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Feeding and digestion in low salinity in an osmoconforming crab, *Cancer gracilis*

II. Gastric evacuation and motility

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Summary

Gastric evacuation and gut contraction rates were followed in the graceful crab *Cancer gracilis* during exposure to low salinity. Crabs were fed a radio-opaque meal and then exposed to 100% seawater (SW), 80%SW or 60%SW; passage of digesta was followed using a fluoroscope. Exposure to low salinity increased the time for food passage through the gut system. Times for emptying of the foregut, midgut and hindgut varied in a dose-dependent manner. In the lowest salinity, crabs regurgitated food from the foregut after approximately 6 h. This may act as a protective response, clearing the gut and avoiding subsequent increases in metabolism associated with digestion. Contraction rate of the cardiac stomach and gastric mill was sporadic and there was no significant change with salinity. In contrast, contractions of the pyloric region were more constant and rapid. Pyloric contractions decreased at each salinity within 2–4 h after feeding. Contraction rates of the pyloric chamber were significantly lower in 60%SW compared with 100%SW and 80%SW. During a salinity cycle there was also slowing of gut contractions and food passage through the gut system. Pre-treatment levels were only regained slowly when the animals were returned to 100%SW. *Cancer gracilis* was able to slow digestion during low salinity exposure, which may spare resources for other systems. However, the crabs could not halt digestion completely and may be committed to protein synthesis once intracellular digestion has begun.

Key words: *Cancer gracilis*, crab, digestion, gastric evacuation, feed, salinity.

Introduction

The decapod crustacean gut is essentially an internal tube, opening at the oesophagus and ending as the anus in the telson of the abdomen. The oesophagus leads into a large foregut that is separated into an anterior cardiac chamber, with a smaller pyloric chamber lying posteriorly (Barker and Gibson, 1977; Barker and Gibson, 1978; Icely and Nott, 1992; Heeren and Mitchell, 1997). The cardiac chamber contains calcified ossicles, which form the gastric mill apparatus (Maynard and Dando, 1974). Its primary function is mastication of food (Heinzel, 1988; Heinzel et al., 1993); enzymatic breakdown of food also starts in this region (Icely and Nott, 1992). The pyloric chamber acts to regulate movement of material into the midgut region. The midgut starts at the junction with the pyloric sac and ends in a coiled tube, the posterior midgut caecum, at the junction between the carapace and abdomen (Smith, 1978). The paired anterior midgut caeca arise from the midgut close to the junction with the pyloric chamber and branch extensively within the hepatopancreas. It is here that enzymatic digestion continues and absorption of food by the hepatopancreas occurs (Hopkin and Nott, 1980). The hindgut arises behind the posterior midgut caecum and runs the length of the abdomen to the anus (Maynard and Dando, 1974). It functions in expelling the muco-peritrophic membrane and its contents by rhythmic contractions along its length (Dall and Moriarity, 1983).

The duration of food passage through the gut is variable among different species of decapod crustaceans, ranging from as little as 3–6 h to as long as 48 h (Haddon and Wear, 1987; Hill, 1976; Hopkin and Nott, 1980; Joll, 1982; Sarda and Valadares, 1990; McGaw and Reiber, 2000). Release of enzymes from the hepatopancreas and subsequent digestion occurs within 30–60 min of food ingestion (Dall, 1967; Barker and Gibson, 1977; Barker and Gibson, 1978; Hopkin and Nott, 1980) and intracellular digestion and protein synthesis can start within 2 h and continue for 2–3 days (Houlihan et al., 1990; Mente, 2003; Mente et al., 2003). The whole system is cleared of food between 12 h and 48 h after feeding (Dall, 1967; Hopkins and Nott, 1980; Joll, 1982; Choy, 1986; Sarda and Valladares, 1990). Movement of material through the digestive

system is highly dependent on temperature (Haddon and Wear, 1987). In addition, gastric rhythms are modulated by hypoxia (Clemens et al., 1998a) and low salinity slows egestion rates in mysid shrimps (Roast et al., 2000). Otherwise there are few other reports of environmental effects on gastric processing in crustaceans.

Feeding and digestion and the subsequent assimilation of nutrients causes an increase in metabolic parameters, a process known as specific dynamic action. This is due to food handling and mechanical breakdown in the gut (Carefoot, 1990), and the subsequent intracellular protein synthesis (Houlihan et al., 1990; Mente, 2003). In crustaceans the specific dynamic action is associated with a two- to threefold increase in oxygen uptake that persists for up to 3 days (Houlihan et al., 1990; Burggren et al., 1993; McGaw and Reiber, 2000; Robertson et al., 2002; McGaw, 2006b). To support the increase in metabolic rate and to facilitate the uptake and distribution of absorbed nutrients, changes in cardiac function and haemolymph distribution also occur during the digestive process (McGaw and Reiber, 2000, McGaw, 2005; McGaw, 2006a; McGaw, 2006b).

There is a growing body of literature on the cardiovascular and respiratory responses of decapod crustaceans to low salinity (see McGaw, 2006b). However, these previous studies were conducted on unfed animals to ensure they were in a similar metabolic state and to avoid stimulatory effects associated with feeding and digestion (Wang, 2001). Recent work on decapod crustaceans shows that digestion can pose additional demands on physiological systems, leading to an increased mortality rate of postprandial crabs in low salinity (Legeay and Massabuau, 2000; McGaw, 2006a). Therefore, the ability of an animal to balance the demands of physiological systems by either prioritizing or summing metabolic effects is important (Bennett and Hicks, 2001).

The graceful crab *Cancer gracilis* is classed as an osmoconformer and does not survive prolonged exposure below 55%SW (D. L. Curtis, E. K. Jensen and I. J. McGaw, manuscript submitted for publication). In contrast to work on unfed osmoregulating crabs, which exhibit an increase in oxygen uptake and heart rate during low salinity exposure (King, 1965; Engel et al., 1975; Hume and Berlind, 1976; Taylor, 1977; McGaw and McMahon, 1996; McGaw and Reiber, 1998), unfed *Cancer gracilis* react with a pronounced decrease in cardiac and respiratory parameters. However, when postprandial *Cancer gracilis* encounter low salinity, the bradycardia is absent and the reduction in oxygen uptake is of shorter duration than in unfed crabs (McGaw, 2006b). This suggests that either digestive events are prioritized and digestion continues unabated in low salinity or, because of the elevated metabolism due to the specific dynamic action, increases in respiratory and cardiovascular parameters are necessary to sustain basic life functions. Since digestive events can pose an additional burden on an already taxed system (Legeay and Massabuau, 2000; McGaw, 2006a; McGaw, 2006b) the ability to alter or delay digestion may be an advantage. Therefore, the present study sought to determine how gastric processing and evacuation are affected or modulated by exposure to low salinity in an osmoconforming species of crab.

Materials and methods

Adult male, intermoult graceful crabs *Cancer gracilis* Dana of mass 180–215 g were trapped in the Bamfield Inlet, British Columbia, Canada, from July to September 2005. They were transferred to the Bamfield Marine Sciences Centre and held in running seawater (SW; 31–32‰ at 11±1°C) for a week prior to experimentation. Crabs were fed fish every other day, but were isolated from the general population and starved for 3 days prior to experimentation. This time period allowed all food to be evacuated from the digestive system, but avoided large-scale physiological changes associated with starvation (Wallace, 1973).

During experiments the crabs were housed in individual chambers of 0.2 m×0.2 m. They were allowed to settle for 12 h in the chambers before experimentation. All experiments were performed in constant light, which helped standardize activity levels, and the tanks were surrounded by black plastic to avoid visual disturbance to the animals. The crabs were fed a radio-opaque meal consisting of the following by mass: 65% liquefied fish muscle (*Sebastes* species), 25% gelatin solution and 10% electrolytic iron powder. This produced a homogenous solid food that could be cut into cubes and was readily consumed by the crabs. Electrolytic iron powder was a better tracer than barium sulphate, which had to be used in such a high concentration that it made the meal unpalatable (Talbot and Higgins, 1983) and ballotini lead glass beads are no longer commercially available. In the first set of experiments, passage of the particulate digesta through the digestive system and contraction of the foregut was followed in 100%SW, 80%SW and 60%SW (N=10 per salinity). These salinities were chosen because they were within the survivable range for this species (D. L. Curtis, E. K. Jensen and I. J. McGaw, manuscript submitted for publication). The crabs were offered 5 g cubes of food (approx. 2% of body mass) in 100%SW (32‰). Once they had finished feeding (approx. 15 min), the salinity was lowered by draining a portion of tank, without aerially exposing the crab, and adding a known volume of freshwater at ambient temperature and oxygen levels. Salinity was checked using a YSI 30 conductivity meter (Yellow Springs, OH, USA). New steady states of salinity were reached within 10 min and did not vary by more than 0.1‰ during experiments. In a second experiment, food passage was followed during a salinity cycle, representative of those experienced in the field (McGaw, 2006b). The crabs (N=10) were fed in 100%SW and allowed to digest for 3 h in 100%SW. The salinity was then lowered to 65%SW for 6 h, before being raised back up to 100%SW.

For X-ray analysis, a plastic box (120 mm×180 mm×80 mm) was submerged in the chamber and the crabs were coaxed into the box and allowed to settle for 30 s. The box was then placed under a LIXI PS500 OS X-ray system (Huntley, IL, USA) and images were recording using LIXI image processing

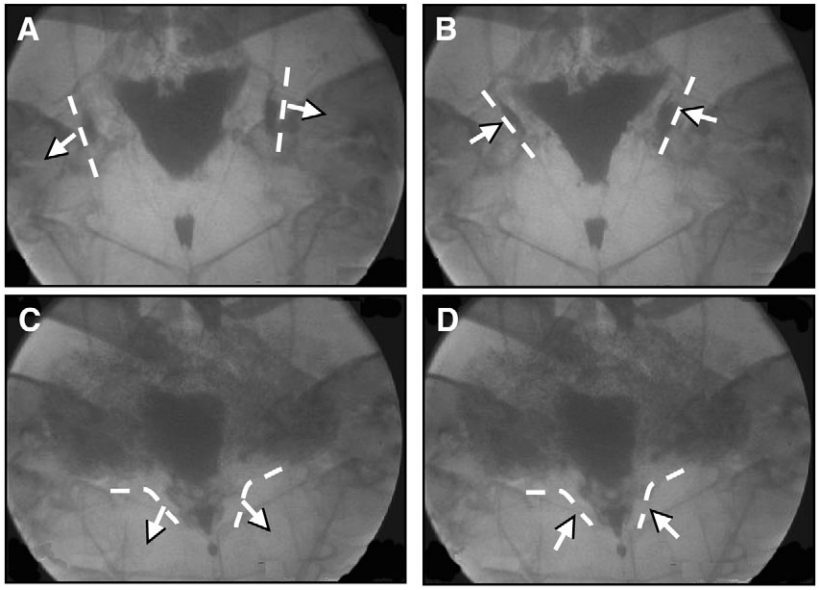

Fig. 1. Methodology for quantifying food processing in the foregut. Large contractions were counted as (A) opening and (B) closing of the gastric mill apparatus and relaxation and contraction of the cardiac sac. Minor gut contractions were quantified as (C) relaxation and (D) contraction of the pyloric region of the foregut.

software. Technical specifications for the X-ray were 35 kV tube voltage, 155 μA tube current with a 5 cm focal window. At hourly intervals following feeding a still image and 15 s segment of video were captured. The passage of food through the gut was measured by outlining the boundaries of each gut region and estimating the percentage fullness of each region, separately. The movement of the digesta and marker were followed until the latter had been voided in the faeces and the time of emptying of the foregut, midgut and hindgut regions was calculated (Edwards, 1971; McGaw and Reiber, 2000). Contraction rates of the cardiac region and pyloric region of the foregut were also recorded (Fig. 1). Cardiac movements were counted as opening and closing of the gastric mill and contraction and squeezing of the cardiac stomach (Heinzel, 1988). Contractions of the pyloric region were counted as small-scale pulsating movements (Heinzel, 1988). Only crabs that appeared to have a full stomach after feeding were used in analysis.

Repeated-measures ANOVAs were used to test for significant differences in gut parameters. Data showing a significant effect, were further analyzed by a Fisher's LSD multiple comparison test ($P<0.01$) to determine at which time periods significant effects were observed.

Results

Gastric evacuation times

In *Cancer gracilis*, hyposaline exposure increased the time for gastric evacuation and also reduced the amount of digesta passing into the midgut and hindgut regions (Fig. 2). There were significant differences in foregut emptying times at each salinity (ANOVA, $F=3.09$, $P<0.01$). Food material started to exit the foregut and move into the midgut, 2 h after feeding. At this time in 100%SW and 80%SW, the foregut was approximately 85% full, while in 60%SW it remained almost 100% full. In each salinity treatment a large proportion of the food had exited the foregut by 12 h. After 12 h in 100%SW less than 5% of the food remained in the foregut. In contrast, after 12 h in 80%SW and 60%SW the foregut was 20% and 27% full, respectively. The foregut emptied between 14 h and 16 h in 80%SW, while it was not until 48 h in 60%SW that the foregut content dropped below 10%.

In 100%SW and 80%SW, once food was processed in the foregut it passed into the midgut and subsequently into the hindgut (Fig. 3). In 60%SW only small amounts of food passed into the midgut. Between 6 h and 12 h in 60%SW there was a sharp drop in foregut content, without a subsequent increase in midgut filling (Fig. 2C, Fig. 3). Approximately 65% of the crabs regurgitated the food from the foregut. In some cases a small amount was regurgitated over several hours, in others the entire contents of the foregut were regurgitated within a matter of minutes. Regurgitation occurred by way of several strong contractions of the cardiac stomach and gastric mill; these increased in frequency with time and in several animals rates of 50–65 min^{-1} were observed. These waves of contraction forced food up into and out of the oesophagus. There also appeared to be contraction of the oesophagus, but this could not be verified from X-ray videos alone (Fig. 4).

Digesta started to appear in the midgut after 2 h (Fig. 2A,B). In 100%SW there was a rapid filling of the midgut that began at 2 h and within 7–8 h it had reached maximal levels of 66.1±4.5%. There was a rapid clearance of digesta thereafter and by 18 h the midgut was less than 10% full. In 80%SW there was a trend towards less digesta entering the midgut and also longer passage through this area of the gut. There was no significant decline in midgut content until 24 h and the midgut was emptied between 48 h and 72 h. Despite this apparent inconsistency compared with 100%SW, no statistically significant difference could be demonstrated (Fisher's LSD, $P>0.05$). There was a significant difference in amount of digesta entering the midgut in 60%SW compared with 100%SW and 80%SW treatments (ANOVA, $F=8.08$, $P<0.001$). Due to regurgitation of food, much less digesta entered the midgut in 60%SW. Maximal levels of 12% full were reached at 9 h. There was a slow decrease thereafter and the midgut was emptied between 36 h and 48 h.

There were also significant differences in evacuation of the hindgut as a function of salinity (ANOVA, $F=7.86$, $P=0.001$). Digesta did not appear in the hindgut in significant amounts until 6 h in 100%SW, 8 h in 80%SW and 11 h in 60%SW. In 100%SW the digesta moved relatively slowly through the hindgut, reaching maximal levels at 18 h and declining slowly

thereafter. The hindgut was cleared between 48 h and 72 h (Fig. 2A). In 80%SW less digesta was apparent in the hindgut. Maximal levels were reached at 24 h and digesta was still evident in the hindgut at 72 h. In contrast in 60%SW only a small amount of digesta entered the hindgut. Levels started to rise after 12 h and continued to increase up to 72 h. The mean time of emptying of the hindgut was 96±4 h (Fig. 5) although in some cases digesta was still visible at 108 h.

Mean emptying times of each area of the gut are shown in Fig. 5. There was a dose dependent increase in time of foregut emptying as a function of salinity (ANOVA, F=17.01, P<0.001). The mean time for foregut emptying in 100%SW was 9.4±0.65 h, which was significantly faster than times measured during exposure to both 80%SW and 60%SW (Fisher's LSD, P<0.01). During exposure to 80%SW the foregut was cleared at 23±3.73 h, which was significantly faster than the 47.89±8.08 h recorded in 60%SW (Fisher's LSD, P<0.05). There was a similar pattern in emptying times of the midgut (ANOVA, F=28.89, P<0.001). In 100%SW the midgut was cleared after 21.4±2.7 h, which was significantly faster than times measured in 80%SW and 60%SW. Midgut emptying times of 45.6±3.48 h were significantly faster than the 72.67±8.4 h recorded in 60%SW (Fisher's LSD, P<0.001). In 100%SW *Cancer gracilis* cleared the entire system after

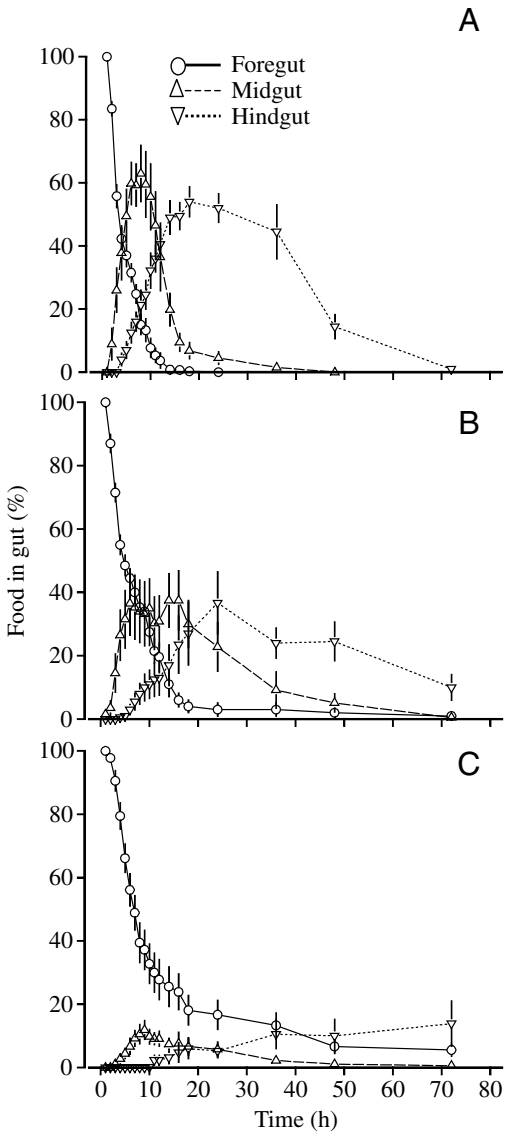

Fig. 2. Passage of digesta through the foregut, midgut and hindgut regions of 10 *Cancer gracilis* maintained in (A) 100%SW, (B) 80%SW and (C) 60%SW. Values represent means ± s.e.m. In some cases standard errors were small and do not show clearly on the graphs.

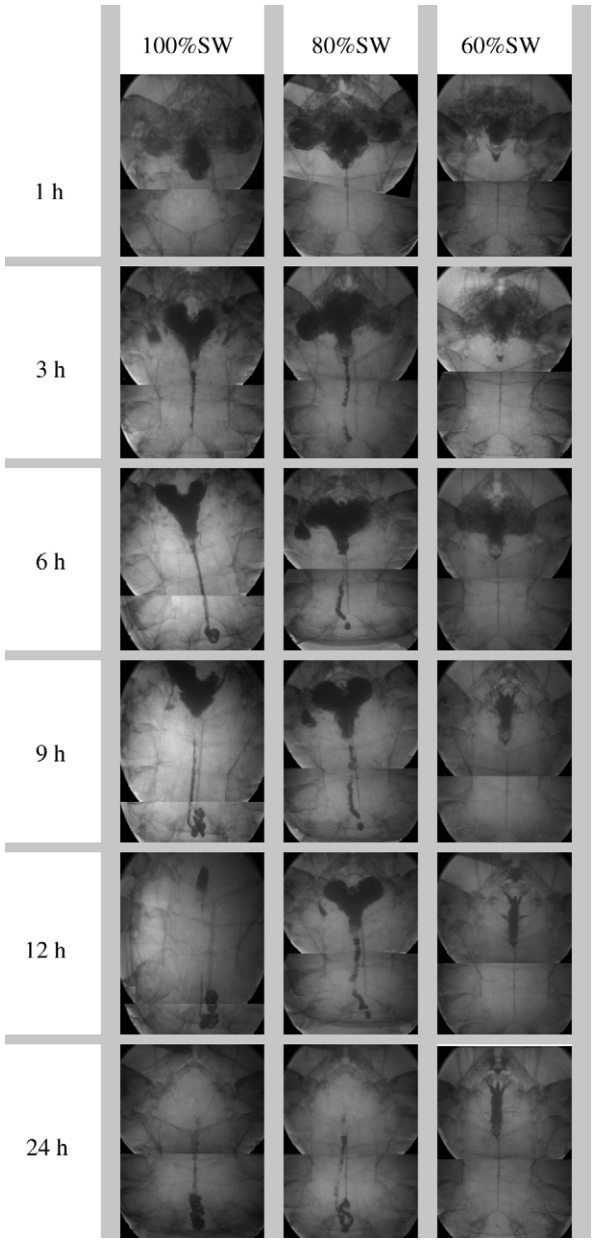

Fig. 3. Radiographs showing passage of digesta through the gut system of *Cancer gracilis* in 100%SW, 80%SW and 60%SW. The foregut appears as a heart shaped mass in the upper portion of each radiograph. Digesta then passes into the midgut region, and finally appears in coil formation in the hindgut.

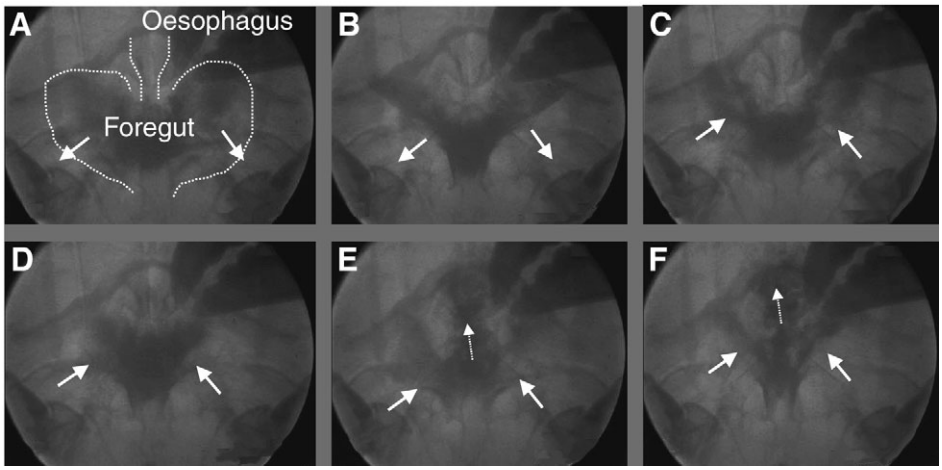

Fig. 4. Radiographs showing the regurgitation of food from the stomach of a *Cancer gracilis* in 60%SW. The boundaries of the foregut and oesophagus are shown. (A,B) The cycle starts with a relaxation of the foregut (C,D), followed by a strong contraction of the cardiac sac (solid arrows). (E,F) Increasing waves of contraction expel food up and out of the oesophagus (dotted arrows).

49.2±2.8 h, which was significantly faster than gut emptying times recorded in 80%SW and 60%SW (ANOVA, F=32.01, P<0.001). Gut emptying times of 84.1±5.34 h in 80%SW and 96±4 h in 60%SW were not significantly different from one another (Fisher's LSD, P>0.05).

Foregut contraction rates

Foregut contractions were also quantified (Fig. 1) and contractions were evident even once the foregut was emptied. Contractions of the cardiac stomach and gastric mill occurred sporadically, with a large variation between individual animals (Fig. 6A). The number of cardiac gut contractions varied between mean levels of 5 and 15 min^{-1}. There was no significant change in the number of contractions as a function of salinity (F=0.07, P>0.5) or time (F=0.89, P>0.5). Contractions of the pyloric region of the foregut were more frequent and stable (Fig. 6B). Contraction rates decreased with time in each salinity treatment (ANOVA, F=11.84, P<0.001). Contraction rates in 100%SW and 80%SW were similar to one another (Fisher's LSD, P>0.05). Initial gut contraction rates varied between mean levels of 70.2±5.6 and 83.6±4.5.min^{-1}.

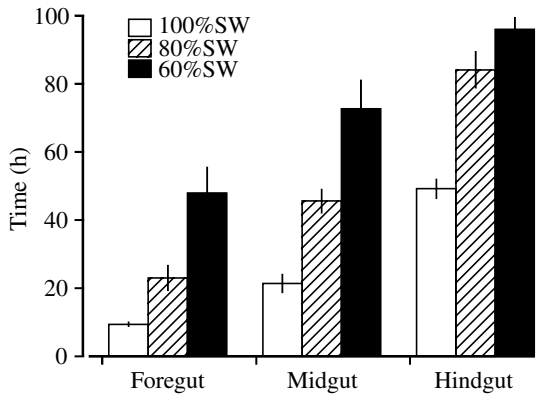

Fig. 5. Mean times (± s.e.m.) for emptying of the foregut, midgut and hindgut of 10 *Cancer gracilis* maintained in 100%SW, 80%SW and 60%SW.

At 5 h in 100%SW and 6 h in 80%SW there was a significant drop in foregut contraction rates, leveling off at between 51 and 59 min^{-1}. These levels were sustained for the remainder of the experimental period (Fisher's LSD, P>0.05). In 60%SW the number of pyloric contractions was significantly lower compared with 100%SW and 80%SW (ANOVA, F=7.58, P<0.01). Contraction rates decreased from approximately 60 min^{-1} at 1 h to 40 min^{-1} at 4 h. There was a further

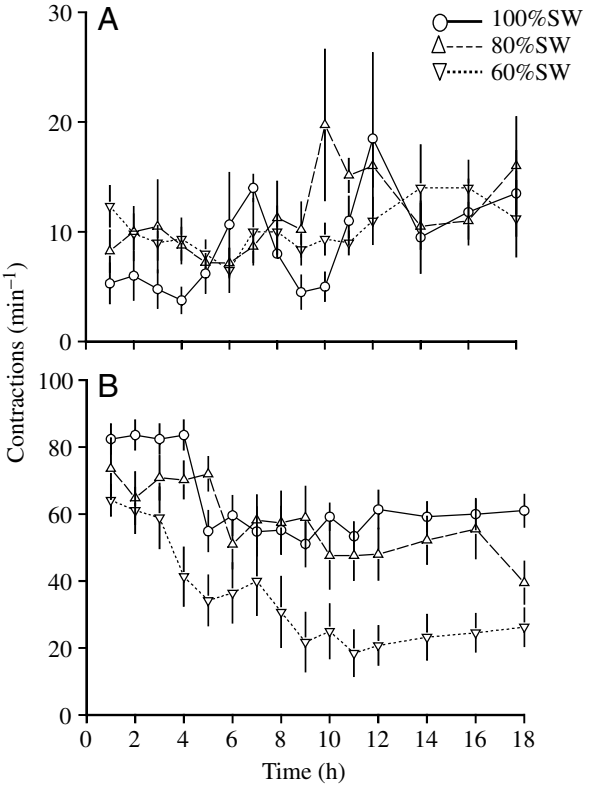

Fig. 6. Gut contraction rates (mean ± s.e.m.) of 10 *Cancer gracilis* measured at various times after change of salinity to 100%SW, 80%SW and 60%SW. (A) Contractions of the cardiac sac and gastric mill. (B) Contractions of the pyloric stomach region.

significant decrease to 18–25 contractions min^{-1} between 11 h and 16 h.

Gastric processing during a salinity cycle

Food passage through the gut, and gut contraction rates were also followed during a salinity cycle (Fig. 7). There was a rapid emptying of the foregut in 100%SW (ANOVA, F=109.48, P<0.001). A significant change in foregut emptying continued during the first few hours of low salinity exposure. At 7 h it slowed down and there was not a significant hourly change in gut emptying (Fisher's LSD, P>0.05). This slowing of foregut emptying continued when the crabs were returned to control conditions.

In 100%SW food started to move into the midgut after 2 h (Fig. 7A; ANOVA, F=8.68, P<0.001) and the midgut was 50±7.76% full when 65%SW was initiated. There was no further significant change in the percentage of food within midgut region during low salinity exposure (Fisher's LSD, P>0.01); at 9 h the midgut was still 38.5±5.87% full. The midgut emptied slowly on return to 100%SW and after 3 h in 100%SW the percentage of food within the midgut had dropped below pre-treatment levels.

Entry of digesta and marker into the hindgut region was routinely slow; only a small amount of digesta (6.6±2.1%) had entered the hindgut when low salinity was initiated. There was no further significant change in hindgut filling during low salinity exposure. Movement of digesta into the hindgut remained slow when 100%SW was reinstated. Hindgut filling only increased significantly (ANOVA, F=12.07, P<0.001) to 11.17±3.5% after 5 h in 100%SW.

In 100%SW the number of cardiac gut contractions ranged between 5 and 8 min^{-1} (Fig. 7B). Towards the end of the time period in 65%SW (8–9 h), the number of contractions reached 19±13 min^{-1}. Theses were higher than levels recorded during 100%SW and during the first few hours of low salinity exposure (Fig. 7B; ANOVA, F=2.77, P<0.01). After 2 h recovery in 100%SW levels had dropped to pre-treatment rates (Fig. 5B). There were also significant changes in pyloric gut contractions (Fig. 7B; ANOVA, F=8.04, P<0.001). There was a sharp drop in contraction rates in the first 3 h in 100%SW, from 86.4±9.1 to 52.4±4.1 min^{-1}. Apart from a slight decrease during the first hour of low salinity exposure, there was no further change in contraction rates until 8 h. At this time contraction rate had dropped below pre-treatment levels. On return to 100%SW there was a slow increase in contraction rates. Pre-treatment rates started to be regained after 3 h recovery in 100%SW.

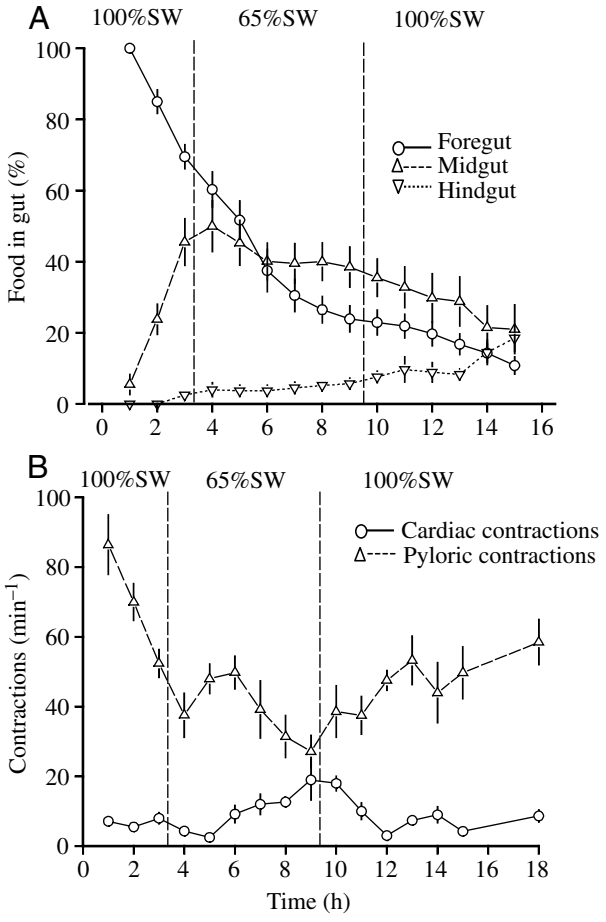

Fig. 7. (A) Passage of digesta through the foregut, midgut and hindgut and (B) gut contraction rates in 10 *Cancer gracilis* exposed to a salinity cycle. Crabs were fed in 100%SW and allowed to digest the food for 3 h. The salinity was then lowered to 65%SW for 6 h, before being raised back up to 100%SW.

Discussion

No radio-opaque marker is ideal, although many fit within acceptable criteria (McCarthy et al., 1993; Bucking and Wood, 2006). Electrolytic iron powder has been used with success to measure food intake and gastric evacuation in fish. It is easily incorporated into feed in relatively low concentrations, it is not absorbed across the epithelium of digestive tract (Talbot and Higgins, 1983) and it is readily eaten by the crabs. Addition of electrolytic iron appeared to have no effect on time for food passage; the first faeces appeared at similar times to iron-free food. Barium sulphate was found to be an unsuitable marker because it had to be used in such high concentrations that it made the meal unpalatable (Edwards, 1971; Jobling et al., 1995). Ballotini lead glass beads have been used previously to measure food uptake in lobsters (Thomas et al., 2002). However, crabs can sort large particles in the pyloric setae (Dall, 1967; Smith, 1978) (I. D. McCarthy, personal communication). This sorting did not occur with the iron powder. The only drawback was that individual particles could not be counted in order to quantify food intake (Talbot and Higgins, 1983; Thomas et al., 2002). This was not a problem, however, since the present study investigated gut contraction rate and gastric evacuation, and not actual intake levels.

Food appeared in the stomach as soon as the animals had fed. Only crabs with stomachs that appeared full were used in the analysis to avoid any inconsistencies associated with meal size (Clemens et al., 1998b; Bernard and Doreau, 2000). In

100%SW seawater, food started to move into the midgut region within 2 h. Similar processing times have been reported for *Metapenaeus bennettae* (Dall, 1967), *Scylla serrata* (Hill, 1976) and *Callinectes sapidus* (McGaw and Reiber, 2000). The finger-like midgut caeca did not appear in the radiographs. Inert material is not found in this region because it is filtered out at the entrance of the midgut caeca ducts and only liquid and particles less than 100 nm in diameter enter the hepatopancreas (Smith, 1978; Hopkin and Nott, 1980). The particulate digesta continued its path along the midgut and reached the hindgut after approximately 6–7 h. This is comparable to the rate measured for *Callinectes sapidus* (McGaw and Reiber, 2000). The foregut was emptied at around 9 h, again this is comparable to similar sized *Callinectes sapidus* (McGaw and Reiber, 2000). The gut system was completely emptied after 49 h. This is somewhat longer than times reported for other species (Dall, 1967; Barker and Gibson, 1978; Joll, 1982; Sarda and Valladares, 1990; McGaw and Reiber, 2000). However, this time period took into account a small amount of digesta that remained in the posterior part of the hindgut. This also occurs in *Carcinus maenas*, and this digesta may not even be voided until a subsequent meal is ingested (Hopkin and Nott, 1980).

Postprandial *Cancer gracilis* exhibit different cardiovascular and respiratory responses to low salinity, compared with unfed individuals (McGaw, 2006b). Instead of a decrease in ventilatory and cardiac function, the levels remain unchanged or even increase (McGaw, 2006b). Thus, there is a prioritization of cardiac and ventilatory responses to digestion, suggesting that food processing is continuing unabated (McGaw, 2006b). However, the results of the present study refute this assumption. Salinity slowed digestive processes and increased the time for gastric evacuation (Fig. 2). Although the crabs could slow digestive processes, they could not halt it completely: the particulate digesta eventually moved into the midgut, and presumably the liquid phase would then enter the midgut caeca where digestion would take place (Icely and Nott, 1980). Once intracellular digestion begins the crabs may be committed to it and have to adjust their physiological responses accordingly (Houlihan et al., 1990; Mente, 2003; Mente et al., 2003). Thus, changes in cardiac and respiratory parameters in postprandial crabs in low salinity probably function to maintain efficient oxygen delivery associated with the increased postprandial metabolism, rather than to support mechanical digestion.

Two types of movements of the cardiac stomach have been described (Heinzel, 1988): chewing and grinding by the gastric mill, and contraction or 'squeezing' by the stomach muscles. In the present study these movements were not differentiated. Movements of the cardiac region of the foregut were sporadic and were not observed in every animal. This sporadic pattern of contraction is also reported for *Homarus americanus* (Morris and Maynard, 1970), *Cancer magister*, *Cancer productus* (Powers, 1973), *Panulirus interruptus* (Heinzel, 1988) and *Cancer pagurus* (Heinzel et al., 1993). Because movements were sporadic and the crabs were only observed under the fluoroscope for about 60 s at a time, this probably accounted for the lack of a discernible pattern of cardiac stomach activity in low salinity (Fig. 6A). The foregut was usually emptied within 12 h. However, contractions of the empty stomach were still evident; this has been reported previously, and it functions for mixing and pumping of digesta through the midgut (Clemens et al., 1998a). Pyloric contractions of the gut regulate movement of the food into the midgut (Heinzel, 1988). In the X-ray preparations these were seen as small amplitude 'pumping' type contractions. The contractions were more stable and rapid than those of the cardiac region; rates of 60–85 min^{-1} agree with data for other species (Morris and Maynard, 1970; Powers, 1973; Heinzel, 1988; Heinzel et al., 1993). Pyloric contractions varied with both time and salinity. Contraction rates decreased significantly, 2 h after feeding (Fig. 6B). A rapid processing of food during the first hour after feeding, followed by a decrease in pyloric contractions, are also reported for *Homarus americanus* (Morris and Maynard, 1970) and *Jasus lalandii* (Rezer and Moulins, 1983). In 60%SW contraction rates were lower than those measured in 100%SW and 80%SW. This decrease in foregut processing, coupled with regurgitation of food (Fig. 4), resulted in significantly less food being moved into the midgut (Fig. 2C). It is known that the stomatogastric ganglion, which innervates the foregut, can be modulated by exogenous sources (Powers, 1973). These include the presence of food material (Powers, 1973; Rezer and Moulins, 1983; Clemens et al., 1998b), internal oxygen levels (Massabuau and Meyrand, 1996; Clemens et al., 1998a) and a variety of neurohormones (Weimann, 1992; Heinzel et al., 1993). Other than these, there are very few reports on modulation of mechanical digestion in crustaceans. The results from the present study now add low salinity as a possible modulator of the stomatogastric ganglion.

Exposure to 60%SW, which is just above what is considered a survivable salinity for this species (55%SW) (D. L. Curtis, E. K. Jensen and I. J. McGaw, manuscript submitted for publication), induced a vomiting response. Rapid contractions of the gastric mill and cardiac stomach muscles expelled food out through the oesophagus. This occurred between 6 h and 12 h after feeding. The exact function is unclear, but in animals that regurgitated food, very little was processed in the midgut. It could be a 'protective response', preventing digestion of the food and subsequent metabolic increases associated with protein synthesis. Alternatively, it could be a simple stress response to low salinity exposure. To argue against the latter, a stress response might be expected to occur more rapidly, because crabs exhibit the greatest changes in activity (McGaw et al., 1999) (D. L. Curtis, E. K. Jensen and I. J. McGaw, manuscript submitted for publication) and cardiovascular and respiratory responses (McGaw and McMahon, 1996; McGaw and Reiber, 1998; McGaw, 2006a; McGaw, 2006b) within the first hour or two of low salinity exposure. Clearly further investigation on alterations in enzyme activity and protein synthesis, before and after regurgitation, needs to be carried out before definite conclusions can be drawn.

Following the digestive processes during a salinity cycle allowed changes in gastric processing to be differentiated more

clearly (Fig. 7). An increase in foregut contraction rate occurred towards the end of the low salinity period. This increase in contraction did not move more food into the midgut (Fig. 7A); rather it was associated with vomiting the stomach contents. A rapid overshoot in cardiac and ventilatory parameters occurs when 100%SW is restored (McGaw, 2006b). Because gut processing and movement of digesta through the gut was only regained slowly upon return to 100%SW this overshoot in cardiac and respiratory physiology is likely to pay off an oxygen debt incurred during low salinity, because low salinity increases haemocyanin oxygen binding affinity [in *Carcinus maenas* (Truchot, 1973)] just at a time when extra oxygen is needed for protein synthesis.

In *Cancer gracilis* low salinity had a dose-dependent effect on gut processing, increasing the time for gastric evacuation and reducing the amount of food processed. It is unclear whether this is an active 'decision' to reduce the specific dynamic effect by processing less food (Wang et al., 1995) thus sparing energy for other systems or, because of the physiological demands associated with hyposaline exposure, there are simply not enough resources to be diverted to digestion. Future studies involving measurement of gastric processing and evacuation in weakly regulating and strongly regulating species are planned to compare and contrast with those of the osmoconformer *Cancer gracilis*. This will allow us to determine whether stronger regulators are able to balance the demands of physiologically competing systems.

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