SHIFTS IN THE FUNCTIONAL RESPONSE OF *BRACHIONUS CALYCIFLORUS* FEEDING ON *SCENEDESMUS OBLIQUUS*?

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Introduction

The subject of my PhD research is on the effects of inducible defences on the dynamics of a freshwater plankton community consisting of *Scenedesmus obliquus* (green alga, phytoplankton) and the herbivorous *Brachionus calyciflorus* (rotifer, zooplankton). With this model system, I will try to evaluate the consequences of inducible defences for the flows of matter and energy through this system. I apologise for the length of this essay, but earlier versions of this essay raised so many question about my system that I decided to give a proper introduction. Anyone who states that the text can be reduced is absolutely right. As I explained, the first few paragraphs of this essay are introductory and may be skipped without loosing too much of the general phenomenon that I would like to address. If you have no time to go through the introduction, try to pick up by the end of the paragraph on functional responses, because here the part starts that I would like to discuss about.

Defences

Life on earth first existed under water, and the microscopically small water creatures have been facing all kinds of threats ever since. The fact that planktonic species are still abundant in any kind of water around us (ranging from water drops to the world's oceans), must imply that they have found means to defend themselves against all kinds of natural enemies. By definition, plankton organisms have no or weak locomotory powers. Lots of traits that have evolved in plankton may potentially act as a defence mechanism against predation, e.g. spine formation (Gilbert, 1967), altered cell wall morphology (Van Donk *et al.*, 1997), reduced recruitment to the pelagic form the sediment (Hansson, 1996) and diel verical migration (DVM: De Meester *et al.*, 1995) . It is good to realise that a trait is being called a defence when one is able to demonstrate the defensive properties of this trait (e.g.: less ingestion by the predator). However, this does not exclude that this particular trait can have other functions for the organism as well.

Inducible defences

The occurrence of the defensive traits may range from no defences at all to being defended permanently. The position of an organism within this spectrum depends on a trade-off between protective benefits and (energetic or fitness) costs. Inducible defences are positioned somewhere between these extremes: with inducible defences, the defensive state of the organism (and therefore the costs) are dependent on the abundance of the enemies that the defense protects against in the environment. Inducible defences are a widespread phenomenon in nature, which occur in different forms in diverse organisms. See for a review Tollrian and Harvell (1999).

Scenedesmus: a morphologically plastic algal genus

Scenedesmus obliquus belongs to the algal genus *Scenedesmus*, a cosmopolitan family of green algae that is being used widely as a food organism for zooplankton. The genus has highly morphologically plastic properties, which makes it difficult for taxonomists to decide which species they are dealing with on morphological properties. For example, *Scenedesmus sp.* usually appear as multicellular colonies in nature. In laboratory culture, however, the multicellularity can disappear. Continuous cultures of *Scenedesmus obliquus* are usually dominated by unicells. Under the influence of zooplankton grazers, unicellular *S. obliquus* develops multicellular colonies, while the reverse occurs in the absence of grazers. Even (1 um) filtrated medium from zooplankton that has been actively grazing on algae will induce this colony formation, while zooplankton or algal extracts alone do not have this inducing property (Von Elert & Franck, 1999). Furthermore, in the absence of these substances, the algae become unicellular again. These observations are often used as an argument to regard this inducible morphological change as a defence against grazing. Although grazing may be important, there are also other factors involved in colony formation in *S. obliquus*, such as nutrients, chemicals and environmental factors, which indicates that colony formation as such is an unspecific response and should not be viewed as a defence against grazers (Lürling & Von Elert, submitted). Inducible colony formation in *Scenedesmus* have recently received a lot of attention. However, the real significance of this phenomenon as an inducible defence in natural ecosystems remains to be investigated. There may be 2 possible benefits of colony formation. The first benefit is that the multicellular colonies may, compared to the unicells, become less easy to ingest. The second is that these colonies settle (sink) faster out of the upper water layer (euphotic zone) than unicells, which can be interpreted as an escape in time. In the darker deeper parts, *Scenedesmus* colonies disintegrate and these unicells may become resuspended again and serve as starting point for new population growth.

The colleagues of one reviewer (ID=33) observed chain formation in the bacterium *Escherichia coli* when exposed to the ciliate grazer *Tetrahymena sp.*. In the absence of *Tetrahymena*, *E. coli* became unicellular again. They attributed this to the release of chemical cues released by *Tetrahymena*, which could induce chain formation in *E. coli* as a defence against grazing. It could be a defence mechanism (but see Crespi (2001) for other reasons of 'social behaviour' in microbes), but it does not necessarily have to be induced by a grazer-released chemical. It could be that the grazing action itself induces or selects (Boraas *et al.*, 1998) for colonies. As described above, there are ways to find out more about this mechanism.

***Brachionus*: variable shapesas well**

B. calyciflorus is a cosmopolitan species, which, as its relatives *B. rubens* (freshwater) and *B. plicatilis* (salt water), is being used a lot in eco(toxico)logical reserach and in aquaculture (mostly to serve as food for fish larvae). *B. calyciflorus* is the fastest growing metazoan, which makes it a suitable species for population dynamics research. *B. calyciflorus* can, like other Brachionids, develop longer spines in reaction to chemicals from predatory zooplankton (for example by the giant rotifer *Asplanchna sieboldii*), and loose them again when no predator is present. This inducible defence makes *B. calyciflorus* an interesting species when studying inducible defences in a multitrophic context.

Functional response models

In literature, people have made a lot of attempts to make a mathematical description of the feeding **response** of organisms as a **function** of the amount of food in the environment. C.S. Holling (1959) was one of the first who tried to relate the functional response to underlying mechanistic processes. On internet you can find several nice decriptions of functional response types. The basic types that were described by Holling are distinguished by numbers, and even without referring to Holling, most ecologists know what these types stand for.

- Type I. A linear increase of feeding with food concentration, where a maximum, constant, feeding rate may be reached at a certain food concentration. In suspension feeder ecology, the latter concentration is often called the incipient limiting level (e.g.: Peters, 1984). Other terms for this type are rectilinear (because of the shape) and Blackman model (see ref. [89] in Kooijman, 2000).

- Type II. Because the animal spends time on both attacking and handling of the prey, the functional response reflects these two. The initial feeding rate (i.e.: feeding at concentraton 0) depends on the attack rate (rate at which an organism searches and finds it food), while the maximum feeding rate is determined by the total handling time of the food (e.g.: time required for capturing, ingesting, emptying of the guts, cleaning). This type is mathematically equivalent to the Michaelis-Menten enzyme kinetics (e.g., see Kooijman (2000), paragraphs 2.4 and 3.1.3). Other names for this model are curvilinear, Michaelis-Menten and Monod (Monod made a model for bacterial growth that can also be described by this model).

- Type III. Here, the searching activity becomes dependent on food concentration: the organism may not feed in times of low food abundance, while it will increase feeding with increasing food concentrations. This type of functional response may involve learning behaviour: with increasing food densities, the organism 'learns' how to handle the food more effectively.

The numbers of these functional responses do not mean anything; they are just different names for different processes of food uptake. All these functional response types require some assumptions to be able to describe feeding processes accurately:

- there is no (significant) depletion of the food during the feeding experiment. In other words: the food concentration has to remain constant. Royama (1971) and Rogers (1972) modified the Holling type II functional response into the so-called 'random predator equation' to account for exponential decay of food during the experiment.

- both food (plant, prey) and organism (herbivore, predator) are homogeneously distributed through space. Interactions between food and organism take place on the basis of the law of mass action, as if they were 2 different types of molecules that can react with each other. The independence of the functional response of the feeder abundance has been questioned by Arditi & Ginzburg (1989), who proposed that the functional response in some cases should be better described by a function of the ratio between food and the feeder. This has received a lot of discussion in ecology. Arditi *et al* (1991) showed, that within cladocera (suspension feeders) with different spatial behaviour, either ratio-dependent (with heterogenous spatial behaviour) or food-dependent (with homogenous distribution through space) functional responses can exist.

- there is an increase in feeding rates with increasing food concentrations up to a certain maximum feeding rate. This sounds logic, but in some cases the feeding rate may even drop above a certain food concentration. This may be interpreted as a confusion effect or as an density-dependent interaction within the food itself (for example: clogging of sticky particles). I shall refer to this as the type IV functional response, although I have not yet been able to locate the exact origin (see for example the reply by Asta on my draft version).

In my opinion, the basic idea of modelling is thinking about how and why a certain mechanism works, and trying to capture the essence of this within a mathematical framework. However, many ecologists seem to be looking for a model that best fits their data, which to my opinion does only lead to satisfaction on the short term, but not to knowledge that might serve ecology. This 'model shopping' may result in the proposal of draconic functional responses that do not seem to relate to any process (e.g.: the power, logarithmic and hyperbolic curves in Porter *et al*., 1982), or to the idea that 'the model that most closely fits the data is the one that most correctly describes the feeding process' (cited from Mullin *et al*., 1975, but still being practiced, e.g. Mohr & Adrian,2000). A side effect of this is that almost unresolvable statistical problems occur in deciding what model too choose when there is a lot of scatter in the data.

Will *B. calyciflorus* eat less when fed with colonies compared to unicells of *S. obliquus*?

The question that I would like to address is on the effectivity of morphological changes in *Scenedesmus obliquus* as a defence against grazing of *Brachionus calyciflorus*. To do this, one has to know more about the functional response (=ingestion rate of food as a function of food concentration) of this grazer on differentially sized algae. The functional responses of an organism on different food sources reflects the preference of the organism for these food sources. They can even be used to predict food preference or the outcome of competition between different organisms on different food sources (e.g.: Rothhaupt, 1988). Ingestion rates correlate strongly to population growth rates (Rothhaupt, 1995), so under steady state food conditions, the functional responses can also be used as a predictor of population growth rates (this is also consistent with DEB theory).

Jeschke and Tollrian (2000) showed that the protective advantage of a defence depends on prey (food) density. They reasoned that defences that affect the ingestion rates, can basically work at two determinants of the functional response: 1. the success rate of the predator (feeder), and 2. the handling time of the prey (food) by the predator (feeder). This can easily be cast in a model structure when the organism has a type II feeding behaviour, but becomes a bit more complicated when the organism has other types or shifts in functional responses.

Shifts in functional responses?

The phenomenon that I am interested in, is described in a very interesting article by K.O. Rothhaupt (1990), which states that the **type** of functional response of two species of *Brachionus* may change from type I (for algae below the optimal size) into type II (for algae above the optimal size). At higher concentrations of very large particles, a gradual transition from type II to type IV was observed. Although I have my doubts about *post-hoc* fitting a model to observations (see last paragraph of *Functional responses*), the data suggest changes in feeding behaviour (functional response) according to the size of the food particles. If these changes do occur, and they could be explained by a likely mechanism, it does not only provide insight in the feeding of *Brachionus*, but also in any size-selective predator-prey relationship. Since the functional response is involved in allmost all ecological models, more mechanistic insight into (general or species-specific) properties that may change the functional response is essential to be able to predict almost any size-dependent interaction between species.

Although the experiments were done in a very reproducible way, there are things in the original article that ask for careful reconsideration:

1. The statistics (I will not go into detail here) show significant differences between the two types of functional responses in only two out of nine cases (=type of algae per type of rotifer). In other words: the conclusions of the article seem to be rather speculative. On the other hand: of there is a good mechanistic reason to choose a certain model, this model can be preferred over “better-fitting-but-less-understanding” models (see again last paragraph of *Functional responses*).
2. The main conclusions of the article are based on a very particular size measure, namely the equivalent spherical diameter (ESD). The ESD is the diameter that an alga would have if its volume would fit into a sphere. It is calculated by taking the cubic root of the mean particle volume (MPV) of the alga, divided by (***pi***/6). An alternative calculation of the ESD (although mathematically not the same!) is by taking the mode from the particle volume-frequency distribution over different ESD classes (perhaps I should give some illustrations to this). Often it is not mentioned in literature how the ESD was calculated, although the different calculations should yield similar results as long as the volume-frequency distribution is not too skewed. However, my greatest problem with this is that the algae that were used in the experiments, had very different shapes (spherical, arcuate, cylindrical, aggregates: Rothhaupt, 1990b). It is not difficult to imagine that for example eating needle-like food particles will lead to other handling times than eating spheres of the same mean particle volume. In the case of such extreme shapes, the ESD may be too different from the real linear dimensions of the food

particle. The conclusions may therefore be artifacts that arose from the differences in shapes of the algae, and not from the ESD's.

3. The rectilinear functional response is explained by no interference between the particles and the feeding process, while the 'plateau' (maximal ingestion rate) is explained by gut packing and the rate of gut evacuation. The curvilinear funtional response is explained by increasing interference between the food particles and the food collecting process as the food particles are getting larger. The transition from a type II into a type IV functional response seems to go more gradually, and here the author also gives a plausible (but a bit rotifer-technical) argument. What I do not understand, is the sudden transition from a rectilinear into a curvilinear functional response above the optimal particle size. If such a process would occur, I would have expected a much more gradual transition. Perhaps this does indeed occur, but the model to describe it may be lacking.

Although these are points of critique, I still hope that these results can be reproduced, because they are very fascinating. That is why I am going to try this, be it under perhaps even more standardised conditions.

Experimental plans

One of the first things that I would do (in order to try to get rid of possible variability) is using isomorphic food particles. In this type of feeding experiments, this could be differently sized plastic or latex spheres (beads), which are available in a large range of sizes. This works only if we can cheat *Brachionus* long enough before it realises that there is no energy gain from ingesting these particles. Beads have been used a lot in size-dependent feeding studies, usually as tracers (e.g.: Rothhaupt, 1990b), but also 'pure', without other food particles (e.g.: Burns, 1968;; Ronneberger 1998). However, it would be preferable to have 'real' food particles that are similarly shaped (isomorphic).

In *Scenedesmus obliquus*, one can play with nutrients to obtain differently sized isomorphs. If for example the inorganic carbon concentration (HCO3-) increases, the algal cells already become larger, and the other way around. Other factors that would increase reproducibility of these experiments are preconditioning of the animals and standardising of/correcting for the size (=food collecting area) of the animals used. Assuming that the results are reproducible, this would probably decrease the variability of the results. Finally, for the link with my own research (inducible defences), I finally want to look at differences between grazing on unicellular algae and (*Brachionus*-induced) colonies of *Scenedesmus obliquus*, and see whether this has simple or more complicated effects on the ingestion.

A more detailed model of the feeding process of *Brachionus calyciflorus*

I think that it should be possible possible to model the transitions between the different types of functional responses. I already tried a very simple two-step model to try and create a transition between the rectilinear and the curvilinear type, based on the explanations given by Rothhaupt (1990). If the ingestion rate exceeds the maximum gut evacuation rate, the model switches from an 'ordinary' type II (curvilinear) functional response to this maximum evacuation rate (if we take the gut volume as constant, it is not difficult to imagine that this is equal to the maximum gut filling rate). However, in my model there is always some handling time involved for the food, so that there is always some curving in the initial part of the functional response (see graph below). However, I use Boolean operators (if, then,else) to describe this process. The model can be described as a type II functional response as long as the maximum ingestion rate does not exceed the gut evacuation rate, and as a constant (ingestion rate = gut evacuation rate) if it does. Since *Brachionus calyciflorus* is able to actively reject food particles, this does not have to be such a strange assumption. However, it works not as simple as presented here.

The mechanisms of particle retention are actually quite complicated: although suspension-feeding organisms are usually being described as 'filter feeders', the organisms do not have any filter inside. Rubenstein & Koehl (1977), Am. Nat. 111: 981-994) give a description of the dynamic options for removing particles from fluid streams. This led to the realisation of many ecologists that viscosity of the water is playing a very important role on microscopic scales. The feeding apparatus of *B. calyciflorus* has three parts that are involved in different steps of the feeding process (Starkweather, 1995)

1. the corona ('wheel organ'), a set of cilia which generates a water current (containing food particles) towards the mouth and also is used for locomotion;

2. the pseudotrochal cirrus, a set of cilia around the mouth opening, which can be bent towards the mouth opening. This creates a water mantle that diverts the incoming water flow from the mouth opening when this is necessary (for example in the case of unwanted particles);

3. the buccal cirri, a set of cilia that can manipulate the food particles in the mouth, for example by putting them I the right position to ingest or by rejecting the food particles when some sensory organs indicate that the food is unsuitable.

Only when the food particles are large enough, they can be detected, and active selection and rejection can occur. Small particles just enter the mouth opening through the water flow, and are being packed into the guts (no handling time, type I functional response with plateau when gut gets full). Many data (the data of Starkweather & Gilbert (1977) on *Rhodotorula glutinis*; Starkweather *et al.*,1979; the reviewed data by Duncan & Gulati, 1983; Rothhaupt, 1990a) suggest this feeding type for small particle densities. Large particles, on the other hand, are handled in some way (type II functional response), for example by being put in the right position or by the chewing of the throat-jaws. This is for example proposed by Walz & Gschloessl (1988), together with the Michaelis-Menten description of the feeding process. Also other data seem to suggest this (Gilbert & Starkweather, 1978; Rothhaupt, 1990a). Knowing that the incidence of closing of the pseudotrochal cirri (=rejection of the water flow coming in) increases with larger particles at higher concentrations (Gilbert & Starkweather, 1978; Starkweather *et al.*, 1979), a type IV functional response can also be imagined, which is suggested by the data by Gilbert & Starkweather (1978) and Rothhaupt (1990a).

The DEB book (Kooijman, 2000: paragraph 7.2.1, p. 235-238) offers an interesting model structure that involves a mantle saturation coefficient, and which could really generate type I functional responses and transitions between type I and type II without having to use Boolean operators (well, perhaps it does when a sudden transition is needed). If we assume some inverse relationship between particle size and mantle saturation coefficient, a whole spectrum of functional responses can be constructed, similar to figure 7.10, p. 236 in Kooijman (2000). By the way, the 3 feeding structures mentioned above have a certain resemblance to figure 7.12 in this book. Kooijman (personal communication) also mentiones that a (transition to a) type IV functional response can be created in several ways. It can for example be created by the effect of excretion products of food on parameter values of the predator (especially maximum feeding rate), e.g. by a negative feedback of toxic excretion products. Another way could be trough competitive inhibition of the assimilation synthesising units, described in similar ways as (Michaelis-Menten) enzyme kinetics. This is a bit more difficult to imagine for me (perhaps by some interference mechanism?), so I have to consult Bas Kooijman on this.

The take-home message of this is that DEB provides a general, non-species specific, framework to produce various types of functional responses. Together with personal observations on feeding, I will try to capture my organisms and their feeding within the (diffusion limited) syntesising units to see whether this can sufficiently describe their feeding and the possible transitions between different feeding modes.

Relevance

Why would I even bother to go into details of feeding of an organism that is as small as my hair is thick (250 µm)? Well, in the first place this is my own curiosity. But specifically, I think it can reveal very much about the relevance of colony formation in *Scenedesmus obliquus* as a defence against grazing. It may help us understanding enemy-induced morphological canges in any organism. And more general, it gives insight into size-selective feeding processes. Of course, it is not only size that matters; there are a lot more factors involved in food particle selection (e.g.: taste, smell, shape, softness and colour). But knowing a bit more of this process will give us tools that can be widely applied in ecology: For example, extensive research is going on ontogenetic niche shifts and the related change in feeding. In aquaculture, such knowledge is very much wanted to design optimal food dosing to growing larvae. Another example is foraging theory, which is based on the energetic gain that an organism gets from its food. What if the energetic content of two different sizes of food particles is similar, but either of these types can be fed on more easily? Well, I could go on like this for a while, but I hope I have convinced you of the relevance of playing with rotifers and algae.

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