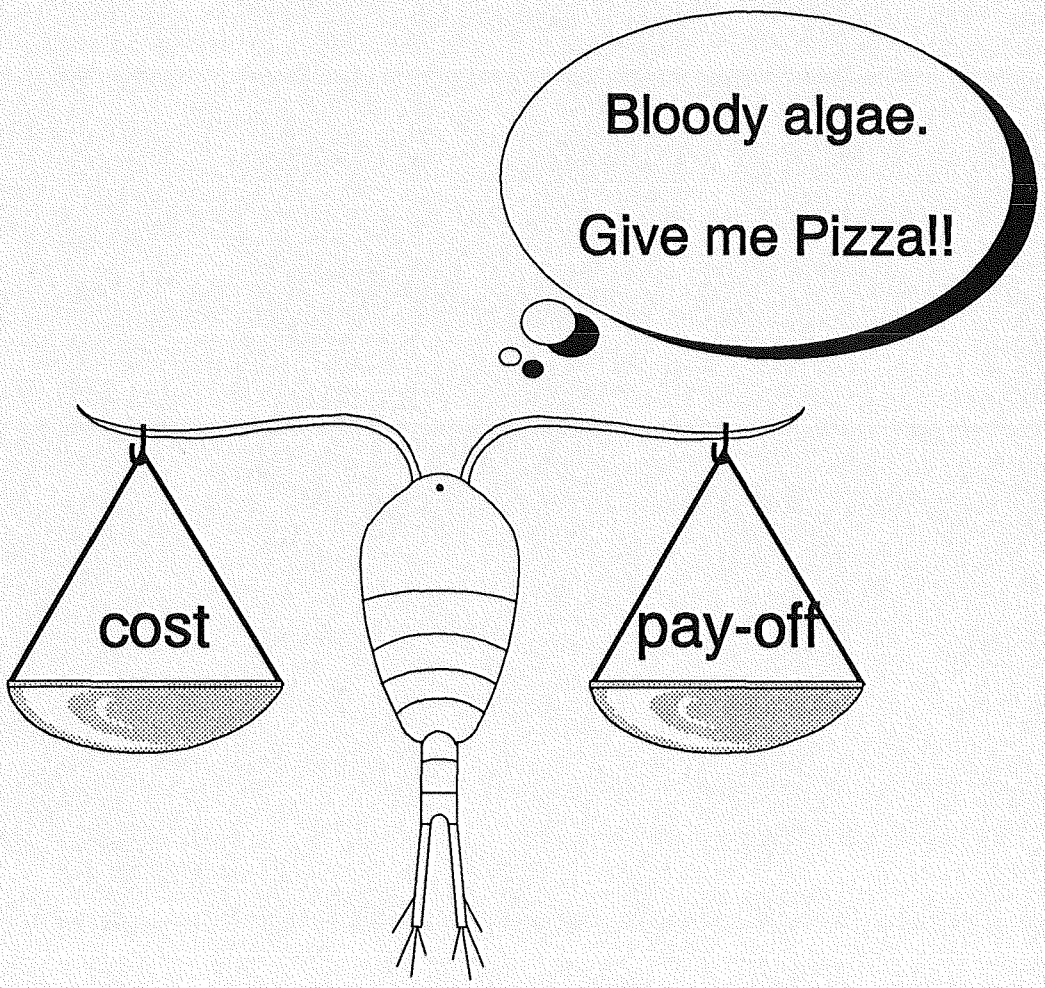
Swimming behaviour related to food concentration in copepods.



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CONTENTS

ABSTRACT

Copepods change their behaviour with changing environments. Experiments show that copepods from laboratory cultures and copepods from the field change their swimming speed at different food concentrations in accordance with the optimal foraging theory and that feeding is an active process of the copepods. More investigation are required to elucidate the relationship between food concentration and swimming path.

INTRODUCTION.

The copepods are a very abundant group of animals and form the largest part of the biomass of the zooplankton.
Ecologically these crustaceans are very important because they are the principal herbivores, feeding on phytoplankton. Therefore, they represent an essential link in the marine food chain. So an understanding of quantitative trophic interactions between phytoplankton and herbivorous copepods is required to gain insight in the amounts of energy transferred between different trophic levels of the marine food web.
In recent years it has become clear that copepods display a wide range of behaviours and that they are able to adapt their behaviour to changing environments (Gerritsen, 1978).
Swimming and feeding are closely related; calanoid copepods create a feeding current with their second antennae. This current is filtered by the plumose setae on the maxillae. Each cell is then seized and eaten (Berner, 1962). Food is perceived by mechano- and chemoreceptors (Buskey,1984).The receptors are located on the first antennae, which are extended during feeding (Gill, 1986). The distance over which an animal can perceive objects will depend on the flow field.
An animal has to solve three major problems during its life:
- increase the probability of encountering food
- increase the probability of encountering sexual partners (in the case of adults)
- decrease the probability of encountering predators.

For small animals in an aquatic environment, encountering food is a major problem. According to the theory of encounter, several factors have an influence on the probability of encounter:
- density of food/ mate/ predator. Copepods can actively search for an area with higher food concentration
- encounter radius. This is the distance over which an animal is capable of perceiving food, and it changes with size and life-stage of the animals. Copepods can actively modify their encounter radius by changing their feeding current
- swimming speed. (Gerritsen and Strickler, 1977)

Research has shown that copepods can alter their speed and feeding rate at different food concentrations (Buskey, 1984; Lam & Frost 1976). This is in accordance with the optimal foraging theory, which predicts that animals should apply a cheaper method of searching for food when food availability is low and a more expensive method when food concentration is high.
An animal has to take each decision in terms of the costs and benefits of alternative courses of action (Krebs & Kacelnik, 1991). The optimal foraging theory predicts an increase in swimming speed at a certain levels of food, because in this way the amount of food a copepod can take in per unit time balances the energy spent in swimming. The optimal foraging theory predicts also, a decrease in swimming speed after a certain level of food. When the food concentration encountered per unit time is higher than the amount of food a copepod can take in per unit time, swimming faster would not result in a higher intake and would therefore be a waste of energy and involve extra predation risk. Apart from changing its swimming speed, an animal can also change its swimming path in order to maximize food intake.
When food concentration is low optimal foraging theory predicts a straight path because the animal should try to find a feeding area where more food is available. When food

concentration is high the animals should stay in this area. Therefore in this case a curvy path would be more profitable (Buskey, 1984).
Our experiments are concentrated on the influence of the food concentration (algae) on the swimming speed of the females of one species of copepods *Temora longicornis*, the most abundant species of copepod in the north sea.
The principal questions are:
- Do copepods change their swimming speed at different food concentrations as predicted by the optimal foraging theory?
- Do copepods change their swimming path at high and at low food concentrations?
- How do feeding experiments with animals from laboratory cultures compare to the field situation?

MATERIAL AND METHODS.

EXPERIMENTAL ANIMALS

2 species of calanoid copepods were used; *Temora longicornis* and *Acartia clausi.*

Temora longicornis.

Temora longicornis was cultured in the laboratory using the same technique as Klein Breteler (Klein Breteler, 1980).
The copepods are fed on a mixture of algae from a continuous culture (*Dunaliella tertiolecta*) and a heterotrophic flagellate (*Oxyrrhis marina*). The flagellate is not cultured but it always present in the copepod cultures, where it feeds of the algae and it is required to keep the concentration on the algae and other unwanted organisms under control.
The algae are siphoned approximately twice per day into the copepod cultures through a soxhlet device. Experience has shown that copepods feed more efficiently when they get a bulk of food once or twice per day then when food is administered continuously (Klein Breteler, pers.comm.).
The algae are cultured on an F2-medium (Admiraal,W. & Werner, 1983). 10 l vessels of this medium are made about once every two weeks. Algal concentration in the continuous cultures and flagellate concentration in the copepod cultures are monitored regularly with a microscope and the presence of nauplii is checked regularly with a stereomicroscope.
Every four weeks a new culture is set up by separating the nauplii from the older stages.
During the experimental period the culture once became infected. The new animals to continue the experiments came from the centre of N.I.O.Z.

Acartia clausi.

The animals were taken from the north sea with a plankton net and left in a tank in a temperature controlled room nor one day before the start of the experiments. They were fed on a different species of algae *Rhodomonas sp.*
The copepods used in these experiments were all of a younger stage (C3-C5). Similar size animals were selected for the experiments.

EXPERIMENTAL APPARATUS

The 3-D filming set up.

Through a set of surface mirrors two orthogonal views of the aquarium are projected side by side into lens of the video camera. Because the copepods do not react to infra-red light, illumination is provided by two infra-red light emitting diodes (L.E.D.'S). These l.e.d.'s, placed in the focus of a lens (diameter 10 cm), produce a parallel light beam which is projected through the mirrors straight into the lens of the camera (I2S 800) (fig.M1).
This whole set up is situated in a dark temperature controlled, room (T=15°C for all the experiments). The camera is connected to a Sony U-matic video recorder and a monitor outside the dark room. On the monitor copepods show up as dark point against a white background.
The speed of the video tape is 50 frames/sec.

At the end of each section of registration an image of the aquarium, with a scaling grid, from the front and from the back, is recorded.

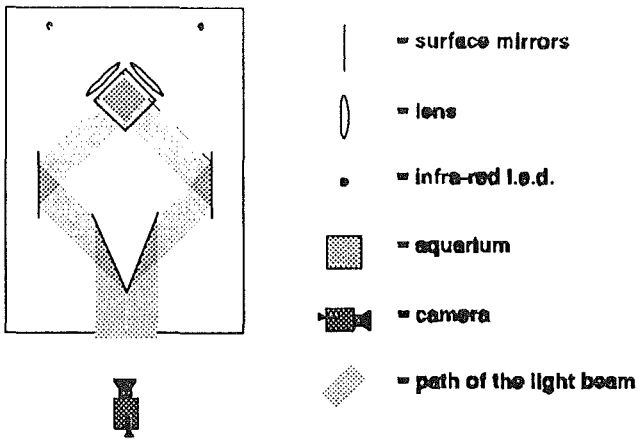

Figure M1: Description of the 3-D filming set-up.

The image analysis system.

The video tapes were analyzed with a computerized image analysis system (TIM; DIFA, Breda).
The system digitizes a video frame and divides it into 768x512 pixels with their own x and y coordinateS. At the start of each sequence of frames the animal is pointed out on either side and its coordinates are stored in a file. In subsequent frames the computer searches for the position of the animal and the coordinates are stored in the same file.
The scale factor in x and y direction is measured with the recordings of the scaling grid, to calculate the size of the pixels.

Calculating the swimming speed (Copsnel).

The program copsnel calculates instantaneous and average swimming speed from the files with pixel coordinates and the scale factors using Lagrange-differentiation and 5-point moving average (Videler, 1981).

Calculating the N.G.D.R.

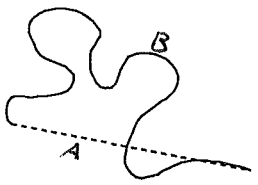

Figure M2: N.G.D.R. = A/B

The N.G.D.R. is a number that gives an indication of the curviness of the swimming path. Through this program the computer looks for the position of the animal in the first and in the last frame and for the position in every 5^{th} frame. The distance between beginning and end of the path divided by the total length of path gives the number of the N.G.D.R. This number therefore has a value between 0 and 1. When it is high it means that the animal is swimming straight, when it is low it means that the animal is following a curvy swimming path (figure M2).

EXPERIMENTAL DESIGN.

Two kinds of experiments were carried out: swimming behaviour measured at different food concentrations and the reaction of animals to an increase in food concentration.

Swimming speed measured at different food concentration.

This type experiment was carried out with *Acartia* and *Temora*.
Their swimming speed was measured at 5 different concentrations (0, 105, 10^6, 10^7, 10^8 cell/l, the most relevant concentrations).
These were chosen through a series of test experiments.

For each test 6-7 animals were selected under a stereomicroscope, the animals were put in the aquarium and left in the experimental set up for 15 minutes. Then algae were added to get the desired concentration and the animals were left for another 15 minutes. At the concentration 0 cells/l the copepods were left in the dark room for 30 minutes. 15 Sequences of 100 or 300 frames were recorded.
For the concentration of 0 and 10^6 2300 frames were processed saving them in 11 files of 100 and 4 of 300 frames each one.
For all the other concentrations, 1500 frames were processed saving them in 15 files of 100 frames each.
For the swimming speed measurement all the files of (100 and 300 frames) were used. For the N.G.D.R. measurement only the files of 300. Each concentration was repeated twice. All the measurements of every concentration of one experiment were carried out within 3 days.

Swimming speed measured immediate after change food concentration.

This kind of experiment repeated three times, only with *Temora.*
After filming 15 series of about 400 frames, algae were added to get the concentration of 10^6 and filming was started 1 minute after the addition of algae.
With the files of 300 frames the N.G.D.R. was calculated and with all the files (100 and 300) the average swimming speed was calculated.
The sequences recorded after the algae were added were divided in two groups: sequences recorded within 15 minutes after adjustment of the concentration and sequences recorded later than 15 minutes.
In total about 80000 frames were processed.

RESULTS.

SWIMMING BEHAVIOUR AT DIFFERENT FOOD CONCENTRATIONS.

Figure R1 shows the data of the pilot experiment. From the data of the first experiment the most important concentrations were established. In this experiment measurements of the

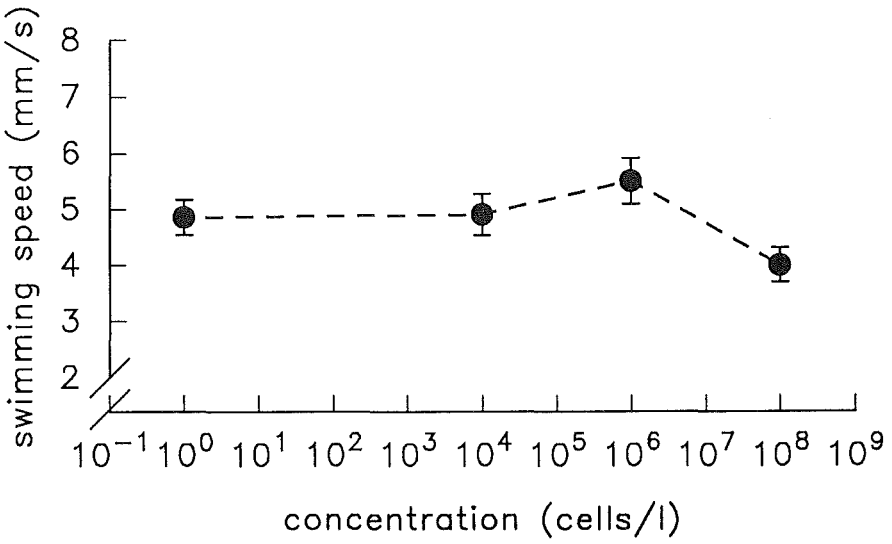

Figure R1: Experiment 1, average swimming speed at different food concentrations. Vertical bars indicate standard error of the mean.

swimming speed were carried out at 4 concentrations: 0, 10^4, 10^6, 10^8.
The swimming speed increased with the increase of the food concentration until the concentration of 10^6, after this value it decreased for each value of the food concentration. The minimum value for the speed is at the concentration of 10^8 and there is a significant difference between the value of the swimming speed at 10^6 (max.) and this value at 10^8 (min.), but there is no significative difference in the value of the swimming speed between 0 and 10^4 and between 10^4 and 10^6 (Student t-test, α=0.05).
In the following two experiments the food concentrations were 0, 10^5, 10^6, 10^7, 10^8 cell/l (fig.R2).
In both of these two experiments the graphs were nearly the opposite of fig.1r; 10^6 was the minimum and 10^8 was the maximum.
Checking the copepod cultures at the time of the third experiment showed that the condition of the cultures was bad: there were very few nauplii and the water inside the cultures was quite green. The reason was probably an infection with a ciliate which in some way interfered with the copepods.
So the experiments were continued with new animals taken from the research centre of N.I.O.Z.
The concentrations used were the same in the second and third experiments, the results are shown in fig.R3. The highest swimming speed is at the concentration of 10^5 and the lowest for 10^8. There is no significant difference between the swimming speed at the concentration

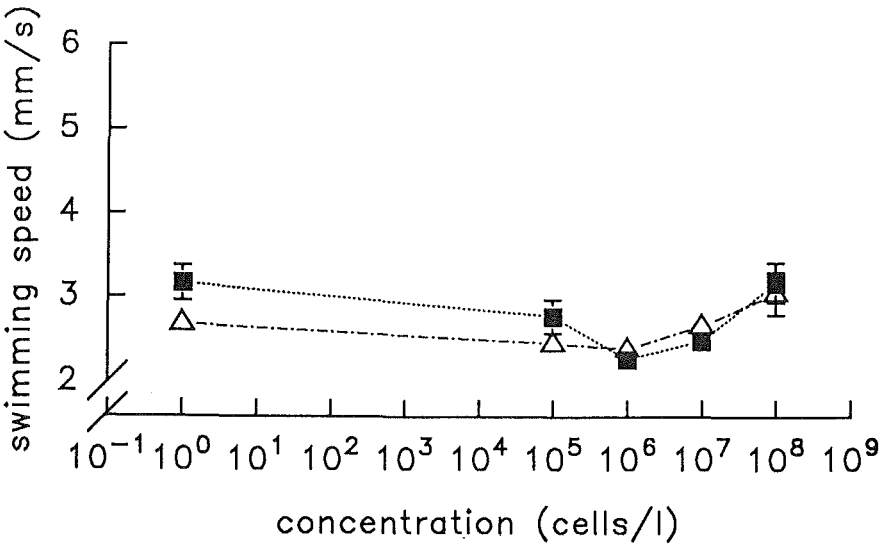

Figure R2: Experiment 2 (solid square) and 3 (open triangle). Average swimming speed at different food concentration. Vertical bars indicate standard error of the mean.

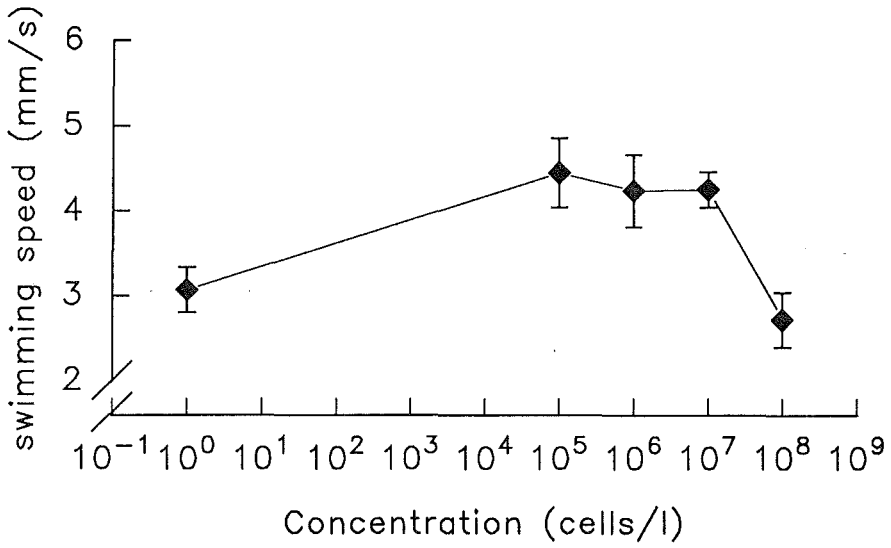

Figure R3: Experiment 4. Average swimming speed at different food concentrations. Vertical bars indicate standard error of the mean.

of 10^5, 10^6, 10^7 cell/l (student t-test α=0.05).
Fig.R4 shows the data of the same kind of experiment with *Acartia clausi*. This species has a swimming behaviour different from *Temora longicornis*; Acartia swims with a lot of stops and Temora swims more constant without apparent big stops (fig.R5).
Also in this case, like for the last experiment with *Temora*, the maximum value of the swimming speed is for the concentration of 10^5 and for the concentration of 0 and 10^8 cell/l the value of the swimming speed was the minimum. Also the absolute values of the swimming speed for these 3 concentrations were very similar. There were differences in the other 2 concentrations. but the general trend of the curve was the same for *Temora* and *Acartia*.

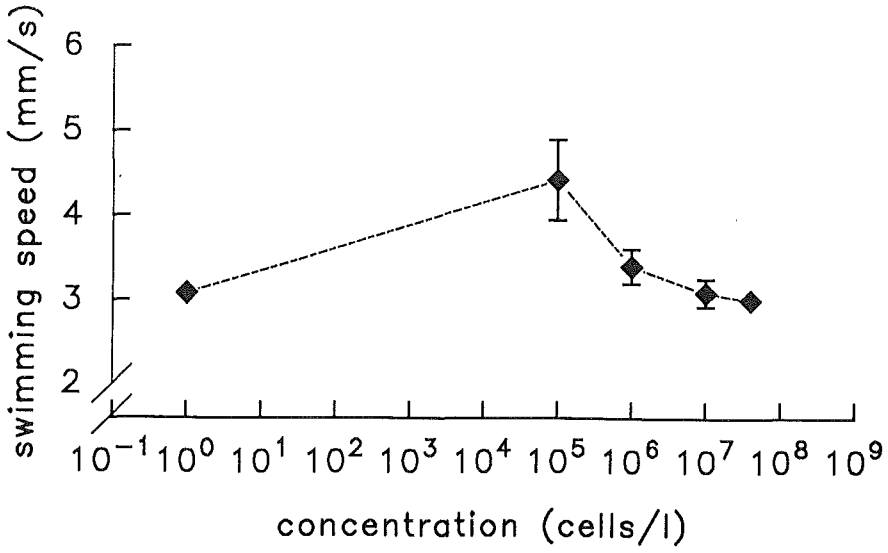

Figure R4: Experiment 5. Swimming speed at different food concentrations. Vertical bars indicate standard error of the mean.

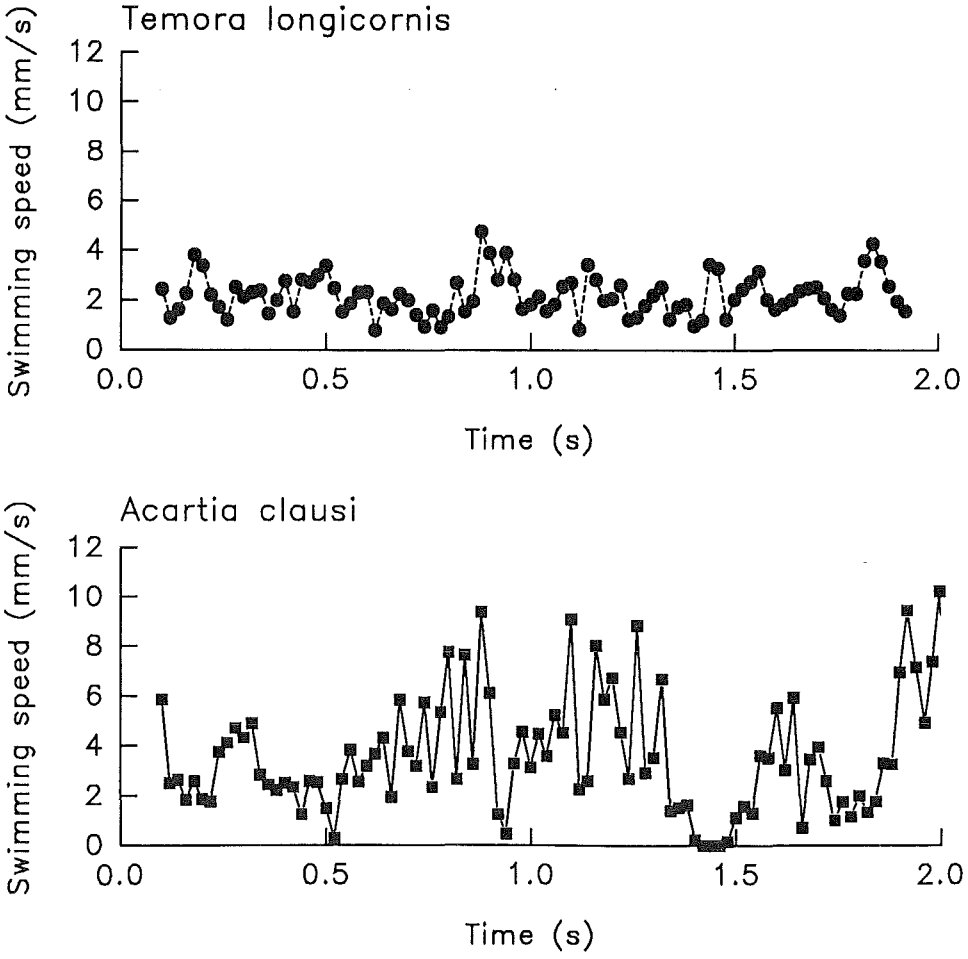

Figure R5: Swimming speed against time for *Temora* and *Acartia*.

Fig.R6 shows the results of the net to gross displacement rate (ngdr) from the experiments with *Acartia* and with *Temora* (the experiment 4). Only two concentrations were chosen: 0 and 10^6. The number of samples at each concentration was very low; for *Acartia* 4 samples and for *Temora* 3 at each concentration. This is too low for statistical comparison.

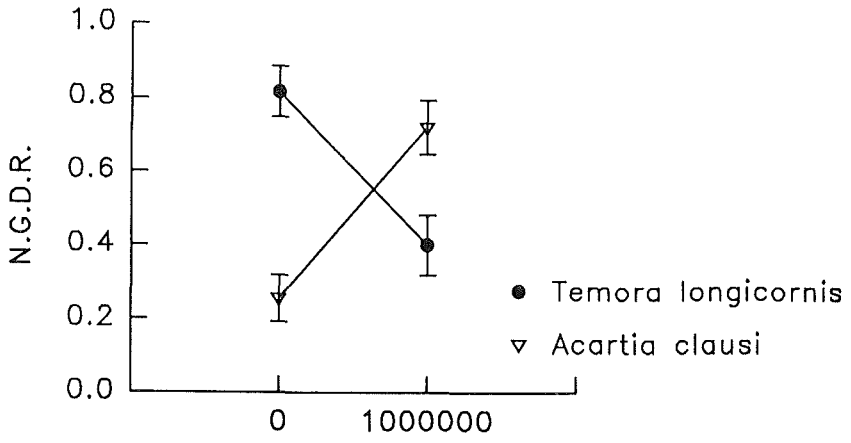

Figure R6: N.G.D.R. at two concentrations.

THE REACTION TO AN INCREASE IN FOOD CONCENTRATION.

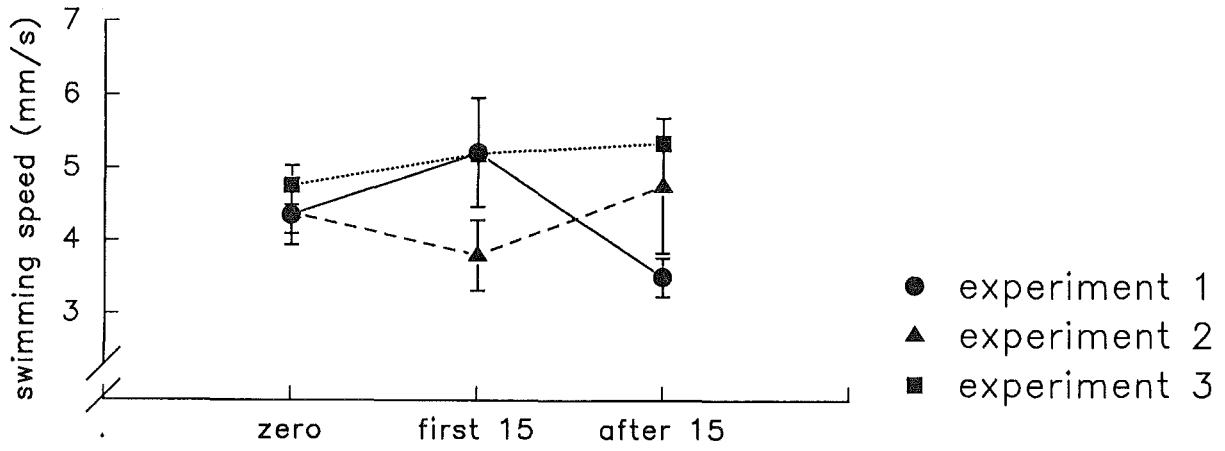

Figure R7: Experiment 6, Average swimming speed. Vertical bars indicate standard error of the mean.

In fig.R7 the results of the swimming speed for 3 experimentS are shown (see table 1 for the number of sequences of frames processed). The results of the swimming speed measurements immediately after the change in food concentration were very different for the 3 experiments. The first part of the graph (the difference between 0 and first 15 minutes) of the first and third experiment were according to the optimal foraging theory; an increase of the swimming speed with the increase of the food concentration. The value of swimming speed after 15 minutes in the third experiment is not significantly different from the value at first 15 (Mann-Whitney u-test).In the first experiment the swimming speed decreases after 15 minutes. The results of the second experiment are completely different from the predictions of the optimal foraging theory; a decrease of the swimming speed between zero and first 15 and an increase

between first 15 and after 15 minutes.

Table 1: number of samples in the three experiments about the reaction in swimming speed to an increase in food concentration.

experiment	zero	first 15	after 15
1	14	7	8
2	15	10	5
3	15	7	8

Table 2: number of samples in the same three experiments of tab.1 about the reaction in swimming path.

experiment	zero	first 15	after 15
1	10	5	6
2	10	7	3
3	10	6	4

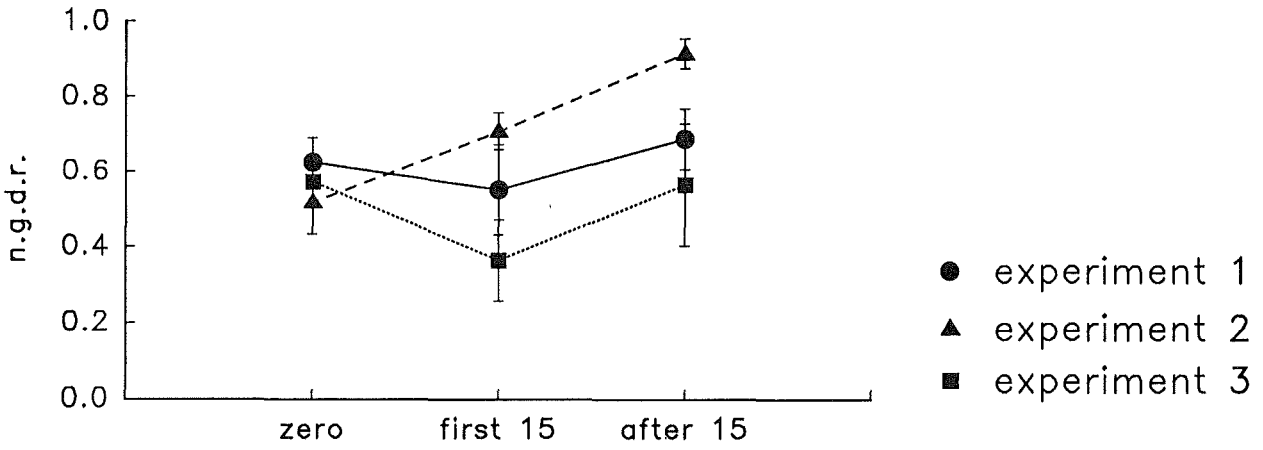

Figure R8: Experiment 6, N.G.D.R. Vertical bars indicate the standard error of the mean.

Fig.R8 shows the results for the same three experiments of the N.G.D.R. (see table 2 for the number of sequences of frames processed). The experiment 1 and 3 show the same trend; a decrease of the N.G.D.R. between zero and the first 15 minutes and a decrease between first 15 and after 15 minutes. The difference between the values of the N.G.D.R. at zero and at after 15 in the experiment 1 and 3 are not significant but if we put the results of these two experiments together the difference between this two points are significant (Student T-test $p<0.05$).

Like for the swimming speed, the results of the N.G.D.R. for the second experiment were very different from the predictions; a constant increase of the N.G.D.R.

DISCUSSION.

SWIMMING BEHAVIOUR AT DIFFERENT FOOD CONCENTRATIONS.

Other authors have shown a relationship between feeding behaviour of the copepods and concentration of food. The volume swept clear by the copepods increases linearly with cell concentration up to a maximum rate. As the cell concentration increases after this value, the volume swept clear decreases (Frost, 1972).
From these experiments can not be concluded whether this is a 'passive' process which depends on the food concentration , or if it is an 'active' process because the copepods are able to adapt their behaviour to changing environmental conditions.
The results of experiment 1,4,5 show that the copepods change their swimming speed exactly as predicted by the theory. The swimming speed of the copepods at the difference food concentration is the result of an active choice of the animals and is not a passive consequence of the changing condition of the environment.
This is most clearly demonstrated from the second part of the graphs when the swimming speed after the maximum value starts to decrease. This shows that the animals actively choose to decrease their speed to balance the amount of energy.
Comparing the graphs of these 3 experiments, it is easy to see that in each experiment the values of the swimming speed at the same food concentrations were different every time. This means that the values of the swimming speed are influenced by a lot of factors and so the data are to be considered relative and not absolute.
A possible explanation for the strange results in experiments 2 and 3 (fig.R2) is that the strange organisms found in the culture react with the receptors of the copepods and interfere with their perceptive ability. So when the food concentration is relative low (between 0 and 10^6 cell/l) they may not increase their swimming speed because they are not able to perceive the food, and therefore are not able to feed efficiently. Only at much higher concentrations they are able to take in enough food so they can afford to increase their swimming speed.
The results of the experiments with *Acartia* are not directly comparable with the results of the same kind of experiments with *Temora*. First of all the species have a different way of swimming. Secondly the conditions between these experiment were very different (the kind of algae used and the life stage of the copepod) but the trend of the curve is indicative for the swimming behaviour. These results for the two species show that the copepods are able to change their behaviour to the different food concentration to balance their amount of energy in the way predicted by the optimal foraging theory and that this conclusion is the valid for animals from laboratory cultures and animal for caught from the sea.
In general we can conclude that copepods modify their swimming speed according to the optimal foraging theory but that it is impossible to establish a quantitative relationship between swimming speed and the difference food concentration because of the large number of factors that influence the swimming speed such as: condition of the animals, different species of animals,different life stages and different species of algae.
The results of the measurement of N.G.D.R. are inconclusive, but the behaviour of Acartia seems the opposite of what we expect on the basis of the theory.
The number of samples used to calculate the N.G.D.R. are too low (n=3 and n=4). This sample size is too small for an accurate estimate of the N.G.D.R. and so they are not useful to give an indication about how the animals change their swimming path at low and high food concentration.

THE REACTION TO AN INCREASE IN FOOD CONCENTRATION.

From the results of the swimming speed experiments no conclusions can be drawn. Because of the limited number of samples it can be not established how copepods change their swimming speed to a change in food concentration. More of this kind of experiments with healthy animals are needed.
The results of the N.G.D.R. for experiment 1 and 3 suggest that the change of swimming path of the copepods is more influenced by the change of the food concentration rather than the absolute concentration.
But this conclusion is rather speculative for a number of reason. First of all, the number of samples is limited. Secondly, the length of 300 frames for these analysis were chosen arbitrarily, possibly longer sequences need to be analyzed for accurate estimates of the N.G.D.R. Thirdly, also the period of acclimatization of 15 minutes was chosen arbitrarily. Also this period may be longer or shorter. More research has to be done in the future to understand this aspect of the swimming behaviour of the copepods.

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