J Comp Physiol B (2004) 174: 299–308
DOI 10.1007/s00360-004-0415-7

ORIGINAL PAPER

Joanne E. Wilde · Stuart M. Linton · Peter Greenaway

Dietary assimilation and the digestive strategy of the omnivorous anomuran land crab *Birgus latro* (Coenobitidae)

Accepted: 15 January 2004 / Published online: 4 February 2004
© Springer-Verlag 2004

Abstract On Christmas Island, Indian Ocean, the diet of robber crabs, *Birgus latro* (Linnaeus) was generally high in fat, storage polysaccharides or protein and largely comprised fruits, seeds, nuts and animal material. The plant items also contained significant amounts of hemicellulose and cellulose. In laboratory feeding trials, crabs had similar intakes of dry matter when fed artificial diets high in either fat or storage polysaccharide, but intake was lower on a high protein diet. Assimilation coefficients of dry matter (69–74%), carbon (72–81%), nitrogen (76–100%), lipid (71–96%) and storage polysaccharide (89–99%) were high on all three diets. *B. latro* also assimilated significant amounts of the chitin ingested in the high protein diet (93%) and hemicellulose (49.6–65%) and cellulose (16–53%) from the high carbohydrate and high fat diets. This is consistent with the presence of chitinase, hemicellulase and cellulase enzymes in the digestive tract of *B. latro*. The mean retention time (27.2 h) for a dietary particle marker (^{57}Co-labelled microspheres) was longer than measured in leaf-eating land crabs. The feeding strategy of *B. latro* involves the selection of highly digestible and nutrient-rich plant and animal material and retention of the digesta for a period long enough to allow extensive exploitation of storage carbohydrates, lipids, protein and significant amounts of structural carbohydrates (hemicellulose, cellulose and chitin).

Keywords *Birgus latro* · Land crab · Digestion · Diet · Robber crab · Dietary assimilation

Communicated by I.D. Hume

J. E. Wilde · S. M. Linton · P. Greenaway (✉)
School of Biological, Earth and Environmental Sciences,
The University of NSW, 2052 Sydney, NSW, Australia
E-mail: p.greenaway@unsw.edu.au
Fax: + 61 2 9385 1558

Introduction

The anomuran land crab *Birgus latro* (Linnaeus 1767) is the largest extant terrestrial invertebrate. It has an Indo-Pacific distribution (Hartnoll 1988) and is found in particularly high numbers in the rainforest on Christmas Island, Indian Ocean. Here, *B. latro* reaches 3 kg in body mass and occurs sympatrically with other land crabs including the coenobitids *Coenobita brevimanus*, *C. rugosus* and *C. perlatus* and the gecarcinids *Gecarcoidea natalis* and *Discoplax hirtipes* (Hicks et al. 1990).

Like other terrestrial coenobitids, *B. latro* is omnivorous and has been reported to eat seeds, fruits and carrion and to predate on conspecifics and sympatric crabs (Grubb 1971; DeWilde 1973; Alexander 1979; Barnes 1997). Digestive strategies and the efficiency of nutrient assimilation have been studied in only a few herbivorous species of land crabs, and the ability of omnivorous coenobitids such as *B. latro* to digest and assimilate nutrients from the diet is unknown. Feeding and digestive strategies in herbivores range between two extremes. The first of these is associated with a high food intake, a short retention time for digesta and a low assimilation of dry matter, largely restricted to readily digestible nutrients. In the second, there is a low food intake, long retention time and high assimilation of dry matter. Of the two species of herbivorous land crabs that have been studied, *Discoplax hirtipes* (Dana 1852) is more reliant on the former strategy and *Gecarcoidea natalis* (Pocock 1888) on the latter (Greenaway and Raghaven 1998). Both species, however, consume large amounts of nutrient-poor leaf litter, have relatively short mean retention times (3.5–11.6 h on brown fig leaves) and assimilate substantial amounts of the soluble cell contents, hemicellulose and cellulose (Greenaway and Linton 1995; Greenaway and Raghaven 1998). Equivalent studies have not been conducted on carnivorous land crabs, but given the high nutrient quality and digestibility of animal material and by analogy with

other carnivorous animals, a low food intake associated with a short retention time and high assimilation would seem a likely scenario.

The diet of *B. latro* is different from that of *G. natalis* and *D. hirtipes*, as the main nutrients in the reported diet (storage polysaccharides, fats and protein) are both concentrated and readily digestible. Given this situation, it might be predicted that *B. latro* would display a low dietary intake, a short retention time of digesta and a high level of assimilation of dry matter. The plant component of the diet, however, may contain significant amounts of structural polysaccharides, and the ability of the animal to digest these compounds and their effects on assimilation and retention time are of interest. This is particularly relevant given the presence of endogenous cellulases and the ability to digest cellulose in other evolutionarily diverse crustaceans, such as the crayfish *Cherax quadricarinatus* (von Martens) and the herbivorous gecarcinid land crabs *G. natalis* and *D. hirtipes* (Greenaway and Linton 1995; Greenaway and Raghaven 1998; Byrne et al. 1999; Xue et al. 1999; Linton and Greenaway, unpublished data).

The objectives of this study were:

1. to determine common dietary items eaten in the field by *B. latro*,
2. to examine the nutrient composition of these items as a precursor to the formulation of artificial diets of an appropriate range of nutrient composition,
3. to determine the ability of this species to assimilate nutrients from these artificial diets,
4. to assess the potential of *B. latro* to digest plant structural compounds, and
5 to formulate an hypothesis for the digestive strategy used. In order to establish the nutritional quality of the diet, common dietary items observed to be eaten by crabs in the field were collected, identified and their nutrient contents determined.

Objectives 1–4 were achieved by laboratory trials in which captured animals were fed artificial diets high in either fat, storage polysaccharide or protein. Assimilation of nutrients (nitrogen, fat, storage polysaccharide, cellulose, hemicellulose) from these diets was then measured. Radioactive microspheres were used as a particulate marker to estimate the mean retention time of digesta.

Materials and methods

Collection and maintenance of animals

Birgus latro were collected from rainforest on Christmas Island, Indian Ocean (105°40′ E 10°35′ S) and air freighted to Sydney. In the laboratory, the animals were maintained individually in plastic boxes (64×26×40 cm) at a constant room temperature of 25 ± 1 °C and 80% humidity with a 12:12 h light:dark cycle. They were fed on fresh fruit, vegetables, nuts and cereals and were given tap water for drinking. Outside the experimental period, food was supplied once a week and animals were healthy and maintained or gained mass on this regimen. Animals used in the experiments described below were of both sexes and averaged 395 g (range 346–474 g) and had been maintained in captivity for 1 month before experiments commenced.

Diet of field animals

Observations of the natural diet of *B. latro* were carried out on Christmas Island between 23 June and 7 July 1997. Food items were taken from feeding crabs and identified using the Flora of Australia, Volume 50, Oceanic Islands (Australian Government Publishing Service, Canberra 1993). Further samples of these common dietary items were then collected, dried at 60 °C and transferred to Sydney where they were stored for several weeks over silica gel prior to analysis. Samples of each food type (4–12 individual fruits) were pooled, milled to a fine powder (Fritsch Pulverisette 14 rotor speed mill with a 1-mm screen) and analysed as described below for food and faecal samples. This provided a snapshot of preferred food types and composition at the study time and supplemented information in the literature concerning the diet of the species.

Dietary assimilation experiments

Composition and preparation of the artificial diets

Feeding trials were conducted to determine the assimilation of nutrients from the diet. Animals were fed a sequence of three artificial diets (designated carbohydrate, fat and crab; Table 1) in random order. The composition of these diets reflected the range of nutrient composition of food items collected from the field. The components of the artificial diets were milled to a fine powder using a Fritsch Pulverisette 14 rotor speed mill with a 1-mm screen. Crab powder was prepared from *G. natalis* which had been killed by chilling and dried at 60 °C prior to milling. Diets were prepared by adding agar and the powdered dry ingredients to boiling water. The resultant gel was allowed to set in shallow trays before being cut into squares. The squares were then dried at 60 °C to form hard biscuits of homogeneous composition.

Feeding trials

Twelve crabs were selected for feeding trials and fed each of the three diets sequentially in a randomised block design. Each crab

Table 1 Components of the artificial diets (g kg^{-1} dry mass)

Component	Mass (g kg^{-1})
Carbohydrate diet	
Cornflower	282.4
Brown rice	211.8
Sunflower seeds	46.9
Bran	317.7
Agar	141.2
Dry ingredients: Agar	6.1
Fat diet	
Coconut	480
Hazelnut	200
Bran	160
Agar	160
Dry ingredients: agar	5.3
Crab diet	
Crab powder	875
Agar	125
Dry ingredients: agar	7

was placed in a plastic box (as above) and sequentially fed three artificial diets in random order. Animals were acclimated to each experimental diet for 1 week before commencement of data collection and the amount of food eaten and faeces produced were recorded daily for the next 11 days. For each animal, intake and output were stable over this collection period, but rates varied up to six-fold between individuals in treatment groups.

Uneaten food and faeces were collected each morning, dried at 60 °C and weighed. A known dry mass of fresh food was supplied daily. Faeces were generally deposited in a corner of the box, and the faecal strings, in their peritrophic membranes, could be removed intact with forceps. Any faeces smeared by the crab were scraped from the box using a razor blade. A complete series of samples was obtained from 11 of the 12 crabs used, but one crab entered premoult during the experiment and ceased eating. Data from this individual were not included in the analyses. Prior to analyses, food and faecal samples were pulverised to a fine powder with a Fritsch Pulverisette 14 rotor speed mill using a 1-mm screen.

Assimilation coefficients for dry matter and other nutrients were calculated using the following equation: Assimilation coefficient = 100×(1−output/input). Rates for intake and output of food are expressed per unit body weight as the allometric relationship between food intake and body size is not known.

Analyses

Food and faecal samples were analysed for nitrogen, carbon, chitin, storage polysaccharides, lipid, fibre and the purines urate, guanine, hypoxanthine, and xanthine.

Nitrogen

The nitrogen (N) contents of dry milled samples were determined using a Leco CHN-1000 analyser. The N content of the faeces was corrected for N in the chitinous peritrophic membrane (which is secreted by the midgut and envelops the faeces) and faecal purine (the main vehicle for excretion of nitrogenous waste in *B. latro* (Greenaway and Morris 1989). The peritrophic membrane was estimated to contain 38.71 μmol N/g^{-1} dry faeces.

Chitin nitrogen

Chitin levels were determined using a modification of the method of Hornung and Stevenson (1971). Chitin was first extracted from the samples and the N content of the extracted chitin was then determined. As chitin is a polymer of *N*-acetyl glucosamine, the molar N content could be expressed as μmol of *N*-acetyl glucosamine, and chitin was quantified in this way.

Aliquots of faecal samples (0.2 g) were digested with 10% KOH for 5 h at 100 °C. This procedure hydrolysed all of the N compounds except chitin and the N released was lost as ammonia. The remaining material (chitin) was allowed to settle, the supernatant was discarded and the precipitate washed three times with water. The N content of the purified chitin was determined with a micro-Kjeldahl system (Gerhardt Kjeldatherm and Vapodest 3C. Gerhardt, Bonn) as described by Greenaway and Linton (1995).

Storage polysaccharides

Storage polysaccharides within the samples were first solubilised by heating an aliquot in 10 ml of dimethyl sulphoxide (DMSO) and 2.5 ml of 8 mol/l HCl at 55–60 °C for 30 min. Samples were neutralised with 8 mol/l NaOH and diluted with citrate buffer, pH 4.0. A commercial starch test kit (Boehringer Mannheim Cat. No. 207748) was then used to determine the concentrations of storage polysaccharides (starch/glycogen).

Lipid content

The lipid content of samples was measured gravimetrically using a modified Soxhlet procedure. Briefly, lipid was extracted from a 0.2 g aliquot by heating it at 60–65 °C for 2 h in petroleum spirit. Extracted samples were then filtered through pre-weighed filter paper and washed three times with excess petroleum spirit. The filter plus residue was dried at 105 °C for 1 h and weighed. The amount of lipid was calculated as the decrease in mass of the sample.

Fibre content

The fibre contents of food and faecal samples were determined using the sequential gravimetric method of Robertson and Van Soest (1982). Given the sequential nature of the method, minor errors in the early extraction steps may affect subsequent extractions and, if the amounts of material in the last few steps are relatively small, this may result in major errors. For this reason, data have not been presented for the small lignin (1.2–1.5% dry diet) and even smaller cutin fractions in these experiments.

C:N ratio

The C and N contents of the samples were measured with a Leco CHN-1000 analyser (Leco Inc, St Joseph; USA), and the values obtained were then used to determine the molar C:N ratios.

Purine content of the faeces

Birgus latro excretes waste N as purines in the faeces (Greenaway and Morris 1989), and it was necessary to correct the N content of faecal samples for any purine N they contained in order to correctly determine assimilation of N from the diet. Purines were extracted from faecal samples with 68 mmol/l $LiCO_3$ at 60 °C and then analysed by HPLC as described previously (Linton and Greenaway 2000).

Food retention time

A radioactive marker (15 μm ^{57}Co microspheres) was used to estimate the residence time for food particles in the gut of *B. latro*. Micro-spheres were incorporated into a batch of the carbohydrate diet and 5×5-mm squares were supplied to crabs previously acclimated to the diet for 1 week. The time at which ingestion occurred was established by checking the animals every hour until the food was consumed. Crabs were then observed at 2, 4, 6, 8, 10, 15, 20, 25, 30, 38, 46, 58, 70, 82, 94, 106 h, and at longer time intervals to 204 h, after the consumption of the marker, and any faeces that had been produced were collected. Marker levels in the faeces were determined with a gamma counter (Bicron γ detector and E.G. & G. Ortec modular scaling equipment). Of the marker ingested, 91.51 ± 1.99% was recovered in the faeces. The times for excretion of 0.5% ($t_{0.5}$), 50% (t_{50}) and 95% (t_{95}) of the radioactive marker were then calculated and the mean retention time (MRT) was determined by the method of Warner (1981). Data are presented as the percentages of total marker recovered from the faeces.

Statistics

Data are expressed as mean ± SEM. Homogeneity of the variances was tested for using Bartlett's test. If the variances were homoscedastic then means were compared using repeated measures ANOVA and a posteriori hypothesis tests (general linear contrasts C matrix). If the variances were heteroscedastic then Friedman's

non-parametric ANOVA and Dunn's multiple comparison a posteriori tests were employed to compare means. Percentage values were normalised using an arcsine transformation before statistical analysis. Unless otherwise stated, a 95% probability level was used to designate significantly different means. Statistical probabilities were calculated using the statistical computing package SYSTAT 7.0 for Windows.

Results

Diet in the field

B. latro was observed to prey upon the gecarcinid land crabs *Gecarcoidea natalis* and *Discoplax hirtipes* and to scavenge the carcasses of dead conspecifics, other crabs and birds. It also consumed plant products such as the fleshy fruit of *Ochrosia ackeringae* (Teijsm and Binn. 1869), the seeds of *Annona reticulata* (Linnaeus 1753) (a custard apple species), the hard-shelled nuts of *Aleurites moluccana* [(Linnaeus) Wild. 1805] and *Calophyllum inophyllum* (Linnaeus 1753) and the fibrous seed coats of *Inocarpus fagifer* (Parkinson 1941). Other dietary items included the inner flesh of coconuts (*Cocos nucifera*) (Linnaeus 1753), the fruit and pith of the arenga palm [*Arenga listeri* (Becc. 1891)] and the fruits of *Pandanus elatus* (Ridl. 1906) and *P. christmatensis* (Martelli 1905). They were not observed to eat low-grade plant materials such as foliage, browse or litter.

Nutritional composition of some dietary items eaten by *B. latro*

Plant material

Samples of the plant materials mentioned above were collected and their nutritional composition determined (Table 2). High lipid contents were found in the seeds, particularly those of *A. moluccana*, and even the fleshy fruit of *O. ackeringae* and the fibrous coat of *I. fagifer* fruit had substantial lipid levels (Table 2). Starch was present in all of the plant material analysed but only reached significant levels in the flesh of the fruit of *O. ackeringae* (Table 2). Carbon levels in the three seed types analysed were high due to their high densities of lipid and protein whilst N levels were relatively low except in the seeds of *A. moluccana* (Table 2). Consequently the C:N ratios, with the exception of the seeds of *A. moluccana* (C:N ratio of 14.79), were generally high and ranged between 24.9–70.2 (Table 2). The N levels, expressed as crude protein, varied between 5.6-28% of the dry plant material (Table 2).

The levels of the structural polysaccharides, hemicellulose and cellulose, were much higher than the concentrations of starch but similar in the dietary samples analysed (Table 2). These plant structural compounds clearly represent a significant part of the dietary intake of *B. latro* and an ability to utilise them is potentially advantageous to the species.

Animal material

The powdered crab diet contained high levels of N (Table 3). Storage polysaccharide, in the form of glycogen, was low compared with levels in the high carbohydrate diet and within the range measured in plant material (Tables 2, 3). Although the lipid level was less than that of nuts and seeds, it was still present in significant quantities (Tables 2, 3). At 8.0 ± 0.0, the C:N ratio of the crab carcasses was the lowest of all of the diets and dietary items examined (Tables 2, 3). Structural polysaccharide was present in significant amounts as chitin.

Dietary assimilation

Dry matter

Dry matter intake, output and retention were similar for crabs fed on the fat and carbohydrate diets, but animals fed the crab diet had a lower intake, output and retention of dry matter (Fig. 1A). Assimilation coefficients for dry matter were high (range 69–74%) and similar for animals on all three diets (Table 4).

Nitrogen

N was well assimilated on all three diets and assimilation coefficients from 76.3 to 99.8% were achieved (Table 4).

Table 2 Nutritional composition of items eaten by *Birgus latro* and collected from Christmas Island. All values expressed per gram dry mass

	Ash	Nitrogen	Crude protein[a]	Carbon	C:N	Lipid	Starch	Soluble cell contents	Hemicellulose	Cellulose
	(mg g^{-1})	(μmol g^{-1})	(mg g^{-1})	(mmol g^{-1})		(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})	(mg g^{-1})
Inocarpus fagifer (seed coat)	99	1450	127	36.1	24.8	94.2	2.4	571	164	174
Calophyllum inophyllum (seed no-shell)	17	708	62	49.6	70.1	554.8	3.42	593	194	61
Aleurites moluccana (seed no-shell)	52	3305	289	48.9	14.8	633.8	2.41	788	196	29
Annona reticulata (seed and seed coat)	19	1267	111	44.8	35.4	325.3	2.55	303	324	219
Ochrosia ackeringae (fruit flesh)	71	638	56	32.8	51.4	154.1	84.6	769	87	101

[a]Crude protein = N×6.25

However, assimilation of N was higher by animals fed the fat diet than by crabs fed the carbohydrate and crab diets, which were statistically similar (Table 4), and output of N via the faeces was higher in the latter two groups (Fig. 1C).

Carbon

Crabs fed the carbohydrate and fat diets had similar intake, output and absorption rates for carbon, but the respective value was much lower in animals fed the crab diet (Fig. 1B). The assimilation coefficients for carbon were high for crabs on all three diets (range 71.6–80.8%; Table 4), and crabs fed the fat diet had a mean assimilation coefficient higher than those for crabs fed the other two diets (Table 4).

Lipid

Intake of lipid was highest for crabs fed the fat diet, whilst lipid intakes by animals maintained on the other two diets were similar to each other and much lower (Fig. 1D). Crabs on all diets had a similar rates of faecal output of lipid (Fig. 1D) and consequently the assimilation coefficients of lipid were highest for crabs eating the fat diet (96.0%), intermediate for animals fed the carbohydrate diet (87.4%) and lowest for crabs consuming the crab diet (70.8%) (Table 4).

Storage polysaccharides

Intake and retention of the storage polysaccharides (starch or glycogen) were highest for crabs fed the carbohydrate diet, followed by crabs maintained on the fat diet and then animals fed the crab diet (Fig. 1E). Assimilation coefficients for storage polysaccharides were very high and ranged from 89.4% for the crab diet to almost complete for the carbohydrate and fat diets (Table 4).

Chitin

Chitin was not detectable in the carbohydrate or fat diets, but was present in substantial amounts in the crab diet (Table 3). However, small amounts of chitin were detected in the faeces of crabs on the carbohydrate and fat diets; the source was endogenous production of the chitinous peritrophic membranes that envelop the faecal strand (Peters 1991). This endogenous production of chitin was corrected for in the calculation of chitin assimilation by crabs on the crab diet.

The mean rate of intake of chitin by animals fed the crab diet was 629 mg kg^{-1} day^{-1}, output was 46.1 mg kg^{-1} day^{-1} and the assimilation coefficient was 92.7%.

Hemicellulose

Hemicellulose was the largest fibre component in the fat and carbohydrate diets (Fig. 1F), and intakes were similar for animals fed these two diets (Fig. 1F). The assimilation coefficients for hemicellulose were lower than values for N, carbon, lipid and storage polysaccharides, but approximately half of the ingested hemicellulose was retained and the assimilation coefficient was higher for crabs eating the fat diet (Fig. 1F; Table 4).

Cellulose

Cellulose comprised a small proportion (3–4%) of the dry matter of the carbohydrate and fat diets (Table 2). Significant quantities of cellulose were assimilated by *B. latro* from both the carbohydrate and fat diets but assimilation was 4.3 times greater from the fat diet (Fig. 1G, Table 4).

Food retention time

Given the relatively low intake and high assimilation coefficients for dry matter, faecal production and the

Table 3 Nutrient composition of food and faeces of *B. latro* fed three artificial experimental diets (mean per g dry mass ± SEM, $n = 11$)

	Carbohydrate diet		Fat diet		Crab diet	
	Diet	Faeces	Diet	Faeces	Diet	Faeces
Non-purine nitrogen (μmol g^{-1})	870.4 ± 3.7	738.2 ± 91.9	1170.1 ± 7.9	639.5 ± 118.2	3161.5 ± 4.0	1363 ± 341.8
C:N (molar ratio)	41.6 ± 0.1	25.1 ± 12.4	36.2 ± 0.3	38.8 ± 6.2	8 ± 0.0	3.5 ± 4.1
Carbon (mmol g^{-1})	36.2 ± 0.04	33.8 ± 0.5	46.6 ± 0.1	33.1 ± 0.7	25.2 ± 0.1	18.4 ± 1.0
Hemicellulose (mg g^{-1})	142 ± 6	247 ± 8	110 ± 1	159 ± 10		
Cellulose (mg g^{-1})	30 ± 1	88 ± 2	40 ± 2	75 ± 4		
Lipid (mg g^{-1})	120.2 ± 5.5	52.7 ± 7.2	431.3 ± 5.2	68 ± 8.2	165.3 ± 2.0	154.4 ± 16.7
Starch/glycogen (mg g^{-1})	339.5 ± 3.9	12.5 ± 2.7	22.4 ± 1.0	1.9 ± 0.6	14.6 ± 1.6	2.6 ± 1.2
Chitin as *N*-acetyl glucosamine (μmol g^{-1})	–	38.3 ± 4.6	–	47.6 ± 9.8	727 ± 12.5	945.8 ± 132.9
Urate (μmol g^{-1})	–	156.3 ± 60.6	–	164.3 ± 92.1	–	1288.4 ± 269.3
Guanine (μmol g^{-1})	–	78.3 ± 24.8	–	76.8 ± 38.7	–	577.8 ± 105.3
Ash (mg g^{-1})	25 ± 1	66 ± 2	29 ± 1	80 ± 3	423 ± 2	322 ± 31

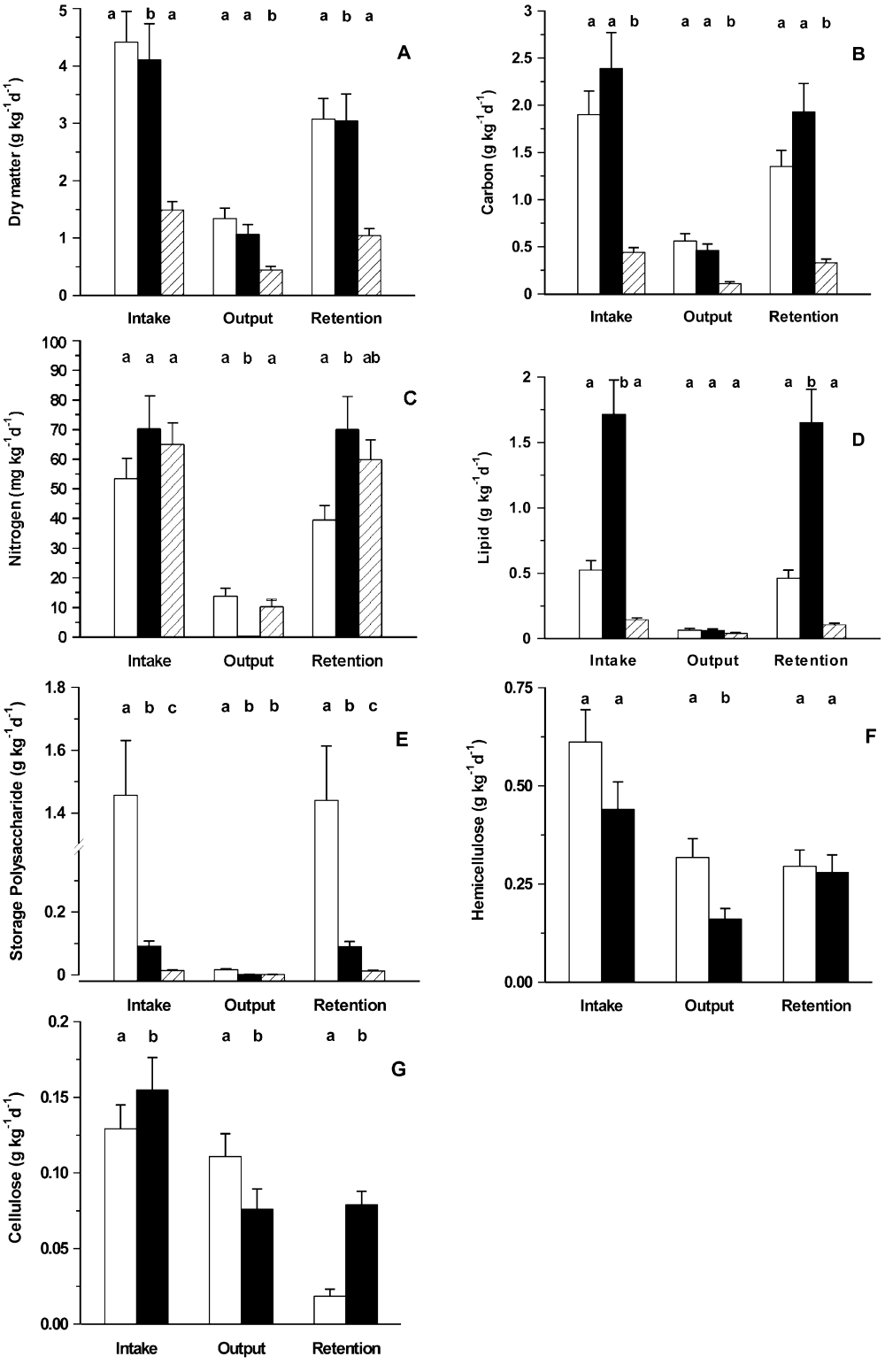

Fig. 1 Intake, output and retention of dry matter and major dietary components by *Birgus latro* fed three artificial diets. **A** Dry matter; **B** carbon; **C** nitrogen; **D** lipid; **E** storage polysaccharides; **F** hemicellulose; **G** cellulose. *Open bars* represent values for crabs fed the carbohydrate diet, *closed bars* represent values for the fat diet and *hatched bars* represent values for the crab diet. Values are expressed as mean ± SEM, $n = 11$. *Different letters above the bars* indicate statistically different means within either the intake, output or retention categories in each panel

frequency of defaecation events were low (1.4 ± 0.1 days). The long interval between each elimination of faeces created difficulty in accurate assessment of the timing of marker appearance for individual animals. The data for marker appearance are therefore presented in mean form for the ten experimental animals used (Fig. 2) and values for retention times have been determined from these mean data.

Radioactive particle marker (^{57}Co-labelled microspheres) ingested with the diet first appeared in the faeces from 1–20 h after ingestion ($t_{0.5} = 0.5$ h) and maximal marker concentration (highest appearance in

Table 4 Assimilation coefficients (mean percent ± SEM, $n = 11$) for *B. latro* fed carbohydrate, fat and crab diets. Within a row, different superscript letters indicate significantly different means ($P < 0.05$)

	Carbohydrate diet	Fat diet	Crab diet
Dry matter	70.4 ± 0.7^{a}	74.1 ± 1.4^{a}	69.4 ± 3.2^{a}
Nitrogen	76.3 ± 3.8^{a}	99.8 ± 0.0^{b}	83.9 ± 4.0^{a}
Carbon	71.6 ± 0.9^{a}	80.8 ± 0.8^{b}	74.0 ± 3.1^{a}
Lipid	87.4 ± 1.7^{a}	96.0 ± 0.7^{b}	70.8 ± 3.7^{c}
Starch or glycogen	99.0 ± 0.2^{a}	98.1 ± 0.7^{a}	89.4 ± 4.5^{b}
Hemicellulose	49.6 ± 3.0^{a}	64.8 ± 2.9^{b}	–
Cellulose	16.1 ± 4.3^{a}	53.9 ± 3.5^{b}	–

1 h) occurred between 11 and 74 h ($t_{max} = 29.2 \pm 5.9$ h) (Fig. 2). The MRT for the marker was 27.2 h. Of the microspheres eliminated, 50% appeared within 20.3 h and 95% appeared within 74.6 h of ingestion (Fig. 2).

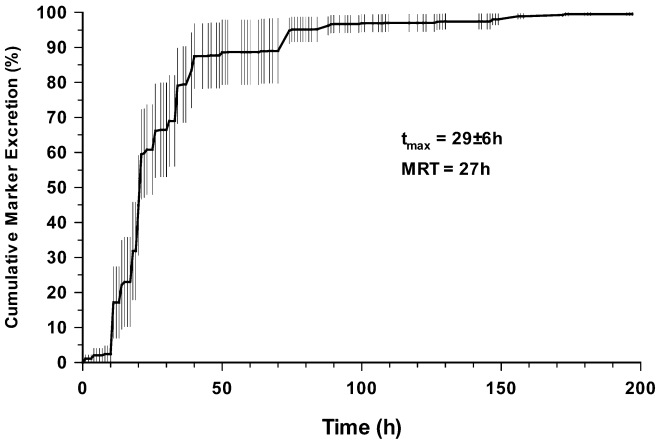

Fig. 2 Cumulative appearance of the inert marker ^{57}Co labelled microspheres by *B. latro* fed the artificial carbohydrate diet. Data are expressed as the percent recovery of the ingested marker. *Solid line* indicates the mean percentage of the recovered marker appearing over time (± SEM, $n = 10$). *MRT* Mean retention time

Discussion

Dietary selection

There are two genera in the family Coenobitidae; *Coenobita* includes the terrestrial hermit crabs and the Robber or Coconut crab is placed in the monotypic genus *Birgus*. The hermit crabs are reported to eat fruits, seeds, nuts, and a wide range of plant material and plant detritus as well as carrion (fish, giant tortoises, birds, goats and donkeys) and the faeces of vertebrates (Grubb 1971; DeWilde 1973; Alexander 1979; Barnes 1997). The robber crab, *Birgus latro,* is more selective and specialises in seeds, fleshy fruits and animal material but is not reported to eat significant amounts of detritus or low-grade plant material. It hunts other crabs and small animals such as tortoise hatchlings and attacks injured animals such as seabirds and scavenges carcasses (Greenaway 2001, 2003). The items selected are normally rich in protein, storage polysaccharides or fat (Tables 2, 3), and there is clearly a predilection for dietary items that are high in these nutrients. This contrasts with other sympatric species of land crabs on Christmas Island; *G. natalis* and *D. hirtipes* consume mainly leaf litter (Greenaway and Raghaven 1998) and *Geograpsus* spp. are carnivorous.

The C:N ratio of plant material eaten by *B. latro* (Table 2) was higher than that normally considered necessary to maintain nutrition and growth (C:N = 15:1) (Russell-Hunter 1970). The C:N for animal material is much more favourable, and it seems likely that *B. latro* must obtain a significant amount of its N requirements from animal material. Whilst the relative proportions of animal and plant material eaten by the species in the field are unknown, high sodium turnovers measured for the species on Christmas Island were only explicable in terms of a high intake of animal material (Greenaway 2001).

Digestive processes in *B. latro*

Fragmentation of the food by *B. latro* is a three step process. Firstly the major chela is used to hold food items whilst the minor chela tears small pieces off or scrapes off the surface layer of fibrous seed coats. Particles are passed to the third maxillipeds and thence to the mandibles which cut the food into smaller portions which are ingested. The third phase of mastication involves the gastric mill in the anterior chamber of the stomach. The structure of the gastric mill of *B. latro* has not been described, but by analogy with other terrestrial decapods crustaceans, it is likely to utilise dorsal and lateral teeth to grind the food and mix it with the digestive enzymes that are passed forward from the midgut gland in the digestive juice (Giddins et al. 1986; Icely and Nott 1992). Undigested particulate matter is routed through the midgut where it is enveloped in peritrophic membranes and compacted in the long, cuticle-lined hindgut until voided in the faeces. Fine particles (less than 1 μm) and solubilised food materials enter the extensive midgut gland (which fills the abdomen and part of the thorax) for completion of digestion and absorption.

Dietary assimilation

As indicated above, *B. latro* selects a diet rich in storage polysaccharide, fat and N, and it is evident, from the high assimilation coefficients obtained in this study (Table 4), that *B. latro* exploits these materials efficiently. To achieve this, amylases, lipases and endo- and exo-peptidases are required in the digestive juice to hydrolyse storage polysaccharides (starch and glycogen), protein and lipid, and these enzymes are near universally present in crustaceans (Dall and Moriarty 1983). Emulsification of lipids is also needed, but exactly what

compounds crustaceans use for this is unclear, although bile salts, taurine, sarcosine and taurochenodeoxycholic acid have been suggested in decapod crustaceans (reviewed Gibson and Barker 1979). After hydrolysis, the resultant nutrient monomers, amino acids, sugars and fatty acids are taken up by the midgut gland before being metabolised and/or stored. Lipids are stored within the R cells of the midgut gland, presumably as triglycerides and these cells also store glycogen (Dillaman et al. 1999). Such lipid stores may exceed 50% of the dry mass of the midgut gland (Lawrence 1970).

The high assimilation coefficients of N on all three diets (76.3 ± 3.8 to $99.8 \pm 0.0\%$; Table 4)) were similar to values reported for *Scylla serrata* fed a shrimp diet ($>95\%$, Sukardi 1994). These high values probably reflect the high digestibility of protein present in animal diets and they contrast with the much lower values (15–60%) reported for herbivorous crabs fed leaf litter diets (Greenaway and Linton 1995; Greenaway and Raghaven 1998) in which N is associated with both protein and lignin.

N intakes by *B. latro* were similar on the three experimental diets despite considerable differences in dry matter intakes (Fig. 1A, C), and it is possible that food intake may have been regulated to maintain N intake. Other explanations are possible, however, e.g. the artificial crab diet may have been relatively unpalatable to *B. latro* or its high content of chitin may have required increased retention time for digestion and thus restricted intake. In the field, *B. latro* tears crab carcasses apart and preferentially consumes the midgut gland and muscle tissue and may thereby minimise chitin intake. The crab diet contained powdered whole crabs and a high chitin intake was inevitable. Additionally, dry matter intake is clearly not regulated by the N concentration of the diet in certain other invertebrates as N intake rises substantially if dietary N is high (Simpson and Abisgold 1985; Linton and Greenaway 1997).

Structural polysaccharides

B. latro exhibited considerable ability to digest structural polysaccharides of both animal and plant origin. The assimilation coefficient for chitin from the crab diet was high ($92.8 \pm 0.9\%$) and an assimilation coefficient of 96.4% has been recorded for the mud crab *Scylla serrata* (P. Sukardi and P. Greenaway, unpublished data). Chitinase is probably a ubiquitous enzyme within the Crustacea, as it is essential during moulting, and its presence in the gut is not surprising as many crustaceans feed on other arthropods. The enzyme has been isolated from the midgut of prawns (Kono et al. 1990).

B. latro regularly encounters the plant structural polysaccharides hemicellulose and cellulose in its diet (Table 2) and when presented with these compounds in artificial diets the crabs were able to digest a substantial proportion (Fig. 1F, G; Table 4). Thus, while selection of dietary items by the crabs is based on the contents of storage polysaccharide, fat and protein, they also gain significant amounts of metabolisable substrates from the structural polysaccharides ingested with the more readily digestible materials.

The assimilation of cellulose from the fat diet was considerably higher than from the carbohydrate diet (Table 4). A possible explanation for this is that a longer digestion time is needed in order to emulsify fat in foods with a high fat content and then to digest and absorb the products. The increased time of residence of food in the digestive region of the gut would also enhance digestion of cellulose and result in a higher assimilation coefficient.

Assimilation of hemicellulose and of cellulose are consistent with the presence of hemicellulase and cellulase activity within the digestive tract of *B. latro*. These enzymes may be either produced endogenously by the midgut gland or by micro-organisms within the gut. The occurrence of hemi-cellulases has been poorly studied in crustaceans, although the leaf-eating land crab *G. natalis* has significant levels of laminarinase and lichenase but not xylanase in the digestive juice (S. Linton and P. Greenaway, unpublished data). Cellulases are widely reported from crustaceans (Dall and Moriarty 1983; Wolcott and O'Connor 1992) and are known to be produced endogenously in the crayfish, *Cherax quadricarinatus*, and the herbivorous gecarcinid crabs *G. natalis* and *D. hirtipes* (Byrne et al. 1999; Xue et al. 1999; S. Linton and P. Greenaway, unpublished data). However, endogenous cellulases have not yet been demonstrated in *B. latro,* and the involvement of microbes in digestion of cellulose should be considered. Successful fermentation requires a large population of bacteria, fungi or protozoans in a region of the gut that is not accessed by digestive enzymes, commonly a chamber anterior or posterior to the digestive region. Whilst such an arrangement is probable in the complex gut of terrestrial isopod crustaceans (Hames and Hopkin 1989; Zimmer and Topp 1998), the foregut and extensive midgut gland (hepatopancreas) of *B. latro* and other decapods both contain digestive juice that is secreted by the midgut gland, circulates between the midgut and foregut and frequently has bacteriolytic capability (Dall and Moriarty 1983). These chambers are thus unsuited to high levels of microbial activity. The only other large chamber is the hindgut which is concerned with compaction and temporary storage of the faeces. As the faecal strands in the hindgut are relatively dry, and there is no evidence of coprophagy, it seems unlikely that significant hindgut fermentation takes place or that its products could be utilised by the crabs. The likelihood of cellulose digestion by microbial activity in robber crabs, therefore, seems remote.

Marker retention time and digestive strategy

The use of radioactive microspheres to estimate the retention time of food in the gut is relatively novel, and it is worthwhile to consider whether its appearance in the

faeces accurately reflects the movement of digesta in the crustacean system. In the decapod gut, digestive fluid is secreted by the midgut gland and passed forwards to the cardiac stomach. The gastric mill in the cardiac stomach assists the digestive process by reducing the size of food particles. Foregut fluid and small food particles (generally less than 1 μm diameter) are cycled back to the midgut gland where digestion is completed and food materials absorbed (reviewed in Icely and Nott 1992). Larger and undigested particles are compacted in the pyloric stomach and pass to the hindgut for temporary storage before elimination. Microspheres of the size used in this study (15 μm) would be expected to move with the particulate fraction and, as the documented appearance of the marker in the faeces (Fig. 2) is consistent with this expectation, the microspheres used may be regarded as satisfactory markers of the particulate phase of the digesta. Fluid markers are also commonly used in digestive studies on animals, but conventional fluid markers might be expected not to provide useful information on digesta movement in crustaceans as the digestive fluids circulate between the midgut gland and the foregut. This is borne out by studies on the portunid crab *Scylla serrata* (P. Sukardi and P. Greenaway, unpublished data). Four weeks after introduction of ^{51}Cr-EDTA (a fluid marker commonly used in vertebrates) into the foregut of this species, less than 10% had appeared in the faeces. Sampling of the foregut fluid at that time revealed that most of the marker was still present in the gut and evidently it circulated between foregut and midgut gland with only a small amount escaping in the compacted faecal strands.

In comparison with other terrestrial crabs, *B. latro* retained the particle marker within its gut for a long time. The mean retention times (MRT) for the marker in *B. latro, G. natalis* and *D. hirtipes* were respectively 27 h, 11.6 ± 5.4 h and 3.5 ± 0.3–7.3 ± 1.1 h (Greenaway and Linton 1995; Greenaway and Raghaven 1998). The longer MRT for *B. latro* potentially permits more time for digestion of the food than in the other two species, and the near complete utilisation of fats, storage polysaccharide and protein and considerable digestion of the structural polysaccharides in the diet was consistent with this. However, the location of the digesta during the retention period was not known, and the frequency of faecal elimination was so low that it was not possible to partition retention time between the digestive phase and faecal storage. For this reason, it was not considered worthwhile to measure MRT time on all three diets. By comparison, the higher intake and lower retention of dry matter by the two gecarcinid crabs provided a much more reliable indication of MRT.

It is evident that *B. latro* selects dietary items that are nutrient rich. Nutritious fruits, carrion and prey animals are not evenly distributed in space or time, and animals that rely on these food sources must be well equipped both to locate them and to survive periods of limited availability. The ability of *B. latro* to store large amounts of fat enable it to capitalise on periodic abundance of food and to survive long periods of starvation (Greenaway 2003). The abdomen, which is flexible and can be expanded some 3–4 times, is packed with midgut gland tissue in which the fat is stored.

This strategy contrasts with that of the herbivorous gecarcinid crabs which chiefly exploit leaf litter that, although readily available, has low levels of N, storage polysaccharide and fat and high levels of hemicellulose, cellulose, lignin and tannins (Greenaway and Linton 1995; Greenaway and Raghaven 1998; Sherman 2002; Wolcott and Wolcott 1984, 1987). Gecarcinid crabs pass food through their guts relatively quickly and digest the soluble cell contents and substantial amounts of cellulose and hemicellulose (Greenaway and Linton 1995; Greenaway and Raghaven 1998). Digestion of cellulose is accomplished by endogenous cellulases (Linton and Greenaway, unpublished data).

Whilst further specific information is needed to fully understand the digestive strategy of *B. latro*, notably the residence time for food in the digestive region of the gut and the identification and characterisation of the enzyme complement, other larger ecological and evolutionary questions beg for answers. With a body mass up to 3 kg, *B. latro* is by far the largest of the terrestrial invertebrates and is believed to grow slowly and to take 40–60 years to reach maximum size (Greenaway 2003). It is important to gain information on feeding rate and frequency and metabolic rate in the field for the species in order to place the digestive details revealed in this study in perspective. Amongst non-endothermic, terrestrial animals, the large body size is only matched by certain of the reptiles and the very different growth patterns, morphologies, feeding and digestive systems offer fascinating opportunities for comparison between the arthropod and reptilian evolutionary lines.

Acknowledgements We are grateful to Dr. F. Stoddard at the Department of Crop Sciences, Sydney University, for use of the Leco CHN analyser. This work was supported by an Australian Research Council grant 942611 (P.G.). All experiments complied with Australian and NSW animal ethics legislation.

References

Alexander HGL (1979) A preliminary assessment of the role of the terrestrial decapod crustaceans in the Aldabran ecosystem. Philos Trans R Soc Lond B 286:241–246

Barnes DKA (1997) Ecology of tropical hermit crabs at Quirimba Island, Mozambique: a novel and locally important food source. Mar Ecol Prog Ser 161:299–302

Byrne KA, Lehnert SA, Johnson SE, Moore SS (1999) Isolation of a cDNA encoding a putative cellulase in the red claw crayfish *Cherax quadricarinatus*. Gene 239:317–324

Dall W, Moriarty DJW (1983) Functional aspects of nutrition and digestion. In: Mantel LH (ed) The biology of Crustacea, vol 5. Internal anatomy and physiology. Academic Press, New York, pp 215–261

De Wilde PAWJ (1973) On the ecology of *Coenobita clypeatus* in Curaçao with reference to reproduction, water economy and osmoregulation in terrestrial hermit crabs. Studies on the fauna of Curaçao and other Carribean islands, vol 144. Martinus Nijhoff, The Hague, pp 1–138

Dillaman RM, Greenaway P, Linton SM (1999) Role of the midgut gland in purine excretion in the robber crab, *Birgus latro* (Anomura: Coenobitidae). J Morphol 241:227–235
Gibson R, Barker PL (1979) The decapod hepatopancreas. Oceanogr Mar Biol Annu Rev 17:285–346
Giddins RL, Lucas JS, Neilson M J, Richards GN (1986) Feeding ecology of the mangrove crab *Neosarmatium smithi* (Crustacea: Decapoda: Sesarmidae). Mar Ecol Prog Ser 33:147–155
Greenaway P (2001) Sodium and water balance in free-ranging robber crabs, *Birgus latro* (Anomura: Coenobitidae). J Crust Biol 21:317–327
Greenaway P (2003) Terrestrial adaptations in the Anomura (Crustacea: Decapoda). Mem Mus Vic 60:13–26
Greenaway P, Linton SM (1995) Dietary assimilation and food retention time in the herbivorous terrestrial crab *Gecarcoidea natalis*. Physiol Zool 68:1006–1028
Greenaway P, Morris S (1989) Adaptations to a terrestrial existence by the Robber Crab, *Birgus latro*. III. Nitrogenous excretion. J Exp Biol 143:333–346
Greenaway P, Raghaven S (1998) Digestive strategies in two species of leaf-eating land crabs (Brachyura: Gecarcinidae) in a rain forest. Physiol Zool 71:36–44
Grubb P (1971) Ecology of terrestrial decapod crustaceans on Aldabra. Philos Trans R Soc Lond 260:411–416
Hames CAC, Hopkin SP (1989) The structure and function of the digestive system of terrestrial isopods. J Zool Lond 217:599–627
Hartnoll RG (1988) Evolution, systematics and geographical distribution. In: Burggren WW, McMahon BR (eds) Biology of the land crabs. Cambridge University Press, New York, pp 6–53
Hicks J, Rumpff H, Yorkston H. (1990) Christmas crabs, 2nd edn. Christmas Island Natural History Association, Christmas Island, Indian Ocean
Hornung DE, Stevenson JR (1971) Changes in the rate of chitin synthesis during the crayfish molting cycle. Comp Biochem Physiol B 40: 341–346
Icely J, Nott J (1992) Digestion and absorption: Digestive system and associated organs. In: Harrison F, Humes AG (eds) Microscopic anatomy of invertebrates, vol 10. Wiley-Liss, New York, pp 147–201
Kono M, Matsui T, Shimizu C, Koga D (1990) Purification and some properties of chitinase from the liver of a prawn, *Penaus japonicus*. Agric Biol Chem 54:2145–2147
Lawrence JM (1970).Lipid content of the organs of the coconut crab, *Birgus latro* (L.) (Decapoda: Paguridea). Crustaceana 19:264–266
Linton SM, Greenaway P (1997) Urate deposits in the gecarcinid land crab *Gecarcoidea natalis* are synthesised de novo from excess dietary nitrogen. J Exp Biol 200:2347–2354
Linton SM, Greenaway P (2000) The nitrogen requirements and dietary nitrogen utilization for the gecarcinid land crab *Gecarcoidea natalis*. Physiol Biochem Zool 73:209–218
Peters W (1991) Peritrophic membranes. Springer, Berlin Heidelberg New York
Robertson JB, Van Soest PJ (1982) The detergent system of analysis and its application to human foods. In: James WPT, Theander O (eds) The analysis of dietary fiber in food. Dekker, New York, pp 123–158
Russell-Hunter WD (1970) Aquatic productivity: an introduction to some basic aspects of biological oceanography and limnology. Collier MacMillan, London
Sherman PM (2002) Effects of land crabs on seedling densities and distributions in mainland neotropical rain forest. J Trop Ecol 18:67–89
Simpson SJ, Abisgold JD (1985) Compensation by locusts for changes in dietary nutrients: behavioural mechanisms. Physiol Entomol 10:443–452
Sukardi P (1994) Digestive physiology of the mud crab *Scylla serrata* Forskål. PhD Diss, The University of NSW, Sydney. Australia
Warner ACI (1981). The mean retention times of digesta markers in the gut of the tammar, *Macropus eugenii*. Aust J Zool 29:759–771
Wolcott DL, O'Connor NJ (1992) Herbivory in crabs: adaptations and ecological considerations. Am Zool 32:370-381
Wolcott DL, Wolcott TG (1984) Food quality and cannibalism in the red land crab, *Gecarcinus lateralis*. Physiol Zool 57:318–324
Wolcott DL, Wolcott TG (1987) Nitrogen limitation in the herbivorous land crab *Cardisoma guanhumi*. Physiol Zool 60:262–268
Xue XM, Anderson AJ, Richardson NA, Anderson AJ, Xue GP, Mather PB (1999) Characterisation of cellulase activity in the digestive system of the redclaw crayfish (*Cherax quadricarinatus*). Aquaculture 180:373–386
Zimmer M, Topp W (1998) Microorganisms and cellulose digestion in the gut of the woodlouse *Porcellio scaber*. J Chem Ecol 24:1397–1408