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ORIGINAL PAPER

Effect of starvation on the energy budget of two Asian horseshoe crab species: *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda* (Chelicerata: Xiphosura)

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Abstract Energy budget is one of the most studied parameters in aquatic animals under environmental challenge. To examine how prolonged starvation would affect their energy budget, respiration rate (RR), ammonia excretion rate (ER), oxygen consumption to ammonia–nitrogen excretion (O:N) ratio and scope for growth (SfG) representing the balance between energy intake and metabolic output, two Asian horseshoe crab species, *Tachypleus tridentatus* and *Carcinoscorpius rotundicauda*, were investigated in two feeding regimes (fed and starved) over a period of 7 weeks. No significant effects of species and time course, as well as their interaction, on absorption efficiency were observed in the fed treatments. For both species, RR and ER of the starved treatments significantly decreased, while their O:N ratio significantly increased during the experiment. However, such values for the fed treatments remained relatively stable over the study period. A rapid reduction in SfG was only apparent in the first week of the starved treatments for both species; thereafter, their SfG remained relatively constant. In the fed treatments, SfG of *T. tridentatus* was significantly lower than that of *C. rotundicauda* throughout the experiment. In general, *C. rotundicauda* showed a greater decrease in SfG under starvation than *T. tridentatus*, suggesting that they may have a more competitive life-history strategy for adjusting to poor nutritional conditions.

Introduction

Food ingestion, ammonia excretion and oxygen consumption rates are the governing factors for growth in aquatic animals because they reflect energy utilization. In particular, scope for growth (SfG) elicited from these physiological parameters is useful for estimating the energy budget of animals under environmental challenge (Beiras et al. 1994).

Horseshoe crabs are an ancient animal group, often referred to as living fossils. There are only four species of horseshoe crabs living in the world. Of the two Asian species, *Tachypleus tridentatus* (Leach, 1819) is distributed from North Borneo and Malaysia, along the coast of China and northward towards Japan (Sekiguchi 1988; Shin et al. 2009; Hu et al. 2010), whereas *Carcinoscorpius rotundicauda* (Latreille, 1802) occurs further to the west in Indonesia, Malaysia, the Philippines, Thailand and the Bay of Bengal, India (Sekiguchi 1988; Chatterji 1994). The morphological characteristics of these two species are different, adult *T. tridentatus* being larger in size and having three distinct immovable spines above the insertion of the postanal spine, while *C. rotundicauda* is relatively smaller with a sub-triangular postanal spine in cross section and a weakly recurved auriculate process (Mikkelsen 1988; Chiu and Morton 2003). However, these two species can co-exist in the same habitat (Hu et al. 2009; Shin et al. 2009).

Horseshoe crabs are benthic feeders which primarily consume bivalve molluscs, such as clams and mussels (Patil and Anil 2000; Botton et al. 2003; Carmichael et al.

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2004). Botton (1984) examined the population of the adult American horseshoe crab *Limulus polyphemus* and concluded that bivalves are their most favourable diets. In marine environments, the amount of food available for animal consumption can be highly seasonal (Clarke 1988; Wassmann 1991). Hence, horseshoe crabs show a considerable diversity of feeding mechanisms and specialization, such as limiting energy expenditures or increasing foraging effort, so as to enable them to survive in an apparently food deficient environment (Botton et al. 2003).

Previous studies have revealed that animals tend to accumulate energy reserves when nutritional conditions are favourable for use in maintenance, growth and reproduction during periods of low food availability (Nakaoka 1992; Brockington and Clarke 2001). Related strategies may include physiological adaptations, such as high absorption efficiency, and behavioural plasticity (Lopez and Levinton 1987; Taghon and Greene 1992). Stead et al. (2003) indicated that the estimation of energy budget in aquatic species could contribute towards an overall comprehension of their feeding strategies and energy utilization, as well as their physiological responses to seasonal changes in food availability. However, such information is lacking in horseshoe crabs, as there are limited physiological data on how these animals respond to different food conditions in the field.

The aim of the present study was to compare, under totally different nutritional conditions (i.e., fed or starved), the physiological responses of *T. tridentatus* and *C. rotundicauda*, in terms of respiration rate, ammonia excretion rate and O:N ratio. The physiological parameters measured at different observation time points were also compared in an attempt to construct a relationship between starvation time course and metabolism. Finally, the energy budgets, as represented by SfG, were estimated for *T. tridentatus* and *C. rotundicauda* in order to determine which species is more energetically efficient when suffering from poor nutritional conditions.

Materials and methods

Experimental animals

Adult male *T. tridentatus* (prosomal width: 162–176 cm) and *C. rotundicauda* (prosomal width: 92–99 cm) were obtained from a local fish market in Hong Kong.

Experimental design

A three-way orthogonal experimental design, with two species (*T. tridentatus* and *C. rotundicauda*), two food treatments (fed and starved) and five observation time points (Week 0, 1, 3, 5, 7), was adopted in the present study. Horseshoe crabs in the fed treatment were offered clam (*Ruditapes philippinarum*) meat ad libitum. There were six individual replicates per experimental treatment (24 total individuals) and the experiment lasted for 7 weeks. The experiment was conducted in two temperature-controlled, re-circulating systems, and each system contained 12 tanks. As the body size of *T. tridentatus* is some two times larger than that of *C. rotundicauda*, different tank size and water flow rates were adopted to ensure that the water volume occupied by each individual and flow of water exchange in the tanks were similar for the two species. One system, housing the larger tanks (length: 66 cm, width: 46 cm, height: 50 cm, water volume: 60 L), was assigned to *T. tridentatus*, while the other system, housing the smaller tanks (length: 50 cm, width: 40 cm, height: 30 cm, water volume: 20 L), was assigned to *C. rotundicauda*. Each tank was connected to a filter tank filled with zeolites and activated carbon. Filtered seawater was re-circulated through the filter at a rate of 0.8 L min^{-1} in the *T. tridentatus* system and 0.25 L min^{-1} in the *C. rotundicauda* system. During the experiment, water temperature was maintained at 21–22°C, salinity 33–34 ‰ and pH 8, which corresponded to the general seawater quality conditions in Hong Kong. Continuous aeration was supplied to each tank, with dissolved oxygen (DO) ranging from 6 to 8 mg L^{-1}. Photoperiod was maintained at 12D:12 L with the light period from 07:30 to 19:30 throughout the experiment.

Physiological measurements

Absorption efficiency (AE), representing the efficiency at which the organic matter is absorbed from the ingested food, was determined as follows. The food was dried in an oven (110°C) for 24 h, weighed, burned in a muffle furnace (450°C for 6 h) and reweighed. Faeces were collected with a pipette from the experimental tank 24 h after feeding, and the organic content of the faeces was determined by the same method as described previously. AE was calculated according to Conover's (1966) formula:

$$\text{AE} = (F - E)/[(1 - E) \times F]$$

where AE = absorption efficiency (%), F = ash-free dry weight:dry weight ratio in the food, and E = ash-free dry weight:dry weight ratio in the faeces.

Food consumption rate (FC) was estimated according to the following procedures. The fed animals were offered clam meat ad libitum. The clam meat, with pre-determined wet weight, was placed in tanks daily (17:00 h), and the wet weight of the remaining clam meat left from the previous day was collected and weighed. A control with clam meat but without horseshoe crab was set up, in order to determine the weight change of clam meat after 24 h immersion in the seawater. The dry weight of consumed food was estimated using a regression relating the wet weight and the dry weight of clam meat. The gross energy of the dry clam meat was measured by using a bomb calorimeter (model Parr 1281, USA). The consumed food energy was then converted to joules using a conversion factor of 18.37 J mg^{-1} dry weight estimated for clam meat from the bomb calorimeter measurements.

Respiration rate (RR) was measured every 2 weeks at the same hour before the horseshoe crabs were fed, approximately 20 h after their previous meal, since feeding can evoke an increase in respiration rate due to mastication and movement of the animals (Hirata and Yamasaki 1987; Pedrosa et al. 2002). Each horseshoe crab was placed in a separate tank filled with aerated seawater, and the tank was sealed for 1 h. Initial and final DO levels of the seawater in each tank were measured using an oxygen probe (model YSI5000, USA). Three tanks without animals served as the control. Measurements on the 24 individuals were made simultaneously. The volume of each horseshoe crab was subtracted from the volume of the tank to obtain the volume of water. Oxygen concentrations in each tank were not allowed to drop below 75% of saturation throughout the experiment (Norkko et al. 2005). Energy expended on respiration was calculated using a conversion factor of 13.98 J mg O_2^{-1} (Ivlev 1934).

Excretion rate (ER) was estimated as follows. After respiration rate measurement, the ammonia excretion rate of the horseshoe crabs was measured. The concentration of ammonia excreted was determined after 1 h using the phenol–hypochlorite method (Solorzano 1969). Three tanks without animals served as the control. Triplicate samples (5 mL) were collected and treated with sodium hypochlorite and phenol in the presence of sodium nitroprusside. The amount of ammonia in the sample was measured by the absorbance of the blue indophenol colour developed using a spectrophotometer at $\lambda = 640$ nm. ER was converted to energy terms using a conversion factor of 0.025 J μg^{-1} NH_4–N (Elliott and Davison 1975).

The ratio of oxygen consumption to ammonia–nitrogen excretion in atomic equivalents (O:N) was calculated to determine the proportion of protein relative to carbohydrate and lipid catabolized for energy metabolism, with the following formula (Widdows 1985):

$$\mathrm{O:N} = (\mathrm{mg\,O_2h^{-1}}/16)/(\mathrm{mg\,NH_4 - N\,h^{-1}}/14)$$

where mg O_2 h^{-1} was the rate of oxygen consumption and mg NH_4–N h^{-1} was the rate of ammonia–nitrogen excretion for the same horseshoe crab during the same period.

SfG was calculated using the energy balance equation given by Bayne and Newell (1983):

$$\mathrm{SfG} = \mathrm{Ab} - (R + U)$$

where SfG = scope for growth (J h^{-1}), Ab = the absorbed rate (J h^{-1}), R = energy lost in respiration (J h^{-1}) and U = energy lost in ammonia excretion (J h^{-1}). Ab was calculated as FC (J h^{-1}) × AE (%).

Statistical analysis

Results were expressed as mean ± standard error (SE). Prior to statistical analysis, assumptions for normality of distribution of the data and homogeneity of variances were tested with the Shapiro–Wilk's *W* test and Levene's test, respectively. Data that did not meet the above assumptions were transformed, as follows. The data of *T. tridentatus* respiration rate were divided by 100 and then cosine transformed. The data of *C. rotundicauda* O:N ratio were cosine transformed. The data of SfG of the two horseshoe crab species were divided by 100 and then arcsine transformed.

Two-way repeated measures analysis of covariance (ANCOVA) was employed to analyse species and observation time point effects on AE of fed horseshoe crabs, with body size (prosomal width) as covariate. Three-way repeated measures ANCOVA was used to analyse species, food treatments and observation time point effects on RR, ER, O:N ratio and SfG of horseshoe crabs, with body size (prosomal width) as covariate.

Tukey's honestly significant difference (HSD) post-multiple range tests or Student *t*-tests were used to compare treatment means in cases where factors did not significantly interact. When interaction between or among factors was significant, the influence of each factor on treatment means was tested at fixed levels of the other factors. Differences were considered significant at $P < 0.05$. All statistical analyses were undertaken using SPSS version 16.0.

Results

No significant effects of species ($P = 0.259$) and observation time points ($P = 0.315$), as well as their interaction ($P = 0.518$) on AE were observed in the fed horseshoe crabs (Table 1, Fig. 1).

Significant interactions between species and food treatments, between species and time course, between food treatments and time course, and among species, food treatments and time course were detected for RR, ER, O:N ratio and SfG throughout the experiment ($P < 0.05$; Table 2).

RR of fed *T. tridentatus* was significantly lower than that of the fed *C. rotundicauda* from Week 0 to Week 5 ($P < 0.05$). No significant difference between species was

Table 1 Summary of two-way repeated measures ANCOVA results for effects of species (S) and observation time points (T) on absorption efficiency of fed adult horseshoe crabs, with body size as covariate

	Factor	Degrees of freedom	Mean square	*F*	*P*
Absorption efficiency	*S*	1	0.002	2.433	0.259
	T	4	<0.001	1.406	0.315
	$S \times T$	4	<0.001	0.878	0.518

Species: *T. tridentatus* and *C. rotundicauda*; observation time points: Week 0, 1, 3, 5, 7

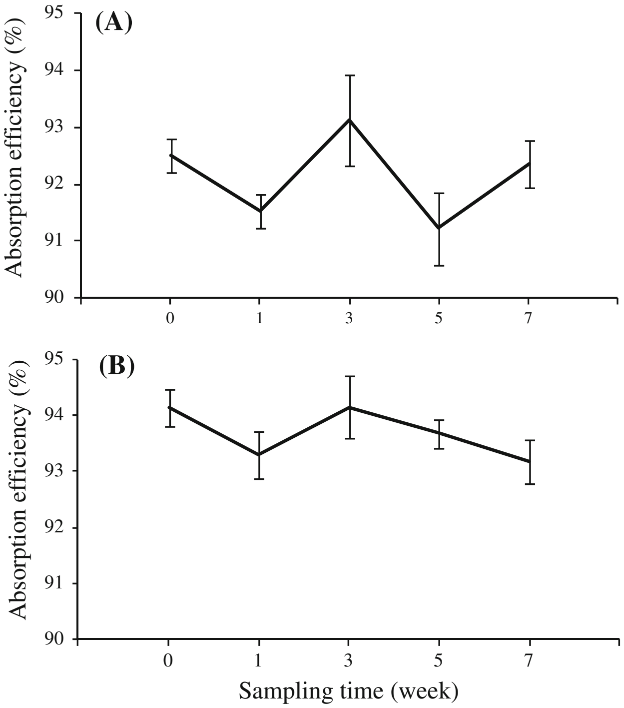

Fig. 1 Absorption efficiency (*AE*) of two horseshoe crab species fed with clam meat: **a** *T. tridentatus* and **b** *C. rotundicauda* ($P > 0.05$)

found in fed treatments in Week 7 ($P = 0.056$), as well as in starved treatments from Week 1 to Week 7 ($P > 0.05$). A continued drop in RR in the starved treatment of each species was evident over the experimental period ($P < 0.05$; Fig. 2). For each species, RR of the fed treatment was significantly higher than that of the starved treatment from Week 1 to Week 7 ($P < 0.05$). For both species, time course had a significant influence on RR ($P < 0.05$), except for the fed *T. tridentatus* group ($P = 0.247$).

ER of fed *T. tridentatus* was significantly lower than that of fed *C. rotundicauda* throughout the experiment ($P < 0.05$), as well as starved *T. tridentatus* and *C. rotundicauda* in Week 7 ($P = 0.026$). No significant difference between species was found in starved treatments from Week 1 to Week 7 ($P > 0.05$). For each species, a continued decrease in ER was apparent in the starved group ($P < 0.05$), but no significant temporal difference was observed in the fed group ($P > 0.05$; Fig. 3). ER of the fed *T. tridentatus* was significantly higher than that of the starved group from Week 3 to Week 7, while ER of the fed *C. rotundicauda* was significantly higher than that of the starved group from Week 1 to Week 7 ($P < 0.05$).

O:N ratio of fed *T. tridentatus* was significantly higher than that of fed *C. rotundicauda* from Week 0 to Week 3 ($P < 0.05$), as well as starved *T. tridentatus* and *C. rotundicauda* in Week 1 ($P = 0.037$). No significant difference between species was found in fed treatments in Week 5 ($P = 0.132$) and Week 7 ($P = 0.072$), as well as starved treatments from Week 3 to Week 7 ($P > 0.05$). For each species, a significant increase in O:N ratio was noted in the starved treatment ($P < 0.05$), but temporal changes in O:N ratio were minimal in the fed treatment ($P > 0.05$) over the experimental period (Fig. 4). O:N ratio of the fed *T. tridentatus* was significantly lower than that of the starved counterparts in Week 5 ($P < 0.001$) and Week 7 ($P = 0.046$), while O:N ratio of the fed *C. rotundicauda* was significantly lower than that of the starved ones from Week 3 to Week 7 ($P < 0.05$).

SfG of fed *T. tridentatus* was significantly lower than that of fed *C. rotundicauda* throughout the experiment ($P < 0.05$). No significant difference between species was found in starved treatments from Week 1 to Week 7 ($P > 0.05$). For each species, a rapid reduction in SfG was apparent in the starved treatment from Week 0 to Week 1 at the start of the experiment; thereafter, their SfG remained relatively stable over the study period (Fig. 5). SfG of the fed treatment was significantly higher than that of the starved treatment from Week 1 to Week 7 in both species ($P < 0.05$). In the fed treatments, only significant temporal changes in SfG were noted in *T. tridentatus* ($P = 0.001$), but not in *C. rotundicauda* ($P = 0.353$).

Discussion

In the present study, mortality was not observed for the two Asian horseshoe crab species during prolonged starvation of 7 weeks. However, a decrease in RR and ER but an increase in O:N ratio was noted in both starved species over the experimental period. A rapid reduction in energy

Table 2 Summary of three-way repeated measures ANCOVA results for effects of species (S), food treatments (F) and observation time points (T) on respiration rate, excretion rate, O:N ratio and SfG of adult horseshoe crabs, with body size as covariate

	Factor	Degrees of freedom	Mean square	F	P
Respiration rate	S	1	33516.552	2334	<0.001
	F	1	2124.553	259.600	0.004
	T	4	207.012	23.673	<0.001
	$S \times F$	1	2139.320	257.796	0.004
	$S \times T$	4	208.206	23.765	<0.001
	$F \times T$	4	103.787	19.631	<0.001
	$S \times F \times T$	4	104.500	19.861	<0.001
Excretion rate	S	1	47.489	142.833	0.007
	F	1	50.781	1997	0.001
	T	4	5.526	43.014	<0.001
	$S \times F$	1	10.450	468.898	0.002
	$S \times T$	4	0.923	11.804	0.002
	$F \times T$	4	4.303	108.497	<0.001
	$S \times F \times T$	4	0.849	14.992	0.001
O:N ratio	S	1	8753.605	337.510	0.003
	F	1	599.854	99.613	0.01
	T	4	133.476	13.092	0.001
	$S \times F$	1	636.418	49.329	0.02
	$S \times T$	4	129.011	15.219	0.001
	$F \times T$	4	126.809	16.291	0.001
	$S \times F \times T$	4	99.556	14.204	0.001
SfG	S	1	0.027	8205	<0.001
	F	1	0.046	3577	<0.001
	T	4	0.002	128.322	<0.001
	$S \times F$	1	0.013	242.577	0.004
	$S \times T$	4	0.001	34.308	<0.001
	$F \times T$	4	0.003	545.110	<0.001
	$S \times F \times T$	4	0.001	79.039	<0.001

Species: *T. tridentatus* and *C. rotundicauda*; food treatments: fed and starved; observation time points: weeks 0, 1, 3, 5, 7

budget, as estimated by SfG, was apparent in the first week of starvation, but thereafter, the SfG was maintained at relatively low levels for both horseshoe crab species. The present findings also revealed species-specific differences in response to food treatments, in which RR, ER and SfG of fed *T. tridentatus* were significantly lower than those of the fed *C. rotundicauda* for most of the experimental period. On the contrary, *C. rotundicauda* showed a greater decrease in SfG than *T. tridentatus* under starvation.

Most taxa suffering from prolonged food limitation could ultimately succumb to severe starvation and eventual mortality. There are various physiological strategies which allow different species to survive starvation (McCue 2010). Many previous studies have shown that metabolic rates in fed animals are higher than those of the starved animals (Widdows 1973; Hagerman and Szaniawska 1994; Hervant et al. 1997; Wood 2001). Similar observations were noted in the present study. While both horseshoe crab species survived in the 7-week starvation treatment, the fed animals showed a higher metabolic rate than the unfed ones. Widdows and Hawkins (1989) divided the total metabolic expenditure of well-fed mussels into costs of maintenance, digestion-absorption and growth in the proportion 3:1:2. They demonstrated that costs of growth considerably enhanced the metabolic rate of well-fed mussels. In the present experiment, no significant differences of body weight between both fed and starved treatments were observed in both species (see Hu et al. 2010). This is in agreement with other studies which showed that body weight reduction is not necessarily the response to starvation, for example, some crustaceans (Hervant and Renault 2002; Comoglio et al. 2004) tend to replace organic mass lost from tissues with water so that they exhibit virtually no net change in body weight during prolonged starvation. The reason for this specific physiological mechanism is still unclear. McCue (2010) suspected that the increased tissue water content results from increased osmotic pressure in tissues, which stems from increased metabolite levels, and cells somehow replace lost organic matter with water in order to maintain their size, and ultimately

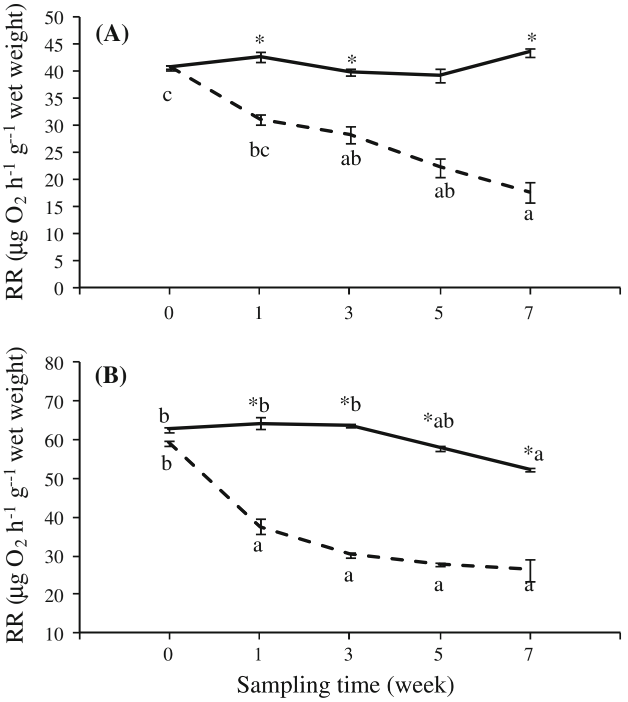

Fig. 2 Effects of treatments on respiration rate (*RR*; mean ± SE) in two horseshoe crab species: **a** *T. tridentatus* and **b** *C. rotundicauda* (fed *solid line*; starved *dashed line*). For each species, values in the same food treatment with different superscripts are significantly different among different observation time points ($P < 0.05$)* Means significant differences were detected between fed and starved groups in the same observation time point ($P < 0.05$)

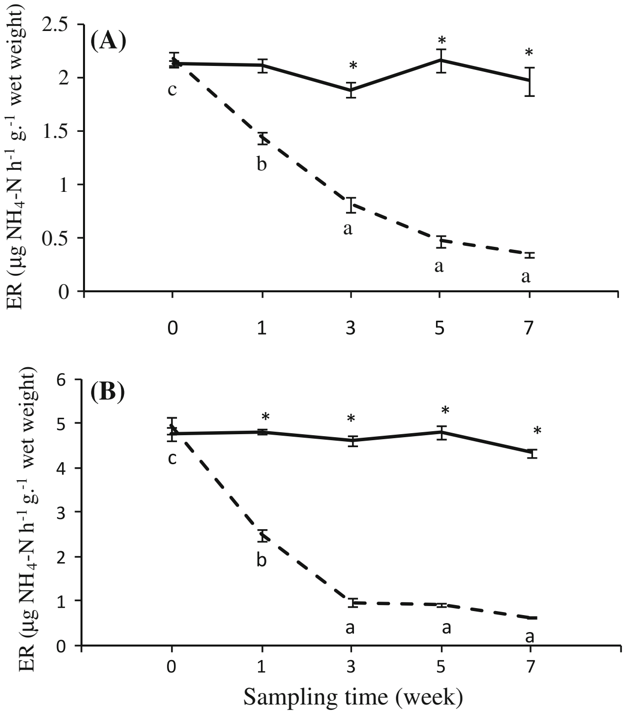

Fig. 3 Effects of treatment on excretion rate (*ER*; mean ± SE) in two horseshoe crab species: **a** *T. tridentatus* and **b** *C. rotundicauda* (fed *solid line*; starved *dashed line*). For each species, values in the same food treatment with different superscripts are significantly different among different observation time points ($P < 0.05$)* Means significant differences were detected between fed and starved groups in the same observation time point ($P < 0.05$)

functionality, during starvation. Nevertheless, at a minimum, successful starvation strategies must enable an animal to mobilize endogenous physiological fuels such as proteins, lipids and carbohydrates to meet the energetic demands required to carry out the basic processes of life. Previous studies have indicated that horseshoe crabs can withstand a long period of starvation by consuming nutrients from their yellow connective tissues (e.g., fat, glycogen and protein) for their basic survival maintenance (Weng et al. 2001; Weng and Hong 2003; Wei et al. 2007). The present study could not verify this statement, but did find that the energy reserves in horseshoe crabs might be able to maintain them through a long period of starvation (at least 7 weeks).

Aquatic animals differ in their abilities to tolerate starvation (McCue 2010). Some animals, such as the blue mussel *Mytilus edulis* (Bayne and Widdows 1978), European frog *Rana esculenta* (Grably and Piery 1981) and hypogean crustaceans (Hervant et al. 1997), are able to survive on energy reserves despite negative SfG over several months of starvation, suggesting that they may have sufficient capacity to balance the competing demands of energy intake, storage and utilization to permit survival for prolonged starvation (Helson and Gardner 2007). Further studies should be conducted to elucidate how much energy a horseshoe crab needs to expend to withstand long-term starvation.

The present findings only reflected the response of adult horseshoe crabs under a single diet of clam meat in the laboratory. While field observations have reported that these animals can forage on a wide range of prey available locally (Botton et al. 2003; Carmichael et al. 2004), adult horseshoe crabs tend to exhibit a strong feeding preference for clams in the wild (Botton 1984; Chatterji et al. 1992). Such a diet preference has also led to the use of clams as feed for adult horseshoe crabs reared in captivity (Smith and Berkson 2005). In the present single-diet experiment, the mean AE of clam meat by both horseshoe crab species was high (over 90%). In the natural environment, however, such high AE of food by horseshoe crabs might not be achievable as adult horseshoe crabs ingest prey containing a variety of organic compounds with a potentially different, but mostly lower degree of digestibility (Chatterji 1992; Botton et al. 2003). The poorer food quality could result in a lower AE (Stead and Thompson 2003), hence, affecting growth and body reserves of the animals for fighting against starvation in the wild.

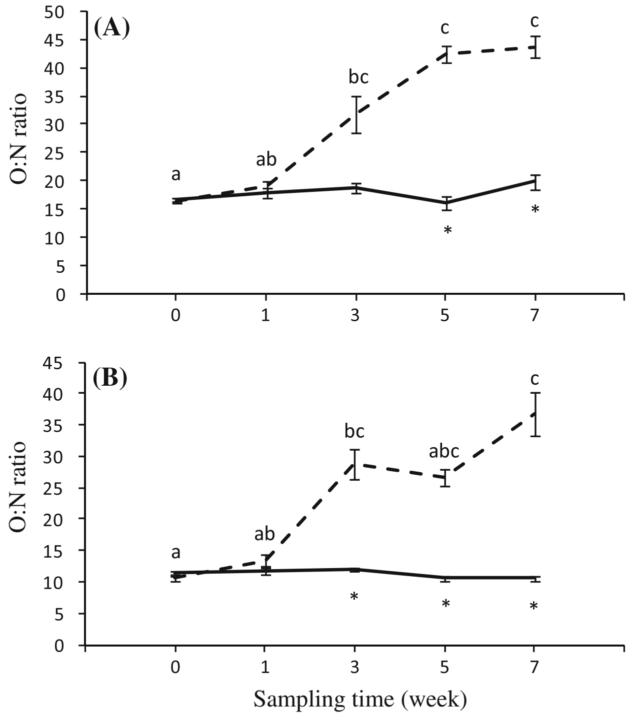

Fig. 4 Effects of treatment on O:N ratio (mean ± SE) in two horseshoe crab species: **a** *T. tridentatus* and **b** *C. rotundicauda* (fed *solid line*; starved *dashed line*). For each species, values in the same food treatment with different superscripts are significantly different among different observation time points ($P < 0.05$)* Means significant differences were detected between fed and starved groups in the same observation time point ($P < 0.05$)

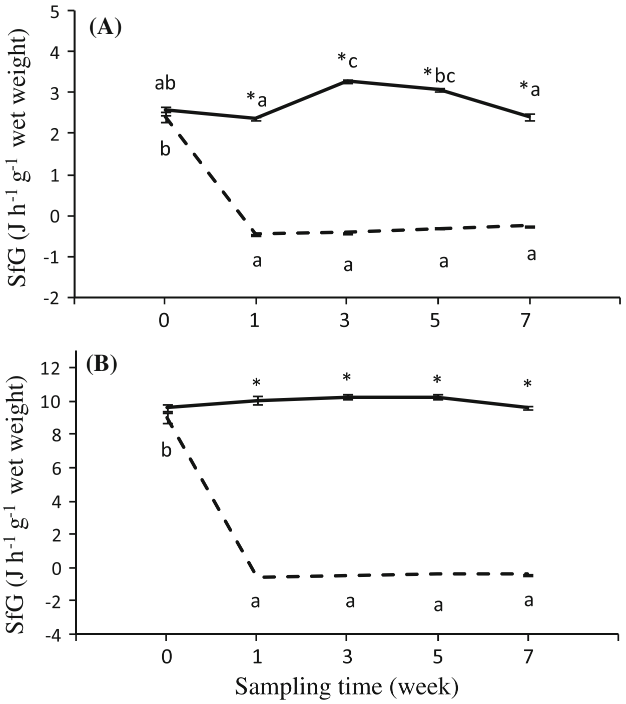

Fig. 5 Effects of treatment on SfG (mean ± SE) in two horseshoe crab species: **a** *T. tridentatus* and **b** *C. rotundicauda* (fed *solid line*; starved *dashed line*). For each species, values in the same food treatment with different superscripts are significantly different among different observation time points ($P < 0.05$)* Means significant differences were detected between fed and starved groups in the same observation time point ($P < 0.05$)

Adaptive mechanisms for facing the energetic limitations caused by starvation may involve a reduction in energy expenditure (Westerterp 1977; Dunlap 1995; Hervant et al.1997; van Dijk et al. 2002; Nagashima et al. 2003). Energy allocated to respiration is used for anabolism and maintenance. The higher respiration rate found in fed horseshoe crabs in the experiment could result from the active feeding process (Hirata and Yamasaki 1987; Pedrosa et al. 2002), anabolism of products of digestion or higher metabolic activity in these animals (Hill and Lawrence 2006). The present study noted that starvation significantly reduced the energy allocated to respiration in both horseshoe crab species. Such differences in respiration rate caused by starvation were also found in similar studies on prawns (Dall and Smith 1986), oysters (Beiras et al. 1994) and mussels (Helson and Gardner 2007).

Besides respiration, ammonium excretion could also be affected by both maintenance and anabolism (Hill and Lawrence 2006). For both horseshoe crab species, starved groups had significantly lower excretion rates than fed groups. Previous studies have indicated that ammonia excretion is able to offer an integrated means to analysing the protein metabolism of aquatic animals (Dall and Smith 1986; Hagerman and Szaniawska 1994; Chen and Lin 1995; Schmitt and Uglow 1997). Regnault (1987) demonstrated that in crustaceans most ammonia excreted is a product of the catabolism of amino acids from the metabolic pool (body reserves) derived from diet. Wood (2001) further reported that protein is a major metabolic fuel in fed fish, whereas oxidation of lipids and carbohydrates is found to be predominant in starved animals.

Atomic O:N ratio is related to the availability of energy stores and the utilization of body protein. This ratio of oxygen consumption to nitrogen excretion by atomic equivalent produces an index of the relative amounts of protein, as compared to carbohydrates and lipids, catabolized by the organism. Theoretically, the minimum value for an O:N ratio is around 7 (when only protein is being catabolized). Mayzaud and Conover (1988) calculated that organisms should have an O:N ratio of 24, catabolizing equal proportions of lipids and proteins. Therefore, when the O:N value of an animal is higher than 24, lipids or carbohydrates could be identified as the major components in its catabolism. As Mayzaud and Conover (1988) suggested, O:N ratios could be variable, rising or falling in response to starvation, depending on the biochemical composition of the organism. In the present study, significant effects of food treatment or sampling time on the O:N ratio of the two horseshoe crab species were found. In fed *T. tridentatus*, the mean O:N ratios ranged from 16.2 to 19.9, indicating mainly protein catabolism, while ratios ranging from 19.1 to 43.7 were recorded in starved animals, denoting a change in utilization of protein to lipid or carbohydrate stores (Bayne et al. 1985). Similar observations were found in *C. rotundicauda*. The present findings were consistent with a previous study, conducted by this team, which showed that starved horseshoe crabs prefer to use glucose or lipids, rather than protein, as energy sources (Hu et al. 2010).

Helson and Gardner (2007) pointed out that positive SfG values are indicative of growth potential, while negative SfG values indicate that energy expenditure is greater than energy acquisition. Many studies have shown that diet is the main factor affecting SfG (Beiras et al. 1994; Kesarcodi-Watson et al. 2001; Navarro et al. 2002). For both horseshoe crab species, positive SfG values were found in the fed treatment, while negative SfG values were recorded in the starved treatment after 1 week of the experiment. SfG remained fairly constant in the starved groups, thereafter, over the time course of the study period.

SfG values of fed *C. rotundicauda* were significant higher than that of fed *T. tridentatus*, which implies that *C. rotundicauda* might have acquired more energy for growth when the two species were fed ad libitum. It is hypothesized that the energy content, per gram of wet weight, of *C. rotundicauda* is much higher than that of *T. tridentatus*, because its soft tissue versus shell content is relatively higher. Therefore, *C. rotundicauda* needs to devote more SfG energy to soft tissue growth. For both species, the starved groups showed a rapid reduction in SfG at the start of the experiment (Week 0 to Week 1), and then no significant difference in SfG between species was found thereafter. *Carcinoscorpius rotundicauda* showed a greater decrease in SfG under starvation than *T. tridentatus*, which suggested that they may have a more competitive life-history strategy than *T. tridentatus* for adjusting to poor nutritional conditions.

Conclusions

The present study demonstrated that the two Asian horseshoe crabs (*T. tridentatus* and *C. rotundicauda*) exhibited a similar trend of responses in energy budget under prolonged starvation. For both species, respiration rate, excretion rate and SfG of the starved groups significantly decreased, while their O:N ratio significantly increased throughout the 7-week experiment. However, species-specific differences could be observed between the two horseshoe crab species, in which *C. rotundicauda* showed a greater decrease in SfG under starvation than *T. tridentatus*. The present study was consistent with previous reports in that sympatric species occupying similar habitats may elicit different responses to environmental challenges for survival (Qvarnstrom et al. 2009). The present findings suggest that for these two Asian horseshoe crabs, *C. rotundicauda* may have a more competitive life-history strategy than *T. tridentatus* for adjusting to starvation in nature.

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