Hydrobiologia (2005) **534:** 239–244

Short Research Note

Macrotrachela quadricornifera featured in a space experiment

Claudia Ricci*, Manuela Caprioli, Chiara Boschetti & Nadia Santo
Department of Biology, Università degli Studi di Milano, via Celoria, 26, Milan, MI 20133, Italy
*(*Author for correspondence)*

Received 2 July 2004; accepted 19 July 2004

Key words: bdelloid rotifer, anhydrobiosis, stress resistance, embryogenesis, International Space Station

Abstract

Macrotrachela quadricornifera, a bdelloid rotifer, is the animal model of an experiment scheduled on the International Space Station (ISS). The focus of the experiment is the role of the cytoskeleton during oogenesis and early development. Bdelloids will fly desiccated, be rehydrated on the ISS and cultivated for five generations. We present the outline of the ISS experiment, the expectations and the state-of-the-art of ground-based research run to date on the major topics of the planned experiment: anhydrobiosis and embryogenesis. Anhydrobiosis focuses on two major aspects, storage conditions that enhance recovery rate and comparison of the resistance between dormant and active rotifers to UV radiation and other environmental injuries. Embryogenesis has been approached at the morphological level under ground conditions: developing embryos have been studied by light and confocal microscopes.

Introduction

During the last century, some biological research addressed the role of gravity on the development of organisms, in view of long-term exposure of living organisms to weightless conditions, or, better, to microgravity. Most experimental animal models used to date belonged to the so-called Radialia, organisms with radial pattern of cleavage (Echinoderms and Vertebrates) (e.g., Ubbels et al., 1995; Marthy, 1997; Ubbels, 1997). This modality of cleavage implies an indeterminative pattern of development, that means that damage or mistakes occurring in the first part of development can be adjusted in a later stage of development, without leaving evidence on the animal. In contrast, animals belonging to the so-called Spiralia possess a determinative pattern of development in which the fate of the blastomeres is fixed very early during development. Each embryonic cell is committed early to a given fate in the developing body, and damage occurring at any time during embryogenesis could be long-lasting and could produce consequences on the future animal. Several taxa of invertebrates belong to the Spiralia, among them the rotifers. In addition to possible mistakes during embryogenesis, another potential source of damage to the rotifers must be considered, since the cytoplasm of the maturing egg is entirely provided by the mother's glands, called vitellaria (Amsellem & Ricci, 1982). So, anomalies in synthesis and in assembly of cytoskeletal elements could occur both during oogenesis and during early developmental stages.

NASA and ESA issued a 'call for proposals' addressed to investigators willing to propose experiments suitable for the International Space Station (ISS) facilities. The present experiment was proposed and approved in 1996, and is scheduled on ISS in 2005. The research project focuses on the role of the cytoskeleton during oogenesis and early development events and on the effect of microgravity on the developmental process. It has promoted new research efforts in areas that were almost neglected for rather a long time. Here we wish to present some results obtained in preparation for the space experiment.

The species

A bdelloid rotifer, *Macrotrachela quadricornifera* Milne, 1886 is proposed as an experimental model. The species is very common, widespread and relatively large in size (400–500 μm). It is easily cultivated under laboratory conditions, and at 22 °C it lives about 40 days and lays approximately 30 eggs (Ricci, 1991, 1995). It is eutelic and reproduces parthenogenetically through apomictic telytoky. The eggs have a modified spiral pattern of cleavage with a mosaic determinative development (e.g., Gilbert, 1989; Boschetti et al., in press). As in most bdelloids, it is able to survive desiccation of the habitat by entering a dormant state called anhydrobiosis (Keilin, 1959; Ricci, 1998).

Outline and rationale of the ISS experiment

M. quadricornifera will be desiccated at the age of 8 days (Ricci et al., 1987) under laboratory conditions. The specimens will be flown to ISS on the Shuttle in dry conditions. In this state the dry animals can be kept dormant. When suitable, the animals will be activated by adding the culture medium. The first batch of experimental animals, when recovered, will be cultivated allowing them to reproduce; these animals will form the P (=parental) generation. At prefixed times, their eggs will be moved to a second culture chamber to start the second population, which will constitute the F1 generation. The same protocol will be followed for subsequent populations in order to perform a multigenerational experiment for a total of five generations. After egg transfers, the remaining animals will be desiccated and maintained dormant until return to Earth. In the laboratory, they will be re-hydrated and cultivated or processed for further studies. Three replicates will run simultaneously. The experiment samples will be paralleled by a flight control in a centrifuge at $1 \times g$ and by a ground control on Earth to provide reference data.

While in space, the rotifers will experience microgravity and this condition is expected to affect the cytoskeletal organization of the embryo and hence its role in establishing the division furrow of the cells. Anomalies in synthesis and assembly of cytoskeletal elements could occur both during oogenesis and during early developmental stages. The 'mosaic' development pattern of the rotifers makes repair of embryo damages almost impossible. In addition, the eutely of rotifers does exclude repair mechanisms involving cell reproduction during the life time of the animals. Thus the damage during development is expected to be maintained in the adult.

Two topics were investigated in detail in preparation for the ISS experiment: (1) anhydrobiosis resistance and (2) embryogenesis. Here we report the results achieved in the two areas to date.

Anhydrobiosis

Bdelloid rotifers are able to survive desiccation of the habitat by entering a dormant state called anhydrobiosis (Keilin, 1959). When dry, the anhydrobionts shut metabolism down and, when water again becomes available, resume active life regardless of the time spent dry (Ricci et al., 1987; Ricci & Covino, in press). Several conditions during anhydrobiotic time affect bdelloid recovery; the desiccation procedure, duration of dryness and storage during the anhydrobiotic period. We tested four desiccation protocols that differed in the rate of water evaporation, in the timing of drying and in the substrates provided to the animals. Morphological changes of rotifers by SEM and recovery rates after dormancy were recorded. The highest recovery rates corresponded to well organized morphologies of anhydrobiotic bdelloids obtained with slow desiccation procedure and with a filter paper as substrate (Ricci et al., 2003).

The length of the anhydrobiotic period is known to affect viability of anhydrobiotic rotifers: longer dryness produces higher mortality rates (Jacobs, 1909). *M. quadricornifera* was kept dry in lab conditions for different periods and no animal recovered from 60-day anhydrobiosis (Caprioli & Ricci, 2001). In order to improve the recovery rate, several storage conditions were tested. A group of dry rotifers was kept in a freezer (−20 °C), others in a container where the atmosphere was either N_2 or CO_2, or normal, but rarefied. The experiments lasted 30, 60, 90 and 180 days. The results indicate that low temperature is the most efficient condition to improve the recovery of *M. quadricornifera* (Fig. 1).

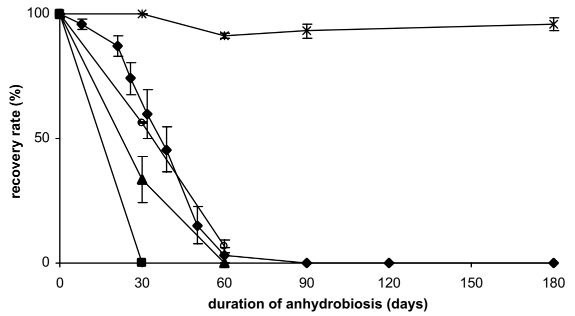

Figure 1. Recovery rates of *M. quadricornifera* kept under different storage conditions during anhydrobiosis lasting different times. (■) CO_2; (▲) N_2; (○) rarefied atmosphere; (*) −20 °C; (♦)control. Bars are ± SE of three or more replicates.

While dormant, animals are known to tolerate conditions that cannot be successfully faced when active (Gilbert, 1974). Anhydrobiotic bdelloids were found to resist extreme temperature and pressure (Raham, 1923; Becquerel, 1950; Caprioli et al., 2002). The exposition of active and anhydrobiotic rotifers to ultraviolet rays (180 nm) resulted in a higher survival and recovery of the anhydrobiotic animals (Fig. 2).

As a prelude to proposing these organisms as suitable models for ISS experiment, anhydrobiotic *M. quadricornifera* were exposed to space conditions in the Shuttle. The animals were flown dry and experienced strong vibrations during take off and landing, and microgravity while in space. The bdelloid recovery was not affected by space flight conditions since the flown rotifers recovered as much as their ground controls, and subsequent life history was similar in the two groups (Ricci & Caprioli, 1998).

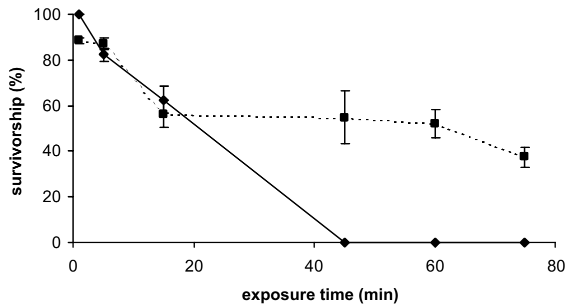

Figure 2. Survivorship rates of hydrated (continue line) and anhydrobiotic (broken line) *M. quadricornifera* exposed to ultra violet radiation (180 nm) for different times. Bars are ± SE of three or more replicates.

Embryogenesis

Because of the parthenogenetic reproduction, the eggs of bdelloids do not undergo meiosis but only equational divisions (=mitosis) (Hsu, 1956a,b). Most rotifers lay unsegmented eggs that undergo a modified spiral pattern of cleavage with a mosaic determinative development where each blastomere is committed early to a given fate (e.g., Gilbert, 1989). Our knowledge of the pattern of embryo development of rotifers, and of bdelloids in particular, is very poor, since the only detailed study on bdelloid embryogenesis was published at the end of XIX century (Zelinka, 1891). We studied the development of *M. quadricornifera* egg under light and confocal microscopy. Light microscopy observations of the rotifer egg development '*in vivo*' are possible because of the transparency of the egg shell, and can provide interesting results on cleavage pattern. However, when the *in vivo* observation is insufficient to reveal the arrangement of embryo cells, a more informative approach is preferred. The major problem we faced was the staining of some components of embryo cells, because of the impermeability of the egg shell. Beside, the chemical composition of the shell of rotifer eggs is very little known; it seems to be made of a mixture of proteins and chitin (Depoortere & Magis, 1967; Piavaux, 1970; Piavaux & Magis, 1970). Nevertheless, we could stain cytoskeletal elements (tubulin and filamentous actin) of blastomeres and observed them using a confocal laser scanning microscope (Boschetti et al., in press). Evidencing the nuclei with DAPI and cytoskeleton with either falloidin (for filamentous actin) or antibody anti-α-tubulin (for tubulin), we could observe different developmental stages of *M. quadricornifera*'s embryo (Fig. 3).

Since the rotifers are Spiralia, we decided to perturb the organization of the cytoskeleton and to study the effect on embryo morphology and on life history of the hatched rotifers. A possible way to perturb cytoskeletal components is to modify the gravity force, assuming that the assembly of long polymeric structures such as the microtubules and microfilaments can be affected. We tested the effects of increased (hyper-gravity) and decreased (hypo-gravity) gravity on embryo development.

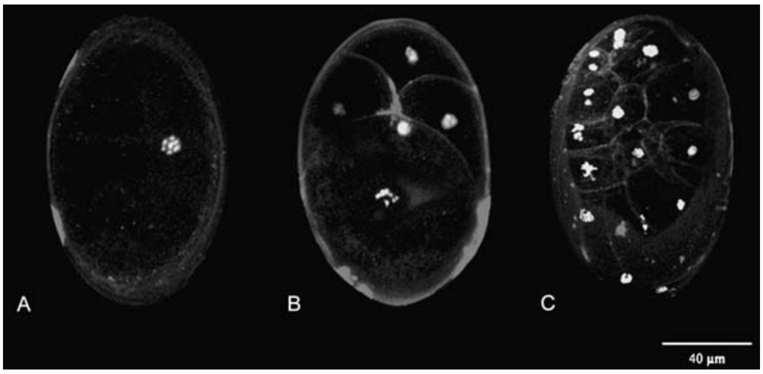

Figure 3. M. quadricornifera embryos at different stages stained with DAPI and falloidin observed at CLSM: (A) unsegmented; (B) 4 cells; (C) 18 cells.

Gravity was experimentally increased by placing the developing embryo in a fine-tuned centrifuge, suitable for culturing small organisms, called Rotifuge. Reduced gravity was simulated with a desktop random positioning machine (RPM) (Dutch Space), that is a rotating platform that randomizes Earth gravity vector. Since the cytoskeleton formation of bdelloid rotifers can be affected both during egg formation inside the mother gonad and during development, we tested the effects of gravity force exposing both unsegmented eggs and rotifers undergoing oogenesis. Similar to what has been found on other experimental animals (Marthy, 2002), the preliminary results show that both embryo morphology and life history traits of *M. quadricornifera* seem to be unaffected by exposure to high (up to 20 ***g***) or low (as low as 0.0001 ***g***) gravity force (Ricci & Boschetti, 2003).

Experimental containers, bioreactors

An Italian factory (Laben) is responsible for the design and development of hardware and software of a bioreactor that will contain this experiment on ISS (Freddi et al., 2002). Laben is going to provide the automatization of all processes, rehydration, animal culture, transfer of eggs, separation of different generation, dehydration. A critical point was the small volume available to each experiment ($160 \times 60 \times 60$ mm) on ISS, that makes compulsory to miniaturize the hardware. However the complexity of the actions to be performed automatically and the need of replicate experiments makes the hardware design more difficult. Every replicate is performed following a back and forth technique between two different cultivation cells. At each generation the eggs are moved by means of an appropriate water flow, while the adults remain in the previous cultivation cell. After eggs hatching, the previous generation is moved to a proper substrate and desiccated under controlled conditions.

Very critical points from biology point of view were the biocompatibility of all materials present in the hardware, and the survival of the rotifers in a closed environment.

Discussion and conclusions

The rotifers proposed for the ISS experiment are a suitable model for space investigation because they have several characteristics that fit the space experiment requirements. (A) Complexity. They are metazoans, made of about 1000 cells, and have tissues and organs, a complete gut, yet they are very simple animals. This simplification allows analysis of complex problems through easier approaches. (B) Miniaturization. Rotifers are small in size and need small volumes to be cultivated. (C) Life span. Rotifers have short life cycles, and can be studied in a reasonable time period. (D) Density. They reproduce parthenogenetically: this allows them fast population growth because all animals are females, then contribute to next generation. (E) Genetic homogeneity. The progeny are the result of an ameiotic parthenogenesis, thus all rotifers are identical to their ancestor. (F) Resistance. Rotifers can easily enter dormancy, and under such conditions they are endowed with increased resistance to a wide range of physical stresses.

The project for running the ISS experiment challenges our knowledge on several topics of the bdelloids, that deserve more research efforts. In particular, of the two major aspects presented here, embryogenesis has been neglected for a very long time and our knowledge is based on studies run more than one century ago. The old results are still valid, but methodology, techniques and instruments have improved since then, and much more information is still to be gained.

Apart from the exciting adventure of space research itself, the experimental design proposed on the bdelloids has promoted ground-based researches, providing funds and opportunities for training young people.

Acknowledgements

Some colleagues (Umberto Fascio, Giulio Melone, Cristina Sotgia) have been involved into this project and we gratefully acknowledge their contributions. A particular thank is due to Alberto Musetti for designing and building the Rotifuge and minor bioreactors, and for technical support. Thanks to everybody for giving us this remarkable opportunity.

Thanks are due to Russ Shiel, University of Adelaide (Australia) for reading an earlier draft of the paper and correcting the English.

Financial support for the study came from ASI grants to C.R. for VErmi SPAzio (=WOrm SPace) project.

References

Amsellem, J. & C. Ricci, 1982. Fine structure of the female genital apparatus of *Philodina* (Rotifera, Bdelloidea). Zoomorphology 100: 89–105.

Becquerel, M. P., 1950. La suspension de la vie au-dessous de 1/20 K absolu par démagnétisation adiabatique de l'alun de fer dans le vide le plus élevé. Comptes Rendus Académie Sciences 231: 261–263.

Boschetti, C., C. Ricci, C. Sotgia & U. Fascio, in press. The development of a bdelloid egg: a contribution after 100 years. Hydrobiologia.

Caprioli, M. & C. Ricci, 2001. Recipes for successful anhydrobiosis in bdelloid rotifers. Hydrobiologia 446/447: 13–17.

Caprioli, M., N. Santo & C. Ricci, 2002. Enhanced stress resistance of dormant Bdelloids (Rotifera). Proceedings of "Life in Space for Life on Earth", ESA SP-501: 319–320.

Depoortere, H. & N. Magis, 1967. Mise en evidence, localisation et dosage de la chitine dans la coque des oeufs de *Brachionus leydigii* Cohn et d'autres rotifères. Annales de la Société Royale Zoologique de Belgique 97: 187–195.

Freddi, M., M. Covini, C. Tenconi, C. Ricci, M. Caprioli & V. Cotronei, 2002. Automated Culture System Experiment Hardware: developing test results and design solutions. Proceedings of "Life in Space for Life on Earth", ESA SP-501: 247–248.

Gilbert, J. J., 1974. Dormancy in rotifers. Transactions of the American Microscopical Society 93: 490–513.

Gilbert, J. J., 1989. Rotifera. In Adiyodi, K. G. & R. G. (eds), Reproductive Biology of Invertebrates. Fertilization, Development, and Parental Care. Vol. IV, Part A. Oxford & IBH Publishing Co. Pvt. Ltd.: 179–199.

Hsu, W. S., 1956a. Oogenesis in the bdelloidea rotifer *Philodina roseola* Ehr. La Cellule 57: 283–296.

Hsu, W. S., 1956b. Oogenesis in *Habrotrocha tridens* (Milne). Biological Bulletin 111: 364–374.

Jacobs, M. H., 1909. The effects of desiccation on the rotifer *Philodina roseola*. Journal of Experimental Zoology 6: 207–263.

Keilin, D., 1959. The problem of anabiosis or latent life: history and current concept. Proceedings of the Royal Society of London 150: 149–191.

Marthy, H. J., 1997. Sea urchin eggs under microgravity conditions. ESA SP-1206: 81–91.

Marthy, H. J., 2002. Developmental biology of animal models under varied gravity conditions: a review. Vie Milieu 52: 149–166.

Piavaux, A., 1970. Origine de l'enveloppe chitineuse des oeufs de deux rotifères du genre *Euchlanis* Ehrenberg. Annales de la Société Royale Zoologique de Belgique 100: 129–137.

Piavaux, A. & N. Magis, 1970. Données complémentaires sur la localisation de la chitine dans les enveloppes des oeufs de rotifères. Annales de la Société Royale Zoologique de Belgique 100: 49–59.

Raham, P. G., 1923. Biologische und physiologische Beiträge zur Kenntnis der Moosfauna. Zeitschrift für allgemeine Physiologie 20: 1–34.

Ricci, C., 1991. Comparison of five strains of a parthenogenetic species, *Macrotrachela quadricornifera* (Rotifera, Bdelloidea). I. Life history traits. Hydrobiologia 211: 147–157.

Ricci, C., 1995. Growth pattern of four strains of a Bdelloid Rotifer species: egg size number. Hydrobiologia 313/314: 157–163.

Ricci, C., 1998. Anhydrobiotic capabilities of bdelloid rotifers. Hydrobiologia 387/388: 321–326.

Ricci, C. & C. Boschetti, 2003. Bdelloid rotifers as model system to study developmental biology in space. In Marthy, H. J. (ed.), Developmental Biology in Space. Elsevier, 25–39.

Ricci, C. & M. Caprioli, 1998. Stress during dormancy: effect on recovery rates and life-history traits of anhydrobiotic animals. Aquatic Ecology 32: 353–359.

Ricci, C. & C. Covino, in press. Anhydrobiosis of *Adineta* sp. 1: costs and benefits. Hydrobiologia.

Ricci, C., L. Vaghi & M. L. Manzini, 1987. Desiccation of rotifers (*Macrotrachela quadricornifera*): survival and reproduction. Ecology 68: 1488–1494.

Ricci, C., G. Melone, N. Santo & M. Caprioli, 2003. Morphological response of a bdelloid rotifer to desiccation. Journal of Morphology 257: 246–253.

Ubbels, G. A., 1997. Fertilisation and development of *Xenopus* eggs on sounding rockets and in a clinostat. ESA SP-1206: 25–36.

Ubbels, G. A., M. Reijnen, J. Meijerink & J. Narrawaj, 1995. The role of gravity in the establishment of the dorso-ventral axis in the amphibian embryo. ESA SP-1162: 175–185.

Zelinka, C., 1891. Studien über Räderthiere. III. Zur Entwicklungsgeschichte der Räderthiere nebst Bemerkungen über ihre Anatomie und Biologie. Zeitschrift für wissenschaftliche Zoologie 53: 1–159.