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Gut expansion and contraction in the predatory soil mite *Pergamasus longicornis* (Mesostigmata: Parasitidae) - a stiff system

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Abstract Mite digestive processes are inferred from gut expansion and contraction time in the free-living predatory soil mite *Pergamasus longicornis* (Berlese) estimated using a temporal series of histological sections. Gut regions (bar the rectal vesicle) behave broadly in unison for rapid initial filling (ingestion half-life about 2 - 3mins : max 8mins), but behave heterogeneously when slowly emptying (digestion/egestion half-life from about 2 - 3h : max 8.5h). Anterior gut regions fill and empty the earliest. Posterior gut regions take the longest to fill and to empty. Switching first from filling-predominating to emptying-predominating in the gut occurs around 2h from the start of feeding. Median time for the initial completion of gut filling and for the commencement of gut emptying are 10 mins and 12.5h respectively from the start of feeding. Three phases of gut changes are critically discussed: - rapid filling; concentration by fluid loss (via coxal glands); and, slow emptying. Independent corroboration of coxal droplet formation is included. Predictions to confirm or refute postulated mechanisms of salivary, coxal or rectal water balance are given. Overall total gut filling (ingestion) plus gut emptying (digestion/egestion) time in this poikilotherm is approximately 29 to 52.5h (1^+–2^+ days) at room temperature from the start of feeding on large dipteran prey ($\equiv$ 0.46 - 0.83 gut emptyings per day). *Pergamasus longicornis* exhibits the stiff digestive system of an intermittent 'bolus' feeder.

Keywords Bayes · censored 'broken-stick' model · coxal gland · FIFO · LIFO · pulse-chase

Introduction

All animals must eat to survive, and most foods that are ingested will need digesting. Understanding invertebrate digestion means tracking food during its processing either directly within an individual or by inferring change processes indirectly over the destructive sampling of different individuals. The trophic ecology

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and digestive processes of predatory mites is a neglected field and their details needs generally more attention. Studies of predators are difficult due to the poorly observed structure of the food bolus in the gut. The general consensus in the cryptozoan literature (Gist and Crossley (1975)) is that micro-predators (like soil mesostigmatids) may consume about 100% of their body weight per day. Many such predators are thus effectively digestion limited (van Rijn et al (2005)), with satiation being a key factor in understanding their behaviour. The feeding process is a particular challenge for acarine carnivores who may have to deal with prey of even bigger size than themselves that must therefore be concentrated during and after ingestion. In phytoseiids ingestion is conceived as a single 'gulp' of food per prey (Sabelis (1990)) and such 'bolus' volumes may take a long time for a voracious poikilotherm to process. Microarthropod ingestion rates in the field are low (0.014-0.1 prey per predator per day w/w) on average (Kowal and Crossley (1971)) and indeed for a soil dwelling predator, like the very abundant polyphagous mite *Pergamasus longicornis* (Berlese) of the family Parasitidae, the time period required to digest a larval dipteran prey has been claimed to be as much as one week (albeit without detailed justification by Bowman (1984)). This is equivalent to as little as 0.14 gut emptyings per day and is a substantial time for a mite - but it is not incongruent with such soil mesostigmatids producing only 1-5 generations per year (Hartenstein (1962), Luxton (1982)). This one week period is three to four times that reported by Sabelis (1990) for the smaller specialised predator *Phytoseiulus persimilis* Athias-Henriot of the related mesostigmatid family Phytoseiidae. In that tetranychid predator, a fully satiated gut is half emptied in 8-9h and is virtually emptied after 36-48h - being equivalent to a rate of gut emptying of 1.84-2.07 /day (Sabelis (1990)). In *Neoseiulus barkeri* Hughes and *Neoseiulus cucmeris* (Oudemans) the rate of gut emptying is similarly rapid – 1.65 and 2.40 /day respectively (van Rijn et al (2005)).

A study to generate definitive estimates for the total ingestion/digestion/egestion time in pergamasids is needed. This paper, as part of a wide ranging investigation of pergamasid digestive physiology, presents detailed data on the digestive status of gut regions (see Figure 1) scored from a temporal series of serial histological sections of the *P. longicornis* at regular times up to 14 days from the onset of feeding. Histology is used as the integument of this parasitid precludes clear visibility of the gut from outside. The underlying concept is the fluctuation of the shape of the gut as the response to filling and emptying by food. i.e. that a mite goes through a gut expansion (filling) stage and then a gut contraction (emptying) stage. Accordingly, the histological data is segmented into predominately gut-filling and predominately gut-emptying phases using a censored maximum likelihood 'broken stick' method. Poikilotherms often show very large differences in the time scales of their physiological processes (they are 'stiff' systems - see Strogatz (2001)) so a fast input and a slow output phase is expected. Accordingly a log-spaced time frame is used for the destructive sampling of the mites. Gut expansion is taken to subsume growth in lumen, growth in cells and the input of food. Gut contraction is taken to subsume decline in lumen, shrinkage/loss of cells and clearance of food (by various processes). In decomposing the acarine feeding processes one expects most gut regions to be empty (contracted) early on, then more and more to become full (expanded) until nearly all are full; followed by a reduction, so that by an extended time period from the start of feeding, most are empty again. The

explicit hypothesis to be probed is that this mite has a one week (= 168h) cycle for feeding and digestion claimed without substantiation by Bowman (1984). To that end, posterior Bayesian estimates of the time for each region of the gut to initially fill and initially empty are estimated to compare each region with each other and with data from other mites. Critical explanation of gut changes is offered together with independent corroboration and predictions to confirm or refute the postulated mechanisms involved.

Materials and Methods

Mites were collected by hand from leaf litter sampled at a variety of deciduous woodland sites in Merseyside and Hertfordshire, UK in a short season during 1977. Mites were kept individually at room temperature and >90% rh throughout. Mites were starved for 1 week and then fed one final instar larva of the fruit fly *Drosophila melanogaster* (vestigial wing strain). At pre-specified log-spaced elapsed times from the commencement of feeding, a total of thirty four mites were destructively fixed in cold Susa, dehydrated through graded isopropyl alcohol, into xylene and double embedded in celloidin and paraffin wax. Sections were taken at 7μ, stained with Mallory's Triple Stain and mounted in DePeX. Each of 15 gut regions (see Figure 1) was scored as expanded by food contents or contracted (see Figure 2). An expanded gut was taken to indicate filling-predominating, a contracted gut was taken to indicate emptying-predominating. As such the expansion/contraction status is a summary surrogate for ingestion, digestion and egestion. Gut filling (expansion) is driven by ingestion, gut emptying (contraction) is driven by concentrative and metabolic digestion and egestion (together with any excretory processes). Due to collecting constraints, no distinction was made between male (elapsed time after the start of feeding: 0, 2, 5, 5, 10, 25, 60, 90 mins, 6 hours, 12, 48, 96, 168, 192, 240, 288 hours) and female (0, 15, 20, 20, 30, 30, 60, 90 mins, 2 hours, 4, 8, 18, 24, 72, 120, 144, 216, 336 hours) mites in this study. Biomass conversion in females is indeed usually much faster than in males in phytoseiids (Sabelis (1990)) where frequent and rapid oogenesis is required to keep pace with the generational processes of their tetranychid prey. However, the K-strategist *P. longicornis* is typified by only infrequent seasonal oviposition (see references in Bowman (1985)), accordingly this first approximation by pooling over gender in this study is not considered to induce significant bias. All data were coded (0 = gut contracted, 1 = gut expanded), stored and manipulated in Access97. Censored regression was carried out by using the censorReg procedure in SPLUS2000 (Meeker and Duke (1981)). Diagrams were produced in SAS 6.12, Excel for OSX and GIMP. Bayesian modelling was carried out in WinBugs 1.3 (see http://www.mrc-bsu.cam.ac.uk/bugs).

Estimating switch point from whole gut filling to gut emptying

To estimate the point of change in time from net gut-filling (i.e. "moving-to-full") to net gut-emptying (i.e. "moving-to-empty"), a discontinuous 'broken stick' or two-segment model was used. This separates the mites before and after the time k into two sets.

Let y_{ij} be an elapsed time (on a continuous scale after appropriate transformation), then:-

- For the net gut-filling phase, $y_{ij}^{F} \sim$ Normal(μ_1, σ_1^2) for all gut regions j=1...15 and for all elapsed times i < k; and,
- For the net gut emptying phase, $y_{ij}^{E} \sim$ Normal(μ_2, σ_2^2) for all gut regions j=1...15 and for all elapsed times i $\geq$ k,

where k is the position of the discontinuous knot between the two processes - gut filling and gut emptying; and, superscripts F and E denote "moving-to-full" and "moving-to-emptying" respectively. μ_1 simply represents the average time for the gut to expand fitted to the first set of mites, μ_2 the average time for the gut to contract fitted to the second (later) set. The aim is to optimally estimate k taking into account the whole gut behaviour and the destructive sampling (censoring) of each mite. In that way, i=1...34 indexes the individual mites, with the ith mite sacrificed at time t_i where $t_i \leq t_{i+1}$ for all i=1...33. This artificial segmentation was employed for the convenient estimation of the characteristics of the two phases expected to have markedly different rates. The normal distribution was used as a convenient robust estimable symmetrical distribution (logistic and extreme value distributions gave similar results albeit resulting in estimated knot values slightly earlier in time - results not shown). This stochastic model ignores the repeated nature of measuring multiple gut regions within individual mites.

Data i<k was used as originally coded to model gut filling. Data for i$\geq$k was inverted and an offset of k mins was deducted from the elapsed time when modeling the gut emptying process. No data was used more than once. A discontinuity was allowed as there was no need for a smooth constraint – the two processes were not to be modelled simultaneously, although it is assumed that both gut-filling and gut-emptying physiological processes occur contemporaneously *in vivo* to an extent. Elapsed time from zero (or from offset adjusted zero) was $\log_e$ transformed (i>0). A logarithmic scale for elapsed time was chosen within each model upon the simplest assumption that both gut filling and gut emptying represent first order rate processes. Natural ($\log_e$) logarithms were used throughout.

As the mites were destructively sampled at any one time point, data was treated as censored (note that the methods used by censorReg are parametric models so the distinction between open and closed intervals is unimportant). For the ith mite, each of the 15 gut regions can then be classed as in state Z_{ij} taking values 0 (contracted) or 1 (expanded). Then,

- For gut filling:- Gut expansion observed at time zero (Z_{0j}=1) was taken to have occurred instantly at time i=0. Lack of expansion at any time i (Z_{ij}=0) was treated as right censored (i.e. expansion was still to occur at time > i). Expansion at any time i$\neq$0 (Z_{ij}=1) was treated as interval censored (i.e. expansion had occurred at time i or somewhere before in the interval 0 $\leq$ i as mites were singly destructively sampled).
- For gut emptying:- Gut contraction at (offset) adjusted time zero (Z_{0j}=0) was taken to have occurred instantly at adjusted time i=0. Lack of contraction at any adjusted time i (Z_{ij}=1) was treated as right censored (i.e. contraction still to occur at adjusted time > i). Contraction at any adjusted time i$\neq$0

(Z_{ij}=0) was treated as interval censored (i.e. contraction occurred at adjusted time i or somewhere before in the adjusted interval $0 \leq$ i as mites were singly destructively sampled).

Pairs of censored models (one gut-filling, one gut-emptying) were run for knot values k taken at the data sampling points from 5 mins through to 24 hours. Log likelihood values were taken as an assessment of overall model fits. For each knot value k, the log likelihood for each gut filling model was added to the respective log likelihood for its respective gut emptying dyad to produce a profile log likelihood. The maximum of this profile was taken as indicative of the optimal knot position (see Figure 4). Sufficient curvature was detected in using knots up to 24h to obviate the use of larger knot values. All pairs of models had the same total number of degrees of freedom and the same number of parameters estimated (four).

Estimating the filling and emptying characteristics for each gut region

Given the 'best' knot position (k), a random effects across-the-regions model framed as a simple Bayesian normal hierarchical model was constructed (separately for gut-filling and -emptying processes predominating) in order to estimate regional specific characteristics, where:-

$y_{nt} \sim$ Normal(a_t, $1/\tau_c$). I(0,cen)

$a_t \sim$ Normal(a_c, $1/\tau_a$)

where τ represents the precision (1/variance) of a normal distribution, I(0,cen) is an indicator variable for right censoring (upper bound = cen), n is the number of mites (1 to 34) and t is the number of gut regions (1 to 15). Left censoring was taken to occur at (adjusted) time zero. No interval censoring was used. This is a repeated measures model where the gut regions a_t (t=1 to 15) are considered as independent samples from an underlying normally distributed population of regions (i.e region is a random effect - or σ_a around an overall gut value a_c) and mites are considered as censored independent draws (replicates) of each region with residual experimental error (σ_c). Due to the small replication an additional random effect (and Bayesian prior) for each mite was not included but subsumed into the between region variation. y is $\log_e$(time) of being full or being empty. If a region at a time point was not expanded (for the filling model) then y was given NA and right censored at that time point (i.e. yet to have occurred). Similarly, if a region at a time point was not expanded (for the emptying model) then y was given NA and right censored at that (adjusted) time point (i.e. yet to have occurred). a_t is the typical time to initially fill or empty per gut region. a_c is the typical time to initially fill or empty for the overall gut.

a_t , a_c, τ_a, τ_c were given independent very flat 'non-informative' priors as follows:-

$\tau_c \sim$ Gamma(10^{-3}, 10^{-3})

$a_c \sim$ Normal(0,$1/10^{-6}$)

$\tau_a \sim$ Gamma(10^{-3}, 10^{-3})

in order to estimate gut regional characteristics with little bias.

Conjugate distributions were used for convenience (Congdon (2001)). Initial values were given or generated automatically by Winbugs1.3. Five thousand iterations of the Gibbs sampler were used for 'burn in' to empirical stability of estimates and discarded. Bayesian posterior distributions for a_c, τ_a, τ_c and each a_t (t = 1 to 15 gut regions) given the priors and the observed data were estimated from a further 5000 samples and summarized. For display purposes precisions were inverted and square rooted to yield typical error values:- $\sigma_c = 1/(\text{sqrt}(\tau_c))$ and $\sigma_a = 1/(\text{sqrt}(\tau_a))$. For σ_c , σ_a , a_t , and a_c, values were exponentiated during iterations before summary and display. The censored Bayesian hierarchical model allows for the physically destructive ascertainment of mites and takes more notice of consistent results, down-weighting excessively variable data. Whilst fixed effects estimates may be shrunken they do have explicit posterior support using this non-frequentist method (see Tables 1 and 2). No explicit hypothesis testing was carried out.

An alternative model would be to consider that there are two different events both driving whether Z is in state 0 or 1 i.e. to simultaneously model the *hidden* or latent variates Y_{ij}^F and Y_{ij}^E; where Z_{ij}=0 if $Y_{ij}^F > t_i$ or if $Y_{ij}^E < t_i$, that is filling has not started or emptying has completed (at time t_i), and Z_{ij}=1 if $Y_{ij}^F < t_i < Y_{ij}^E$. Modeling $P[Z_{ij} = 1]$ via the number of sections expanded and a negative binomial (DBERN) could be carried out in Winbugs. Whilst this obviates the need for k, it is left for future work on a follow-up experiment. Similarly, one could perhaps take advantage of the spatial order (proximity) of the sections using some sort of spatial 'kriging' smoother rather than a random region effect.

Results

Typical contracted and expanded gut sections in *P. longicornis* are shown in Figure 2. Scoring of the gut regions is presented in Figure 3.

Expansion of the gut begins immediately (by 2mins) and proceeds quickly. Inspection of the raw data (Figure 3) suggests that all regions of the gut in *P. longicornis* fill and empty broadly in unison. Empirically, there is no obvious marked anterior to posterior differences in filling or emptying bar a tendency for posterior regions to be more frequently expanded later in time than anterior gut regions. Left and right parts of the gut appear to be synchronised. Only the behaviour of the rectal vesicle appears perhaps different looking possibly 180^o out of phase with the other gut parts (i.e. being full when they are empty, or, being empty when they are full). The rectal vesicle thus may be simply a passive storage vessel before gut waste products and the excretory materials from the Malpighian tubules, which debouch into it, are voided. That is, the rectal vesicle is constricted when other regions are full retaining prey matter, and the rectal vesicle is expanded open when other gut regions expel their waste products into it.

Estimating switch point from whole gut filling to gut emptying

The profile total log likelihood for the pairs of gut filling and emptying models is presented in Figure 4.

Although the maximum of the profile likelihood occurred at the knot <120, $\geq$ 120 mins, the likelihood surface is fairly flat, spanning a zone from 20 mins to 4h (see Figure 4). The histological data would thus support a knot between gut filling-predominating and gut emptying-predominating over a range of early time values from the commencement of feeding in *P. longicornis*. The surface is not smooth due to the discrete nature of the sampling (and thus the knot) points. Most of the curvature in the likelihood surface was attributable to data from the (larger) females in the sample. However, there was insufficient replication to allow the analysis of males and females separately. Targeted data in perhaps a folllow-up gender-specific study is needed to unequivocally place the true biological position of any clear physiological switch from net gut filling to net gut emptying. Dense sampling around 2h post-feeding would be needed.

Estimating the filling and emptying characteristics for each gut region

Adopting a Bayesian model allows for the paucity of data but produces consistent results mindful of the pattern of the whole data and full probbility distributions for *ad hoc* comparisons. The Bayesian posterior distributions for σ_c , σ_a , a_c, values in mins (after exponentiation) for the gut filling and emptying processes (given censoring and a knot at <120, $\geq$ 120 mins) are shown in Figure 5. The Monte Carlo MC error and the centiles for σ_c , σ_a , a_t (t=1 to 15), and a_c (all after exponentiation) in mins for the gut filling and emptying processes are summarized in Tables 1 and 2. NB for the gut emptying process no re-adjustment in time values for the knot at 120 mins has been made in Table 2.

Throughout, the Monte Carlo estimation (MC) error values were low for all posterior estimates (see Tables 1 and 2) showing that the Bayesian approach used on this data here is reasonable. A left truncated distribution could have been used rather than left censoring and would have produced different results if the bulk of the gut filling or emptying values had been very low, which they were not. Graphs of the posterior Bayesian distributions (see Figure 5) show that the centiles in Tables 1 and 2 are good summaries of the shape of the distributions. Whilst σ_a has a long upper tail for its posterior distribution in both the cases of gut filling-predominating and gut emptying-predominating, the probability of these extreme values (given the data) is vanishingly small. The modest estimates of σ_c for both gut filling and emptying are expected due to the low replication and high homogeneity in this study and may not be typical of a larger study. More replication may first produce larger σ_c variation before a subsequent reduction in estimated MC error magnitudes is achieved. The method has produced reasonable, consistent estimates from this data set (see *Middle Central* Figure 6).

Discussion

The gut in *P. longicornis* is shown to be a 'stiff' dynamical system with markedly different rate constants for filling (ingestion) and emptying (digestion and egestion) – for an explanation of stiff systems see Strogatz (2001). Pergamasids have multilobate midgut systems filling out most of the internal space in their idiosoma - see Figure 1 - much like as in Opilionids (Kästner (1934), Dumitrescu (1976)). They have a continuous lumen between the midgut and hindgut (unlike prostigmatids - see Mitchell and Nadchatram (1969), Moss (1962)). Faecal residue remaining after digestion can leave the gut and enter the rectal vesicle directly. No hepatopancreas (as in scorpions - Mishra (1968)) or tissue clearly analogous to a hepatopancreas was observed. If one simply takes the data in Figure 3 and counts the number of sections which are expanded (i.e. sum over j of $Z_{.j} = 1$) one gets the following series over time: *3.5, 10, 3, 11, 9, 12, 11, 12, 12, 10.5, 14, 14, 7, 14, 11, 9, 12, 8, 11, 2, 9, 9, 2, 1, 1 , 0, 5, 6*. This pattern summarises nicely what is temporally going on (confirming the conceptual basis of this study) - the gut expands, then the gut contracts.

Gut filling

Median values for the posterior Bayesian distribution of the typical time for initial filling to be complete for the gut overall (a_c) was around 10 mins with a typical posterior population error σ_a of 1 minute (see Table 1). The posterior median typical initial filling time for each of the fifteen individual gut regions (a_t) varied from 9.2 to 10.3 minutes with a typical residual error σ_c of 4.5 mins. Anterior parts of the gut (i.e. left hand and right hand anterodorsal caeca, anterior mesenteron and ventriculus) expand marginally faster than posterior parts (see Figure 6). The hindgut and rectal vesicle show intermediate values. However, even the most extreme 97.5% posterior centile for filling an individual gut region was only 15.2 mins (c.f. the posterior mesenteron) with twice a typical residual σ_c error of just over 5 mins. Thus, the gut in *P. longicornis* rapidly fills in a very homogeneous manner (this consistency is also supported by the very small magnitude of inter-regional variation σ_a). The behaviour of the rectal vesicle on filling appears to be an impossibly early outlier (see Figure 3) given the topology of the gut (see Figure 6), suggesting that it is being filled by the digestive remnants, or by the Malpigihian tubule waste products, of the previous meal.

Assuming that the population median gut filling time is equivalent to 3 - 5 half lives of a first order rate physiological process, then the initial overall gut filling half life is of the order of 3 mins. A first order rate process is the simplest assumption concerning the kinetics of filling (see Lister et al (1988)) and is the same as the negative exponential assumption in the models of Sabelis (1990). This 3 mins, I suggest, is a reasonable estimate of the true biological speed of gut filling and, given that this mite only ingests fluid material (Bowman (1984)), it suggests a very quick initial imbibition of prey material by the predatory soil mite (a 'gulp'). Pharyngeal pumping as in phytoseiids (see Flechtmann and McMurtry (1992)) is assumed to be the filling mechanism. Even under an extreme case (c.f. the posterior mesenteron 97.5% centile + 2 typical residual σ_c errors), this half-life would

still only be around 8 mins. This makes sense given the copious haemocoel in the mite's dipteran prey that must be immediatedly handled efficiently by this liquid feeding arthropod on integumental breakage by the cheliceral chelae. These numerical values are congruent with the description of Bowman (1984), where *P. longicornis* is described as quickly rupturing the prey on attack, causing a large clear droplet to appear in the mite's gnathosomal region which slowly vanishes as the prey volume is substantially reduced through imbibition during the first phase of feeding.

What are the correlates of this?

- If gut stretching on ingestion is the signal for digestion to commence as in ticks (see Gabbay and Warburg (1976)) then two possibilities arise:-
  - Digestion commences at the point of maximal stretching (estimated by the maximum of the derivative of the smooth solid curve in Figure 7 to be at $\log_e$(time)=1.23 or approximately 3.5 mins post commencement of feeding), or
  - Digestion commences around the time that essentially maximum expansion is reached i.e. about at the initial completion time of gut filling - 10 mins ($\log_e$(time)=2.30).
- If stopping feeding is the signal for digestion to commence as it is in ticks (Galun and Warburg (1968)), then it should start at between 56-96mins after *P. longicornis* begins feeding (Bowman (1987)).

Inspection in a separate study of gut lumenal and cellular changes during feeding in *P. longicornis* is needed to decide which is the case above.

Gut emptying

The median value for the posterior Bayesian distribution of the typical time for net overall gut emptying to commence (a_c) was around 10.5h (from the knot at 2h from the start of feeding) with a typical posterior population error σ_a of 1 minute (see Table 2). The posterior median gut emptying typical time for each of the fifteen individual gut regions (a_t) varied from around 9 to 11.5h (from the knot at 2h from the start of feeding) with a typical residual error σ_c of approximately 7 mins. Anterior parts of the gut, (i.e. the left hand and right anterodorsal caeca, anterior mesenteron, ventriculus) together with the left hand and right hand posterior ventral anterior caeca empty faster than the other more posterior parts. The posterior mesenteron, the posterior parts of all caeca, the hindgut and the rectal vesicle take the longest to empty (see Figure 7). The most extreme case (as taken by the 97.5% posterior centile for the hind gut and rectal vesicle) for emptying an individual gut region was approximately 25h (from the knot at 2h from the start of feeding, with a twice typical residual error of just over 9 mins). Thus, the gut in *P. longicornis* empties in a very heterogeneous manner - the most posterior gut parts taking the longest to empty. This heterogeneity is supported by the size of inter-regional variation σ_a.

It is not clear how this overall anterior-posterior axis of delay in being empty is controlled. At one level it appears to be proportionately the same delay on emptying as the delay on filling (i.e. approximately 100 minutes mapping to 1 min

respectively - see Figure 7). Overall and in particular the anterior parts of the gut behave like a FIFO (first-in-first-out) conveyor-belt process (see regressions). However, the posterior parts behave in small part like a LIFO (last-in-first-out) process (see regression equation). It could be that because the posterior gut is much larger in volume compared to the anterior sections (see reconstructions in Bowman (1984)), that it functions also in these regions, in part as a temporary food storage 'buffer' for large amounts of prey material. Digestion then being left until the very last in these regions before the egestion of any residual material via the hind-gut into the rectal vesicle. All other matters being equal, the larger amount of material here when digested at the same rate, this should take longer to disappear. Further kinetic work is needed perhaps using biomarkers.

Assuming that the population median gut commencement of emptying time is equivalent to 3 - 5 half lives from a first order rate physiological process, then the typical gut emptying half life is around 2^{-}h – 3^{+}h. A first order rate process is also the simplest assumption concerning the kinetics of emptying (see Lister et al (1988)) and is the same exponential assumption as van Rijn et al (2005). Bowman (1984, 1987) does not mention major episodes of defecation during feeding. Thus, in the light of no other obvious process, I suggest this approximate 2h – 3h value is a reasonable estimate of the true biological speed of digestion in this free-living soil predatory mite. It indicates a steady disappearance of prey material by digestion in the predatory soil mite at an order of magnitude slower than the original imbibition of the fluidised prey. The digestive system has 'stiff' dynamics. Even under an extreme scenario (i.e. the hindgut 97.5% centile + 2 typical residual σ_c errors) this half-life would be around 8.5h - still an order of magnitude above the equivalent worse case scenario for the imbibition half-life - yet close to that of specialised predatory phytoseiid *Phytoseiulus persimilis* Athias-Henriot where a fully satiated gut is half emptied in 8-9h (Sabelis (1990)).

Gut stasis

The 90% Bayesian credible interval for the population value of the gut filling time a_c (of 8.9 to 11.2 mins) is noticeably shorter than the optimal time (2h knot position) estimated from this histological data set to switch from gut filling-predominating to gut emptying-predominating. What is happening in the intervening period when the gut does not appear to change in size (see Figure 7, especially 20ff mins)?

Bowman (1987) reports the feeding time of *P. longicornis* when fed last instar *D.melanogaster* (vestigial winged strain) for males as 56.3 ± 40.8, 3 (mean ± se, n) mins, and for females 96.0 ± 14.3, 11 (mean ± se, n) mins. This is 3-30 times longer on average than the smaller phytoseiids take to feed on prey, but is of the same order of 1-2h total feeding time to satiation on pollen (Flechtmann and McMurtry (1992)). Females were able to feed longer, although Bowman (1987) casts doubt on the explanation that their expandable idiosoma, unlike that of males, is the reason why - it may rather be their fertility demands as in ticks (Gregson (1943)). Feeding, on average, thus continues for 45 mins – 1h 25mins in excess of the population median time (approximately 10 mins herein) needed to produce

initial gut filling and expansion in this histological study. Moreover, feeding clearly continues during the period that this histological study shows no noticeable gut volume change. How can this occur?

The continued consumption, without apparent expansion of the gut, can only occur if the early ingested material is quickly reduced in volume somehow - either by continual digestion and absorption, or by the loss of water or solutes from the gut (and if appropriate eventually out of the body). With the half life of emptying being estimated as several hours I suggest that mite digestive processes are too slow to be an effective mechanism at this juncture and cannot be the reason for this observed stasis in gut size. With a half life of initial gut filling estimated as only a couple of minutes, and feeding continuing for a considerable period with no further gut expansion, it seems more likely to me that rapid passive water and solute transport (perhaps by cellular filtration) is the mechanism for a concentration of gut contents rather than unrealistically fast digestion. High speed active secretion of water and solutes by cells is a theoretical possibility but comparatively rare in biological phenomena compared to the prevalence of rapid passive filtration mechanisms. Similar reduction of the ingested meal takes place by elimination of water through the coxal organ is known in argasids, where a concentration factor of almost 2 has been measured within 2 h of starting feeding (Smit et al (1977)). A volume measurement over time in future work with *P.longicornis* would help.

This possible filtration mechanism as a balancing process to allow a stasis in gut size can be critically assessed qualitatively by examining three observations during feeding in *P. longicornis* on drosophilid prey made by Bowman (1984). These are:-

- the 'ballooning out' of prey from a wash of clear fluid emanating from the gnathosomal area (putative saliva?) in the early phase of feeding;
- the occasional deposition of small clear droplets (with granular material) from the anus in the later phase of feeding; and,
- the production of watery coxal droplets in the later phase of feeding,

see Figure 8.

Taking the first - Unfortunately Bowman (1984) does not give explicit timings of the first early phase of feeding nor the later second phase however, salivary production is typically a secretory mechanism in animals and not an excretory filtration mechanism (except in ticks). Nevertheless, the production of watery saliva *would* effectively recycle fluids from the prey back into the prey carcass by: - first, ingestion into the mite gut; followed, by fluid movement into the mite's haemocoel; and, then return from the haemocoel, via the salivary glands into the prey (see Figure 8). Some contraction and thus emptying of the mite's gut indicated by some of the sections in this histological study during the feeding period (see Figures 3 and 7) could be via this mechanism. This would produce a temporal concentration of gut contents but no overall net loss of fluid from the predator unless pieces of a resultant wet prey carcass were discarded. In fact, Bowman (1984, 1987) describes the contrary – the prey is totally consumed in one piece except for the cuticle. So even if fluid is moved out of the idiosoma by this salivary method, the gut should fill up and expand again at the next ingestion point during feeding as the prey fluid is re-imbibed, yielding no net loss. Also under this assumption, contraction

of the gut would have to occur on the same time-scales as the production of salivary fluid. Repeated 'ballooning' of the prey with fluid *was* observed by Bowman (1984) occurring in the first early phase of feeding i.e. any salivary production occurs mainly during the gut filling phase (up to say the first 10 mins). However, if such a salivary process is happening to maintain continual feeding, then this histological study, which shows a general synchrony of gut regions during filling, would have to have been extraordinary unlucky not to have seen the whole gut regularly contracted and expanded repeatedly in the early stages of feeding. Whilst gut expansion is variable, Figure 3 does not unequivocally show this required behaviour. Further, Bowman (1984) *only* describes this 'ballooning' out during the first *early* stage of feeding – a phase described as one of prey volume diminution concluded by a substantial reduction in prey volume. I believe that this diminution is only possible if significant imbibition is occurring - the opposite of the salivary dumping back of fluid. Thus, although Bowman (1984) does not give explicit timings, I infer that salivary liquid production extra-corporeally cannot be a major part of the process which ensures the stasis in gut size histologically (over the period 1.5 - 2h from the start of feeding) in this study.

Taking the second - Immediate (if inefficient) discard of substantial amounts of undigested food material imbibed into the gut directly via the anus during feeding (see Figure 8) *is* a possible mechanism to explain these histological results - but Bowman (1984, 1987) does not describe this happening during feeding. Filtration of water and solutes by the Malpighian tubules, debouchment into the rectal vesicle and loss via the anus (see Figure 8) is also a possible mechanism to engender a stasis in gut size during the feeding phase of *P. longicornis*. Bowman (1984) does describe small clear droplets with granular material (guanine?) being frequently deposited by anal 'dabs' on the substrate in the second later phase of feeding. This Malpighian mechanism may well happen as Figure 3 shows the rectal vesicle to be expanded during the early stages of feeding - perhaps containing material filtered out via the tubules. However, this expansion of the rectal vesicle at this time may, in fact, be due not to the consequences of the current meal but due to the retention of remnants of the previous meal - these remnants being mechanically voided by the physical internal space change induced as the gut fills up (c.f. the contracted rectal vesicle shown in the middle period of this study). However, this Malpighian process *could* be just the gut volume balancing mechanism one is looking for as this anal 'dabbing' was only observed up to the termination of feeding (i.e. up to say 1.5h from the start of feeding) and not thereafter. Against this latter interpretation is the observation that only small amounts of fluid are lost via this route (Bowman (1984)) compared to the large coxal droplets also produced during feeding (see photographs in Bowman (1984)). An estimation of the volume of fluid lost via the anal route and its explicit timing is needed, as also confirmation that the granular material deposited anally at this juncture is excreted guanine in order to resolve this question.

Taking the third - More simple and congruent to the earlier observations (Bowman (1984)) is that continual passive filtration via the coxal droplet mechanism is the volume balancing mechanism to produce the stasis in gut size despite continual feeding over this early period (i.e. 10 mins to 1.5h from the start of feeding) - see Figures 8 and 9. These independently produced *anecdotal* photographs confirm the

common existence of coxal fluid production during feeding. The typical lifting of the prey's puncture point above the substrate leaves space for their formation - the hydrophobic lipophilic predatory mite's cuticle ensures their sphericity. I further suggest that coxal droplet production is not just intended to remove unwanted excess water and solutes but is an integral part of the concentration of gut contents to actually *allow* continual feeding in this poorly expandable arthropod and the consequent observed full utilisation of a prey's nutritive content. That the latter entire utilisation occurs is substantiated by the description of the completely digested larval drosophilid carcasses by Bowman (1984, 1987) at the end of feeding and similar observations in phytoseiids (see Flechtmann and McMurtry (1992)). Acarine coxal glands are, at least, involved in ion/water balance and osmoregulation according to Alberti et al (1996) and are associated with the gut in other arachnids too - see Kästner (1934). Water eliminated in this way by pergamasids could be physically manouvered into contact with the gnathosomal groove (postcapitular channel) and courtesy of surface tension partially recycled by capillary action via the deuterosternal groove/tritosternum into the mouth as described by Wernz and Krantz (1976) during times of water need.

Scaling

Published phytoseiid results are plotted in comparison to *P.longicornis* in Figure 10 - for more details of these phytoseiids - see Sabelis (1985), Sabelis (1986) and Metz et al (1988). Why is there such a disparity between mites of common phylogeny and similar body plans? Not only are phytoseiid life-cycles short (of the order of 5-10 days) suggesting rapid food utilisation (neoseiulid prey capture to prey abandonment times are reported between 10-30mins - see van Rijn et al (2005)); but plant inhabiting phytoseiids are much smaller than pergamasids. Their size is around 0.5-0.6mm (see Rosenheim et al (2004)), compared to *P. longicornis* (at >1mm) and thus their total feeding time is expected to be shorter. Furthermore, *P. persimilis* is more of an *r*-strategist feeding frequently with short bout lengths (of the order of 100s - see Bancroft and Margolies (1996)), while pergamasids as *K*-strategists exhibit slow life cycles and long feeding bouts (of the order of 1-1.5h - see Bowman (1987)). The 90% Bayesian credible interval for the population value a_c of gut final emptying time commencement (of 446 to 809 mins), plus 2 typical population errors σ_a (of a minute or two) - all twice over, plus the 2h from the position of the optimal knot, yields a likely minimum total time of 17h for the total feeding cycle time (ingestion, digestion and egestion) in *P. longicornis* and a maximum total time of 29h based upon the gut overall. Compare this with the specialised predatory phytoseiid *Phytoseiulus persimilis* Athias-Henriot, where a fully satiated gut is virtually emptied after 36-48h (Sabelis (1990)). Under the worst-case scenario of using the 97.5% centile a_t for the hind-gut (or rectal vesicle) and its error values gives a total feeding cycle in *P. longicornis* of approximately 52.5h. Murray and Solomon (1978) found that prey esterases could be detected in the gut of the predatory phytoseiid *Typhlodromus pyri* (Scheuten) for at least 50 hours suggesting that this scale of duration could be typical of mesostigmatids. Fifty two and a half hours herein, is much shorter than the one week (= 168h) for feeding and digestion claimed without substantiation by Bowman (1984) for *P. longicornis*. Rather, the latter value matches the begining of a return to a state of

starvation better, as Figure 3 shows an almost total lack of gut expansion occurs by 144h. Bowman (1984) appears to have been incorrect.

It is to be expected that, the speed of digestion appears slow, and the total feeding cycle is prolonged, as *P. longicornis* is a poikilotherm and here is consuming prey of a magnitude bigger than its own body size. What is surprising is the poor congruence with satiated phytoseiids (see Figure 10). Perhaps there is not a broad uniformity in digestive processes over mesostigmatids? The 'gulp' of food in *P. longicornis* is likely to be bigger than that of smaller phytoseiids so digestive rates may be faster in the former. Pergamasid mites were investigated at ambient room temperature but exist all year round as adults (see Bowman (1985)). In the wild, where temperatures can fall dramatically, the total feeding cycle may be even longer - although mesostigmatids (e.g. *Pergamasus*, *Parasitus* and *Veigaia* spp.) are even active under snow (Leinaas (1981)). It would therefore be useful if the half life and duration estimates inferred from this histological study could be physically confirmed by monitoring live mite weights (under constant RH) at field temperatures (say using the method in Dicke et al (1989)) or be biochemically confirmed by direct assay of the appearance and disappearance of prey material in the gut of *P. longicornis* (using say molecular methods derived from Cutherbertson et al (2003), Greenstone (1977), Lister et al (1987), Meng et al (1980), Murray and Solomon (1978), Solomon et al (1996), or Yamanaka et al (1972)). Electrophoretic methods already used on phytoseiids (Dicke et al (1988)) would have the added advantage of indicating the predator's gut state (see Giller (1984)). If such confirmation was not available, then understanding the temporal changes in Malpighian tubules and the time course of the production of waste material in the gut of *P. longicornis* during and after feeding would help confirm or refute the explanations offered in this paper as well as further clarifying the role of the rectal vesicle in digestion.

Conclusion

Pergamasus longicornis shows a stiff digestive system suitable for an intermittent bolus feeder. At 1^{+}-2^{+} days the gut cycle is clearly much less than the 1 week previously claimed. Figure 10 shows that the rate of gut emptying approximately scales with body size in free-living mesostigmatids. Gender-specific estimates could be found if sufficient number of male and female mites were to be studied independently.

In summary, from this 'pulse-chase' experiment, three phases of gut changes in *P. longicornis* during and after feeding are clear (see Figure 7):-

- Expansion due to rapid filling by imbibition (with concomitant salivary processes);
- Continued filling by imbibition with the concentration of gut contents through fluid loss via coxal droplets and anal 'dabbing';
- Contraction due to slow emptying.

Only under the most extreme case scenario (in this histological study) of the filling of the posterior mesenteron (judged by its 97.5% centile + 2 typical residual σ_c

errors totalling 25mins) being typical, together with the earliest possible knot position (k = 20 mins); would there be no need for the middle 'concentrative' phase.

The interpretations above infer certain experimentally testable predictions based upon the results of this histological study:-

- Firstly, that coxal droplets should be most often produced by *P. longicornis* in the period 10 mins to 1.5 hours from the start of feeding (on last instar drosophilid larvae). Further that the droplets should occur more often in the early times of this window than the later times (as Bowman (1984) states that feeding is terminated much later after coxal droplets are observed). Of course, coxal droplets might still be produced even less frequently from 1.5h up to the 2h 'knot point' between gut filling-predominating and gut emptying-predominating but that they should *not* occur after this 2h point (i.e. when digestion proper takes over and predominates).
- Secondly, due to the sclerotised nature of the idiosoma in male *P. longicornis* precluding expansion, it is predicted that males should show coxal droplet formation more often than females. Or that male pergamasids should produce relatively larger droplets size-for-size than females given the same size prey.
- Thirdly, that the propensity for *P. longicornis* to produce coxal droplets should be related to the magnitude of the prey and it's water content. Larger prey and/or more watery prey should induce coxal droplets more often. Small prey (as in phytoseiids feeding on tetranychids - see Flechtmann and McMurtry (1992a)) may produce no excess flow of body fluids needing handling.

Alternatively, if salivary production is an important temporal mechanism for continual gut content concentration then, salivary production should be seen operating repeatedly throughout the whole period - i.e. 10 mins from the start of feeding through to the cessation of feeding (approximately 1.5h) - not just early-on during feeding (when perhaps enzymes and anticoagulants are introduced into the prey to aid extracorporeal digestion). Salivary histology needs examination.

Finally, if a rectal route is the balancing physiological mechanism, then approximately equivalent volumes of fluid material to the original prey volume should be deposited pygidially during the period 10 mins to 1.5h after the commencement of feeding (and not thereafter). Faecal deposition after 1.5h (and in particular after 2h) from the start of feeding could be explained simply by the voiding of post digestion and Malpighian excretory material. Excretion out of the idiosoma should occur 180^o out of phase with the gut being full (although metabolism should produce excretory products throughout), and thus suggests that post digestion, excretory product levels should be high. This can be checked histologically.

Notwithstanding these caveats, *P. longicornis* is a microarthropod physiological system worth exploring further.

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Table 1 Gut filling in *Pergamasus longicornis*. Summary statistics for Bayesian posterior distributions of a_c (exp.alpha.c - typical time of overall gut initial filling), σ_a (exp.sigmaa), a_t (typical time for initial filling to be complete per gut region) and σ_c (exp.sigmac - residual error) in mins (after exponentiation) based upon N=5000 samples for gut filling and emptying processes (given a knot at <120, $\geq$ 120 mins). The quantity reported as 'MC error' is an estimate of σ / $N^{0.5}$, the Monte Carlo standard MC error of the mean. The batch means method outlined by Roberts (1996); p.50, is used to estimate σ.

		Centiles								
	MC error	2.5%	5%	10%	25%	Median	75%	90%	95%	97.5%
a_c	0.1	8.0	8.2	8.5	9.2	9.8	10.7	11.4	11.9	12.3
σ_a	0.01	1.03	1.03	1.04	1.07	1.12	1.21	1.33	1.41	1.49
a_t										
ANTDCLH	0.1	5.9	6.6	7.3	8.4	9.4	10.5	11.4	12.0	12.6
ANTDCRH	0.1	5.2	5.9	6.6	8.0	9.2	10.2	11.2	11.7	12.3
VENTRIC	0.1	6.0	6.7	7.4	8.5	9.5	10.6	11.6	12.2	13.0
MESANT	0.1	5.8	6.6	7.3	8.4	9.5	10.5	11.4	12.1	12.7
MESPOST	0.1	7.5	7.9	8.4	9.3	10.3	11.4	12.8	13.9	15.2
POSDCLHA	0.1	7.5	7.9	8.4	9.2	10.2	11.4	12.7	13.8	14.9
POSDCRHA	0.1	7.0	7.6	8.1	9.0	9.9	11.0	12.1	13.0	14.0
POSVCLHA	0.1	7.4	7.9	8.3	9.2	10.1	11.2	12.5	13.6	14.8
POSVCRHA	0.1	7.4	7.9	8.4	9.2	10.2	11.3	12.7	13.7	14.9
POSDCLHP	0.1	7.6	8.0	8.4	9.2	10.2	11.4	12.7	13.8	15.1
POSDCRHP	0.1	7.5	7.9	8.4	9.2	10.2	11.4	12.7	13.7	14.9
POSVCLHP	0.1	7.4	7.9	8.3	9.1	10.1	11.2	12.5	13.6	14.9
POSVCRHP	0.1	7.4	7.9	8.3	9.1	10.1	11.3	12.6	13.7	14.8
HINDG	0.1	7.0	7.5	8.0	8.9	9.9	11.0	12.2	13.2	14.2
RECTALV	0.1	6.7	7.2	7.9	8.8	9.7	10.8	11.8	12.6	13.5
σ_c	0.00	3.94	4.01	4.09	4.26	4.47	4.69	4.92	5.04	5.15

Table 2 Gut emptying in *Pergamasus longicornis*. Summary statistics for Bayesian posterior distributions of a_c (exp.alpha.c - typical time of overall gut initial emptying), σ_a (exp.sigmaa), a_t typical time for initial emptying to commence per gut region) and σ_c (exp.sigmac - residual error) in mins (after exponentiation) based upon N=5000 samples for gut filling and emptying processes (given a knot at <120, $\geq$ 120 mins). NB No adjustment of gut emptying figures for knot at 120 mins in a_c and a_t (i.e. it is necessary to add 120 mins to convert to a time from the start of feeding). The quantity reported as 'MC error' is an estimate of σ / $N^{0.5}$, the Monte Carlo standard MC error of the mean. The batch means method outlined by Roberts (1996); p.50, is used to estimate σ.

		Centiles								
	MC error	2.5%	5%	10%	25%	Median	75%	90%	95%	97.5%
a_c	8.4	420.9	445.7	485.5	553.5	632.4	717.3	808.9	866.4	905.6
σ_a	0.02	1.03	1.04	1.05	1.09	1.22	1.46	1.74	1.95	2.16
a_t										
ANTDCLH	10.5	205.3	264.8	327.5	443.2	565.1	680.6	791.4	864.3	920.8
ANTDCRH	12.4	156.7	197.7	262.1	389.5	532.5	654.0	754.0	822.7	875.0
VENTRIC	9.6	258.7	310.7	373.4	480.3	595.6	710.7	822.4	903.8	986.4
MESANT	8.7	278.6	331.1	394.4	497.9	607.8	717.9	832.2	911.4	988.5
MESPOST	11.6	404.0	443.4	492.9	580.0	689.2	828.3	1021.0	1203.0	1395.0
POSDCLHA	10.2	397.6	437.4	484.9	575.6	683.6	817.0	1007.0	1179.0	1379.0
POSDCRHA	10.4	389.5	434.9	489.9	574.7	679.8	808.0	987.9	1143.0	1320.0
POSVCLHA	8.6	265.2	312.8	377.1	491.0	604.7	715.3	831.7	908.5	1011.0
POSVCRHA	8.5	299.3	343.6	396.8	499.8	608.6	724.2	848.9	933.1	1043.0
POSDCLHP	9.5	366.0	421.9	468.7	560.9	665.4	791.3	955.6	1110.0	1301.0
POSDCRHP	9.0	375.2	417.8	465.8	553.5	659.5	782.8	945.8	1100.0	1278.0
POSVCLHP	8.3	324.5	373.9	431.2	528.6	634.9	752.6	882.4	1000.0	1156.0
POSVCRHP	8.7	361.3	412.8	459.9	550.0	654.4	773.6	922.0	1052.0	1193.0
HINDG	11.7	401.2	440.1	490.8	575.6	690.1	835.1	1046.0	1247.0	1487.0
RECTALV	12.4	416.7	453.3	502.6	589.9	695.4	854.8	1078.0	1283.0	1493.0
σ_c	0.05	5.42	5.59	5.82	6.28	6.86	7.51	8.24	8.73	9.22

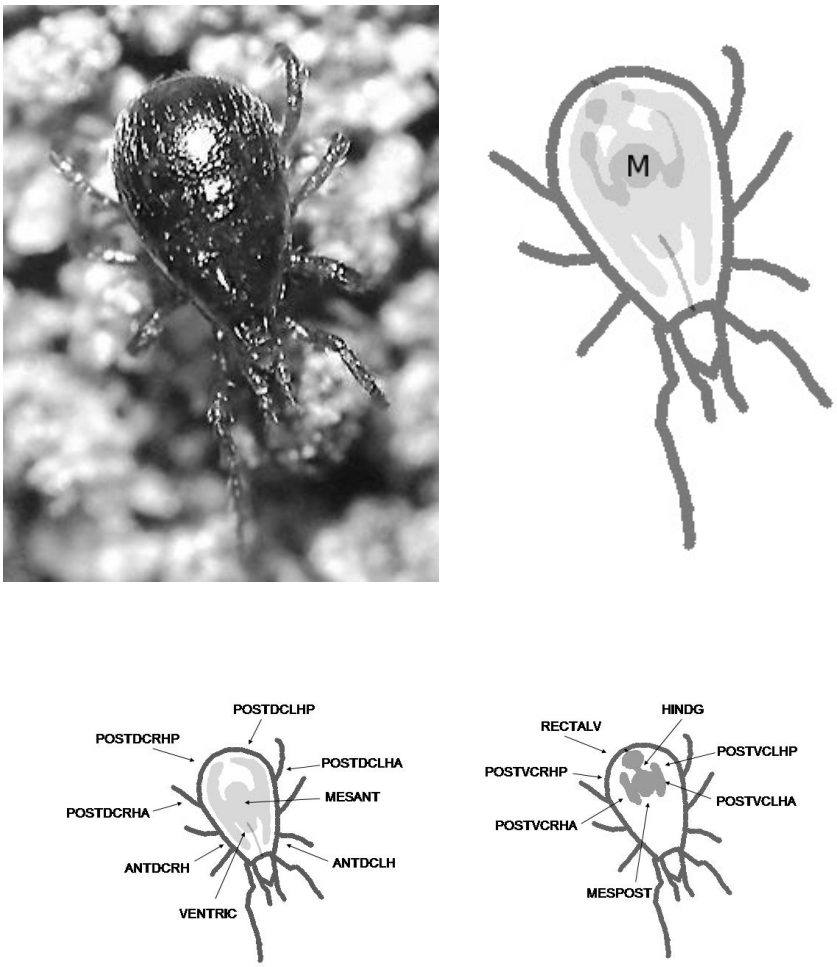

Fig. 1 *Top Left*: A typical forest soil pergamasid (from colour photo of *Pergamasus crassipes* Linnaeus by Maria Minor © Massey University with permission). *Top Right*: *Pergamasus longicornis* whole gut schema overlain at same scale as photograph seen from above (pale grey = dorsal gut, dark grey = ventral gut - see reconstructions by Bowman (1984). M = mesenteron). *Bottom Left*: Dorsal gut schematically labelled from above. Note oesophagus climbs from gnathosoma to enter ventriculus from below almost at junction with mesenteron; *Bottom Right*: Ventral gut schematically labelled from above. Rectal vesicle debouches pygidially. NB: Postero-ventral caeca anteriorly have been exaggerated for illustrative effect simply to aid in showing their distinction from the *posterior* mesenteron (often there are no substantial forward lobes - see Figure 6). *Abbreviations:* Anterodorsal caecum LH = ANTDCLH; Anterodorsal caecum RH = ANTDCRH; Ventriculus = VENTRIC; Mesenteron anterior = MESANT; Mesenteron posterior = MESPOST; Posterodorsal caecum LH anterior = POSDCLHA; Posterodorsal caecum RH anterior = POSDCRHA; Posteroventral caecum LH anterior = POSVCLHA; Posteroventral caecum RH anterior = POSVCRHA; Posterodorsal caecum LH posterior = POSDCLHP; Posterodorsal caecum RH posterior = POSDCRHP; Posteroventral caecum LH posterior = POSVCLHP; Posteroventral caecum RH posterior = POSVCRHP; Hind gut = HINDG; Rectal vesicle = RECTALV.

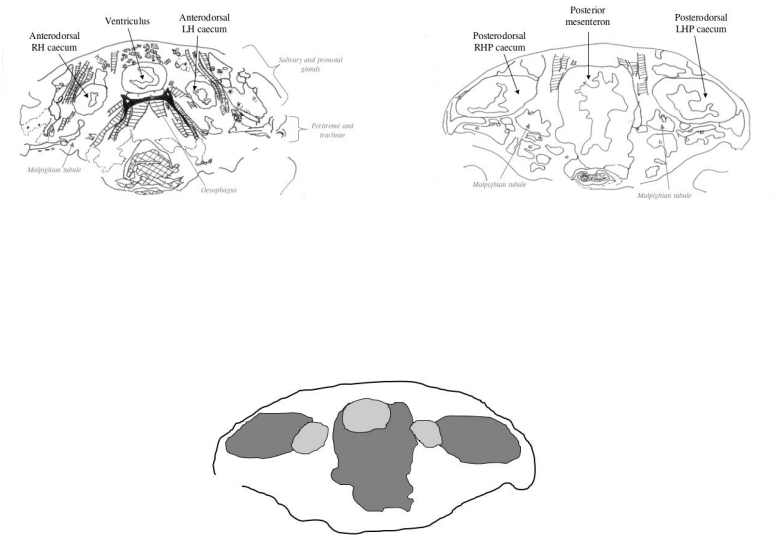

Fig. 2 Sectional drawings of typical:- Contracted (anterior section on left); and; Expanded (posterior section on right) gut regions in female *Pergamasus longicornis* 18h after feeding. When registered and overlain, the anterior gut (pale grey shading) can be seen to be contracted i.e. relatively 'not filled' by prey material, whilst the posterior gut (dark grey shading) can be seen to be expanded i.e. relatively 'filled' by prey material.

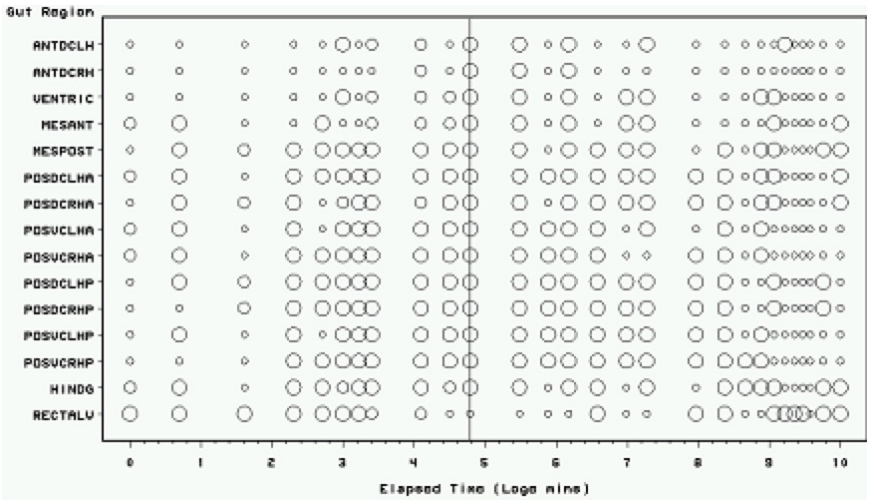

Fig. 3 Schematic of *Pergamasus longicornis* gut (ordered anterior to posterior) as: Expanded with food contents (large circles $Z_{ij} = 1$), or, Contracted without food contents (small dots, $Z_{ij} = 0$). Intermediate size circles represent an ambiguous score over mite replicates ($0 < Z_{.j} < 1$). Time is from the commencement of feeding and is on a natural logarithmic scale. Grey line is at the 120 mins optimal knot position (k) between net gut-filling and net gut-emptying.

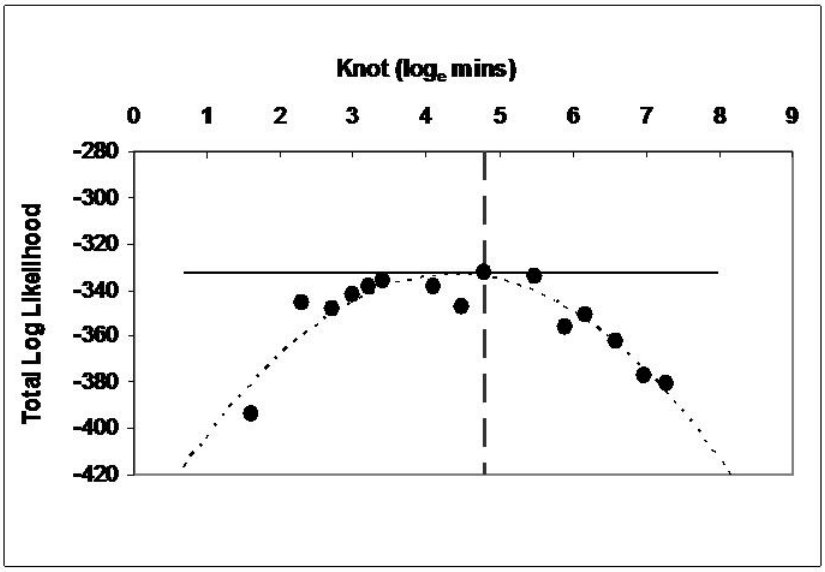

Fig. 4 Profile likelihood of total log likelihood for each pair of gut filling and gut emptying models in *Pergamasus longicornis*. Maximum likelihood (= -332, horizontal solid line) optimal knot is at <120, >=120 mins (vertical grey dash). Dotted line is empirical quadratic fit.

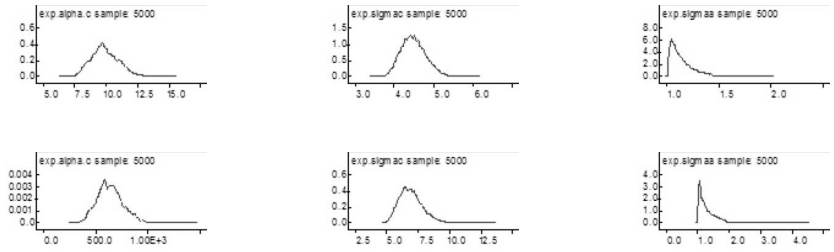

Fig. 5 The Bayesian posterior distributions for:- *Left*, a_c (exp.alpha.c - typical time for gut overall); *Middle*, σ_c (exp.sigmac); and, *Right*, σ_a (exp.sigmaa) in mins (after exponentiation) for the gut filling and emptying processes given a knot at <120, $\geq$ 120 mins in *Pergamasus longicornis* . NB No adjustment of gut emptying process for the knot at 120 mins in a_c. *Top*: Gut filling. *Lower*: Gut emptying.

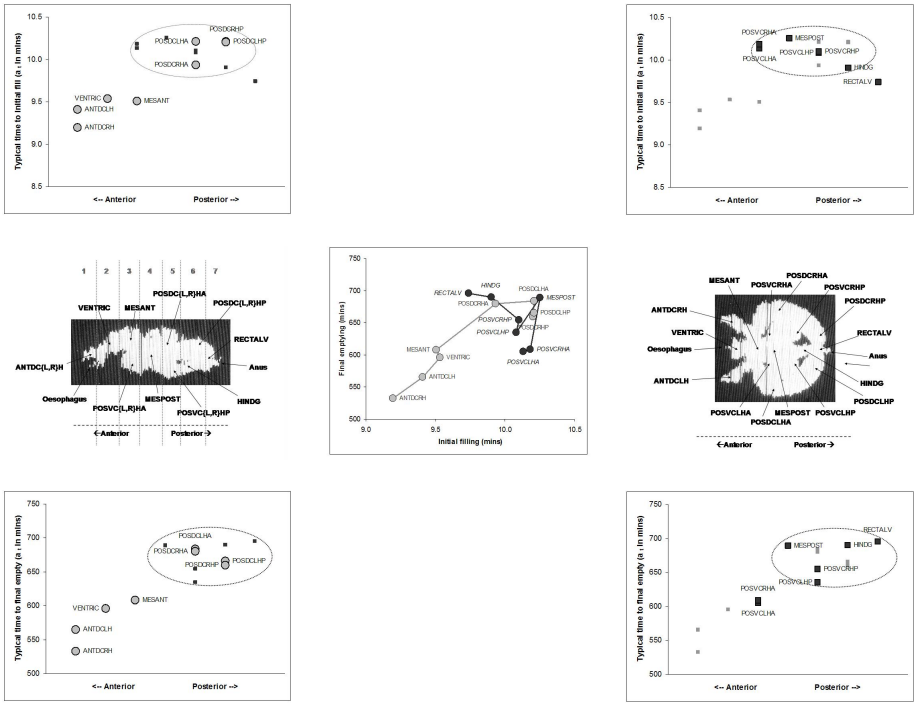

Fig. 6 *Pergamasus longicornis* summary based upon Bayesian posterior median for typical times (a_t) in mins (after exponentiation) for initial-filling and final-emptying processes (given a knot at <120, $\geq$ 120 mins) per gut region. Pale grey symbols - dorsal gut regions. Dark grey symbols - ventral gut regions. Ellipses indicate grouping of approximate equal times. X-axis = nominal scale of antero-posterior order (see *Middle row* reconstructions). NB After adjustment of gut emptying process for the knot at 120 mins in a_c. *Top Left*: Gut filling, dorsal gut regions. *Top Right*: Gut filling, ventral gut regions. *Middle Left*: Annotated lateral view - reconstruction after Bowman (1984) with permission. Dotted vertical grey lines (and numbers) indicate boundaries between (and values for) nominal antero-posterior position of regions used in plots in *Top row* and *Bottom row*. *Middle Central*: Relationships of filling with emptying (plotted in antero-posterior order). Regression for (dark grey) dorsal sections alone y = 129.55x - 642.53 R^2 = 0.8834; Regression for (italic pale grey) ventral sections alone y = -116.49x + 1825.2 R^2 = 0.2753; Regression overall y = 98.397x - 336.84 R^2 = 0.4598 *Middle Right*: Annotated dorsal view - reconstruction from Bowman (1984). *Lower Left*: Gut emptying, dorsal gut regions. *Lower Right*: Gut emptying, ventral gut regions.

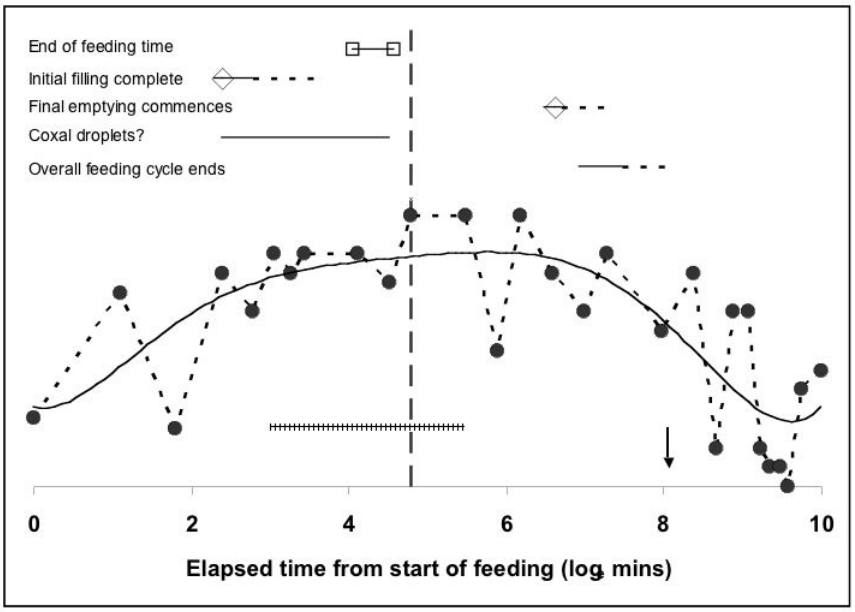

Fig. 7 Re-scaled schematic of gut filling and emptying during feeding and digestion in *P. longicornis* . Overall gut mean score - grey closed circles and dotted data line. Solid line - 6th order polynomial smooth trend. Time is from the commencement of feeding and is on a natural logarithmic scale. Grey dashed line is at the 120 mins breakpoint between ingestion dominating and digestion predominating. Y axis is measure of average gut size and infers relative expansion/contraction between plotted points. *Annotation*: Hashed horizontal line indicates 20 mins to 4 hours period of flat likelihood surface (see Figure 4). Diamond = median; Solid lines = range; Dotted lines = worse-case scenario. Squares = mean of *end* of feeding time for males and females respectively (see Bowman (1987)). Arrow indicates worse-case total feeding cycle time based upon modeling gut expansion/contraction of 52.5h herein, thereafter is egestion.

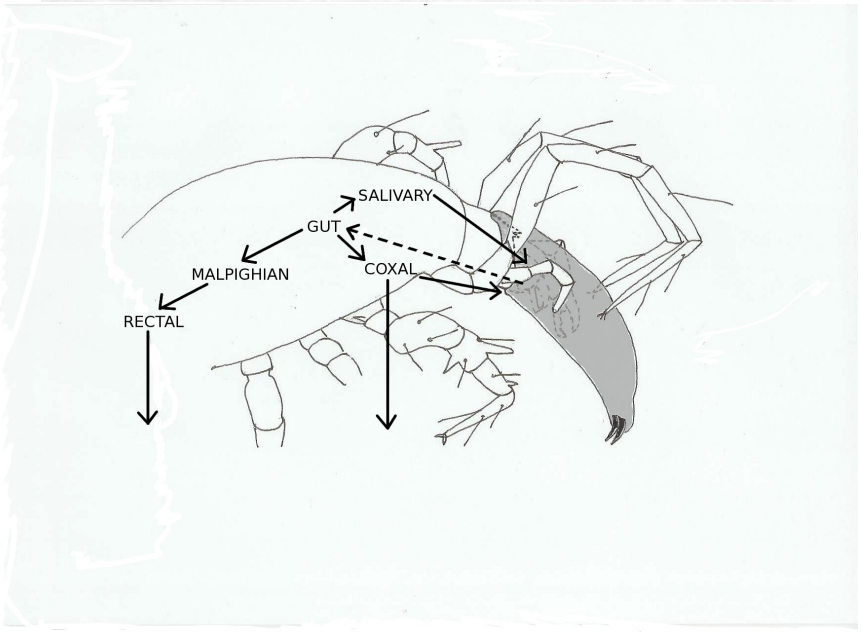

Fig. 8 *Upper:-* Typical pergamasid feeding posture with imbibition point in prey lifted high off substrate. From a colour photograph © Philippe Legros, Villiers sur Marne, 94350 France, November 26, 2011 with permission. *Lower:-* Potential routes of water flow and balance during gut filling in *Pergamasus longicornis* feeding in a similar position upon larval dipteran prey. Dotted line indicates water ingestion from liquidised prey. Solid lines indicate options for recycling or discard of water during feeding (see Discussion).

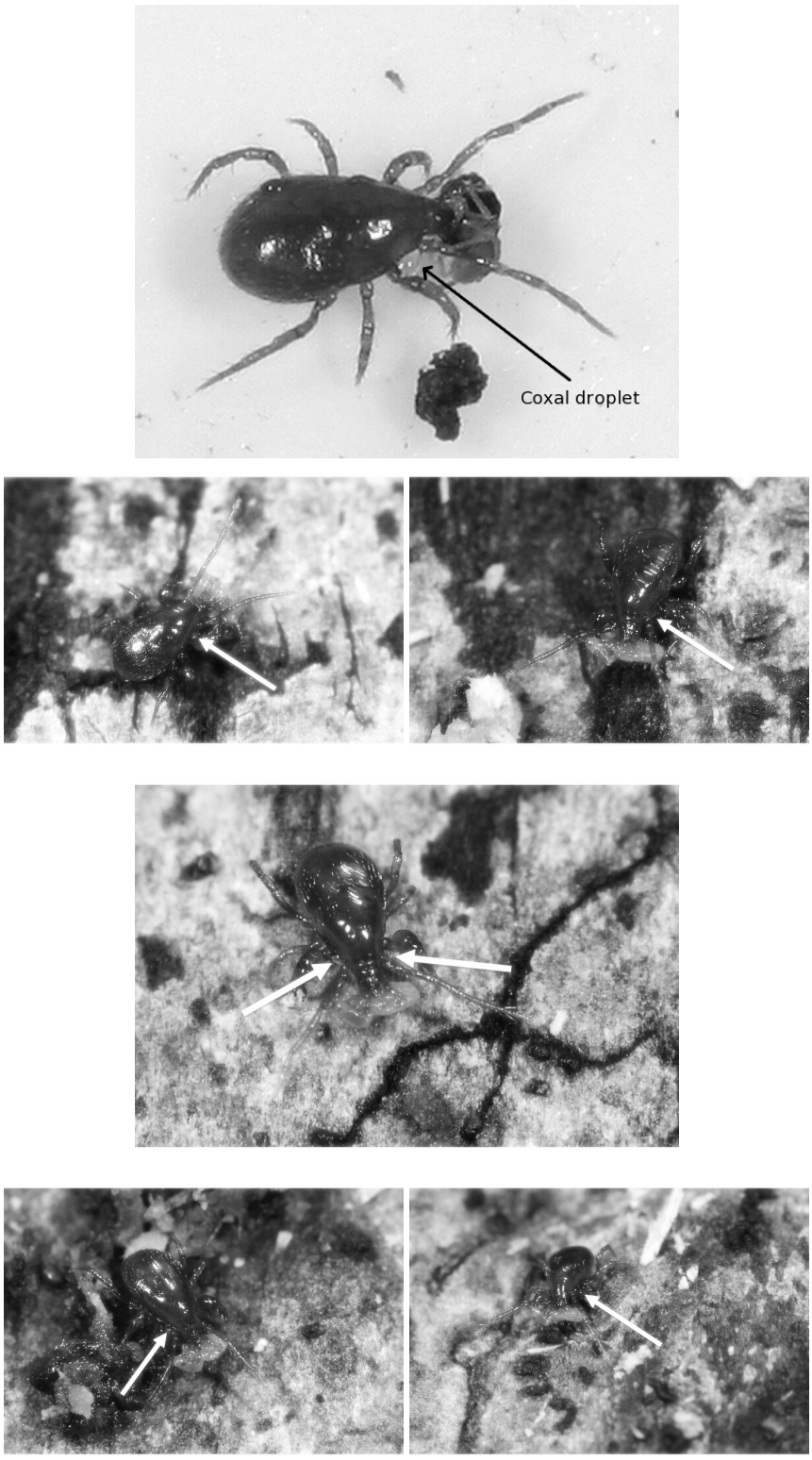

Fig. 9 Independent corroboration of clear coxal droplets of watery fluid forming unilaterally and bilaterally under coxa I and accumulating in front of leg 2 during Pergamasid feeding (indicated by arrows - see Discussion). *Top row:-* Female consuming a globular springtail - Harvard, Worcester County, Massachusetts, USA, December 2, 2006. From a colour photograph by Tom Murray © with permission. *Remaining rows:-* Male consuming a ?collembolan - Ellernbusch, north of Oldenburg, Lower Saxony, Germany, April 13, 2010. From colour photographs by Jörg Pageler © with permission.

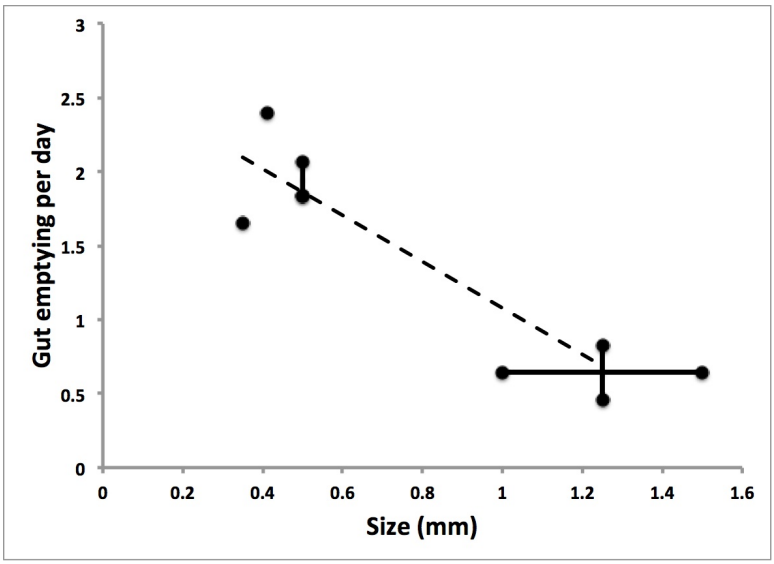

Fig. 10 Plot of gut emptying rates (per day) verus body size. Top left group are *r*-strategists from the family Phytoseiidae (see Introduction). Bottom right is the *K*-strategist *P. longicornis*. Dashed line is simple linear regression showing poor fit. Note how the phytoseiids form a compact cluster showing around 3 times higher gut emptying rates. Bars indicate ranges.