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AS WE SEE IT

Reassessment of the gut pigment method for estimating *in situ* zooplankton ingestion

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ABSTRACT: Correction for chlorophyll pigment destruction has been frequently used in calculation of copepod ingestion rates using the gut pigment method. We argue that tracers in the gut may be either digested and assimilated, or evacuated, and that both processes are taken into account when an evacuation (disappearance) rate curve is determined. As a result, any correction for gut pigment destruction in calculating ingestion rate is inappropriate.

KEY WORDS: Ingestion · Gut pigments · Zooplankton

The use of gut contents to estimate *in situ* feeding rates has been widely applied in studies of fish and zooplankton ingestion. The stomach content of a field caught fish can readily be determined by removing it from the fish and weighing it. This information, together with knowledge on the stomach evacuation rate, makes it possible to estimate the ingestion rate. Elliott & Persson (1978) developed a model based on an assumption of an exponential stomach evacuation rate, R, where the amount of food consumed (I_t) in t hours is given by:

$$I_t = (G_t - G_0 e^{-Rt})Rt/(1 - e^{-Rt}) \quad (1)$$

where G_0 is the stomach content at the beginning of the time interval, and G_t the stomach content at the end. Daily food consumption is estimated by summing the consumption for each time interval over 24 h. Elliott & Persson (1978) also developed a simpler equation:

$$I = 24\bar{G}R \quad (2)$$

where G is the mean amount of food in the stomach over 24 h. These approaches have been used extensively in the fisheries literature.

In zooplankton a somewhat similar approach has been developed using pigments that are derived from chl *a* (Mackas & Bohrer 1976). Details of the method are discussed by Bamstedt et al. (2000). An advantage of the method for zooplankton is that it enables one to estimate *in situ* feeding rates upon phytoplankton without bottle incubations. It does, however, require a separate measure of the rate of decline in pigment contents in the zooplankter, preferably from freshly caught individuals from the field that have been feeding under the same conditions as those for which ingestion is being measured (Irigoien 1998). Ingestion over 24 h, for example, is calculated in a similar manner as outlined above for fish.

This method has been used extensively after it was first proposed, but has recently fallen into disrepute because of what we feel are some erroneous assumptions about the method. As a result, during the past 5 yr there have been few publications where the method has been employed.

Shuman & Lorenzen (1975) suggested that chlorophyll pigments are not destroyed or degraded to nonfluorescent compounds in the guts of copepods. This question was examined by Wang & Conover (1986)

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and Conover et al. (1986). They used the mass balance approach to formulate an equation describing the amount of pigment in the gut, *G*, as the resultant of pigment ingested minus that defecated and that which was degraded to undetectable compounds:

$$dG/dt = I - (E + b) \quad (3; \text{their Eq. 2})$$

where I is the ingestion rate, E the amount defecated, and b the undetected pigment (lost through degradation). E was estimated as RG, where R is the exponential evacuation rate. Ingestion is then measured experimentally using the gut pigment method, where $I = RG_0$ and G_0 is the asymptotic value of gut pigments after the animal has been feeding for some time. It is also measured from changes in chlorophyll concentration in grazing chambers with $I = F\bar{C}$, where F is the filtration rate and C is the average chlorophyll concentration. Ingestion rates measured by the 2 methods differed and these differences were attributed to an undetected loss of gut pigment.

There has subsequently been considerable debate as to whether or not chlorophyll pigments are destroyed in the stomachs of copepods and rendered into colorless compounds. In estimating ingestion rate, corrections have been made based on estimates of pigment destruction (see discussion in Bamstedt et al. 2000). Below we argue that the issue as to whether pigments are destroyed or not does not affect the method of using chlorophyll pigments in the gut to estimate ingestion rates.

We suggest that the term 'evacuation rate' is an inappropriate term for describing the processes occurring in the gut and instead suggest that the term 'disappearance rate' is more appropriate, since this makes no assumptions as to the processes occurring. Food in the gut may either be digested and assimilated, or evacuated as fecal pellets; the disappearance of any tracer in the gut is the resultant of these 2 processes. When an evacuation (disappearance) rate curve is determined, digestion and/or fecal pellet evacuation may be responsible for the disappearance of the tracer. It is this disappearance rate that is needed for the calculation of ingestion.

In measuring gut fullness any tracer may be used and the assumption of inertness is not important. Tracers used in addition to chlorophyll pigments include ^{68}germanium (Ellis & Small 1989) and DNA (E. G. Durbin & M. C. Casas unpubl.). A tracer that is inert and being evacuated from the gut in fecal pellets will have the slowest disappearance rate. If it is broken down through the digestion process then the disappearance rate will be more rapid, since it will occur earlier than the final elimination of food in fecal pellets. In the case of chlorophyll pigments, if they are partially broken down in the gut of copepods, then the disappearance rate will be determined by both the rate of breakdown and the evacuation rate of fecal pellets. In our recent work using the mitochondrial cytochrome oxidase 1 (mtCOI) gene copy number as a tracer of feeding, disappearance rates were a result of digestion, and more rapid than the published disappearance rates of gut pigments (E. G. Durbin & M. C. Casas unpubl.).

It is generally assumed that the processes affecting the disappearance of the tracer from the gut are exponential, and exponential curves have been fitted to these data. The assumption of an exponential disappearance rate has some interesting effects on the stomach filling curve and the asymptotic value of stomach fullness given a constant feeding rate. Assuming exponential disappearance rates, the amount of food in the stomach, S_t, after t minutes is given by Elliott & Persson (1978):

$$G_t = G_0 e^{-Rt} + (1 - e^{-Rt}) I/R \quad (4)$$

In Fig. 1 we used chlorophyll pigments as an example of a tracer and calculated the change in stomach content over time for a copepod feeding at a constant rate (1 ng chl *a* min^{-1}), but with different exponential disappearance rates. The stomach fills over time and its content approaches an asymptotic value where a balance between filling rate and disappearance rate is reached. With faster disappearance rates the gut pigment content reaches this asymptotic value more rapidly than for slower filling rates. Further, the asymptotic value varies with disappearance rate: the more rapid the disappearance rate the lower the asymptotic value of stomach fullness or gut pigment

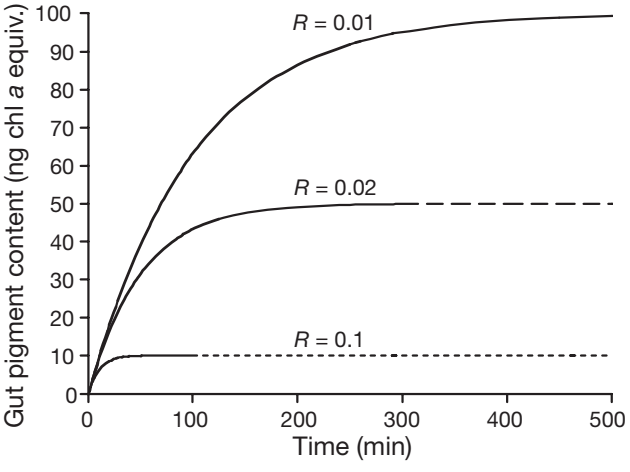

Fig. 1. Change in gut pigment content over time calculated from Eq. (4) for a copepod feeding at a constant rate (1 ng chl *a* min^{-1}) with different exponential disappearance rates of R = 0.01, 0.02, and 0.1, corresponding to chlorophyll degradation rates of 0, 50, and 90% respectively

Table 1. Pigment ingestion calculation from $I = \bar{G}R$, where I is the ingestion rate (ng chl *a* equivalents min^{-1}), G is the gut pigment content (ng chl *a* equivalents), and R is the pigment disappearance rate (min^{-1}). Values of G are based on equilibrium gut pigment contents and R from the assumed pigment disappearance rates reflecting the level of pigment degradation in Fig. 1. Pigment correction factor (PCF) is the correction factor that would be applied to the estimated ingestion rate (I), and I_{corr}, the corrected ingestion estimate based on the level of pigment destruction (PD) in the gut

PD (%)	G	R	I	PCF	I_{corr}
0	100	0.01	1	1	1
50	50	0.02	1	2	2
90	10	0.1	1	10	10

content. From a knowledge of these disappearance rates, the feeding rate used in this example can be calculated from the stomach content data shown in Fig. 1 using Eq. (1). This means that while differing food tracers disappear at different rates, as long as the amount of the tracer in the gut of the copepod and disappearance rate is known, the ingestion rate can be calculated.

Based on this discussion we argue that the gut pigment method is valid and that any pigment destruction that may be occurring is not an issue in the calculation of the ingestion rate.

This also means that any correction to ingestion rate based on estimated pigment destruction rate is inappropriate and that published data where this was done should be adjusted accordingly. Estimates of pigment destruction used in published reports vary from 0 to 90% (Bamstedt et al. 2000). To account for pigment loss, many investigators have applied correction factors. Depending on the amount of pigment degradation being corrected for, ingestion rates can be substantially overestimated. If we assume that the different evacuation rates of 0.1, 0.02 and 0.01 min^{-1} used in the example above are caused by pigment degradation rates of 0, 50 and 90%, and that these correction factors are applied to the estimated ingestion, then these ingestion rates are overestimated by as much as an order of magnitude (Table 1). This has important consequences for models that incorporate mesozooplankton grazing impacts on primary production; grazing impacts will be severely overestimated if a correction for a high rate of pigment degradation is included.

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