Part B :

A simple, seagoing method to determine gut passage time in an appendicularian

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Summary of talk on current progress. EC OMEX-II (Ocean Margin EXchange Processes) project. Lisbon. 27 April 1998. Not to be cited without permission from the authors.

ABSTRACT: Measurement of grazing rates by the gut pigment technique relies on the determination of the individual gut content and gut passage time. We have developed a new technique to determine the gut passage time of an appendicularian without the use of marker particles which are known to present a variety of shortcomes. The technique is based on observation of fecal pellet circulation along the digestive system. In addition we have found the fecal pellet production rate to be highly correlated with gut passage time. This physiological variable can be measured by incubation experiments which do not involve microscopical observation in cold rooms, and that can be used as a proxy to estimate gut passage time during research cruises where microscopical observation is limited by weather conditions and by space availability in cold rooms. Currently, we are working on the development of equations to estimate gut passage time from a set of predictor variables like temperature, food concentration or body size which could be used when it is logistically impracticable to conduct experimental work onboard.

One of the objectives of University of Oviedo within OMEX-II is to measure the grazing rates of gelatinous zooplankton, and more specifically of appendicularians, by in situ techniques, to estimate their grazing impact on phytoplankton prey and on the export of particulate material to the sediments. The gut pigment technique has become a popular approach for measuring zooplankton grazing on phytoplankton under field conditions. This technique is based on the quantification of autotrophic food within the gut of field collected grazers by measurement of their chlorophyll content. The gut pigment content in combination with a measurement of gut throughput time, i.e. the elapsed time between ingestion and defecation of a food item, can be used to calculate individual ingestion rates (Fig.1). Individual ingestion rates times population abundances allows scaling up from individual to population ingestion rates.

Much of our work during this startup phase of the OMEX-II project has dealt with setting up novel techniques to measure gut throughput rates in *Oikopleura dioica* (video). The animal, consisting of a trunk and a long tail, lives inside a gelatinous filter house, which is used as a particle concentration system. This filter house is discarded periodically, and a new one is then secreted by the animal. The gut of oikopleurid appendicularians consists of a bilobated stomach, a vertical intestine, a median intestine and a rectum (video). Captured food particles enter continuously in the stomach and are progressively compacted into a food bolus, which once formed passes into the vertical intestine as a conspicuous fecal pellet, which is then sequentially translocated to the median intestine and to the rectum before its defecation.

So far techniques to measure gut passage time in appendicularians involved the use of marker particles, which are added to the food suspension and then followed by microscopical observation in their passage through the gut. However, this experimental approach involves manipulation of both food quality and concentration, and it is often hard to be sure of the precise moment in which the marker is being ingested. But most important, addition of the marker might happen, by chance, at the beginning, at the middle or at the end of the fecal pellet formation. Thus, our measurement of gut passage time has a systematic error term due to indetermination in the exact moment in which the

marker is added (Fig.2). How can we devise a technique to measure gut passage time of *O. dioica* without the shortcomes of using markers? We sought an answer by carefully observing food circulation along the gut.

To measure gut passage time we need a precise timing of the moment of ingestion and defecation of a given food particle. All particles incorporated into the same fecal pellet are defecated at the same time (Fig.2). It is thus possible to define precisely a defecation time. However, not all particles are incorporated into a fecal pellet in the same moment. Note that, if a food particle has been incorporated to that fecal pellet at the beginning of its formation, it will spend more time within the digestive system than a particle that has been incorporated at the end of its formation. Therefore only an average time of incorporation to a fecal pellet has operational sense.

Here we will define the average time of incorporation of a particle into a fecal pellet as the middle point between the time at the initiation and the time at the end of the fecal pellet formation. For a given fecal pellet, this two moments can be easily timed by visual observation: a new pellet starts to form when the previous pellet leaves the bilobate stomach (t_0), and a pellet is fully formed when it leaves the stomach (t_1) (Fig. 3). Then the average time of ingestion of the particles incorporated into a fecal pellet will be given by $(t_1-t_0)/2$. The time at which a fecal pellet is defecated (t_3) is a precise measurement of the defecation time for all particles belonging to that pellet. Therefore, the average gut passage time will be given by the difference between the time of defecation of a fecal pellet and the average time of incorporation of a particle to that pellet $t_3-(t_1-t_0)/2$ (Fig. 3).

But what if you cannot use a microscope or do not have a cold room onboard a ship to measure gut passage time? It is very easy to do a short bottle incubation and see how many pellets an animal produces per unit time – a fecal pellet production rate. The inverse of the fecal pellet production rate is the time interval between defecation of successive fecal pellets or defecation interval (t_3-t_2). So it is feasible and simple to measure the defecation interval onboard a ship by incubation of wild captured animals. Interestingly, there is a strong correlation between the defecation interval and gut passage time, what is not surprising as they are different aspects of the same physiological process. A linear regression of gut passage time on defecation interval explains more than 90% of the total variance in gut passage time (Fig. 4). This means that, by measuring time between defecation of successive fecal pellets we can accurately estimate gut passage time.

Thus, the way to go onboard a ship would be (Fig 5): net-collected appendicularians are forced to secrete a new filter house, to eliminate previously produced fecal pellets. The animals are then introduced into 20 ml vials filled with seawater collected from the desired location and depth and immersed into a thermo-insulated water bath at the desired temperature. After 30 minutes the animals are forced again out of their filter house and removed from the vials. Pellets are then preserved in formalin to be latter counted in the laboratory. Defecation interval is then calculated as the inverse of the number of pellets produced per minute.

It could well happen, as it usually happens on cruises, that your net to collect animals for experiments does not work or that it is logistically impracticable to conduct experimental work onboard. Then you may want to use well founded equations to predict gut passage time from a set of predictor variables like temperature, food concentration or body size. Much of our latest work has been directed to the characterization of the effects of these variables on gut passage time. We have detected significant effects of food concentration and temperature on the gut passage time of cultured animals. We have found gut passage time to decrease as an inverse function of food concentration (Fig. 6). There is also a strong dependence of gut passage time on temperature over a range of temperature from 10 to 20º C (Fig. 6). The development of these equations will allow estimation of a ingestion rate consistent with the environmental conditions at the sampling site when no experimental data are available.

Our newly developed techniques will be used during routine grazing rate measurements to be conducted at the coming OMEX cruises in summer 1998 and spring 1999 by University of Oviedo.