Prey exoskeletons influence the course of gastric evacuation in Atlantic cod *Gadus morhua* L.

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Running headline:

GASTRIC EVACUATION OF CRUSTACEAN PREY

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ABSTRACT

This study examined the effects of prey exoskeleton characteristics on gastric evacuation patterns in Atlantic cod *Gadus morhua*. Three distinct stages were highlighted in the gastric evacuation of crustacean prey characterized by a robust exoskeleton. The expectation that the three shrimps species (*Pandalus borealis*, *Pandalus montagui* and *Eualus macilentus*) and the crab *Chionoecetes opilio* were evacuated from the stomach at different rates was confirmed. The duration of all stages increased with increasing ash (and carbonate) content of the fresh prey. It was concluded that thickness, chemical composition and morphology of the prey exoskeleton all affected gastric evacuation: duration of initial delay, overall evacuation rate and a decreased evacuation rate at the end of the process. The power exponential function (PEF), with its shape parameter, described well the course of evacuation for these prey types, especially the initial delay. However, the PEF does not allow describing evacuation by the current stomach content mass independently of meal size, which limits its usefulness in estimating consumption rates of wild *G. morhua*. To predict and describe gastric evacuation of prey with a robust exoskeleton, it is therefore suggested to expand the square root function with an initial lag phase, coupled to the mechanistically based cylinder model of gastric evacuation.

Key words: carbonate and ash contents; crustacean prey; energy content; integument thickness and morphology.

INTRODUCTION

Understanding trophic relationships, including consumption rates of predators, is an important component of the science supporting ecosystem-based fisheries management. Bioenergetics models use an energy-balance approach to estimate food consumption based on the energy required for growth, reproduction, and metabolism. Apportion of consumed energy among prey species is subsequently accomplished according to the mass contribution and energy density of the prey in stomachs that are representatively sampled in the field (Benoit-Bird, 2004). Alternatively, the stomach contents data are combined with gastric evacuation rates to determine prey consumption rates using more or less explicit feeding patterns (Elliott & Persson, 1978; Sainsbury, 1986; Jarre *et al.*, 1991) or assuming that on average over time ingestion rate equals gastric evacuation rate (Bajkov, 1935; Jones, 1978; Pennington, 1985). Often, the energy requirement of the predator is not known and only the second approach can be used. In addition, the prey composition in the stomach does not necessarily reflect the consumed one because of differential evacuation rates (Karlsen & Andersen, 2012), which may be especially pronounced for fishes preying on fish and crustacean prey (Bromley, 1994). In this case, knowledge about the nature of evacuation of different prey types is, therefore, decisive to avoid excessive bias even when using the bioenergetics approach to estimate food consumption and prey mortality rates (Andersen, 2001; Andersen & Beyer, 2005*a*).

Although there have been some attempts at estimating the gastric evacuation rate in the field (e.g. Arrhenius & Hansson, 1994; Worischka & Mehner, 1998; Darbyson *et al.*, 2003; Rindorf, 2004), most determinations of gastric evacuation rates have been obtained in the laboratory using a variety of methods (Talbot, 1985; Bromley, 1994). The majority of these studies focussed on commercial species, especially Atlantic cod *Gadus morhua* L. 1758 (Tyler, 1970; Jones, 1974; MacDonald *et al.*, 1982; dos Santos & Jobling, 1988; Temming & Herrmann, 2003; Andersen, 2012). The most examined factors affecting gastric evacuation rate are predator size, meal size, prey type and temperature (Bromley, 1994). The influence of prey characteristics (size, shape, energy density and resistance to digestive processes) have also been described (Jobling, 1987; dos Santos & Jobling, 1991; Singh-Renton & Bromley, 1996; Andersen, 2001, 2012; Karlsen & Andersen, 2012). Including these prey characteristics, the simple, mechanistically founded cylinder model suggested by Andersen & Beyer (2005*a,b*) is the only gastric evacuation model at present that is able to predict properly the evacuation of ingested prey fish. Based on surface considerations, it describes evacuation of ingested prey by the square root of their current mass in the stomach. Further, interactive effects on evacuation rate of size, shape, energy density and resistance to digestion of individual prey are inherent features of the model and have been validated by Andersen & Beyer (2005*b*, 2007) and Karlsen & Andersen (2012). However, a critical assumption is that the individual prey is considered as having constant energy density and resistance to the digestive processes throughout. While prey fish generally fulfil the proviso about prey homogeneity (Andersen & Beyer, 2005*a*), crustaceans with a robust exoskeleton that initially delays the digestive processes are evidently heterogeneous regarding their resistance to digestion. Crustaceans make up a large proportion of the diet of many piscivorous fishes. For instance, shrimp, in particular the northern shrimp *Pandalus borealis* Krøyer 1838, and snow crab *Chionoecetes opilio* Krøyer 1838 are important prey species of *G. morhua* in the Gulf of

St. Lawrence, Canada (Waiwood & Majkowski, 1984; Savenkoff *et al.*, 2006; Chabot *et al.*, 2008).

Depending on the amounts of chitin and minerals (mostly calcium carbonate), invertebrate exoskeletons may be very robust (Welinder, 1974) and act as a chemical and physical barrier against the actions of gastric juices and grinding by gastric muscular contractions (Jones, 1974; MacDonald *et al.*, 1982; Bromley, 1994). Andersen (2001) confirmed that the evacuation rate of brown shrimp *Crangon crangon* with a relatively tough exoskeleton is slower than that of fish prey of similar energy density. In contrast, krill *Meganyctiphanes norvegica* (Sars 1857) characterized by a thin, flexible integument was evacuated at the same rate as fish, which suggests that the digestion rate depends on thickness, composition and morphology of the armour (dos Santos & Jobling, 1992; Andersen, 1999). Evacuation of prey with a tough exoskeleton is typically characterized by an initial period in which decomposition of the prey progresses slowly, followed by a second phase of a significantly higher evacuation rate (dos Santos & Jobling, 1992; Bromley, 1994; Berens & Murie, 2008).

To date, gastric evacuation of different crustacean prey types have never be compared and explained by the nature of the exoskeleton. Using crustacean prey with different levels of armour, the aim of the present study was to examine how the course of gastric evacuation (gastric evacuation pattern) is related to prey exoskeleton characteristics. Four characteristics were quantified (chitin, ash, carbonate contents and energy density) and two functions were used to describe evacuation of the crustacean meals in *G.*

morhua. The power exponential is a flexible function, which enables a mathematical description of initial impediment of gastric evacuation due to prey armament (Elashoff *et al.*, 1982; dos Santos, 1990). The general power function (Temming & Andersen, 1994; Andersen, 1998) was used to examine the overall deviation of the observed evacuation patterns from that of a homogeneous prey (the square root function).

MATERIALS AND METHODS

PREDATOR FISH AND PREY

Gadus morhua were trawled in the Baie des Chaleurs, Québec (48° 21’ N; 64° 24’ W) in July 2002. They were kept indoors under natural photoperiod and artificial light (< 22 lux) in 13 m^3 tanks at the Maurice-Lamontagne Institute, Mont-Joli (Québec, Canada). Water temperature (2–11° C) and salinity (25-31) followed the natural cycle of the water intake. *Gadus morhua* were fed twice a week with a maintenance ration of capelin *Mallotus villosus* (Müller 1776) corresponding to *c.* 3 % of body mass per meal.

Four types of prey representing a gradient in their integument resistance to digestion were used: three species of shrimp having a relatively thin exoskeleton (*P. borealis*, striped pink shrimp *Pandalus montagui* Leach 1814, Greenland shrimp *Eualus* *macilentus* Krøyer 1841) and *C. opilio* with a fairly robust exoskeleton. Shrimps were caught in the St Lawrence estuary (48° 29’ N; 68° 50’ W) in October 2002. They were stored at –80° C. Juvenile *C. opilio* were caught from April to June 2002 in the Baie Sainte-Marguerite, Gulf of St Lawrence (50° 07’ N; 66° 38’ W). They were kept alive in 730 l tanks at 4.0 ± 0.2° C and fed twice a week with chopped *M. villosus* and *P. borealis,* sometimes with the addition of two species of krill (*Euphausia pacifica* Hansen 1911 and *M. norvegica*) kept frozen at –20° C.

The size of the experimental prey was chosen from the size of prey found in *G. morhua* stomachs collected in the field (D. Chabot, unpubl. data). Experimental meals consisted of whole specimens, weighed and measured individually (*E. macilentus* were not measured).

EXPERIMENTAL SET-UP AND PROTOCOL

The experimental set-up consisted of twelve circular 730 l tanks (diameter 1.15 m, depth 0.70 m). The tanks were supplied with sand-filtered recirculated sea water (1–2 l min^{-1}). The water temperature was maintained at 4.0 ± 0.2° C and continuous air bubbling ensured oxygen saturation close to 100 %. Each tank was divided into two compartments by plastic netting. Each compartment contained one cod, providing 24 cod per experiment.

One month before the beginning of the experiments, *G. morhua* of similar size (Table I) were transferred into experimental tanks to acclimatize to the experimental conditions. Here, they were trained for at least two weeks to feed voluntarily on the experimental prey.

Four experiments were completed using one prey type per experimental meal. Shrimps were thawed just before meal preparation. Dos Santos (1990) showed that frozen prey tend to be evacuated slightly more rapidly than fresh ones, but he did not find any significant difference for shrimps. Pre-test showed that snow crabs could not be offered alive as they were hiding away in the experimental tanks, between the plastic netting and the drain pipe. Thus, prior to be offered to cod, snow crabs were plunged into fresh water and killed by osmotic shock without being damaged (unlike freezing, which tends to make the legs brittle). All prey were gently dabbed to remove excess water prior to weighing. Additional meals were prepared and refrozen at –80° C for subsequent analyses of prey composition.

Gadus morhua were fed their meal (t = 0 h) and at predetermined time intervals (t = 6, 12, 24, 48, 72, 96, 120, 144 h) their stomach contents were recovered by dissection or by stomach flushing following anaesthetization (metomidate hydrochloride, 5×10^{-3} g l^{-1}). Stomach flushing was realised with a Tygon® tubing (ID = 5 mm, OD = 7 mm) connected to a sea water supply system (*c.* 1.4 l min^{-1}). The tubing was introduced into the cod stomach while the fish was held inclined above a filter (mesh size 120 µm). The stomach content was collected, rinsed under fresh water, gently dabbed on a paper towel and frozen at –80° C for later analyses on composition. The fish was then weighed and measured. It was generally used for more than one experiment and a minimum of two weeks was allowed for recovery between experimental trials.

Preliminary tests were performed to confirm the efficiency of stomach flushing. Moreover, to ensure that recovery was complete during the evacuation experiments, the number of ingested prey items was noted and flushing was continued until all prey were recovered.

PREY COMPOSITION

Preserved meals and stomach contents were dried 72 h at 65° C or freeze-dried 48 h to determine their water content. Tests showed no difference between these two techniques. Energy density of dried samples was determined by bomb calorimetry (PARR 1261; www.parrinst.com). Values obtained were corrected for carbonates content (Paine, 1966) as well as nitric acid produced during combustion (Golley, 1961). Ash content was determined by weighing the remains of the sample after 24 h at 500° C (Atkinson & Wacasey, 1976). Carbonate content was determined as the difference in mass after an additional 5 h of heating at 900° C (Paine, 1966).

Chitin content was assayed on whole prey (stomach contents did not provide enough material to allow any assay). Chitin was determined in 2012 on *P. borealis* and *P. montagui* captured in summer of 2011 and kept alive until the determinations, and on *C. opilio* captured in 2012, frozen at sea and processed shortly after. No *E. macilentus* were available for chitin determination. The chitin assay method of Spinelli *et al.* (1974) was modified by Martin Poirier (ABK Gaspésie Inc., Quebec). Briefly, samples were dried and reduced to powder by pestle, deproteinated in a 2 % NaOH solution during 90 min at 90° C, and then filtered and demineralised using a 5 % HCl solution during 16 h at ambient temperature. After another filtration, samples were rinsed with acetone to remove lipids and dried at 105° C during 16 h. Chitin content was the difference in mass before and after the treatments.

Water, ash, carbonate and chitin contents were expressed as percentages of wet mass and transformed before statistical analyses (arcsine of the square root values; Sokal & Rohlf, 1995). Differences between prey types were tested using one-way ANOVA followed by post-hoc comparison (Tuckey HSD). Significance was accepted at $P<0.05$. The relationship between ash and carbonate contents was determined by linear regression.

GASTRIC EVACUATION PATTERNS

Gastric evacuation of the four prey species was described by two functions that previously have been used to describe heterogeneous prey. The power exponential function

$$S_t = S_0 2^{-(\eta t)^{\varphi}} \qquad (1)$$

is able to describe deviations from an exponential evacuation including an initial slow evacuation, which is particularly relevant for prey with a robust exoskeleton (dos Santos & Jobling, 1992). S_0 (g) is the mass of the ingested meal, S_t (g) is the recovered stomach content mass, and t (h) is the elapsed time. The two parameters η and φ determine the evacuation rate (η^{-1} represents the time at which S_0 has halved) and the shape of the evacuation curve ($\varphi = 1$: simple exponential evacuation; $\varphi > 1$: initial delay of evacuation; $\varphi < 1$: initial acceleration).

The shape of the evacuation curve described by the general power function

$$S_t = [S_0^{1-\alpha} - (1-\alpha)\rho t]^{(1-\alpha)^{-1}} \ ; \ \ 0 \le t \le S_0^{1-\alpha}(1-\alpha)^{-1}\rho^{-1} \qquad (2)$$

depends on the shape (power) parameter α, while ρ is the rate parameter. An estimate of α close to 0.5 (the square root version) obtained by use of this function to data on evacuation of a prey indicates that the prey can be considered homogeneous as regards the prey characteristics influencing evacuation rate. Lower values have been obtained from crustacean prey with a robust exoskeleton as well as gadoid fish prey with a large, fat liver. In contrast, higher values were estimated from small fish prey with a large proportion of easily digestible parts relative to their more compact trunk (Andersen & Beyer, 2005*b*).

The parameters of equations (1) and (2) were estimated by non-linear regression (the iterative Marquard method; SAS Institute Inc.; www.sas.com) with the modifications for the general power function as described by Andersen (1998). The data set from each experiment was truncated at the time of first occurrence of an empty stomach (Andersen, 1984; Olson & Mullen, 1986).

The goodness of fit of two evacuation functions were evaluated using r^2 values. Candidate functions were compared using Akaike Information Criteria corrected for small sample size (AICc) and normalized relative likelihoods w (Akaike weights; Burnham & Anderson, 2002). As a general rule of thumb, the confidence set of candidate functions (analogous to a confidence interval for a mean estimate) include those with w_i within 10 % of the highest, where w_i is interpreted as the weight of evidence (probability) that model i is the best approximating model given the data and set of candidate models . AICc denotes the amount of information lost when using a model to approximate the "true" model and is estimated as $n \ln(\mathrm{RSS}\ n^{-1}) + 2kn(n-k-1)^{-1}$ (adjusting for small sample sizes), where n is the number of observations, RSS the residual sums of squares from the non-linear regression, and k the number of parameters (including the error term). $w_i = \exp(-0.5\Delta_i)[\sum \exp(-0.5\,\Delta_i)]^{-1}$, where $\Delta_i = \mathrm{AICc}_i -$ $\mathrm{AICc}_{\mathrm{min}}$ and $\mathrm{AICc}_{\mathrm{min}}$ is the lowest AICc value obtained (for a detailed explanation of the methods, see Burnham & Anderson, 2002)

RESULTS

PREY COMPOSITION

Contents of water and ash differed between shrimp and crab, but were relatively similar among the three species of shrimp. Hence, the water content was significantly different among prey types ($F_{[3,37]} = 29.2$; $P<0.001$), being the lowest in *C. opilio* (Table II). Ash and carbonate contents also varied significantly among prey species (ash: $F_{[3,37]} = 3658$; $P<0.001$; carbonate: $F_{[2,22]} = 1341$; $P<0.001$). The contents of ash and carbonates in *C. opilio* were three times higher than the contents found in the three shrimp species. Among the latter, *P. borealis* and *P. montagui* had similar ash and carbonate contents, which were higher than those of *E. macilentus* (Table II). A strong linear relationship was obtained between ash and carbonate contents using the values from the three species:

$$C_c = 0.39A_c - 0.27 \qquad (3)$$

where C_c and A_c are carbonate and ash contents (% wet mass) ($r^2 = 0.998$, $P<0.001$, $n = 25$).

The energy densities of the prey species used in the present study differed ($F_{[3,37]} = 34$; $P<0.001$). The energy density of *C. opilio* was lower than that of the three shrimp species, which showed similar values. The chitin content was measured for three prey species and they differed significantly ($F_{[2,29]} = 426.6$; $P<0.001$): the chitin content of *C. opilio* was twice as high as that of the two shrimp *P. borealis and P. montagui, but P. borealis had a slightly but significantly lower chitin content than P. montagui*.

CHANGES IN STOMACH CONTENT COMPOSITION DURING EVACUATION

The remaining amount of material in the stomach after 72 h of evacuation was usually insufficient to perform analyses of the composition.

The water content of all prey increased slightly during the first 6 h in the stomach (Fig. 1a). Subsequently, the water content kept increasing slightly during digestion for *C. opilio* and *P. borealis* but not for *E. macilentus* and *P. montagui*. Larger changes were observed for the ash content, which decreased exponentially in the stomachs with *C. opilio* (Fig. 1b) dropping sharply to half of the initial value within 48 h and decreasing more slowly during the remainder of the evacuation period. The ash content in fresh shrimp was substantially lower as described above, and it decreased at a much lower rate during evacuation.

The decrease in carbonate of stomach contents (not shown) was similar to that of ash content. Accordingly, the relationship between these two variables remained strong in the stomachs ($r^2 = 0.98$, $n = 57$):

$$C_c = 0.38A_c - 0.03 \tag{4}$$

The energy density of shrimp decreased about 1 kJ g^{-1} during the first 12 h of evacuation, and levelled off until the shrimp was completely evacuated (Fig. 1c). Data on crab fluctuated around the initial value and were more variable, probably due to scarce data.

GASTRIC EVACUATION PATTERNS

Gastric evacuation of *C. opilio* lasted more than 144 h (Fig. 2). An initial period of 24 h with no significant evacuation was followed by an exponential decrease of the remaining prey mass. At the final stage of evacuation, the stomach contents were principally composed of soft pieces of exoskeleton that were most likely composed of chitin. A confirmatory assay was, however, not performed. For the three species of shrimp, the first empty stomach appeared 96 h after food intake (Fig. 3).

For all prey, r^2 values for the power exponential function were higher than the corresponding ones obtained by use of the general power function because the former function was able to describe the initial delay of evacuation (Table III). Accordingly, the normalized relative likelihood w of the general power function was below 10 % of the value obtained by use of the power exponential (crab, < 1%; shrimp, < 3-6%; shrimp combined, < 0.01 %). So, there was a strong weight of evidence in favour of the power exponential function being the best explanatory model for all species (Table III).

The estimates of the shape parameter φ in the power exponential function were significantly higher than 1 (95% C.I. in Table III) for all crustacean prey (i.e. gastric evacuation was initially delayed relative to a pure exponential decay function). The species specific estimates of φ were significantly different (ANOVA: $F_{3,59} = 3.29$; $P < 0.05$). Subsequently, *post hoc* comparison (Tuckey HSD) showed that the value estimated from *C. opilio* was significantly different from those obtained from the three shrimp species indicating a longer lasting initial delay for evacuation of the crab prey. The estimates of the rate parameter η obtained from shrimp prey were likewise similar, and they were significantly higher than the value estimated from crab. Moreover, the estimates of η^{-1} indicated that half the meal is evacuated within 20–24 h for shrimp prey, whereas 57 h was needed for the crab. The value of the shape parameter φ was positively correlated with initial contents of ash and carbonate in the prey.

The shape parameter α of the general power function was negative for *C. opilio* pointing to the long delay period and the poor performance of the general power model to describe the course of evacuation (Fig. 2). In contrast, this function with a power value around 0.5 (square root) gave a fair description of evacuation of the individual shrimp species (Table III and Fig. 3).

DISCUSSION

This study confirmed the expectation that the three shrimps and *C. opilio* were evacuated from the stomach at different rates by *G. morhua*. These prey differ in energy density that is known to influence evacuation rate negatively (Windell, 1966; Grove et al., 1978; dos Santos & Jobling, 1988; Andersen, 1999). However, *C. opilio* had the lowest energy density and should have been evacuated most rapidly if energy density was the only prey characteristic influencing the evacuation rate. In contrast, the protecting effect of the heavy exoskeleton of *C. opilio* is expected to delay the evacuation relative to that of shrimp. The shrimps were totally evacuated in *c.* 72 h, whereas it took twice as long for *C. opilio* to be evacuated. Hopkins & Larson (1990) obtained similar results. They compared gastric evacuation patterns of four prey types in black and yellow rockfish *Sebastes chrysomelas* (Jordan & Gilbert 1881), including two prey species with exoskeleton, the purple shore crab *Hemigrapsus nudus* (Dana 1851) and the Franciscan bay shrimp *Crangon franciscorum* Stimpson 1856. In their study, the shrimp was evacuated in 30 hours, whereas the crab was evacuated in 50 hours, but no data on prey energy density was provided.

The present data demonstrated three distinct stages of evacuation of prey with an exoskeleton. In the first stage, the evacuation was slow, presumably because the exoskeleton prevented stomach enzymes from attacking the prey (dos Santos & Jobling, 1992; Bromley, 1994). Then, the gastric evacuation rate increased significantly, and in the final stage of evacuation, the evacuation slowed down again.

In the first stage of evacuation, the exoskeleton probably has a dual effect on digestion of the prey. Pepsin and other gastric enzymes are activated in an acidic environment (Jobling, 1986). During fasting, pH of the gastric juice is neutral but decreases after intake of food because of HCl secretion. The buffer capacity of carbonates contained in the exoskeleton might raise the pH value and delay enzyme activation, which would explain partly why the delay in the first stage of evacuation seems to be directly correlated with prey carbonate (and ash) contents. To rigorously test this hypothesis, pH should be measured in stomach contents during evacuation, which was impossible in this study, because stomach flushing was used to collect stomach contents.

The other property of the exoskeleton is the barrier effect (Bromley, 1994). It protects the rest of the body from the digestive processes in the stomach, and evacuation seems to begin when breaks occur in the more fragile parts of the exoskeleton, such as the joints. Once gastric juices have penetrated into the internal soft tissues, the digestion and, subsequently, the evacuation rate rapidly increases. For prey without exoskeleton like fish, gastric evacuation begins as soon as the prey is ingested (dos Santos & Jobling, 1992). The initial delay in evacuation of shrimps only lasted for a few hours (Fig. 3). As described above, the shrimp exoskeleton is less resistant (lower ash and chitin contents) than in crab. It was observed that the cephalothorax of shrimp was separated from the abdominal part early in the course of digestion (~6 h). This separation made digestion of flesh possible after only a short lag. The situation was different for *C. opilio*, which did not appear to be altered much during the first 24 h following ingestion (Fig. 2); this could be related to the thicker exoskeleton merged to form a compact carapace and/or to the carbonate heavy encrustations that harden the exoskeleton and perhaps delay enzyme activation by their buffering action as described above. The latter is also indicated by the significant loss of carbonate and ash contents to around half the value of fresh crab during this initial stage of evacuation (Fig. 1). The present results confirm that energy-poor minerals, as estimated by ash contents, are quickly evacuated from the stomach (Hopkins & Larson, 1990).

In the second stage of evacuation, the evacuation rate of crab was significantly lower than that obtained from combined data on the three shrimp species as reflected in the estimates of the power exponential rate parameter η (Table III). The rates for crab and shrimp were 0.26 g h^{-1} and 0.45 g h^{-1} at the time of halving of the prey body mass, and 0.26 g h^{-1} and 0.51 g h^{-1} at their respective maximum values. The energy densities of the prey were quite similar after the initial stage of evacuation (Fig. 1). This could be explained by the initial heavy loss of carbonates in *C. opilio*. The high level of carbonates in fresh *C. opilio* also explain the low energy density of these (Table II). Therefore, differences in energy density could not explain the differential evacuation rates in the second stage of evacuation, which seems to be caused by prey specific resistance to digestion (Andersen, 2001). Whereas the compact carapace of the crab only gradually opened up and did not fragment until late in the evacuation period, the exoskeleton of shrimp quickly broke up in smaller fragments leaving larger surfaces of the inner soft tissues exposed to the enzymatic and mechanical processes in the stomach.

In the final stage of evacuation, mainly chitin was recovered from the stomach. The chitin contents of remaining prey in the stomachs were not quantified. However, visually, it appeared that for both shrimp and crab prey, remains of chitin was the last evacuated prey component. Dissecting stomachs from bluegill sunfish, *Lepomis macrochirus* Rafinesque 1819, Windell (1966) also observed that other organic matter in the exoskeleton was digested first, whereas chitin stayed longer in the stomach, i.e. until the pieces were small enough to pass through the pylorus as chitin was considered a non-digestible material. However, the gastric mucosa of some fish, including *G. morhua*, secretes chitinases and chitobiases to hydrolyse chitin in β-N-acetyl-D-glucosamine (Danulat & Kausch, 1984). Thus, *G. morhua* can digest chitin, but the process is likely slower than for the other prey constituents. At the end of the evacuation, only pieces of softened exoskeleton parts without minerals remained in the stomach. The slow digestion of chitin explains why the estimated evacuation curves level off in the final stage of evacuation and, therefore, are of an exponential nature subsequent to the initial delay.

The duration of all stages increased with increasing ash (and carbonate) content of the fresh prey. Increasing proportionally to the ash content, the duration of the first stage was, however, most affected as indicated by the higher estimate of the shape parameter φ of the power exponential function obtained from crab as compared to those for shrimps

(Table III). Estimates of φ for brown shrimp *Crangon crangon* (L. 1758) fed to whiting *Merlangius merlangus* (L. 1758) (data from Andersen, 2001) and blue-leg swimming crab *Liocarcinus depurator* (L. 1758) fed to *G. morhua* (N. G. Andersen, unpubl. results) corroborated the present findings (Fig. 4). Together with an estimate of 1.33 obtained from *P. borealis* by dos Santos & Jobling (1992), values between 1.30 and 1.60 for *C. crangon* were in line with the estimates of 1.45–1.53 obtained from the three shrimp species in the present study. The estimated value 3.15 of the shape parameter for *L. depurator* of 9.6 g body mass was higher than the estimate of 2.13 obtained from *C. opilio* (Fig. 4). This may be ascribed to the three times larger body mass of *L. depurator* and the consequently thicker carapace. Accordingly, Berens & Murie (2008) found φ values of 2.28 to 3.07 for iridescent swimming crab *Portunus gibbesii* (Stimpson 1859) ranging from 2.8 g to 11.7 g body mass and fed to gag *Mycteroperca microlepis* (Goode & Bean 1879).

Estimates of the shape parameter φ obtained from experiments on *M. merlangus* evacuating krill *M. norvegica* with low ash content, small body size (0.20 g) and a thin flexible exoskeleton (data from Andersen, 2001) were generally lower (1.09–1.26) than those obtained from shrimp (Fig. 4). The values were in most cases significantly higher than 1, which in terms of the power exponential function indicated an initial delay of evacuation. This was not obvious as demonstrated by, for example, evacuation data on *M. norvegica* from which a value of 1.26 ± 0.14 (estimate ± 95 % C.I.) was obtained for φ [Fig. 5(a)]. Similarly, data on lesser sandeel *Ammodytes tobianus* L. 1758 of 4.0 g body mass fed to *G. morhua* from Andersen (2012) produced values between 1.19 and 1.27, all significantly higher than 1 (Fig. 4). This prey fish is particularly homogeneous as regards prey characteristics influencing evacuation rate (Andersen & Beyer, 2005*a*), and it is consistently evacuated according to the square root function without an initial delay. This was also the case for the data on *A. tobianus* as exemplified by the data subset that gave the value 1.25 ± 0.13 (estimate ± 95 % C.I.) of φ [Fig. 5(b)]. The evacuation of *M. norvegica* likewise closely followed the square root function suggesting that this prey type can be considered homogeneous [Fig. 5(a)]. Thus, whereas the evacuation curve described by the power exponential function deviated significantly from that of the simple exponential with regard to an initial delay, it was not necessarily so when it was compared with the square root function (Fig. 5). Values of φ significantly higher than 1, should therefore not uncritically be interpreted as an initial delay of evacuation.

In conclusion, resistance of the exoskeleton (thickness, composition or morphology) was the prey characteristic differentiating the crustacean prey. This was reflected in the duration of the initial delay of evacuation as well as in the overall evacuation rate. The remnants of robust exoskeletons furthermore slowed down the evacuation process towards the end of evacuation relative to what should be expected from the course of the square root function. These effects of robust exoskeletons provided an exponential course of evacuation with an initial delay that was well described by the power exponential function.

Unfortunately, unlike the square root function, the power exponential function cannot describe evacuation by the current mass of stomach contents and, in particular, it cannot work independently of meal size (dos Santos & Jobling, 1995). The general power model with a shape parameter close to that of the square root function provided, however, a reasonable description of the evacuation of shrimp (Table III and Fig. 3). This was in accordance with the results of Andersen & Beyer (2005*b*), who successfully used the cylinder model to predict evacuation of mixed meals composed of shrimp and fish prey. Nevertheless, it is evident that the longer lasting initial delay of evacuation of *C. opilio* (and probably crab and lobster in general) due to its thick carbonate encrusted carapace, should be considered in mathematical descriptions of evacuation patterns and rates. This may be realized by ignoring the very low evacuation rates of minor contents of chitin at the end of evacuation and splitting the course of evacuation into two stages: an initial period with slow evacuation (if appropriate data are available for the quantification) or, alternatively, with no evacuation at all, followed by a period of faster evacuation, probably according to the square root function. Surface considerations, prey characteristics and the square root function being inherent features, the cylinder model of gastric evacuation (Andersen & Beyer, 2005*b*) should qualify for the purpose.

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