

Does Predator Go for Size Selection or Preferential Toxic-Nontoxic Species Under Limited Resource?

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Abstract: Problem statement: Selectivity is common in predator-prey interaction but the selection mechanism is still unexplored and a debatable issue in modern theoretical and experimental ecology for numerous species across the globe. In present investigation we emphasized the hypothesis that the zooplankton is less inclined to opt the food based on size selectivity criteria than the preferential selectivity for the safe non-toxic food species. **Approach:** As a test bed we select one nontoxic phytoplankton (*Chaetoceros gracilis*), one toxic phytoplankton (*Microcystis aeruginosa*) and one zooplankton (*Artemia salina*). Initially the experiment is setup through the small batch cultures of Nontoxic (NTP) and toxic Phytoplankton (TPP). Both the strains of phytoplankton are collected from the deltaic region of river Subarnarekha (87°31"E and 21°37"N) and the isolation is done in the laboratory. Similarly batches of zooplankton (Brand: Red Top, USA) are also hatched and maintained at optimal conditions in the laboratory. We set off the experiments with the physical parameters viz. Photo period: 12: 12 L: D cycle, Temperature: 26-27°C, Salinity: 10 ppt and pH of the medium 7.5. To evaluate our hypothesis in restricted environment we have introduced the zooplankton in a 3 liter beaker with 75: 25 (TPP: NTP) food ratio. Biological activities (feeding) are monitored for each of the species with regular recorded biomass count on each experimental day till the predator population goes to extinct. **Results:** The mean biomass profile of zooplankton remains more or less constant at the initial stage but a sharp decline trend has been observed after the 4th day of the experiment. A similar trend has been observed for the mean biomass profile of NTP leading the population toward extinction after 6th experimental day. The entire mean biomass profile trend of TPP can be interpreted as a convolution of three growth pulses viz., initially positive, followed by a negative and terminating with a positive growth. To evaluate the bias in the result of experiment we have estimated the variance levels of sample biomasses for each of the experimental time points for each of the three species. **Conclusion:** The observed stable nature of the zooplankton biomass may be due to initial NTP uptakes but a sudden decline suggests that they are forced to feed on the TPP for survival. In absence of grazing pressure, TPP initially showed a mild positive growth but when the predator switch to TPP for food it shows a negative growth and finally due to rapid mortality of zooplankton and excretal nutrient input the growth rate again kicks up. In summary we conclude that the zooplankton (*Artemia salina*) can discriminate toxic and nontoxic food species and more inclined toward the non-toxic species if the resource available. But shortage of nontoxic species, force them to feed on toxic one, in spite of drastic adverse effect on its survival.

Key words: Toxic and nontoxic phytoplankton, zooplankton, Artemia, selective predation, limited resource

INTRODUCTION

Plankton is the most abundant food source in any aquatic food chain. Phytoplankton, in particular, occupies the first trophic level of the aquatic food chain. Plankton serves many ecological functions in an aquatic food chain. Many workers have investigated the

numerous role of plankton in an aquatic ecosystem and identified the existence of many avenues from which plankton sustains in a system under limited resources. Every predator in nature has its own signature pattern of predation and species preference may vary to a large extent as nature offers strong competitors among the predator communities and limited resource for survival.

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When numerous prey species were abundant in the predation zone, the predator will opt for random selection. But in a limited resource environment and under low prey density, non-selective predators choose its optimal prey species for survival, by enforcing the food selectivity criteria (Marleen *et al.*, 2007; Stoecker *et al.*, 1981; Yen, 1985).

Selectivity is a complex choice among all the biological species from the tiny virus particle to the largest animal on earth "Blue Whale". Selection is opted for various micro and macro habitat to facilitate the species a more stable and comfortable existence into the battle of survival ranging from food acquaintance to habitat selection, partner selection for future propagation etc. Selection in nature is a very complicated fabric of the ecosystem that holds the key to success among millions of species thriving in this planet with various extreme climates (the polar ice caps, the deserts) to the lush green forest and the scintillating marine world. Basically it is an aggregate of several factors that the species considers itself to be well enough to give itself a fair chance of optimum survival for the struggle of existence.

In nature under extreme condition predator may exhibit variety of switching and selective mechanisms in connections with their feeding behavior. A slight variation in food habits may produce a useful predatory species (Sweetman, 1936). Such deviation in feeding habits probably resulted in modifications of certain useful characters which in the course of further development become functional and permanent. In most cases however, morphological are confined to the mouth parts and the gasping organ only for e.g., Syrphid larvae may be phytophagous, carnivorous or saprophagous depending on the balance of plants and animals food in the environment (Trehan, 1943). Similarly, the switching mechanisms of omnivorous copepod (*Calanus pacifica*) from herbivorous to carnivorous is observed during the decline of phytoplankton bloom (Landry, 1981). Another behavioral flexibility of a marine predator, The common murre (*Uria aalge*) has been observed when food density decreases in the environment. It is known that flexible time budget allows some animals to buffer the effect of variable food availability (Harding *et al.*, 2007). When food densities is low individuals may allocate more time for foraging, whereas when it is abundant they might allocate more time for other activity such as rest, play, courtship (Davies and Lundberg, 1985; Hixon *et al.*, 1983). This type of behavioral plasticity is a useful trait for marine predators living in a variable environment characterized by patchy and ephemeral food resources. Also the

quality of food plays as an additional selectivity criteria of predation. Due to (Silva *et al.*, 2008) the functional feeding of Chironomid larvae (Insecta, Diptera) are probably conditioned by environmental characteristic of the aquatic system, such as the modification of substrate and input of organic matter of allochthonous origin, which reflect directly on the quality of food sources available. Similarly, European Rabbits may compromise for predation with predation risk for the highest quality of food (Bakker *et al.*, 2005). Food quality was highest close to burrows, therefore the species selecting for high quality of food should forage most intensely close to the burrows and only move further away for higher quality items or when the vegetation close to their burrow is depleted.

Aquatic systems are enriched with varieties of high quality foods for the predators. It might be possible that a predator could adopt more than one selectivity criteria at a time for selecting its prey in such natural environment. Obviously it is very difficult to identify such criteria separately from field data. Controlled lab experiments are necessary to establish such hypotheses. Size selective predation was common in nature but what will happen if the predator has to choose from limited resources under controlled environment? Will it opt for an alternate species from the given set of choice rather indulging into its prime food or it will show a buffered consumption with respect to the available food densities (food rationing)? Does it move forward toward a safe food (non-toxic) for its survival?

Before searching possible answers of these questions, we like to narrate briefly an experiment performed by Reeve (1963) to establish size selectivity and filter feeding criteria of a marine zooplankton *Artemia salina*. The experiment showed no signature that *Artemia* could discriminate different species of plant cells presented in mixtures of food. It was also observed that the species showed no appreciable ability to discriminate between nutritious and non-nutritious particles. But in the presence of toxic (unsafe) and non-toxic (safe) mixtures of food particles the behavioral activity of the same species is still unknown and unexplored. The hypothesis that the selection will be driven more naturally toward the safe non-toxic food than the optimum sized food particles. This should be tested in a proper laboratory environment for making a decision on such typical selectivity criteria. By investigating such basic choice patterns among zooplankton we tried to gain some basic knowledge on selectivity. We conducted an experiment in the spirit of Reeve (1963) with a marine zooplankton and two different food items as feed in restricted environment forcing the grazer to opt among a binary choice (either

feed or no feed). This would indeed help us to understand the basics of selection criteria if it exist and try to investigate the interrelations among the food and the consumer (i.e., the zooplankton). So in summary this study aims at to explore a basic choice pattern among the interactions of a zooplankton (*Artemia salina*) and two different algal species with intricate toxic and nontoxic nature to reflect a contrast among themselves as food items. Initial results motivate us to leap further into this aspect of zooplankton which remains quite unrevealed and little explored than it requires.

The materials and methods is attributed to the experimental setup and protocols adopted for the experiment. The results segment deals with the post experimentation data analysis as well as the findings of the experiment undertaken.

MATERIALS AND METHODS

Experimental design and protocol: The test species chosen for our experiments were *Chaetoceros gracilis* (non-toxic strain), *Microcystis aeruginosa* (toxic strain) and *Artemia salina* (zooplankton). In order to carry out the designed experiment we first cultured small batches of Nontoxic (NTP) and toxic Phytoplankton (TPP) in our laboratory environment (Guillard, 1975). Both the strains of phytoplankton are collected from the deltaic region of river Subarnarekha (87°31'E and 21°37'N) and the isolation of the species have been performed in the laboratory. Similarly batches of zooplankton (Brand: Red top, USA) were also hatched and maintained in optimal condition for experiment in the laboratory environment (Provasoli and Shiraishi, 1959). The following protocols were laid down before the experiments were initially performed: Only those zooplankton populations which have just started feeding (i.e., after 36-48 h of hatching) were selected. Determination of individual phytoplankton stock concentrations for mixing equivalent proportions of the food ratio was done before the start of the experiment. Segregation and enumeration of the zooplankton population were performed. After the initial protocols were met we started the experiments with the physical parameters viz. Photo period: 12: 12 L: D cycle. Temperature: 26-27°C, Salinity: 10 ppt and pH of the medium 7.5. The experiment was designed in a chemostat fashion. The experiments were run in 3 liter containers with mild aeration to facilitate a homogeneous mixing of the plankton population and better search rate. In all conducted experiment the toxic strain (*Microcystis aeruginosa*) has a tendency to form patch in high densities so the gentle mixing imparted a counter measure adopted to minimize such occurrence

of patch formations. Daily biomass counts were taken for both the phytoplankton with a hemacytometer and the zooplankton counts were taken with narcotized samples from the test vessel with Sidgwick-rafter chamber. The samples were returned to the test vessel after the counting process is over. During the counting process we modified the counting method to eliminate sampling errors and patch formations by the toxic strain. Counts were taken for the entire Neubauer rulings with 10 samples. For enumeration of patch we adapted the following protocol as described below: Each small square of the central grid has been divided into 16 further squares or grids with equal sizes. Now, each tiny square could be able to accommodate approximately 10 cells within a patch. Now the volume of the patch could be determined by eye estimate through the patch occupying the tiny grid area. Again from each tiny square of the central grid estimates of individual cells were taken by direct counting.

RESULTS

Data analysis and inference: We have recorded the mean biomass levels of the three species, for each of the experiment day. The experiment was terminated after the eleventh day due to massive fall of zooplankton biomass almost toward extinction. The mean biomass and Relative Growth Rate (RGR) profile curves of the three species are depicted in Fig. 1. It is to be observed that each of the profile curves are composed of several small growth pulses.

In the first panel of Fig. 1, RGR values are plotted only for first four time points. This is due to mathematical intractability of RGR metric when NTP population goes to extinction. For other two species it is defined for all the experimental time points which are depicted in second and third panel of Fig. 1. RGR profiles for NTP and ZOO exhibit some unusual trends, which are not analogous with the RGR trends of some known growth laws. The objective of the feeding experiment was to determine the feeding response of zooplankton (*Artemia salina*) when presented with mixtures containing one toxic (5 µm) and one non-toxic phytoplankton (average diameter 7 µm) already established in existing literature (Demott, 1989; Logan *et al.*, 1994). Following the same protocol as Frost (1972; 1977) we deliberately made the small cell (toxic) more abundant (75%) than the large cell (non-toxic, 25%). Therefore if *Artemia* was inclined to concentrate its feeding effort only based on the size selectivity and abundance criteria, it should not have any preference for the large NTP. But in our experiment drastic initial fall of NTP biomass (0-4 days) indicates that the zooplankton primarily was

more inclined towards the predation of NTP rather than the TPP although according to size selectivity criteria it should be more biased towards the small cell particles. The mean biomass profile of zooplankton remains more or less constant at the initial stage but a massive sharp decline trend has been observed after the 4th day of the experiment. A similar decreasing trend has been observed for the mean biomass profile of NTP which leads toward an extinction of the population after 6th experimental day. The entire mean biomass profile trend of TPP can be interpreted as a convolution of three growth pulses viz., initially positive, followed by negative and end with a positive growth.

Table 1: Mean biomass, SD of the three species against experimental time points

Time points	Species					
	ZOO		TPP		NTP	
	Mean	SD	Mean	SD	Mean	SD
1 (initial)	3.65	2.50	2.06	0.13	0.51	0.02
2	3.70	2.14	2.29	0.09	0.44	0.02
3	3.60	2.59	3.14	0.05	0.27	0.02
4	3.62	3.02	3.97	0.36	0.04	0.01
5	3.42	1.33	3.35	0.23	0.01	0.02
6	3.30	2.25	2.11	0.23	*	*
7	2.65	2.95	2.15	0.37	*	*
8	1.50	2.35	2.41	0.21	*	*
9	1.00	1.05	3.32	0.20	*	*
10	0.85	1.34	4.63	0.27	*	*
11 (final)	0.40	0.96	5.33	0.18	*	*

*: Denotes extinct population from the system

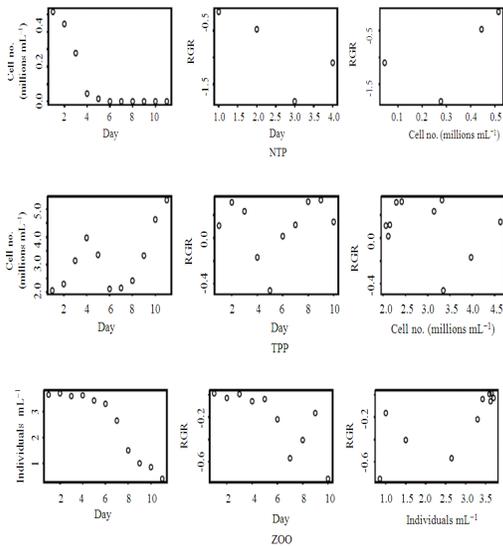


Fig. 1: Biomass levels of NTP, TPP, ZOO in correspondence to time points, Relative Growth Rate (RGR) and RGR against mean population respectively for each species (row wise)

It is quite interesting to study mean biomass profile of the zooplankton after the NTP was terminated from the system (time point 6 onwards). Sudden decline of the biomass profile suggests that they were forced to feed on the TPP for survival and it has a significant negative impact on the population by causing a rapid mortality due to adverse toxic effect. The observed rapid growth of TPP might be due to the recycling nutrient input from the zooplankton (donating nitrogenous excreta) and less predation due to the massive fall of zooplankton biomass. These results were depicted in Fig. 1. These findings supported our hypothesis, a priori, of the experiment. We infer that with available resources *Artemia salina* could discriminate the toxic and non-toxic food species. *Artemia* is more inclined to opt for non-toxic species which is comparatively a bigger size than the toxic one. In the long run shortage of non-toxic species, forced them to feed on toxic one although it has a drastic negative impact on its survival. To evaluate the bias in the result of experiment we have plotted (Table 1 and Fig. 2) of the variances level of sample biomass for each of the experimental time points separately for three species.

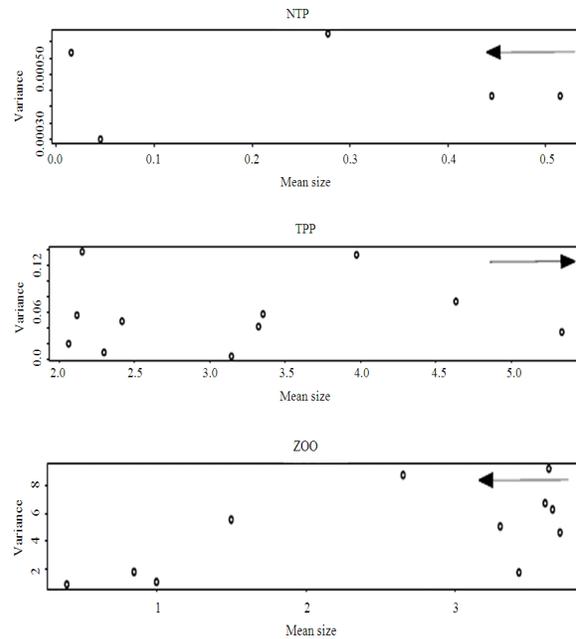


Fig. 2: Sampling fluctuations in terms of variance levels for each of the three species. Left and right arrows depicted in each panel of the figure indicate that the species biomasses are decreasing and increasing respectively

The variance of NTP remains almost constant with the population size representing less bias in sample measurement. On the other hand TPP shows a moderate sample fluctuations initially might due to few small patches in the sample. But as time progress, the size of the patches will increase leading to high sample fluctuations. Initially the sample fluctuations of zooplankton are high due to biased measurement of collected samples. The non-toxic food provides more energy to the zooplankton at the initial stage leading to erratic movement of the species resulting in a biased measurement. As soon as the zooplankton starts to feed on toxic species, it becomes less reactive due to the adverse effect of such predation. This negative impact helps in minimizing the sample fluctuations.

DISCUSSION

To observe the food selectivity of *Artemia* we performed a controlled lab experiment. In the conducted test, food ratio shows larger impact on zooplankton resulting to mortality of a significant population due to the toxic nature of the food to which the grazer is forced to prey upon. In the test bed we deliberately use toxic species as more abundant in the mixture than the non-toxic one (i.e., 75% toxic and 25% nontoxic food source). Initially we observe a stable biomass of zooplankton population but in the presence of toxic phytoplankton only, the species almost goes to extinct. A very small proportion of individuals were able to survive with induced morbidity and stunted growth dynamics. Our results are in agreement with the hypothesis mediated through temporal fluctuation driven species co-existence and consumer-resource interactions (Chesson, 2000; Tilman *et al.*, 1982). Growth patterns of the TPP (*Microcystis* sp.) support the research of Raven (1994, 1998); Carpenter and Kitchell, (1993) which elucidate that smaller cell size have a natural small package effect in contrast to larger cell size organisms in terms of resource utilization in a limited environment. The apparent growth of TPP in the first 2-3 days may be due to the higher grazing pressure on NTP. As a result TPP population gains some advantage for patch formation. Our experimental results reveal that sometimes the consumer behaves dynamically with the available circumstances they encounter during their life cycle and adapt accordingly. It could add in a new insight in biological adaptations manifesting toward the survival prospect of the species. The experiments we organized are an initial attempt in these directions. A similar type of earlier experiment Reeve (1963) suggests that the predator *Artemia salina* does not have the ability to discriminate different

phytoplankton species presented in mixtures of food. The species also was unable to discriminate nutritious and non-nutritious food particles.

CONCLUSION

But from our experiment we conclude that *Artemia* could discriminate toxic and non-toxic food species and more inclined toward the non-toxic species if the resource available. But shortage of nontoxic species, force them to feed on toxic one, in spite of drastic adverse effect on its survival.

ACKNOWLEDGMENT

We are grateful to Dr. B.C. Patra for his valuable advice. We also thank Mr. Bapi Saha for his untiring support and suggestions during the experiment.

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