reflects habitat choice which, in turn, is sex-specific. Male crabs in the forest equally feed on detritus and invertebrate prey that they

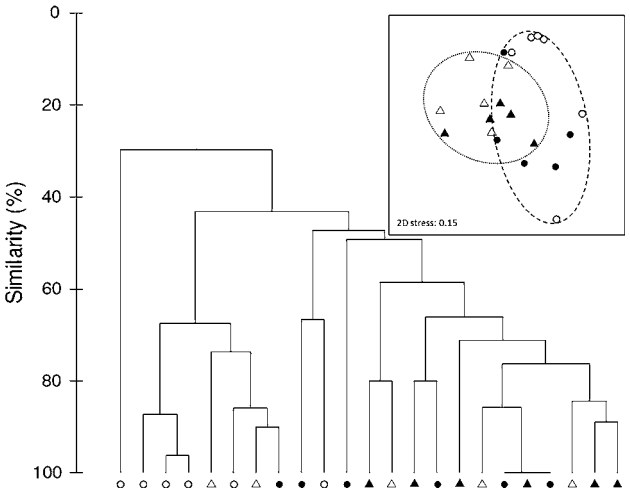

Fig. 7 Cluster analysis (Euclidean distance) and non-metric MDS (*inset* Bray-Curtis similarity) of gut bacterial community composition in male (*filled symbols*) and female (*open symbols*) marsh crabs (*triangles*) versus forest crabs (*circles*)

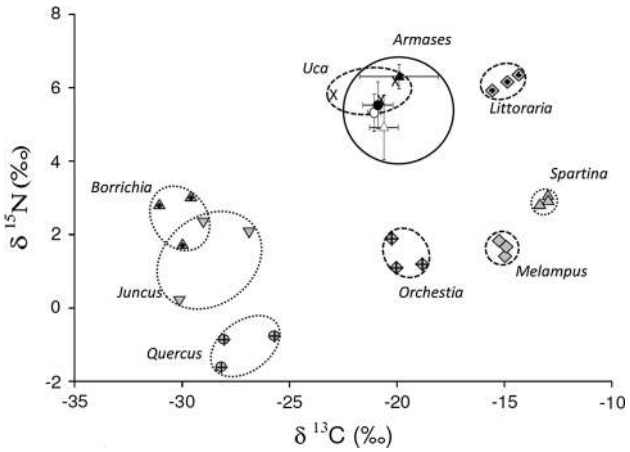

Fig. 8 Stable isotope (^{15}N and ^{13}C) signatures of male (*filled symbols*) and female (*open symbols*) *Armases* from marsh (*triangles*) versus forest (*circles*) habitat and selected potential animal (*dashed elipsoid*) and vegetal (*dotted elipsoid*) food sources

can handle with their larger claws. Females, particularly gravid ones, in the marsh feed more actively, both on detritus and those more protein-rich animals that are easy to handle (amphipods).

Previous feeding experiments demonstrated that *Armases* feeds, and is able to grow on, a wide variety of food sources (Pennings et al. 1998; Buck et al. 2003; Zimmer et al. 2004). For other grapsid saltmarsh crabs (*Paragrapsus laevis* and *Helogarapsis haswellianus*), microphytobenthic food sources have been shown to play a primary dietary role (Alderson et al. 2013). Our present results, however, clearly indicate that *Juncus* detritus was both the preferred and one of the major food sources of *Armases* individuals from the upper marsh and females from the forest at this study site. This distinct preference may be due to the specific habitat that we used to collect and study *Armases*. This site had large stands of *Juncus* but relatively little *Spartina* (that is more common at the low marsh), with *Armases* frequently being encountered underneath mats of *Juncus* litter (authors' personal observations). *Quercus* litter, on the other hand, although common, appears to be a detrital food source that *Armases* prefers not to eat in food preference tests (c.f. Zimmer et al. 2004; Ewers et al. 2012). Zimmer et al. (2004), by contrast, found that *Armases* ate similar amounts of *Juncus*, *Spartina*, and *Quercus*, and much more of *Borrichia* than reported here. Similarly, the present stable isotope data indicate, in contrast to what we would have concluded from food preference tests, that *Quercus* (along with *Juncus* and *Borricchia*) makes up a major proportion of the diet of all crabs but forest males. These previous food preference experiments (Zimmer et al. 2004), however, were performed over 30 days, and were no-choice rather than multiple-choice feeding trials, in which animals offered low-quality diets may have been making the best of a bad situation by compensatory consumption. Additionally, *Armases* had poor survival on *Borrichia* in these experiments, possibly due to toxic compounds released from *Borrichia* litter during decomposition. Such effects of *Borrichia*, however, are unlikely to occur in the field (Zimmer et al. 2004), where leached compounds would be rapidly diluted. Both *Littoraria* and *Uca* contributed insignificantly to the nutrition of crabs, except for forest males, partly corroborating preference tests with (dead) animal prey. While the ability to capture and handle prey will drive food preference in the field, we were not interested in prey capture abilities in our preference test in the laboratory. Hence, we offered dead prey items, possibly explaining why marsh crabs preferred *Uca* that was only rarely eaten in the field.

Among the animal prey items offered, *Littoraria* were preferred by males. Female *Armases* have smaller claws than males, and therefore have difficulty crushing the hard shell of *Littoraria* (Buck et al. 2003). Males residing in the forest, on the other hand, would be expected not to be used to capturing and eating *Uca* (as corroborated by food preferences), since they hardly ever move into the area of the low marsh where *Uca* is common. Both females and males that do move into the marsh are experienced in preying upon *Uca* by first cutting off the large claw upon attack and then killing the undefended fiddler crab (personal observation). However, according to our stable isotope data, *Uca* was not among the preferred food sources of marsh crabs or forest females, whereas they contributed to the nutrition of forest males as much as all other food taken into account herein.

Changes in an individual's diet seem to mediate individual enzyme activities when proportions of components (protein, cellulose, etc.) in the diet change. Such a link between

dietary composition and the activity of digestive enzymes was shown in many previous studies (e.g., Johnston and Freeman 2005) and has been hypothesized for other crustaceans, such as the mud crab, *Scylla serrate* (Pavasovic et al. 2004). In contrast to stable isotope signatures that integrate over a longer time, this link is short term and indicates most recently consumed food types. Male and female *Armases* differed from each other in that females and crabs captured in the marsh exhibited higher enzyme activities than males and crabs captured in the forest, respectively. Protease activity is expected to be high in individuals consuming large amounts of protein-rich diet and was expected to be found in *Armases*, because these crabs are considered omnivorous, feeding on both animal and vegetal tissue (but see below). The higher protease activity in crabs captured in the marsh corroborates reports of *Armases* feeding on invertebrate herbivores of saltmarsh plants (Ho and Pennings 2008; Marczak et al. 2011) or on worms, insect larvae and other invertebrates that are present in the mud of saltmarshes (Buck et al. 2003, Zimmer et al. 2004), all being rich in protein. This assumption supports our hypothesis that crabs move into the marsh for feeding because these protein-rich food sources are abundant and easier to find in muddy marsh sediments than in forest soils. High protease activity in crabs sampled in the marsh suggests that they had been feeding on marsh invertebrates shortly before they were captured, but among the animal prey tested here, only *Orchestia* significantly contributed to the nutrition of marsh crabs in the long term. Along the same line, (gravid) females that tended to be more abundant in the marsh would make use of a higher proportion of invertebrate prey items, and, thus, exhibit higher protease activity. Buck et al. (2003) showed that sexual dimorphism in claw size influences predatory ability in *Armases*. Although male crabs are able to feed on better-defended prey (here *Littoraria*), females may feed on other prey (insect larvae, etc.) that are also rich in protein. However, δ^{15}N-values of male versus female crabs suggest that females make their living based less on predation than males. Corroborating this, Johnston and Freeman (2005), investigating dietary preference and digestive enzyme activities in six crab species, showed that the highest protease activity occurred in omnivorous crabs feeding on large amounts of microalgae in contrast to the expected highest protease activity in carnivorous crabs. However, benthic diatoms did not significantly contribute to the nutrition of female crabs. Kyomo (1992) investigated differences in feeding habitats and feeding modes between males and females of the crab *Sesarma intermedia* de Haan (Crustacea; Grapsidae) and demonstrated two-times higher feeding activity (based on claw movement) in females than in males. Possibly, reproductively active females (as here) are in need of higher input of nutrients. Such different feeding abilities and strategies may contribute to the spatial segregation between forest and marsh that we observed among males and females, with females trading-off the availability of food sources higher than the risk of being preyed upon by saltmarsh predators, but males using the forest as shelter from predation as pay-off for reduced food availability in the forest.

Pavasovic et al. (2004) demonstrated that cellulase activities in mud crabs, *Scylla serrata*, that fed on starch or cellulose were up to four times higher than in crabs feeding on other diets, reflecting a general ability to modulate individual enzyme activities in response to changing proportions of dietary materials. Plant material of high cellulose content is available in both habitats, and *Armases* feeds on fresh leaves and leaf litter of various plants common either in the coastal forest, at the terrestrial border between marsh and forest, or in the marsh (Buck et al. 2003; Zimmer et al. 2004; Ho and Pennings 2008). The major vegetal food sources seem to be *Juncus*, *Borricchia* and *Quercus* that can be found in the marsh and forest. Like cellulases, amylases are required for the digestion of plant material (starch), but, in contrast to cellulose, they also break down glycogen in animal tissue. Similar to proteases and in contrast to cellulases, amylase activity was highest in crabs captured in the marsh. Thus, amylase in *Armases* seems to be induced upon feeding on invertebrate prey, such as *Orchestia*, insects, snails or nematodes, for cleaving α-1,4 linkages in glycogen, as has been proposed for the mud crab, *Scylla serrata* (Pavasovic et al. 2004) and slipper lobsters (Johnston and Yellowlees 1998). Differences between males and females may, thus, be due to sex-specific feeding strategies, with females (in the marsh) consuming more of these food sources than males.

Feeding on vegetal food sources is often constrained by high tannin contents. One way for consumers to deal with tannins in vegetal tissue is to feed on leached leaf litter (Giddins et al. 1986; Schofield et al. 1998; Zimmer 2002). *Armases* showed a clear preference for leaf litter over fresh leaves (author's own observation; Buck et al. 2003), but preferences among different decomposition stages were not tested, and Nordhaus (2004) reported that most herbivorous crabs have no preference for consuming aged leaves (with phenolic compounds leached off) over fresh ones, and therefore use other mechanisms to deal with phenolic compounds. Esterases are important digestive enzymes for detritivorous, herbivorous or omnivorous species that have to deal with these compounds. Esterase activities did not differ among crabs captured in the marsh versus the forest but were higher in females than in males, supporting our conclusion that females were generally more engaged in feeding than males. The relatively lower contribution of animal prey to the diet of (female) crabs captured in the marsh would then correspond with the higher phenol oxidase activities in these crabs that encounter large amounts of vegetal detritus of both forest and marsh that accumulates in the upper marsh (Zimmer et al. 2002, 2004).

Although individual crabs that had been captured in the same region of our study area differed remarkably in the composition of their gut bacterial communities, differences among crabs that had been captured in different regions (forest vs. marsh) were even bigger. Taking into account that detrital matter of different plants differ in their bacterial communities (Myers et al. 2001) and different vegetal food sources of saltmarsh detritivores shape their gut bacterial communities (Dittmer et al. 2012), this finding suggests that food sources of crabs captured in the marsh differed from those of crabs captured in the forest. Crabs that were feeding in the marsh harbored more diverse bacterial communities than crabs in the forest. Along the same line, females differed from males in their gut bacteria, with males being more similar to each other than females, suggesting either a higher proportion of sex-specific indigenous bacteria in less actively feeding males than in heavily feeding females, or a homogenizing effect of staying in the forest versus migrating back and forth and feeding on more variable food sources as females seem to do.

The preference of males for *Littoraria* is surprising, taking into account previous findings on preferences for *Orchestia* (Buck et al. 2003) and *Melampus* (c.f. Ewers et al. 2012). However, stable nitrogen isotope signatures indicate that neither *Uca* nor *Littoraria* contribute significantly to the nutrition of *Armases* in the field (except for forest males). While both are preferred nitrogen sources when accessible (as in our laboratory assay), the hard shell of *Littoraria* presents a formidable defense in nature, and *Uca* may escape by fleeing or hiding in their burrows (which was prevented in the laboratory by our experimental design). Hence, although *Armases* is capable of preying upon these prey items, their actual contribution to the nutrition of *Armases* is insignificant, because *Armases* choose to feed on easier prey.

The carbon signature of *Armases* suggests that it actually feeds on a mixture of C4-based (*Spartina*) and C3-based (*Juncus*, *Borrichia*, *Quercus*) vegetal sources. Taking into account that they may additionally prey on *Orchestia* (c.f. Buck et al. 2003) and *Melampus*, these would then make a contribution of *Spartina* to their diet less likely, based on the predictions of the mixing model applied. One problem in estimating carbon sources in saltmarsh systems that is discussed in different studies (Haines 1976a, b; Currin et al. 1995; Créach et al. 1997; Fantle et al. 1999) is that benthic microalgae, and particularly diatoms, have carbon isotopic values (~−15 to −20 ‰) that are similar to values of a mixture of terrestrial and saltmarsh plants (Deegan and Garritt 1997) and have been found to play a key role in supporting saltmarsh food webs (Fantle et al. 1999). These authors concluded that the majority of consumers appear to have a mixed diet of *Spartina* and microalgae, and Sullivan and Moncreiff (1990) reported microalgae to be even more important. Our present data, however, overall suggest an insignificant role of both *Spartina* and benthic diatoms in the diet of saltmarsh crabs of Sapelo Island.

Armases can frequently be observed sieving through the saltmarsh sediment (authors' personal observations), possibly foraging for small detrital particles (POM) or small invertebrate prey (snails, nematodes), but—according to our present findings—probably less so for microalgae, possibly because of their small size. They exert indirect positive effects on saltmarsh plants by feeding on their invertebrate herbivores (Ho and Pennings 2008; Marczak et al. 2011). On the other hand, *Armases* is known to feed on terrestrial plants (Ho and Pennings 2008), and to contribute to the mass loss of saltmarsh litter of terrestrial origin (Zimmer et al. 2004; but see Ewers et al. 2012; Treplin et al. 2013). Earlier studies that reported $\delta^{13}C$ values from −12.2 to −19.1, depending on the study region, suggest that crabs differ substantially in what they feed on among sites (Haines 1976a; Haines and Montague 1979). In the present study, admittedly, numerous additional or alternative food sources may have been used by *Armases* for which we did not obtain isotope data.

Whereas male crabs tended to exhibit higher $\delta^{15}N$-values than females, suggesting a greater contribution of animal versus vegetal matter to their diet, it was impossible to distinguish between crabs captured in the marsh versus the forest based on $\delta^{15}N$-values. Taking food preferences in the laboratory also into account, we conclude that crabs, both males and females, move into the marsh to feed on *Juncus*, *Borrichia* and *Quercus*, as well as on *Orchestia* and *Melampus* (Buck et al. 2003) and probably also other invertebrates. The better suitability of male claws for predatory feeding is reflected by the higher $\delta^{15}N$-values of males. Hence, the appearance of *Armases* as an omnivore seems to reflect the females' preference for vegetal food sources, with males occasionally consuming animal tissue.

Conclusions

We conclude that *Armases* has the potential to act as a biotic vector of spatial subsidies. These motile link organisms frequently migrate between the marsh and the adjacent forest, but forest crabs seem to be more stationary, at least during summer. According to this and to our findings on crabs mostly feeding in the marsh, the forest would be a net recipient fed from the marsh as a net donor for spatial subsidy vectored by crabs. The resulting net flux of matter, nutrients and energy from the upper saltmarsh into coastal forests may depend on seasonal changes in migratory and/or feeding behavior, though. Hence, detailed studies on whether this subsidy actually occurs are warranted.

Females show higher migration activity than males and appear to be more active in feeding, and the more stationary males may form locally stable sub-groups of crabs. Food sources encompass both vegetal and animal

matter, being reflected by enzyme activities and stable isotope signatures. However, the potentially most preferred prey items, *Littoraria* and *Uca*, do not contribute to the natural diet of *Armases* in the marsh. The most abundant type of detritus is also one of those preferred by *Armases*. Hence, *Juncus* (and to a lesser degree *Borrichia*) strongly contributes to the vector-based intertidal spatial subsidy to the terrestrial realm. By contrast, spatial subsidies from coastal forests into the marsh are based on *Quercus* but will play a minor role, due to forest crabs migrating less frequently into the marsh. Intraspecific variation with respect to feeding seems to be sex-specific rather than habitat-specific, but habitat choice also largely depends on the sex (and reproductive status).

As can be deduced from most studies that showed sex-specific behavior in migrating species (e.g., Breed et al. 2006; Thaxter et al. 2009; Helfer et al. 2012), male and female *Armases* will contribute to subsidies in different ways, or to different extents. Hence, any change in population structure or sex ratio will have profound effects on ecosystem connectivity and functioning. This aspect of motile link ecology has essentially been neglected in previous studies but should be taken into account when studying biotic vectors of spatial subsidies. Further, as is obvious from our partly contrasting results, several measures of spatial subsidies through motile link organisms should be taken into account in studies of habitat connectivity.

Author contribution statement M.Z. and L.H. conceived and designed the study. L.H. and S.C.P. performed the study. L.H. and M.Z. analyzed the data. M.Z. and S.C.P. wrote the manuscript.

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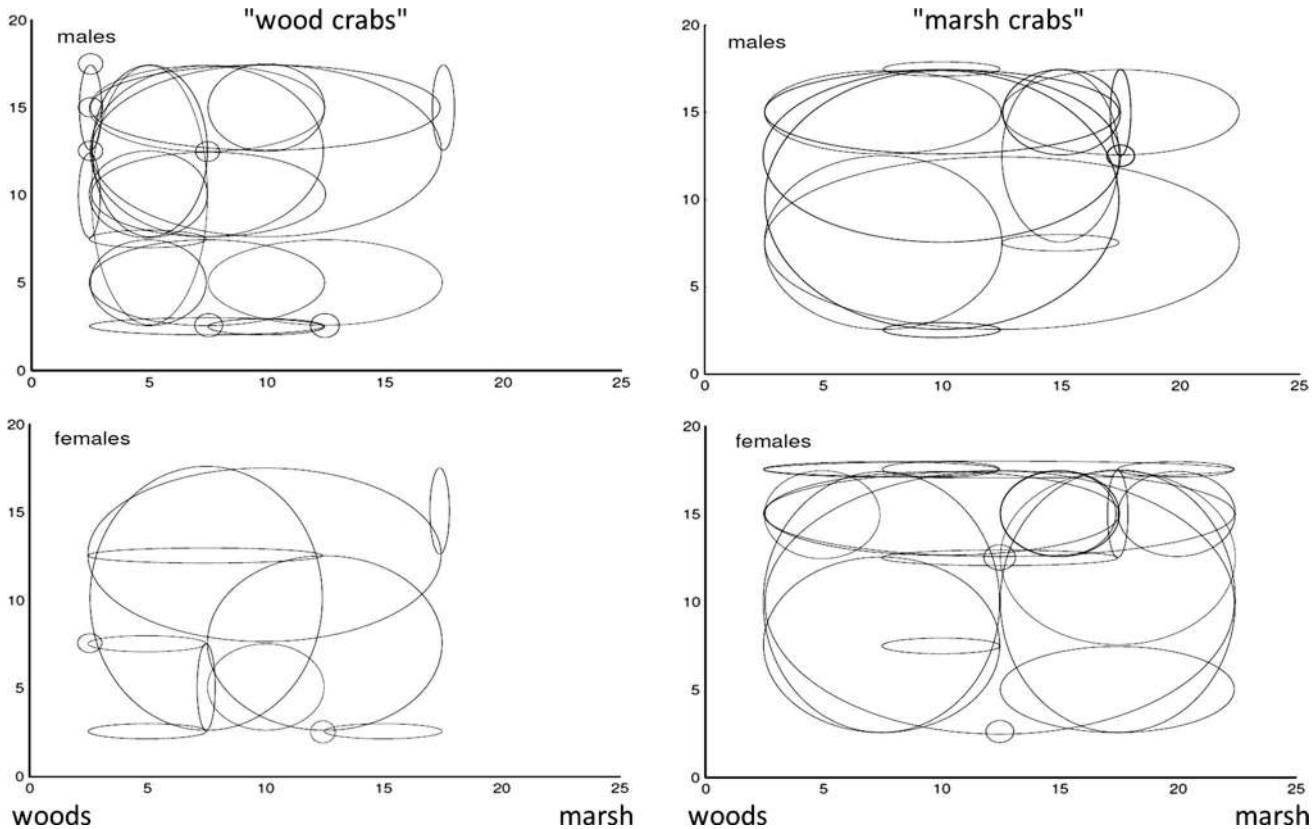
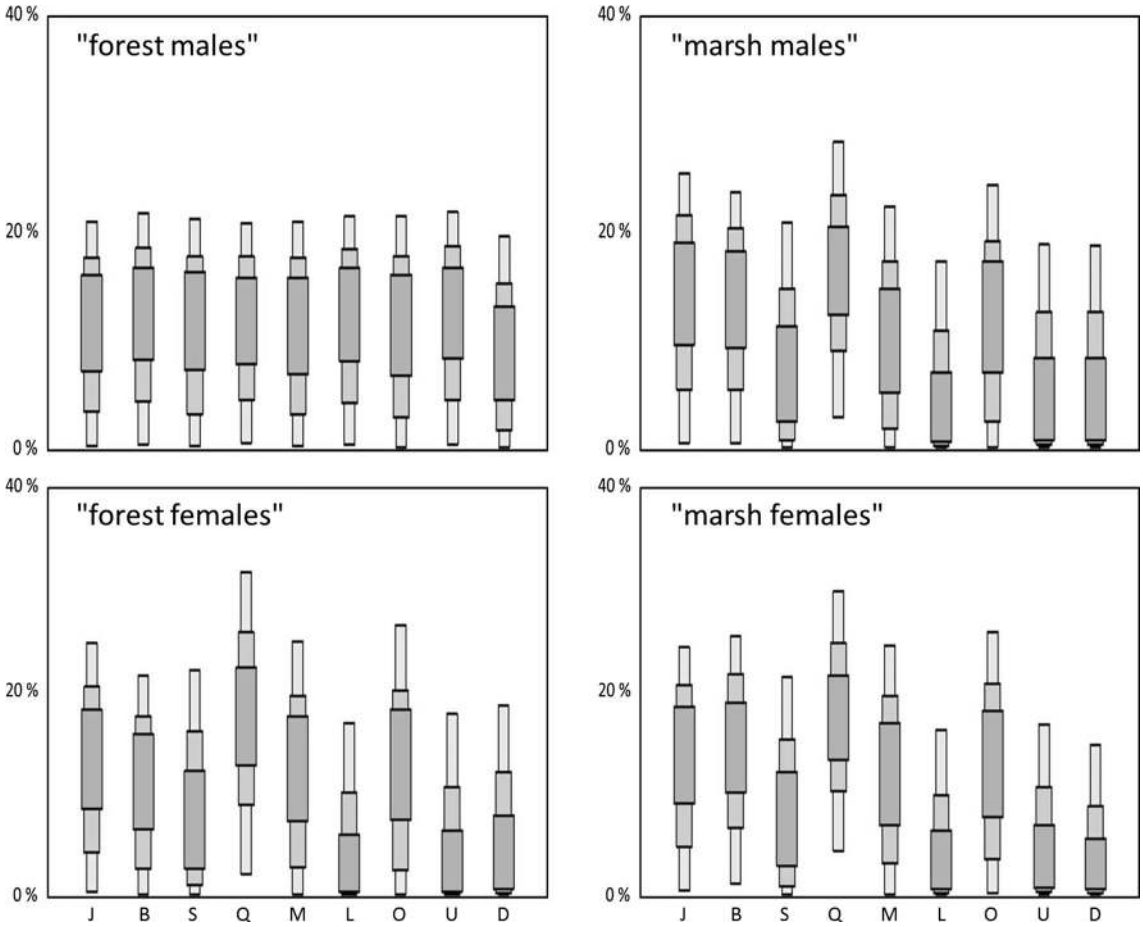
Appendix 1: Spatial distribution of individual male and female crabs

Spatial distribution of individual male and female crabs according to recapture-based migration patterns within the marsh–forest ecotone. Elipsoids reflect the individual range of recapture spots along the two axes parallel versus perpendicular to the coast line.



Appendix 2: Estimated proportional contribution of different food sources

Estimated proportional contribution of different food sources (J, *Juncus*; B, *Borrichia*; S, *Spartina*; Q, *Quercus*; M, *Melampus*; L, *Littoraria*; O, *Orchestia*; U, *Uca*; D, benthic diatoms) to the nutrition of male and female *Armases* from marsh versus forest habitat. Box plots represent minimum, 5, 25, 75 and 95 % percentiles, and maximum.



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