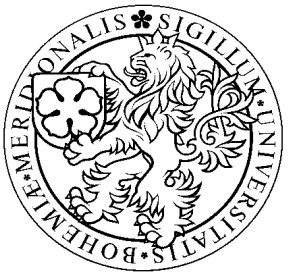
UNIVERSITY OF SOUTH BOHEMIA
FACULTY OF SCIENCE

České Budějovice 2008



Ph.D. Thesis

Effect of food quantity and quality on population growth rate and digestive activity in planktonic rotifers

Martina Štrojsová

Supervisor: Doc. RNDr. Jaroslav Vrba[1,2], CSc.
Tutor: RNDr. Jaromír Seďa[2,1], CSc.
[1]University of South Bohemia, Faculty of Science and [2]Biology Centre AS CR, v.v.i., Institute of Hydrobiology

Štrojsová, M., 2008: Effect of food quantity and quality on population growth rate and digestive activity in planktonic rotifers. Ph.D. Thesis, in English. – 65 pp., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic.

Annotation

As homeostatic organisms, rotifers have to use the mechanism to cope with nutrition unbalance in their food. The regulation of digestive enzyme activities as a possible physiological mechanism involved in maintaining of rotifer homeostasis was studied. This study further explored the effect of food quantity and quality on rotifer population growth rate and reproduction.

Financial support

The studies described in this thesis were supported by the Grant Agency of ASCR (A6017202 awarded to J. Vrba, IAA600170602 awarded to J. Nedoma, 1QS600170504 and AV0Z60170517 both awarded to J. Matěna), by the Czech Ministry of Education, Youth and Sports (MSM 6007665801 awarded to H. Šantrůčková) and by the Japan Society for the Promotion of Science (PE 07026 awarded to M. Štrojsová).

Prohlašuji, že jsem svoji disertační práci vypracovala samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury.

Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejně přístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách.

V Českých Budějovicích, 11. září 2008

Martina Štrojsová

Acknowledgements

To begin, I would like to gratefully acknowledge the enthusiastic supervision of Jaroslav Vrba during this work. Jaroslav Vrba has been generous with his help; he taught me how to write scientific papers and provided me with excellent facilities for my study. He left me space when needed and pushed me when necessary. His encouragement and constant guidance were necessary to finish this thesis.

I highly appreciated valuable discussions on the zooplankton issue with Jaromír Seďa. Many thanks also to Jiří Nedoma for intensive tutoring on the Lucia image analysis software and for other constructive advice. I thank Miloslav Devetter for introduction to world of rotifers. I am very grateful to my colleagues from the Institute of Hydrobiology, who helped me in many ways. I acknowledge Hana Šantrůčková for being a really good leader of the Department of Ecosystem Biology throughout my study and I also thank Karel Šimek for the successful and employee-friendly leading of the Department of Aquatic Microbial Ecology. I would like to acknowledge the Faculty of Science and the Institute of Hydrobiology for continuous financial supports and for their generosity in making their equipment available. I highly value the cooperation with Atsushi Hagiwara from Nagasaki University and I am also thankful the members of the Aquaculture Biology Laboratory who made my time in Nagasaki very inspirational. I thank Wilko Ahlrichs who taught me how to prepare rotifers for SEM.

I would like to express special gratitude to my parents Marie and Zbyněk, for their confidence and years of support. A big thanks to my sister Alena and brother Jan for their friendship and kindness. Apart from that, I am grateful to Alena for her help in scientific field, especially in manuscript corrections. Finally, I would like to thank Tomáš Konvička for his love, empathy and cheer.

TABLE OF CONTENTS

The PhD thesis is based on three original articles and two manuscripts, which are listed below. Co-authors participated in the design of the experiments, read and improved the manuscript. I declare that I did the major contribution to each of the papers.

Štrojsová, M. and Vrba, J. (2005) Direct detection of digestive enzymes in planktonic rotifers using enzyme labelled fluorescence (ELF). *Marine and Freshwater Research*, 56, 189–195.

Štrojsová, M. and Vrba, J. (2007) Rotifer digestive enzymes: direct detection using the ELF technique. *Hydrobiologia*, 593,159–165.

Štrojsová, M., Seďa, J., Nedoma, J. and Vrba, J. (2008) Diet quality impact on growth, reproduction and digestive activity in *Brachionus calyciflorus*. *Journal of Plankton Research*, issue 10(30), *in press.*

Štrojsová, M. and Ahlrichs W. H. Epizooic bacteria on *Brachionus calyciflorus*: possible relationship with rotifer enzyme activity. *Manuscript.*

Štrojsová, M., Suga, K., Hagiwara, A. and Vrba, J. Effect of food quantity and quality on growth, reproduction and digestive activity in the euryhaline monogonont rotifer *Brachionus plicatilis* Müller. *Manuscript.*

CHAPTER 1

pages 1–8

Introduction

GENERAL INTRODUCTION

Rotifers

Phylum Rotifera is a group of primary freshwater invertebrates. Rotifers are characterized by an anterior ciliated corona, a rigid lorica (may be missing in some species) and a mastax containing trophi. Rotifers are tiny animals (50 – 2000 μm), but they often occur in abundance up to 1000 ind. L^{-1}. Cosmopolitan rotifers can be found in almost all types of freshwater ecosystems, where they have crucial importance (Segers, 2008). Though rotifers are not the keystone species as are considered the cladocerans (De Bernardi and Peters, 1987), rotifer population density and productivity could sometimes be higher than other metazoan populations (Yan and Geiling, 1985). Consequently, they may have a large impact on nutrient recycling in freshwaters (Ejsmont-Karabin, 1983; Walz, 1995). Their success can be explained by the high rate of reproduction and fast adaptation to changing conditions of the environment (Herzig, 1987).

Rotifer digestion

Feeding is an important interaction of rotifers with their environment (Walz, 1997; Ooms-Wilms *et al.*, 1999; Merriman and Kirk, 2000; Yoshida *et al.*, 2003). Rotifers are active predators of other plankton, including members of their own phylum; they are important feeders mainly on algae and bacteria (Arndt, 1993). Since rotifers are not able to take up mineral nutrients directly, they are dependent only on their food to obtain the needed amounts of essential elements (Jensen and Verschoor, 2004). Therefore, the ability to digest sufficient food is a key factor for rotifer maintenance metabolism, growth and reproduction. Rotifers often face a different food quantity and quality and can be limited by food via energy, when carbon (C) assimilation is low because of low quantity of edible food, via essential elements such as nitrogen (N) and phosphorus (P) or biochemical compounds (e.g. fatty acids) and also via algal toxins that can limit rotifers by feeding inhibition and direct toxicity.

Nutrition is a multiple step process, which involves ingestion, gut transit time, digestion, absorption, excretion and egestion. Digestion of food particles and nutrients is through cooperative function of the digestive system, which consists of mouth, buccal tube, mastax, esophagus, salivary and stomach glands, stomach, intestine, cloaca and anus (Ruttner-Kolisko, 1974). Two types of digestive enzymes were identified in rotifers (Kühle and Kleinow, 1989; 1990): (i) membrane bound enzymes, which act on the luminal side of plasma membranes of stomach cells, and (ii) secrete enzymes, which are released from glands of digestive tract.

Enzyme assay

Hydrolytic enzymes, involved in rotifer digestive processes, could be assayed either directly at the site of enzyme action in the intact rotifers or in the rotifer homogenates. We used both approaches, (i) the Fluorescently Labelled Enzyme Activity (FLEA; previously wrongly called the ELF technique) technique (Gonzáles-Gil *et al.*, 1998), which gives information about the localization and, with the help of image cytometry, the quantity of enzyme activity (Nedoma *et al.*, 2003), and (ii) spectrofluorimetric method (Hoppe, 1983) using 4-methyllumbelliferyl phosphate sodium salt (MUFP) as a substrate, which enables to measure bulk enzyme activity.

The FLEA technique was employed for direct localization or measurement of enzyme activities of rotifers from the field and from the non-axenic cultures. The fluorogenic ELF®97 substrates are soluble in water and could be ingested by the rotifers with their food. After enzymatic hydrolysis, the non-fluorescent substrate turns into insoluble fluorescent ELF alcohol that precipitates and tags the enzyme activity outside or inside the rotifer body. It has been focused on phosphatases, β-N-acetylhexosaminidases and lipases. Because non-axenic cultures were used, the contribution of enzyme activities of other organisms than *B. calyciflorus* had to be considered.

For the assay of bulk activity in the rotifer homogenate, fluorogenic MUFP was used. MUFP is hydrolyzed by phosphatases yields a fluorescent soluble product, its fluorescence is measured using the spectrofluorometer. Because the localization of enzyme action is not possible using this technique, axenic rotifer and algal cultures were used.

Stoichiometric regulation

Ecological stoichiometry uses the balance of energy (C) and nutrients (N, P) in organisms to explain the relationships between organisms and biogeochemical cycles (Sterner and Elser, 2002). Algae, a main rotifer food source (Arndt, 1993), can vary extensively in C:nutrient ratios (Hessen *et al.*, 2004). On the other hand, rotifers have a more fixed stoichiometry and commonly lower C:nutrient ratios and have to take up their nutrients in ratios as supplied by their food. Consequently, when the C content of rotifer food increases, a larger fraction of C will be in excess relative to the rotifers demands, and will be lost as excretion, egestion or respiration. This could lower the growth efficiency, and thus limit the rotifer growth.

Rotifers have to use the complex strategies to deal with unbalanced food quantity and quality. Stoichiometric regulations dealing with not balanced food operate either pre- or postabsorption and include changes in uptake, incorporation and release of elements (Frost *et al.*, 2005). Physiological solution, which operates before absorption involves changes in the rate of ingestion, which could be either decreased that leads to an increase in a gut transit time (Sibly, 1981; Horn and Messer, 1992) or increased that leads to an decrease in a gut transit time (Darchambeau, 2005; Mitra and Flynn, 2007). Animals could also regulate the secretion of gut enzymes (Darchambeau, 2005). On the other hand, consumers could maintain their homeostasis by using postabsorptive mechanisms that involve transferring of excess C via respiration and excretion of dissolved organic C (Darchambeau *et al.*, 2003). Likewise, model by Anderson *et al.* (2005) suggested that consumers maintain their homeostasis mainly by using postabsorptive mechanisms rather than using regulation before absorption by the gut.

N and P limitation

Traditionally, studies have been focused on food quantity and the studies of food quality mainly focused on the effects of particle size and morphology. Recent studies have shown that not only food quantity but also food quality affects the rotifer growth. As N and P represent the main limiting elements in freshwater systems (Sterner and Elser, 2002), N- and P-depleted algae could reduce the rotifer growth (Rothhaupt, 1995; Conde-Porcuna, 2000; Ramos-Rodríguez and Conde-Porcuna, 2003; Jensen and Verschoor, 2004; Lürling, 2006; Hessen *et al.*, 2007).

The key idea of ecological stoichiometry is the growth rate hypothesis (GRH), which states that there are positive relations between organismal growth rate, the body content of RNA and P (Elser *et al.*, 1996). Ribosomes, sites of proteins synthesis, are composed of P-rich rRNA needed for the rapid growth. This suggests that fast growing organisms may have high P demands and low C:P atomic ratio (Frost *et al.*, 2006). However when N-limitation occurs, connection between growth rate, RNA and P may be relaxed (Elser *et al.*, 2003). As proteins are N-rich, their ribosome synthesis could also be limited under N-deficiency. Thus more P for RNA does not enhance growth under N-deficiency (Hessen *et al.*, 2007).

OBJECTIVES AND OUTLINE

The main objectives of this thesis were to: (i) optimize the FLEA technique for assessing rotifer enzyme activities, (ii) set up the rotifer cultures needed for the use in feeding experiments, (iii) localize the activities of phosphatases, β-N-acetylhexosaminidases and lipases within rotifer body, (iv) determine the effect on population growth rate, reproduction and phosphatase activity in digestive tracts of *B. calyciflorus* when feeding on P-replete and P-depleted algal food and (v) evaluate the effects of both nutrient quantity and quality, i.e. nutrient-replete, P-depleted or N-depleted food on population growth, reproduction and phosphatase activity of *B. plicatilis*.

Chapter 2 focuses on localization of activities of phosphatases, β-N-acetylhexosaminidases and lipases, which were investigated in planktonic rotifers from natural assemblages from a eutrophic reservoir. Activities of all

studied enzymes were regularly inspected using the FLEA technique that allowed the localization of enzyme activities in the intact rotifer bodies during two seasons.

Chapter 3 summarizes the investigation of phosphatases, β-N-acetylhexosaminidases and lipases in *Brachionus angularis*, *B. calyciflorus*, *Keratella cochlearis* and *Lecane closterocerca* from the fed-batch cultures. Suitability of using the FLEA technique for study of rotifer enzyme activities was determined. The sites of enzyme activity within rotifer body were investigated. The enzyme activities were studied at different incubation times and the most appropriate length of the incubation was chosen.

Chapter 4 describes the effect of different P nutrition on the rotifer *B. calyciflorus*. Population growth rate, reproduction and phosphatase activity in digestive tracts of the individual rotifers fed by P-replete or P-depleted algal food supplied at three non limiting C concentrations were examined. The activities of phosphatases were measured using the FLEA technique. The individual amount of chlorophyll *a* was considered as a proxy for the ingested algal food occurring in the rotifer's gut and phosphatase activity in the gut likely reflected variable individual assimilation of P under different food treatments. The hypothesis that P-deficient rotifers use preabsorptive regulation by changing activity of the digestive enzymes was questioned.

Chapter 5 focuses on the bacteria attached on the lorica of *B. calyciflorus*, which were examined by scanning electron microscopy (SEM). Epizooic bacteria were not previously observed on the rotifer body stained with DAPI, however, because of possible overlap of the fluorescence of ELF alcohol and DAPI, we were not completely convinced that enzyme activities were exclusively of rotifer origin. To resolve this problem, we used SEM and inspected the same culture of *B. calyciflorus*, which was used for studying rotifer digestive enzymes.

Chapter 6 evaluates the effects of food quantity (supplied in limiting and non limiting quantities) and quality, i.e. nutrient-replete, N-depleted or P-depleted food on population growth, egg ratio and phosphatase activity in *B. plicatilis*. Phosphatase activity was assayed in the rotifer homogenate with the spectrofluorimetric method using MUFP as a substrate. This study further explores effect of food stoichiometry on the RNA content in rotifers.

Chapter 7 summarizes the result of this thesis.

SUMMARY OF RESULTS

At the beginning, the digestive enzymes were investigated in the rotifers from a natural assemblage from the epilimnion of Římov reservoir. The activities of phosphatases, β-N-acetylhexosaminidases and lipases were detected in twelve rotifer species mostly in the stomach area, at the corona and, less often, in the mastax area. Lipases were active only in the stomach area and rarely in the mastax. In one species phosphatases and β-N-acetylhexosaminidases were active at the posterior part. The result suggested that most of the detected enzymes were connected with the digestive tracts of rotifers. The function of enzyme activities at the corona and the posterior remained unknown.

Though FLEA technique was successfully employed in localization of enzyme activity within the rotifer body, there were some methodological difficulties in the use of the rotifers from the field, such as a low number of inspected rotifers of some species and low percentage of rotifers with enzyme activities. Moreover, rotifers could change their feeding activities as a result of some stress during transport from the sampling site to the laboratory and because of the sample concentration.

B. angularis, *Keratella cochlearis* and *Lecane closterocerca* were isolated from a pond near České Budějovice and a stock culture of *B. calyciflorus* originally isolated from Lake Constance was obtained from the Leibniz-

Institute of Freshwater Ecology and Inland Fisheries (Berlin, Germany). Fed-batch cultures of rotifers were fed at two-day intervals with fresh algae *Chlorella kessleri* Fott et Novakova. Introduction of rotifer culture made possible to determine the effect of food quantity and quality on population growth rate and activity of digestive enzymes in planktonic rotifers. Rotifers from the culture were under controlled condition and rotifers were available at sufficient densities for feeding experiment.

We investigated the enzyme activities in the several incubation times from 0.5 to 5 h. Incubations of < 1 h were in most cases insufficient for the detection of the enzyme activities. On the other hand, incubations > 4 h led to an increase in enzyme activities of the background particles that impeded proper localization of the enzymes in rotifers. For the following experiments, we chose the length of incubation from 1 to 3 h.

More than 6 thousand of rotifers were inspected for the presence and localization of enzyme activity. *B. calyciflorus* was the most inspected species because it had the highest growth rate, thus there were high numbers of individuals available for enzyme assay. We detected activities of phosphatases, β-N-acetylhexosaminidases and lipases mainly in the stomach and intestine of rotifers. *L. closterocerca* was the only species showing enzyme activity at the mastax. Lipase activity was observed only in the stomach and intestine of all species and in the mastax of *L. closterocerca*. Beside localization of enzyme activity in the stomach and intestine, phosphatases were frequently located at the corona of all investigated species and β-N-acetylhexosaminidases were active at the corona of *B. calyciflorus*. Both phosphatases and β-N-acetylhexosaminidases were detected on the lorica of some species.

Enzyme activities were observed after 1-, 2- and 3-h incubations. Longer incubation time did not positively influence the proportion of the presence of the enzyme activity at the corona and on the lorica, where the enzymes were directly accessible for the diffuse ELF substrates. On the other hand, the proportion of enzyme activity in the digestive tract increased with the longer incubation time. The delay of the presence of the enzyme activity in the digestive tract was most likely due to slow rotifer uptake of the dissolved ELF substrate.

Epizooic bacteria were not observed on the rotifer bodies stained with DAPI, however, we were not completely convinced that enzymatic activities at the corona and on the lorica were exclusively of rotifer origin. We used more sensitive method, SEM for detecting possible epizooic bacteria on *B. calyciflorus* surface. Using SEM bacteria were observed on the surface of rotifer lorica. Comparing rotifer images from SEM and from the epifluorescence microscope, we concluded that attached bacteria most likely produced enzymes observed on the lorica of *B. calyciflorus*. While the phosphatase and β-N-acetylhexosaminidase activities were detected at the corona of *B. calyciflorus*, bacteria were never observed at the corona using SEM. Hence, enzyme activities detected at the corona were most probably of pure rotifer origin.

We observed a significant effect of the P content of algal food on population growth and reproduction of *B. calyciflorus*, whereas the effect of food quantity was significant only for the egg ratio of rotifers fed by P-replete food. The population growth rate and reproduction of *B. calyciflorus* was reduced on the diet composed of P-depleted algae as compared to P-replete algae. There was no significant difference in phosphatase activity between the P-replete and P-depleted treatments. The results indicate that *B. calyciflorus* does not regulate nutrient balance via preabsorptive mechanisms such as an optimization of digestive activity and thus postabsorptive mechanisms offering an alternative.

Similar experiments as were done on *B. calyciflorus* were repeated on *B. plicatilis*. Besides the P effect, the effect of N-depleted food was also investigated. Population growth rate and egg ratio of rotifers significantly increased with increasing food quantity of nutrient-replete algal food, while phosphatase activity significantly decreased with increasing food quantity. The growth rate of *B. plicatilis* was reduced on the N- and P-depleted diet compare to the nutrient-replete diet (all types of food were supplied at the same food quantity). PA^{RH} and rotifer RNA content were not influenced by different food quality. The results indicate that *B. plicatilis* do not use an optimization of digestive activity to regulate nutrient limitations of their food.

REFERENCES

Anderson, T. R., Hessen, D. O., Elser, J. J. and Urabe, J. (2005) Metabolic stoichiometry and the fate of excess carbon and nutrients in consumers. *Am. Nat.*, **165**, 1–15.

Arndt, H. (1993) Rotifers as predators on components of the microbial web (bacteria, heterotrophic flagellates, ciliates) – a review. *Hydrobiologia*, **255/256**, 231–246.

Conde-Porcuna, J. M. (2000) Relative importance of competition with *Daphnia* (Cladocera) and nutrient limitation on *Anuraeopsis* (Rotifera) population dynamics in a laboratory study. *Freshwater Biol.*, **44**, 423–430.

Darchambeau, F. (2005) Filtration and digestion responses of an elementally homeostatic consumer to changes in food quality: a predictive model. *Oikos*, **111**, 322–336.

Darchambeau, F., Færovig, P. and Hessen, D. O. (2003) How *Daphnia* copes with excess carbon in its food. *Oecologia*, **136**, 336–346.

De Bernardi, R. and Peters, R. H. (1987) Why Daphnia? In Peters, R. H. and De Bernardi, R. (eds), *Daphnia*. Memorie Dell'Istituto Italiano Di Idrobiologia, Pallanza, **45**, pp. 1–9.

Ejsmont-Karabin, J. (1983). Ammonia nitrogen and inorganic phosphorus excretion by the planktonic rotifers. *Hydrobiologia*, **104**, 231–236.

Elser, J. J., Acharya, K., Kyle, M., Cotner, J., Makino, W., Markow, T., Watts, T., Hobbie, S., Fagan W., Schade, J., Hood, J. and Sterner, R. W. (2003) Growth rate-stoichiometry couplings in diverse biota. *Ecol. Lett.*, **6**, 936–943.

Elser, J. J., Dobberfuhl, D. R., MacKay, N. A. and Schampel, J. H. (1996) Organism size, life history, and N:P stoichiometry. *Bioscience*, **46**, 674–684.

Frost, P. C., Benstead, J. P., Cross, W. F., Hillebrand, H., Larson, J. H., Xenopoulos, M. A. and Yoshida, T. (2006) Threshold elemental ratios of carbon and phosphorus in aquatic consumers. *Ecol. Lett.*, **9**, 774–779.

Frost, P. C., Evans-White, M. A., Finkel, Z. V., Jensen, T. C. and Matzek, V. (2005) Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. *Oikos*, **109**, 18–28.

Gonzáles-Gil, S., Keafer, B., Jovine, R. V. M., Aquilera, A., Lu, S. and Anderson, D. M. (1998) Detection and quantification of alkaline phosphatase in single cells of phosphorus-starved marine phytoplankton. *Mar. Ecol. Progr. Ser.*, **164**, 21–35.

Herzig, A. (1987) The analysis of planktonic rotifer populations: A plea for long term investigations. *Hydrobiologia*, **147**, 163–180.

Hessen, D. O., Ågren, G. I., Anderson, T. R., Elser, J. J. and Reuter, P. C. (2004) Carbon sequestration in ecosystems: the role of stoichiometry. *Ecology*, **85**, 1179–1192.

Hessen, D. O., Jensen, T. C., Kyle, M. and Elser, J. J. (2007) RNA responses to N- and P-limitation; reciprocal regulation of stoichiometry and growth rate in *Brachionus*. *Ecology*, **21**, 956–962.

Hoppe, H.G. (1983) Significance of exoenzymatic activities in the ecology of brackish water: measurements by means of methylumbellyferyl-susbtrates. *Mar. Ecol. Prog. Ser.*, **11**, 299–308.

Horn, M. H. and Messer, K. S. (1992) Fish guts as chemical reactors: a model of the alimentary canals of marine herbivorous fishes. *Mar. Biol.*, **113**, 527–535.

Jensen, T. C. and Verschoor, A. M. (2004) Effects of food quality on life history of the rotifer *Brachionus calyciflorus* Pallas. *Freshwater Biol.*, **49**, 1138–1151.

Kühle, K. and Kleinow, W. (1989) Localization of hydrolytic enzymes within cellular fractions from *Brachionus plicatilis* (Rotifera). *Comp. Biochem. Physiol.*, **93**, 565–574.

Kühle, K. and Kleinow, W. (1990) Glycosidases in *Brachionus plicatilis* (Rotifera). *Comp. Biochem. Physiol.*, **95**, 393–402.

Lürling, M. (2006) Investigation of a rotifer (*Brachionus calyciflorus*) - green alga (*Scenedesmus pectinatus*) interaction under non- and nutrient-limited conditions. *Ann. Limnol. - Int. J. Lim.*, **42**, 9–17.

Merriman, J. L. and Kirk, K. L. (2000) Temporal patterns of resource limitation of natural populations of rotifers. *Ecology*, **81**, 141–149.

Mitra, A. and Flynn, K. J. (2007) Importance of interactions between food quality, quantity, and gut transit time on consumer feeding, growth, and trophic dynamics. *Am. Nat.*, **169**, 632–646.

Nedoma, J., Štrojsová, A., Vrba, J., Komárková, J. and Šimek, K. (2003) Extracellular phosphatase activity of natural plankton studied with ELF97 phosphate: fluorescence quantification and labelling kinetics. *Environ. Microbiol*., **5**, 462–472.

Ooms-Wilms, A. L., Postema, G. and Gulati, R. D. (1999) Population dynamics of planktonic rotifers in Lake Loosdrecht, the Netherlands, in relation to their potential food and predators. *Freshwater Biol.*, **42**, 77–97.

Ramos-Rodríguez, E. and Conde-Porcuna, J. M. (2003) Nutrient limitation on a planktonic rotifer: Life history consequences and starvation resistance. *Limnol. Oceanogr*., **48**, 933–938.

Rothhaupt, K. O. (1995) Algal nutrient limitation affects rotifer growth but not ingestion rate. *Limnol. Oceanogr*., **40**, 1201–1208.

Ruttner-Kolisko, A. (1974). Plankton rotifers: biology and taxonomy. Die Binnengewässer, 26 (Suppl.), pp.1–146.

Segers, H. (2008) Global diversity of rotifers (Rotifera) in freshwater. *Hydrobiologia*, **595**, 49–59.

Sibly, R. M. (1981) Strategies of digestion and defecation. In Towsend, C. R. and Calow, P. (eds), *Physiological Ecology: an evolutionary approach to resource use*. Blackwell Scientific Publication, Oxford, pp.109–139.

Sterner, R. W. and Elser, J. J. (2002) *Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere*. Princeton University Press, Princeton.

Walz, N. (1995) Rotifer populations in plankton communities – Energetics and life history strategies. *Experientia*, **5**, 437–453.

Walz, N. (1997) Rotifer life history strategies and evolution in freshwater plankton communities. In Streit, B., Städler, T. and Lively, C. M. (eds), *Evolutionary Ecology of Freshwater Animals*. Birkhäuser Verlag, Basel, pp. 119–149.

Yan, N. D. and Geiling, W. (1985) Elevated planktonic rotifer biomass in acidified metal-contaminated lakes near Sudbury, Ontario. *Hydrobiologia,* **120**, 199–205.

Yoshida, T., Urabe, J. and Elser, J. J. (2003) Assessment of 'top-down' and 'bottom-up' forces as determinants of rotifer distribution among lakes in Ontario, Canada. *Ecological Research*, **18**, 639–650.

CHAPTER 2

pages 9–17

Direct detection of digestive enzymes in planktonic rotifers using enzyme labelled fluorescence (ELF)

Martina Štrojsová and Jaroslav Vrba

Marine and Freshwater Research (2005) 56, 189–195

Štrojsová, M., Vrba, J., 2005: **Direct detection of digestive enzymes in planktonic rotifers using enzyme-labelled fluorescence (ELF).** Marine and Freshwater Research, 56 (2): 189–195.

Abstract

A novel enzyme-labelled-fluorescence (ELF) method was applied to natural populations of planktonic rotifers from a eutrophic reservoir. Direct visualisation of rotifers by this new method provided new information about enzymatic activities in situ, including detection and location of enzyme activities. Three fluorogenic substrates were used for the enzyme assay in concentrated (20–60×) samples of the rotifers. After a short (1–3 h) incubation in test tubes, samples were filtered and the rotifers on polycarbonate filters were examined using an epifluorescence microscope. Activity of phosphatases, β-N-acetylhexosaminidases and lipases were detected in some species that were regularly inspected during two seasons – most frequently in the stomach area, at the corona and, less often, in the mastax area. The results suggest that most of the detected enzymes are connected with the digestive tracts of rotifers. Also, autofluorescence of chlorophyll a enabled visualisation of the digestive tracts of the rotifers and provided additional information on the food (phytoplankton). Enzyme expression did not show any clear seasonal trend. Detection of specific enzymes varied considerably between species of rotifers and between individuals. This variability could be a result of change of feeding behaviour of rotifers in the concentrated samples and also could reflect individual differences among the rotifers in a population, such as feeding activity, age or life stage.

Souhrn

V této práci byla použita „enzyme labelled fluorescence“ (ELF) metoda vizualizace enzymové aktivity na populaci planktonních vířníků z eutrofní údolní nádrže. Metoda poskytla informace o enzymové aktivitě u vířníků v *in situ* podmínkách. Tato metoda umožňuje přímou detekci enzymových aktivit. Během dvou sezón bylo u několika taxonů vířníků detekována fosfatázová, β-N-acetylhexosaminidázová a lipázová aktivita. Nejčastějším místem aktivity byla oblast žaludku a vířivého aparátu, méně často oblast mastaxu. Výsledky naznačují, že většina pozorované enzymové aktivity měla souvislost se zažívacím traktem vířníků. Exprese enzymů v planktonních vířnících neměla jasný sezónní trend. Variabilita v sezónní expresi enzymů může být vysvětlena různým stavem zkoumaných jedinců (odlišná aktivita v příjmu potravy, věk nebo životní fáze).

CHAPTER 3

pages 19–27

Rotifer digestive enzymes: direct detection using the ELF technique

Martina Štrojsová and Jaroslav Vrba

Hydrobiologia (2007) 593, 159–165

Štrojsová Martina and Vrba Jaroslav, 2007: **Rotifer digestive enzymes: direct detection using the ELF technique.** Hydrobiologia, 593 (1): 159–165.

Abstract

Hydrolytic enzymes involved in rotifer digestive processes were investigated directly at the sites of enzyme action using the ELF (Enzyme Labelled Fluorescence) technique. After enzymatic hydrolysis of an artificial ELF substrate, the fluorescent product ELF alcohol (ELFA) marked the sites of enzyme action. The time development of ELFA labelling was studied at different incubation times. Phosphatases, β -N-acetylhexosaminidases and lipases were examined in *Brachionus angularis*, *B. calyciflorus*, *Keratella cochlearis* and *Lecane closterocerca* from fed-batch cultures. We detected activities of all studied enzymes mostly in the stomach and intestine of rotifers. *L. closterocerca* was the only species showing enzyme activity at the mastax. Lipase activity was observed in the stomach and intestine of all species and in the mastax of *L. closterocerca*. Phosphatases were frequently located at the corona of *B. calyciflorus*.

Souhrn

Trávicí enzymy vířníků byly detekovány a lokalizovány pomocí ELF (Enzyme Labelled Fluorescence) metody. Fluorescenční substrát (ELF97) vytváří precipitáty ELF alkoholu (ELFA) na místě enzymatické aktivity a tím umožňuje její přímou mikroskopickou lokalizaci. Vývoj ELFA značení byl pozorován při různě dlouhých inkubacích. K detekci trávicích enzymů byla nejvhodnější doba inkubace tři hodiny. Fosfatázová, β-N-acetylhexosaminidázová a lipázová aktivita byla studována ve vířnících *Brachionus angularis*, *B. calyciflorus*, *Keratella cochlearis* a *Lecane closterocerca* z "fed-batch" kultur. U všech studovaných druhů byla nejčastějším místem aktivity enzymů oblast žaludku a střeva a u *L. closterocerca* oblast mastaxu. Fosfatázy byly často lokalizovány na vířivém aparátu *B. calyciflorus*.

CHAPTER 4

pages 29–39

Diet quality impact on growth, reproduction and digestive activity in *Brachionus calyciflorus*

Martina Štrojsová, Jaromír Seďa, Nedoma Jiří and Jaroslav Vrba

Journal of Plankton Research (in press)

Martina Štrojsová, Jiří Nedoma, Jaromír Seďa and Jaroslav Vrba, 2008: **Diet quality impact on growth, reproduction, and digestive activity in *Brachionus calyciflorus*.** Journal of Plankton Research, issue 10(30), in press.

Abstract

We examined population growth rate, reproduction, and phosphatase activity in digestive tracts of individual *Brachionus calyciflorus* fed by P-replete (molar C:P = 107) or P-depleted (C:P = 920) algal food supplied at three carbon (C) concentrations (1, 2, and 4 mg C L–1). Rotifer growth rate was significantly influenced by P content, whereas the effect of algal C concentration was not significant. Both algal P content and C concentration had a significant effect on the egg ratio. The individual amount of chlorophyll *a* (chl-*a*) was considered as a proxy for the ingested algal food occurring in the rotifer's gut and phosphatase activity in the gut likely reflected variable individual assimilation of P under different food treatments. Both the amount of chl-*a* and phosphatase activity in the rotifers fed by P-replete algae significantly increased with the increasing C concentration in the food suspension. In contrast, the amount of chl-*a* significantly decreased with increasing food concentration, and phosphatase activity did not change significantly over different food quantity in the rotifers fed by P-depleted algae. Thus the hypothesis that P-deficient rotifers use preabsorptive regulation by changing activity of the digestive enzymes is not supported by the data.

Souhrn

Byl studován populační růst, reprodukce a fosfatázová aktivita v trávicím traktu jednotlivců *Brachionus calyciflorus,* kteří byli krmeni fosforem (P) limitovanou (atomový poměr C:P = 107) nebo nelimitovanou řasovou potravou (C:P = 920) dodávanou ve třech různých koncentrací uhlíku (C) (1, 2 a 4 mg C L^{-1}). Populační růst vířníků byl signifikantně ovlivněn obsahem P v řasách, zatímco efekt koncentrace C nebyl signifikantní. Obsah P v řasách i C koncentrace měly signifikantní vliv na poměr počtu vajíček na samičku. Obsah chlorofylu *a* (chl-*a*) v jednotlivých vířnících byl stanoven jako množství přijaté řasové potravy, která se vyskytovala v trávicím traktu vířníku a fosfatázová aktivita v trávicím traktu vyjadřovala různou asimilaci P u jednotlivých vířníků krmených různou potravou. Množství chl-*a* i fosfatázová aktivita ve vířnících krmených P nelimitovanou řasou signifikantně vzrostly se zvyšující se koncentrací C v potravní suspenzi. Překvapivé bylo, že se množství chl-*a* v trávicím traktu vířníků krmených P limitovanou řasou zmenšovalo se vzrůstajícím množstvím přítomné potravy a fosfatázová aktivita ve vířnících krmených P limitovanou potravou se signifikantně neměnila s různým množstvím P v limitované potravě. Tedy hypotéza, že P limitovaní vířníci používají předabsorpční regulaci pomocí změny v aktivitě trávicích enzymů nebyla podpořena našimi daty.

CHAPTER 5

pages 41–46

Epizooic bacteria on *Brachionus calyciflorus*: possible relationship with rotifer enzyme activity

Martina Štrojsová and Wilko H. Ahlrichs

Manuscript submitted to Hydrobiologia (short research note)

Martina Štrojsová and Wilko H. Ahlrichs: **Epizooic bacteria on *Brachionus calyciflorus*: possible relationship with rotifer enzyme activity.** Manuscript submitted to Hydrobiologia.

Abstract

Rotifer *Brachionus calyciflorus* was examined by the scanning electron microscopy (SEM) for the surface-attached, i.e. epizooic bacteria to ascertain their specific localization. Lorica of *B. calyciflorus* was colonized by one distinct type of bacteria, which originated from the algal culture used for rotifer feeding. Corona, posterior epidermis and foot of all inspected individuals were always without attached bacteria. Density of the attached bacteria was higher with increasing age of *B. calyciflorus*, while young individuals were colonized by ~ tens of bacterial cells, older ones had hundreds to thousands attached bacteria in average. We hypothesize that epizooic bacteria might produce ectoenzymes phosphatases and β-N-acetylhexosaminidases on the lorica of *B. calyciflorus*.

Souhrn

Vířník *Brachionus calyciflorus* byl prohlížen pomocí rastrovacího elektronového mikroskopu, zda má na svém povrchu přisedlé bakterie. Krunýř vířníků byl kolonizován jedním typem bakterií, které pocházely z řasové kultury používané ke krmení vířníků. Na vířivém věnci, pokožce trupu a na noze nebyly bakterie nikdy pozorovány u žádného z prohlédnutých vířníků. Množství přisedlých bakterií se zvyšovalo se stářím *B. calyciflorus*; zatímco mladí jedinci měli na svém povrchu jen desítky bakteriálních buněk, starší jedinci měli stovky až tisíce přisedlých bakterií. Předpokládáme, že přisedlé bakterie můžou produkovat ektoenzymy fosfatázy a β-N-acetylhexosaminidázy detekované na krunýři *B. calyciflorus*.

CHAPTER 6

pages 47–61

Effect of food quantity and quality on growth, reproduction and digestive activity in the euryhaline monogonont rotifer *Brachionus plicatilis* Müller

Martina Štrojsová, Koushirou Suga, Atsushi Hagiwara and Jaroslav Vrba

Manuscript submitted to Journal of Plankton Research

Martina Štrojsová, Koushirou Suga, Atsushi Hagiwara and Jaroslav Vrba: **Diet Effect of food quantity and quality on growth, reproduction and digestive activity in the euryhaline monogonont rotifer *Brachionus plicatilis* Müller.** Manuscript submitted to Journal of Plankton Research.

Abstract

The decomposition of particulate and dissolved organic matter by rotifer digestive enzymes plays a crucial role in the rotifer nutrition. Among other enzymes, rotifers produce phosphatases, non-specific enzymes that allow for release of orthophosphate from a variety of organic phosphorus compounds. Phosphatase saturation was measured in *Brachionus plicatilis* homogenates using the spectrofluorimetric method at pH 5.6, 6.6 and 7.6. The enzyme affinity decreased from 48.3 to 107.3 µmol L^{-1} with increasing pH, while the maximum velocity of hydrolysis increased from 0.08 to 0.27 µmol μg^{-1} h^{-1} with increasing pH. We examined population growth rate, reproduction and phosphatase activity in the rotifer homogenate (PA^{RH}) of rotifers fed by nutrient-replete algal food supplied at different quantities. Growth rate and egg ratio of rotifers significantly increased with increasing food quantity, while PA^{RH} significantly decreased. The well-fed rotifers without food limitation likely released a lot of only partly ingested food while rotifers limited by food quantity likely intensified their digestive activity. Response of rotifer population growth rate, reproduction, PA^{RH} and RNA content to different food quality, i.e. nutrient-replete, N-depleted and P-depleted algae supplied at the non-limited quantity was also investigated. The highest population growth rate was reached by rotifers fed by nutrient-replete food, while it did not significantly differ between rotifers fed by the N-depleted and P-depleted food. The egg ratio was more limited by N than P supply. PA^{RH} and rotifer RNA content were not influenced by different food quality. The results indicate that *B. plicatilis* do not use an optimization of digestive activity to regulate nutrient limitations of their food.

Souhrn

Rozklad organické hmoty pomocí hydrolytických enzymů, které jsou produkovány slinými a žaludečními žlázami, hraje základní roli v trávení vířníků. Mezi dalšími enzymy, vířníci produkují fosfatázy, nespecifické enzymy umožňující uvolnění orthofosforečnanů z různých organických sloučenin. Saturace fosfatázy byla měřena v homogenátu *Brachionus plicatilis* použitím spektrofluorometrické metody v pH 5.6, 6.6 a 7.6. Afinita enzymů klesla od 48.3 do 107.3 µmol L^{-1} se zvyšujícím se pH, zatímco maximální rychlost hydrolýzy se zvýšila z 0.08 na 0.27 µmol μg^{-1} h^{-1} se zvyšujícím se pH. Studovali jsme populační růst, reprodukci a fosfatázovou aktivitu v *B. plicatilis*, kteří byli krmeni živinami nelimitovanou řasovou potravou podávanou v různém množství. Populační růst a poměr vajíček na samičku signifikantně vzrostly se zvyšujícím se množství potravy, zatímco fosfatázová aktivita naopak signifikantně klesla. Výsledek naznačuje, že vířníci, kteří nejsou limitováni ani množstvím ani kvalitou potravy uvolňují velký podíl jen částečně strávené potravy. Na druhou stranu, vířníci krmení malým množstvím potravy pravděpodobně zlepší trávení potravy tím, že zvýší aktivitu trávicích enzymů . Dále byla studována odpověd populačního růstu, reprodukce a fosfatázové aktivity v *B. plicatilis* na řasovou potravu dodávanou ve stejném množství ale o různé kvalitě, t.j. živinami nelimitované, dusíkem limitované a fosforem limitované potrava. Nejvyšší populační růst měli vířníci krmení živinami nelimitovanou potravou, zatímco populační růst se signifikantně nelišil u vířníků krmených dusíkem a fosforem limitovanou potravou. Poměr vajíček na samičku byl více limitován dusíkem než fosforem. Fosfatázová aktivita ani množství RNA ve vířnících nebyly ovlivněny různou kvalitou potravy.

CHAPTER 7

pages 63–65

General conclusions

GENERAL CONCLUSIONS

The results of the preliminary experiments showed that the FLEA technique is suitable for the direct detection of rotifer enzymes. Four rotifer species: *Brachionus angularis*, *Keratella*, *Lecane closterocerca* and *B. calyciflorus* were maintained in fed-batch cultures. Activities of phosphatases, β-N-acetylhexosaminidases and lipases were most often localized in the gut of rotifers, but phosphatases and β-N-acetylhexosaminidases were observed also at the corona and on the lorica. Enzymes acting in the gut had likely digestive function, the role and origin of enzyme activities in the area of corona and lorica was unknown. By comparing rotifer images from SEM and from the epifluorescence microscope, we could conclude that attached epizooic bacteria most likely produced enzymes observed on the lorica of *B. calyciflorus*. On the other hand, enzyme activities detected at the corona were most probably of pure rotifer origin.

Rotifer of the genus *Brachionus* responded to feeding on nutrient-depleted food by decreasing of population growth rate and egg production. The result suggests that well-fed rotifers without either food quantity or quality limitation released a lot of partly ingested food. On the other hand, rotifers fed by the low amount of nutrient-replete food likely improved digestion of the food by increasing the digestive activity. Food quality did not have any effect on phosphatase activity, thus rotifers likely do not use an optimization of enzyme activity to compensate for low-quality food. The results indicate that *Brachionus* do not regulate nutrient balance via preabsorptive mechanisms such as an optimization of digestive activity and thus postabsorptive mechanisms offering an alternative.

The results of the research allows for basic understanding on the patterns of homeostatic regulation of chemical resources in rotifers. The connections between elemental supply ratios and physiological processes advance the basic understanding of the interactions between rotifer physiology and the environment. By combining measurements of the activities of rotifer digestive enzymes with different rotifer food quantity and quality, it was added new insights into how rotifers regulate their homeostasis.