An eBook on Marine Biology and Aquaculture

Chapter 3

Aquatic Organisms as Models in Biomedical Space Research

Nikolai V Kuznetsov [*]

Karolinska Institute, SE-171 77 Stockholm, Sweden.

Email: nikkuznetsov@inbox.com

Abstract

From the word go till nowadays, laboratory biologists have been focused mostly on a few practical biological models, which largely determined and narrowed down our modern vision of diverse complex physiological cellular and molecular bioprocesses. The choice of model organism is important issue in experimental biology, particularly, in the space exploration biomedical science. Largely unknown non-classical aquatic model organisms will be advantageous for further developments on the path of humanity into space. Selected perspective hydrobiont models based on old almost-forgotten and new literature data are discussed that could be of future use in the expanding biomedical space science.

Keywords: Hydrobiont Models; Space Research; Microgravity; Gravisensing; Regeneration; Cosmic Radiation.

1. Introduction

Aquatic organisms (hydrobionts) live throughout the Earth's biosphere and count for larger parts of Animalia and Protista kingdoms in Earth biomass [1]. Hydrobionts surprise us by their enormous biodiversity, especially, taking in account that more than 90% of aquatic species have not been described yet [2]. Already in the 21st century, mankind inevitably will head to other planets, carrying in its luggage and reconstructing at new bases, the portion of the Earth's biosphere. One challenge would be to recognize how different organisms of various species will react to the space travel and new conditions on other planets.

Traditionally, molecular biology has been dealt with a few standard model organisms, which largely determined our vision of diverse and complex bioprocesses. Recently developed almost unlimited power of genomes sequencing showed that a picture we observe is incomplete. The use of other, non-classical model organisms could reveal undetected molecular pathways and mechanisms that could be valued in the future as hidden treasures allowing us, for example, to improve the adaptation of astronauts to the weightlessness or to boost their regenerative potential. Therefore, the investigations of new biological models in new experimental conditions become an advisable and necessary step on the path of humanity into the space.

For a number of reasons, aquatic organisms will be most likely our companions in future long-term space missions and are among the most promising objects in terms of understanding the biomedical issues such as combating atrophy of the musculoskeletal system in zero gravity and the regeneration of organs and tissues. Hydrobionts can teach us how to overcome the effects of long-term exposure to the hostile factors of the space: microgravity, radiation and hypomagnetic field. They will be indispensable for the provision the crew with oxygen, nutrients, drugs and probiotics as well as for an arrangement of necessary ecological and psychological environment for future space travelers. Consciously, photosynthetic aquatic species of Plantae, Protista and Monera kingdoms have not been touched here as they deserve to be discussed in a separate future review. In this chapter, several potential and established aquatic models will be mentioned regarding gravisensing, regeneration, response to microgravity and cosmic radiation.

2. Quasi-Microgravity

The lack of gravity is one of the hostile factors, limiting human power during space missions [3]. To train astronauts and to perform microgravity related biomedical experiments, various wet and dry aquatic facilities are usually in use. For example, NASA Extreme Environment Mission Operations (NEEMO) low gravity simulation carry out at the Aquarius Reef Base situated off Key Largo in the Florida Keys [4] **(Figure 1A).** Long-term microgravity had been successfully simulated in the Dry immersion facility at the Institute for Biomedical Problems of the Russian Academy of Sciences (IBMP RAS) that allowed to study human body physiological response to weightlessness [5] **(Figure 1B).**
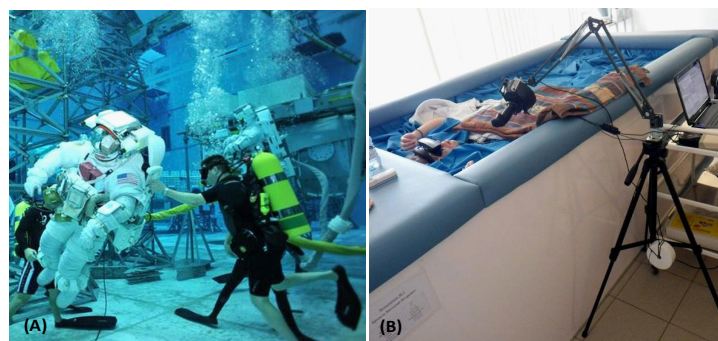

Figure 1: Simulation of microgravity in immersion facilities
A.   **NASA low gravity simulation facility** at the Aquarius Reef Base, Key Largo, Florida Keys.
B.   **Dry immersion facility** at the Institute for Biomedical Problems RAS, Moscow.

Indeed, human body submerged in water loses its weight thanks to the Archimedes' principle. The same Archimedean force acts on all hydrobionts in the natural water basins and all habitants of the World Ocean placing them in the conditions of quasi-microgravity state. That makes certain hydrobionts especially suitable models for studying and modelling the molecular mechanisms of diverse physiological responses to quasi-microgravity.

Highly likely, first life forms have appeared in aquatic surrounding, and, almost certainly that complex unicellular and multicellular organisms have evolved in the ancient ocean [6]. Therefore, genomes and transcriptomes of various buoyant organisms can be used as relevant sources to search for genes of response to quasi-microgravity conditions that could be found among most conserved genes of hydrobionts. Particularly, buoyant plankton microorganisms including prokaryotes (Archaea, Bacteria) [7, 8] and microscopic eykariotes [9, 10, 11] as well as higher eukaryotic aquatic species inhabiting the water column could be selected as models in the gravitation biology research. In Protozoa, the taxon of unicellular marine microorganisms Radiolaria appeared in the Cambrian that were one of the first groups to change from a benthic to free floating mode of life [12]. Usually, radiolarians are of microscopic size (0.1– 0.2 mm), still some giant species exceed dimensions of 6 - 7 mm [13]. In multiple species of Radiolaria, the projecting spines extend the surface of silica skeleton and support the pseudopodia radiating through the perforated shell, that was abundantly illustrated by Ernst Haeckel in his systematic works **(Figure 2)** [14].

These cell structures increase the overall surface and lower the specific gravity of radiolarians. Furthermore, multiple pores in the skeleton and gas vacuoles or fat globules in the ectoplasm, add to the buoyancy of microorganisms [15]. Unique phenotypes, ecology characteristics and the adaptive features make radiolarians suitable models to study primary quasi-microgravity effects on a single cell level.
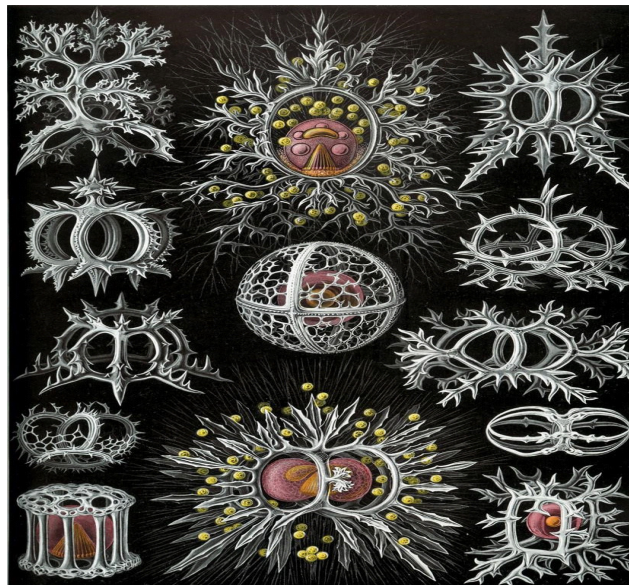

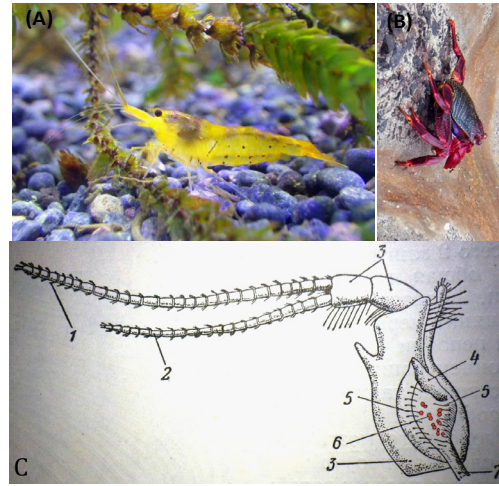**Figure 2: Radiolaria** *Stephoidea* by Ernst Haeckel [16].

Figure 3: Gravisensing in Decapods

A.   **Tangerine tiger shrimp** (*Caridina serrata*) maintain vertical position at the bottom of aquarium;

B.   **Moorish red legged crab** (*Grapsus adscensionis*) climbing walls in supralittoral zone (Canary);

C.   **The anatomy of the statocyst of crayfish** (from Dogel,V.A., 1959). The numbers indicate: **1, 2 -** the antennules; **3 -** basal segments of the antennules; **4 -** opening of the statocyst; **5 -** sensitive hairs on the wall of the statocyst; **6 -** sand particles inside the statocyst functioning as statoliths (red); **7 -** the nerve

3. Gravisensing

The aspect of gravisensing is directly interconnected with the problem of response to microgravity. Several Mollusca, Arthropoda, Echinodermata and Vertebrata species could be used as interesting models to study the gravisensing in the conditions of quasi-microgravity or in real microgravity. In Gastropoda (*Biomphalaria gluhrara*), a simple gravisensing organ, the statocyst with the diameter around 0.15 mm is filled with statolymph and stone-solid statoconia formed of calcium carbonate crystals that are produced and exocytosed by the supporting cells into the statocyst lumen. In normal 1g gravity, the statoconia sink to the bottom of the cyst and interact with the receptor cells in the statocyst wall that leads to their stimulation [17].

On Earth, *Biomphalaria* snails show a preference to crawl downward on an aquarium wall and this behavior is, obviously, controlled by the statocysts. After reaching to the bottom, the snails inflate an air bubble under the shell, and float to the surface where the crawling behavior cycle reoccurs. In spaceflown developed in space snails, the statoconia in their statocysts were formed in microgravity and the total volume of statoconia was found to be 50% greater than in size-matched ground control snails [18].

In Decapoda, the gravisensors - two statocysts locate in the basal segment of the antennules and function as an equilibrium organs helping to navigate in the water column (e. g., Tangerine tiger shrimp (*Caridina serrata*), **(Figure 3A)** and, even through the supralittoral zone (e. g., Moorish red legged crab (*Grapsus adscensionis*), **(Figure 3B).** The statoliths inside the statocysts press on the sensitive hairs or setae **(Figure 3C)** [19].

In shrimps, the opening of statocyst is effectively closed to the external environment by

coarse setae and a thin layer of chitin that extends from the basal segment of the antennules [20]. "When the otocysts [statocysts] are removed, shrimps swim with a more or less rolling motion and may even turn ventral side up. Their equilibration is thus shown to be seriously interfered with" [21]. The role of statocysts was shown experimentally in the 19th century by Kreidl, when shrimps were induced to form statoliths of iron particles instead of sand grains and then the animals became oriented to the lines of force of a magnet as they formerly did to gravity [22].

In crabs, the grains of sand works as statoliths in a deepening of statocysts. During molting, statoliths are lost through the aperture of the statocyst. After molting, some macrurans gathers sand grains in its claws and puts them in the opening of the statocysts. Some crabs in a new shell submerge their cephalothorax in the sand until they get new grains of sand in the statocysts. Several practical questions have to do with how these animals will behave in microgravity conditions. Will their behavior resemble the movement in the absence of statoliths? Will they be able to adapt to long-term weightlessness? Would it be possible to find a way to reconstruct their behavior in zero gravity, perhaps, in "a Kreidl" way? Similar questions should be addressed to the aquatic vertebrate models – fishes and amphibians. Simulated microgravity via clinorotation for seven days in cichlids (*Oreochromis mossambicus*) [23] as well as wall vessel rotation (WVR) for up to six days in early-staged zebrafish (*Danio rerio*) [24] resulted in larger otoliths than in 1g control fishes. In the swordtail fish (*Xiphophorus helleri*), the size of otoliths was compared between ground- and flight-reared larvae of the same size. For later-stage larvae, the growth of the otolith was significantly greater in the flight-reared fish [25]. Therefore, the microgravity induces otolith growth, however, fishes must have passed a certain developmental stage as it has been discussed earlier [26].

The neural readaptation to Earth's gravity after an exposure to microgravity during NASA shuttle orbital flights was studied in four oyster toadfish (*Opsanus tau*) using electrophysiological techniques by recording the responses to inertial accelerations of vestibular nerve afferents supplying the utricular otolith organ [27]. Within the first day postflight, the magnitude of response was on average three times greater than in controls, apparently, due to reduced gravitational acceleration and upregulation of the sensitivity of utricular afferents in orbit. Already, by 30 hours postflight, responses were returned to normal afferent sensitivity that was similar the reported time course in vestibular disorientation in astronauts following return from space [27].

Beside gravisensing via specialized sensory systems, a number of effects have been observed in fishes that were related to the metabolic changes. Among the acute responses in fishes to microgravity onboard ISS, the increase of levels of transcription regulators specific to osteoblasts/osteoclasts was reported that particularly, was accompanied by bone mineral density loss in Japanese rice fish or medaka (*Oryzias latipes*) [28, 29]. In spaceflown developing

larvae of Japanese red-bellied newt (*Cynops pyrrhogaster*) [30] and African clawed frog (*Xenopus laevis*) [31], the otoliths were found be five times larger the volume and 30% larger of those in ground control newts and frogs, respectively. A number of studies were performed to elucidate the importance of gravity for the normal embryogenesis in aquatic Invertebrates and Vertebrates.

Particularly, fertilization of eggs and skeletogenesis in sea urchins (*Paracentrotus lividus, Sphaerechinus granularis*) [32, 33], (*Hemicentrotus pulcherimus*) and early embryogenesis in newt (*Pleurodeles waltl*) [34] and frog (*Xenopus laevis*) [35] have been investigated. Newts fertilised in microgravity showed some abnormalities during embryonic development but were able to produce nearly normal larvae. Mummichog (*Fundulus heteroclitus*) [36, 37] and medaka (*Oryzias latipes*) showed unique behavior in the microgravity including "looping behavior", upside-down swimming, mating behavior and "hypokinesia"-type behavior [28]. Medaka successfully mated in space with normal fertilization and hatching rates implying there are no specific developmental stages during gametogenesis that are susceptible to microgravity [38].

4. Regeneration

The regeneration of tissues and organs is an important area of biomedical research that will certainly lead to further development of regenerative therapies in humans with its broad perspective for application in space biology. Several important regeneration models can be considered to use in BioSpace labs, including cnidarians, planarians, decapods and urodeles.

Freshwater Hydrozoa Cnidaria polyps Hydra (*Hydra vulgaris*) has phenomenal regeneration potential and is able to regenerate the whole organism even from dissociated single cells [39]. Wnt, TGF-β, Notch [40], VEGF and FGF [41] signaling were described to be key pathways in the regeneration in Hydra. Despite *Hydra vulgaris* is referred as an oldest model in the regenerative biology, first described and named in mid 18th century [42, 43, 44], we still miss a deep understanding of Hydra regenerative phenomena at the cellular and molecular level. Thereto, its properties and regenerative potential in the conditions of the space flight are still largely unknown.

Another fascinating emerging cnidarian model, colonial marine *Hydractinia* possess remarkable power of regeneration and demonstrate distinct mechanisms act to regenerate different body parts [45]. Freshwater flatworms planaria can regenerate new heads, tails, or entire organisms **(Figure 4A)** from small fragments containing stem cells (neoblasts) [46]. FoxA family of transcription factors are important key gene regulators shown to be involved in the regeneration of planaria [47].
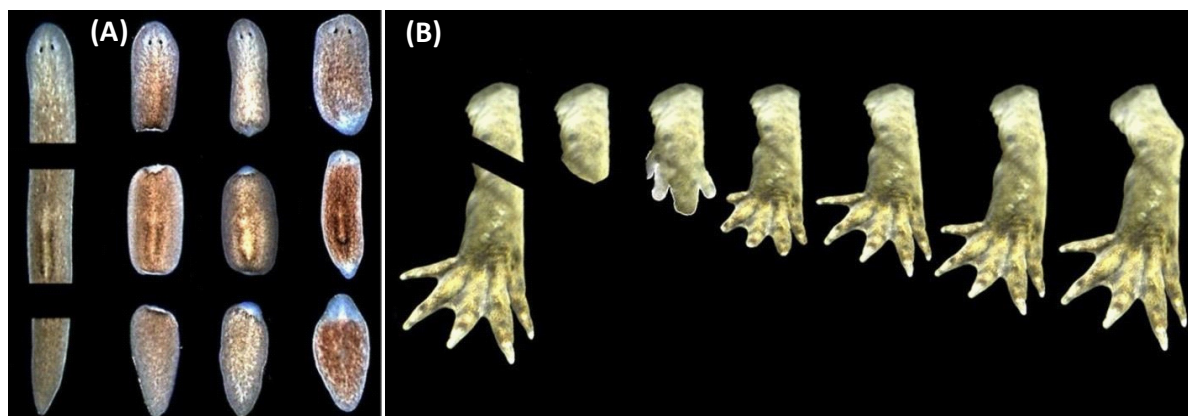
Figure 4: Regeneration biological models
A.   **Flatworm planaria** regenerates new heads and tails
B.   **Larvae of *Ambystoma mexicanum*** - axolotl regenerates an entire lost limb

Number of studies were performed with planarians on board of the International Space Station. Particularly, rare double-headed persisting phenotype was reported in one out of 15 regenerated animals following five weeks incubation of the planaria samples on the ISS. This suggests further space experiments and investigations to clarify whether observed phenotype was due to space travel [48]. Another recent study showed that rocket launch related vibrations and hypergravity can affect the expression of the early stress response genes in planarians during a regenerative process [49]. Regeneration found in many crustaceans. In crabs, limbs that are lost due to injury or predation as a result of the reflexive autotomy response, can be regenerated completely during a single intermolt cycle. Regenerative processes are controlled by FGF-like growth factors and arthropod molting and growth hormones – ecdysteroids [50] and promoted by melatonin [51].

Urodelian amphibians demonstrate unique ability to regenerate limbs, retina, heart, nerves, and spinal cord throughout their whole life. The urodeles respond to amputation of limb by a fast wound closure with re-epithelisation within 12 hours after amputation that is of great interest and importance for space medicine to develop acute management for large open wounds and burns. Nerve regeneration in urodeles results in a fully functional tissue restoration that may have a potential for surgery to improve nerve repair and the functional outcome. Also, an understanding of the molecular processes of the cell dedifferentiation in the amphibian tissue to restore the defects like an entire excised limb could help to design new tissue engineering and treatment protocols [52].

For example, an axolotl - a neotenic larvae of Mexican tiger salamander (*Ambystoma mexicanum*) is capable to regenerate an entire lost limb without any scarring **(Figure 4B).** Wnt signaling pathway has been shown to be essential for proper limb regeneration to occur in both developing and mature animals in axolotls [53, 54]. Interestingly, the administration of stem cells in the axolotl model accelerated even more the process of regeneration of amputated limbs [55]. Other salamander species, most often used in regeneration research, are three species of newts: (*Notophthalmus viridescens*, Eastern red-spotted newt; *Cynops phyrrogaster*, Japanese

fire-belly newt; and *Pleurodeles waltl*, Iberian ribbed newt) which have similar regeneration capacities [56].

Following the genome and transcriptome sequencing of the Iberian ribbed newt, recent findings revealed potential roles of embryonic stem cell-specific miRNAs mir-93b and mir-427/430/302, as well as Harbinger DNA transposons carrying the Myb-like proto-oncogene that have expanded dramatically in the Pleurodeles waltl genome and were found co-expressed during limb regeneration [57]. How effecient will be the regeneration in amphibians in conditions of space flight? Earlier observations showed a positive effect of simulated microgravity on the regeneration in newts [58, 59, 60]. During space flight experiments, the lens regeneration appeared faster in space animals than in controls [61]. After a 2-week space flight, it was observed that retina recover more intensively and the differentiation of regenerate layers was more rapid in flight newts than in ground controls [62]. Also, after a 2-week flight, a higher rate of limb regeneration was detected in flight animals at nine days after landing [61, 63, 64].

Following these primary data, more attention should be paid to observed or similar effects and it will be definitely worth to investigate further various newt models in simulated and real microgravity in more detail.

5. Response to cosmic radiation

Multiple biomodels, including a number of hydrobiont species, have been tested for the effects of cosmic radiation on the survival, developmental processes, genome stability, et cetera, that is of particular importance for the prevention of cancer caused by long-term exposure to the space radiation. Among these models, are tardigrades, branchiopods and several fish species. Tardigrade species *Milnesium tardigradum* is a cosmopolitan hydrobiont in aquatic environments of marine, coastal, and terrestrial areas and it proved to be a valuable model organism in space research [65].
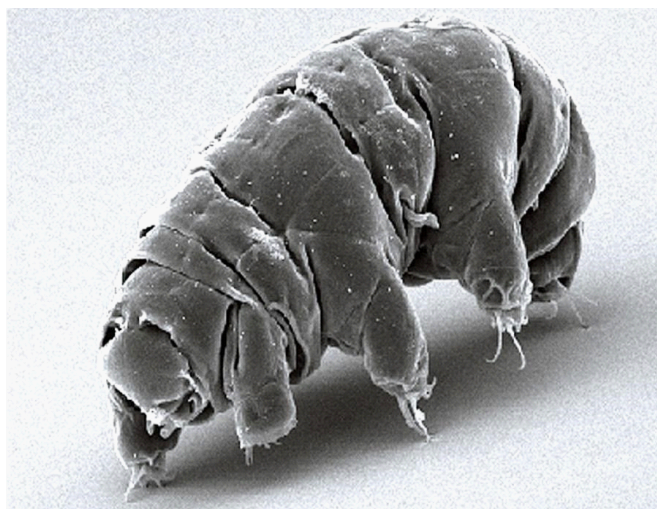

Figure 5: Tardigrade (*Milnesium tardigradum*) [66] can survive long periods of exposure to cosmic radiation.

Tardigrades are the first known animals to survive long exposure to outer space. In 2007 during FOTON-M3 mission, groups of tardigrades (some of them previously dehydrated) were exposed in outer space to the vacuum and solar cosmic radiation for 10 days. Upon return to Earth and rehydration, three of tardigrades *(M. tardigradum)* have survived [67]. The eggs of brine shrimp (*Artemia salina*) have been taken to a number of space missions. They showed high sensitivity to cosmic radiation with 90% of the embryo lethality at different developmental stages [68]. Zebrafish (*Brachidanio rerio*) model for radiation-induced cataracts have been exposed to simulated high energy galactic cosmic rays as well as gamma irradiation at Brookhaven National Lab on Long Island, New York to provide data on threshold limits of galactic cosmic rays on zebrafish and hence to astronauts [69].

Medaka (*Oryzias latipes*) males have been exposed to high-energy cosmic-ray nuclei to estimate the germ cell mutagenesis rate. The relative number of resulted mutant embryos as a function of dose were compared with those induced by γ-rays. Research team concluded that low values compared with those for mutations in somatic cells and for neoplastic transformation, indicated that germ cell mutations arising from exposures to cosmic ray nuclei are not a significant hazard to astronauts [70].

Redtail notho (*Nothobranchius guentheri*) dry eggs were exposed to low orbit radiation on the outer side of the ISS during the Biorisk-MSN mission in 2007. Unfortunately, no data had been available due to temperature sensor failure and overheating [71]. In a ground-based study, zebrafish (*Brachidanio rerio*) embryos were exposed to increasing gamma radiation that increased DNA damage and mortality rate and decreased hatching rate [72]. Fish research data need to be taken in the future consideration in space and at moon base where all habitants should be protected to minimize space radiation hazards. Also it would be valuable to conduct further experiments on the impact of different particles and charges from cosmic radiation on fish [73].

6. Epilogue

The visionary of space travel paradigm, Russian scientist and philosopher Konstantin Tsiolkovsky wrote in one of his works: "The planet is the cradle of the mind, but one cannot live forever in the cradle" [74]. What Tsiolkovsky could not know yet at the dawn of the twentieth century though, perhaps, could foresee that certain celestial bodies have deposits of water ice like Moon [75] or even the oceans of liquid water like Enceladus [76] and Europa [77]. Taking hydrobionts in the spaceship heading to other planets could surprisingly bring not only humans but also hydrobionts to a new home, either an artificial or a natural one. *Et pisces ad astra!*

Table 1: Aquatic models in Space Biology research.

Application	Taxa	Hydrobiont models	References
Quasi-microgravity and gravisensing	Prokaryota	Archaea, Bacteria	7, 8
	Protista	Euglenozoa, Ciliophora, Radiolaria	9-16
	Gastropoda	Snails	17, 18
	Decapoda	Shrimps, crabs	19-22
	Echinodermata	Sea urchins	32-34
	Vertebrata	Fishes, amphibians	23-31, 34-38
Tissue and organ regeneration	Cnidaria	Hydra, Hydractinia	39-45
	Turbellaria	Planaria	46-49
	Decapoda	Shrimps, crabs	50-51
	Vertebrata	Urodelian amphibians	52-64
Response to cosmic radiation	Tardigrada	Water bears	65-67
	Branchiopoda	Artemia	68
	Vertebrata	Fishes	69-73
Note: heterogeneous data, for overview purpose only			

7. Author contributions

Nikolai V. Kuznetsov wrote, approved and submitted the manuscript.

8. Competing Interests

Author declare that he has no conflict of interest.

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