

Tardigrades



Tardigrades Under the Influence of Acidic and Alkaline Solutions, and UV-C Radiation

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Entry to the
Stockholm Junior
Water Prize 2019

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Abstract

Tardigrades are very small animals who live in humid environments. They belong to the taxon of Ecdysozoa, and are found in most water bodies, sediment and moss. They have developed the unique ability to react to rapidly changing environmental conditions by changing their physical characteristics and taking on different stages of resistance. Especially the cryptobiosis, a special form of resistance stages, amazes many scientists. No metabolism can be detected while in cryptobiosis, therefore tardigrades are in a condition close to death but can exit under more favorable environmental conditions.

Given the fact that water ecosystems are most affected by climate change, the adaptability of tardigrades to rapidly changing environmental conditions deserves a closer look. In my research, I conducted two experiments on the tardigrade *Hypsibius exemplaris* regarding their tolerance and vitality under the influence of UV-C light and pH values between 2 and 13.

Based on the experiment with UV-C light I discovered that *Hypsibius exemplaris* dies within 14 days after an irradiation of 1.152 kJ/m² of UV-C light. Furthermore, *Hypsibius exemplaris* does not counteract UV-C light with any kind of resistance stages. Thus, *Hypsibius exemplaris* is not capable of protecting itself against UV-C light. They tolerate an irradiation up to 1.152 kJ/m², but the optimum lies between 0 kJ/m² and 0.288 kJ/m². The LD50 (the amount of a toxic agent such as a poison, virus, or radiation that is sufficient to kill 50 percent of a population of animals usually within a certain time) is in between 0.576 kJ/m² and 1.152 kJ/m², closer to 0.576 kJ/m².

Due to the pH experiment I found out that *Hypsibius exemplaris* reacts differently to acidic and alkaline environments. In pH 3, the tardigrades did osmobiosis, they died in pH 2 and did not use the benefits of osmobiosis from pH 3.5 upwards. The range of tolerance is between pH 2 and pH 12. The cuticle of *Hypsibius exemplaris* probably dissolved in pH 2 and their inner muscle tissue seemed to denature in pH 12. The optimum, regarding their vitality, is between pH 6 and pH 10. The LD50 could not be determined exactly but lies most probably between pH 2 and 3, as well as between pH 11.5 and 12.

According to the findings of the UV-C light experiment, *Hypsibius exemplaris* cannot protect itself with any stage of resistance in contrast to the pH experiment. Here, *Hypsibius exemplaris* only used osmobiosis once in an acidic environment but no further reactions were observed in alkaline fluids.

1 Introduction

The key question of my research is:

"How do liquids with different pH values and UV-C light of different intensities influence the vitality of the tardigrade *Hypsibius exemplaris*?"

The aim is to investigate the tolerance and vitality of *Hypsibius exemplaris* under the influence of different pH values and different dosages of UV-C light and thereby create a combined diagram for their tolerance and vitality for each experiment.

My hypothesis on the pH experiment is that *Hypsibius exemplaris* uses a form of cryptobiosis in strong acidic and strong alkaline environments. The highest activity of the tardigrades should be in liquids with a pH value of 7 to 8, since *Hypsibius exemplaris* is native to fresh water and should therefore be adapted to these pH values.

Regarding the UV-C light experiment, I hypothesize that *Hypsibius exemplaris* dies at a certain dosage and has no possibilities to protect itself with any stage of resistance. Irradiated tardigrades may not die immediately after their treatment but could also die over the course of the following days.

My work delivers information about tardigrades and their adaptation to extreme environments. Research on their mechanisms to withstand and protect themselves against changes in their habitat may contribute to finding solutions against climatic changes. Not only climate change does have an influence on temperatures around the world, but it also affects the physical characteristics of different waters, which results in need of adaptation for all organisms living in aquatic ecosystems. Therefore, I believe it is important to understand how and under which circumstances tardigrades protect themselves and how resistant they are without cryptobiosis.

I would like to express my gratitude to my parents, my older brother and Valérie Piolino for supporting me during my research. Special thanks to Dr. Peter Veit for providing me with microscopes and other essential materials, PD Dr. Samuel Zschokke for his support and Dr. Stefanie Schmid for providing the materials for the pH experiment. I also want to thank Karla Schlie from the Swiss Toilet Organisation for supporting me in preparation for the Stockholm Junior Water Prize. My final acknowledgement goes to Ralph O. Schill for sharing his vast knowledge of tardigrades and supporting me.

2 Resistance stages and Cryptobiosis

2.1 Resistance in general

Tardigrades have the ability to protect themselves against extreme environmental conditions. These conditions may be osmotic fluctuations, dry environment, lack of oxygen or extreme cold. In order to withstand such conditions, tardigrades use various types of cryptobiosis and other resistance stages. Cryptobiosis is a change of their characteristics that minimizes or completely stop growth, reproduction and metabolism. Thus, cryptobiosis is described as a state of an organism which *“no longer shows any visible signs of life and whose metabolic activity is hardly measurable or comes to a reversible standstill [...]”*^{1,2}

2.2 Anhydrobiosis

Anhydrobiosis is a type of cryptobiosis. It is caused when terrestrial tardigrades suffer from a lack of water. As soon as their living space is refilled with water, the tardigrades become active again. Activity in all tardigrades depends on the presence of water. If there is hardly any water, tardigrades fall into a dry state and the body shape resembles a contracted barrel.³ (see Fig. 1)

In order to achieve the dry state, tardigrades retract their legs and reduce their body surface. The cuticle becomes impermeable to water, which reduces evaporation. Until they reach the anhydrobiotic state, certain molecules must be created to prevent irreversible damage within the body. Complementary hypotheses have been proposed to explain this occurrences.

The «water replacement» hypothesis describes proteins which form hydrogen bonds using hydroxyl groups of sugar molecules. The head group of phospholipids counteract the approach of lipids due to the dehydrogenating sugar trehalose. The second hypothesis, the “glassy state” hypothesis, describes the principle of carbohydrates which form a glass-like structure. This glass greatly lowers the kinetic reactions by immobilizing proteins and preventing unwanted reactions within the body.⁴ After about five to seven hours, the tardigrade is in an anhydrobiotic state. From now on it is practically “dead” because there is no metabolism and oxygen consumption has been stopped completely. In addition, the tardigrades are

¹ Greven, Hartmut, (1980) Die Bärtierchen, p.57

² Greven, Hartmut, (1980) Die Bärtierchen, p.57

³ Greven, Hartmut, (1980) Die Bärtierchen, p.57

⁴ Schill, Ralph O., (2010) *Von Biodiversität bis Biotechnologie: Bärtierchen und die Kunst des Überlebens*

now resistant to temperatures from -273°C ⁷ up to 100°C ⁵, various chemicals, high hydrostatic pressure as well as vacuum.⁶

If there is enough water in the environment again, restitution begins. Restitution takes about ten minutes to several hours or even days, depending on the species and environmental conditions.⁷

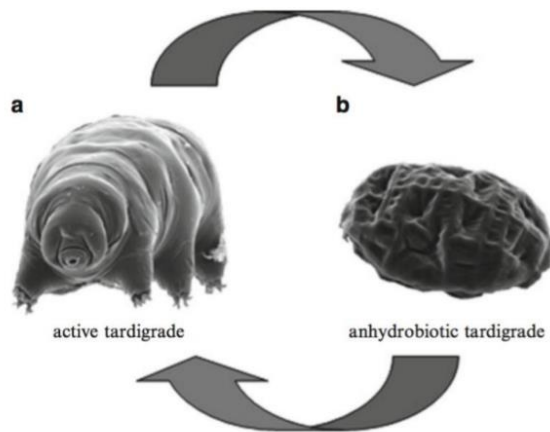


Fig. 1: Change from an active state (a) into the dry anhydrobiotic state (b) and vice-versa

2.3 Osmobiosis

Another type of cryptobiosis is the osmobiosis. Tardigrades fall into osmobiosis when dissolved products reduce the water potential. The process of osmobiosis is similar to the anhydrobiosis. However, the osmobiotic state is not necessary for all species, as tardigrades are already highly resistant to high salt concentrations.⁸ Especially species that live in tidal zones and some that are regularly exposed to osmotic fluctuations do not resort to osmobiosis due to their innate adaptation to increased salt concentrations.⁹

⁵ Hengherr, p. & Worland, M. R. & Reuner, A. & Brümmer, F. & Schill, R. O, (2009) *High-Temperature Tolerance in Anhydrobiotic Tardigrades Is Limited by Glass Transition*

⁶ Wikipedia, (2018) *Bärtierchen*

⁷ Wikipedia, (2018) *Bärtierchen*

⁸ Lindahl, Karen and Professor Balser, (1999) *Species Distribution Project Tardigrade Facts*

⁹ Wikipedia, (2018) *Bärtierchen*

3 Methods

3.1 *Hypsibius exemplaris*

Hypsibius exemplaris (see Fig. 2) is a freshwater tardigrade species and was formerly classified as *Hypsibius dujardini*. The reclassification was officially recognized on 2 February 2018.¹⁰

Today, *Hypsibius exemplaris* is one of the most studied and most common tardigrades. Biogeographically they have been found in the Palearctical, Neotropical, Nearctical, Afrotropical, Antartical and Indomalayan realm.¹¹

Like many aquatic tardigrades, *Hypsibius exemplaris* is white to transparent and its pharyngeal muscles are conical or pear-shaped. Its claw shape belongs to the *Hypsibius* class. The claw base and the secondary branch are separated by a septum less visible relative to other species. *Hypsibius exemplaris*' elongated body shape, the rather forwardly displaced stiletto support, a different shaped macroplacoid (see Fig. 2), as well as a differently shaped claw, grants this species a prominent position in the *Hypsibius* class and allowed it to be reclassified.¹²

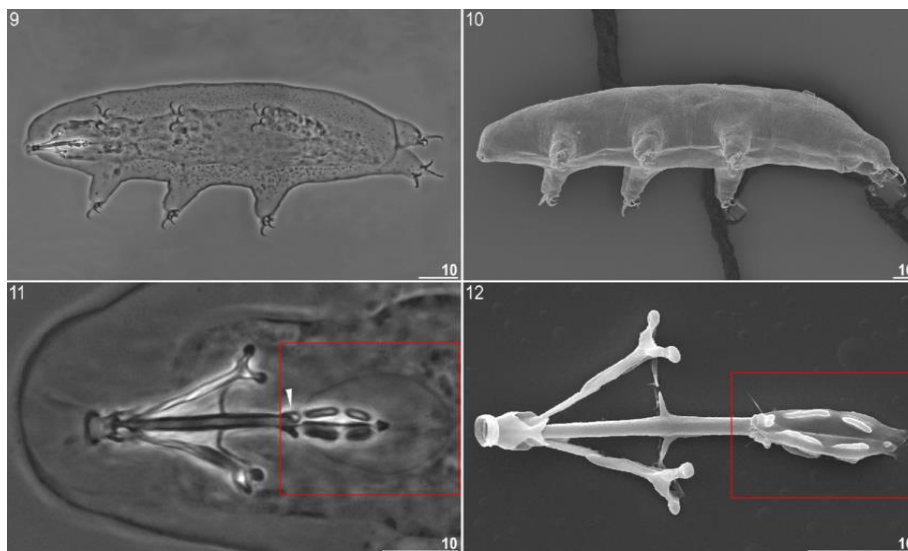


Fig. 2: *Hypsibius exemplaris*;
9: ventrolateral;
10: lateral;
11: macroplacoid (marked in red);
12: macroplacoid (marked in red) connected to the digestive system

¹⁰ Gąsiorek, Piotr et al., (2018) *An integrative redescription of Hypsibius dujardini* (Doyère, 1840), the nominal taxon for Hypsibioidea (Tardigrada: Eutardigrada), p. 45

¹¹ Surette, Fiona, (2014) *Hypsibius dujardini*

¹² Gąsiorek, Piotr et al., (2018) *An integrative redescription of Hypsibius dujardini* (Doyère, 1840), the nominal taxon for Hypsibioidea (Tardigrada: Eutardigrada), p. 56+58+59

3.2 Information about both experiments

In both experiments 50 ml cultures of the tardigrades *Hypsibius exemplaris* of "Sciento.uk" with green algae (A68 Chlorococcum sp.) were ordered. Chapter 3.5 describes the aspects according to which the tardigrades were observed, how the obtained data was evaluated and how it was used further.

3.3 UV-C light experiment

The aim of this experiment was to investigate the tolerance and vitality of tardigrades *Hypsibius exemplaris* under different exposure to UV-C light. The materials used were Petri dishes, small pipettes with a maximum capacity of 5 ml, *Hypsibius exemplaris* tardigrades, a stereomicroscope with 40-fold magnification and a UV-C light sterilizer which does not emit heat (Germix ZX-508 (8W)). The entire experiment was performed in a darkened room so that the tardigrades were not exposed to sunlight, in order to prevent the suspected photoreactivation. In order to avoid carrying out the observations in complete darkness, small light sources such as LED glass fiber light arms were used. Some light was also needed to examine the Petri dishes with the tardigrades on them under the stereomicroscope.

Five tardigrades were distributed to each of 21 Petri dishes. The tardigrades were pipetted into the Petri dishes in small droplets together with green algae, so that the animals had enough water around them, but only enough to be easily found again (see Fig. 4). Subsequently, all Petri dishes were placed in the UV-C light sterilizer and irradiated. Three Petri dishes with five tardigrades each were irradiated for 64, 32, 16, 8, 4, 2 and 1 minute. Another Petri dish with five tardigrades was used as a control experiment, it was placed in a small box for 64 minutes in order not to be exposed to the radiation. The control experiment was only required at the exposure of 64 minutes, as the irradiation had no effect and the remaining dosages were lower. The tardigrades were then observed under the stereomicroscope and evaluated for tolerance and vitality. I examined the number of tardigrades that did not survive the procedure, show some form of resistance, hardly move at all, move more slowly or show no major change compared to their previous behavior. The observations were made immediately after irradiation and repeated 15 minutes, 45 minutes, 75 minutes and 105 minutes after end of irradiation. Afterwards, a time was fixed until a maximum of four hours after irradiation in order to find a similar time for further observations. In my experiment it was at 10 p.m. Further observations were carried out from this time on, after 4, 8 and 20

hours and every day thereafter, at the same time as the observation after the 20 hours mentioned, i.e. 6 p.m. for 14 days. New algae and fresh water were regularly added, so that the tardigrades did not starve or dehydrate.

3.4 pH experiment

In this experiment, the tolerance and vitality of tardigrades *Hypsibius exemplaris* were also investigated, this time in the presence of different pH values. The following materials were required: a beaker (250 ml), Petri dishes, buffer solutions, pH meter (Hanna Instruments HI 991001), deionized water, small pipettes, stereomicroscope (40-fold magnification), sodium hydroxide solution (32%), hydrochloric acid (1M; 0.1M; 0.01M; 0.001M), 15 centrifuge tubes (TPP) and a magnetic stirrer.

I investigated the effect of 16 different pH values on the tolerance and vitality of tardigrades. Three times in a row five tardigrades were exposed to one of the following pH values: 2; 3; 3.5; 4; 4.5; 5; 6; 7; 8; 9; 10; 11; 11.5; 12; 12.5; 13.

To prepare the solutions with the different pH values, a 250 ml beaker was filled with 100 ml deionized water. The pH value in the beaker was measured continuously with the pH meter. Sodium hydroxide solution (32%) was continuously added until a pH value of 13 was reached. As soon as the pH value required for the tests had been reached, 3 to 4 ml of the solution were added to one of the containers. Subsequently, hydrochloric acid was continuously added to the solution and the pH value was lowered until a further measurement data point was reached. The concentration of the hydrochloric acid added depended on the pH value. From pH 13 to 11 1M hydrochloric acid was used. From pH 11 to 9 0.1M hydrochloric acid, from pH 9 to 5 0.01 or 0.001M hydrochloric acid, from pH 5 to 3 again 0.1M hydrochloric acid and from pH 3 to 2 1M hydrochloric acid.

The tardigrades were again pipetted into the petri dishes in small droplets together with the food, so that they had just enough water to remain active. For the actual experiment, the water was carefully removed from the tardigrades using a pipette. The water was then replaced with a solution with the desired pH value. The animals were then observed under a stereomicroscope and their behavior recorded. Again, attention was paid to the number of tardigrades that either could not withstand the solution, had some form of resistance stage, hardly moved at all, slowed down or showed no major change in previous behavior.

The tardigrades were observed after 5, 10, 15, 30, 45 and 60 minutes and their behavior recorded. For tardigrades that were in a resistance stage, the entire solution was replaced by water after 60 minutes to determine how long it took the tardigrades to get out of their resistance stage.

3.5 Evaluation and presentation of data

The tolerance points were distributed as follows: If a tardigrade was alive, the measured value received 2 points; 1 point if a resistance stage of an animal could be determined; if the test animal was dead, no points were given. Finally, for each data point, three measurement series with five tardigrades each would achieve points, which in turn will be added together and represent the common data point as the total number of points. The same applies to the diagram of vitality, except that the distribution of points differs slightly from that of tolerance. The vitality diagram also assesses the activity of the tardigrades. The point distribution is now as follows: active tardigrades received 4 points; slightly slower *Hypsibius exemplaris* were evaluated with 3 points; tardigrades, which were still alive but hardly moved or even stood still, received 2 points; those tardigrades in a resistance stage score 1 point; dead tardigrades receive 0 points.

The points system allows vitality and tolerance to be clearly displayed over time. The points in the diagram which lie on the y-axis therefore serve as indicators for the strength of tolerance and vitality. The x-axis on the other hand serves as an overview of the strength of tolerance and vitality as it changes over time.

In addition to both experiments, another diagram is created in each case. This last line diagram describes the tolerance and vitality with two graphs of its own. On the x-axis, the pH values and the dosages of the UV-C light are lined up. The y-axis describes the achieved points. Here 2 y-axes are needed, because the total points of tolerance and vitality differ. The data used are those measurement data points which were last documented in terms of time. This means that the pH experiment used the data which was observed after 60 minutes of exposure time and for the UV-C light experiment the data which was recorded after 14 days of observation. The reason for this is that the tardigrades, after the chosen times, have adapted to the environmental effects, or at least have been exposed long enough to detect final reactions.

4 Results

4.1 Results UV-C light experiment

The behavior of *Hypsibius exemplaris* differed greatly depending on the dose of irradiation. Despite of behavior ranging from unchanged activity to death, the presence of a resistance stage was never observed. The experiment started on august 14th 2018 and ended on august 28th 2018.

The radiation intensity of the UV-C sterilizer was determined with a UVC-Log measuring device which was 0.06 mW/cm². Using the formula $UV-C \text{ dose (mWs/cm}^2) = \text{intensity (mW/cm}^2) \times \text{time (s)}$ ¹³ the dose of the individual measurement series could be determined and then converted to kJ/m² using $1 \text{ mWs/cm}^2 = 1 \text{ mJ/cm}^2$ ¹⁴ (rounded to three decimals) in order to obtain a comparative value to the article "Analysis of DNA Repair and Protection in the Tardigrade *Ramazzottius varieornatus* and *Hypsibius dujardini* after Exposure to UVC Radiation".¹⁵

The behavior of the tardigrades was measured at the following observation times: immediately after irradiation, after 15 minutes of irradiation, after 45 minutes, after 75 minutes and after 105 minutes, at 10 p.m. on august 14th 2018, at 2 a.m. and 6 a.m. on august 15th 2018, at 6 p.m. on the same day and each subsequent day at 6 p.m. until august 28th 2018. Since the tolerance points in the assessment of activity refer only to living, dead and resistant tardigrades and the vitality points divide the living tardigrades into three further categories, the description of the activity level of the individual tardigrades will follow only the example of the distribution of the vitality points.

The irradiation of 64 minutes with the UV-C light sterilizer corresponds to a UV-C dosage of 2,304 kJ/m². All tardigrades of the three series of measurements, except one, were already dead immediately after the radiation treatment. The animal that had survived could hardly move and was more or less rigid in the water. After 15 minutes the tardigrade had also died. Over the next 14 days, no activity could be determined.

¹³ flow-med medical appliances, (2018) *Über UVC*, p. 3

¹⁴ Horikawa, Daiki D. et al., (2013) *Analysis of DNA Repair and Protection in the Tardigrade *Ramazzottius varieornatus* and *Hypsibius dujardini* after Exposure to UVC Radiation*, p. 2

¹⁵ Horikawa, Daiki D. et al., (2013) *Analysis of DNA Repair and Protection in the Tardigrade *Ramazzottius varieornatus* and *Hypsibius dujardini* after Exposure to UVC Radiation*

An irradiation of 32 minutes in this experiment corresponds to a UV-C dosage of 1,152 kJ/m². Although about half of all tardigrades were still alive immediately after the petri dishes were removed from the sterilizer, most of them were exhausted. However, 2 tardigrades from the first series of measurements showed similar activity as before irradiation. After 15 minutes more tardigrades died. After 45 minutes, an apparently dead tardigrade showed slight signs of life again. Until the measurement at 10 p.m. the still living, exhausted tardigrades had recovered. The next observations at 2 a.m. and 6 a.m. showed how the surviving tardigrades suffered a relapse and eventually all died at 6 p.m. august 15th 2018.

After an irradiation time of 16 minutes and a UV-C dosage of 0.576 kJ/m², the irradiated tardigrades still seemed relatively active. Most of them were as active as before, while a few moved more slowly. Until 10 p.m. most slow tardigrades could recover. At 2 a.m. four tardigrades began to suffer a relapse. On 16 August 2018, the four tardigrades had died. During the remaining days nothing changed in terms of activity.

A further group of tardigrades received a dosage of 0.288 kJ/m² in the UV-C sterilizer for 8 minutes. This was the first measured data point where the tardigrades showed no change in activity over the entire 14 days without exceptions.

In all further measurements with 4 minutes irradiation time and a dosage of 0.144 kJ/m², 2 minutes and 0.077 kJ/m² and 1 minute and 0.036 kJ/m² the tardigrades also showed no changes in their activity level.

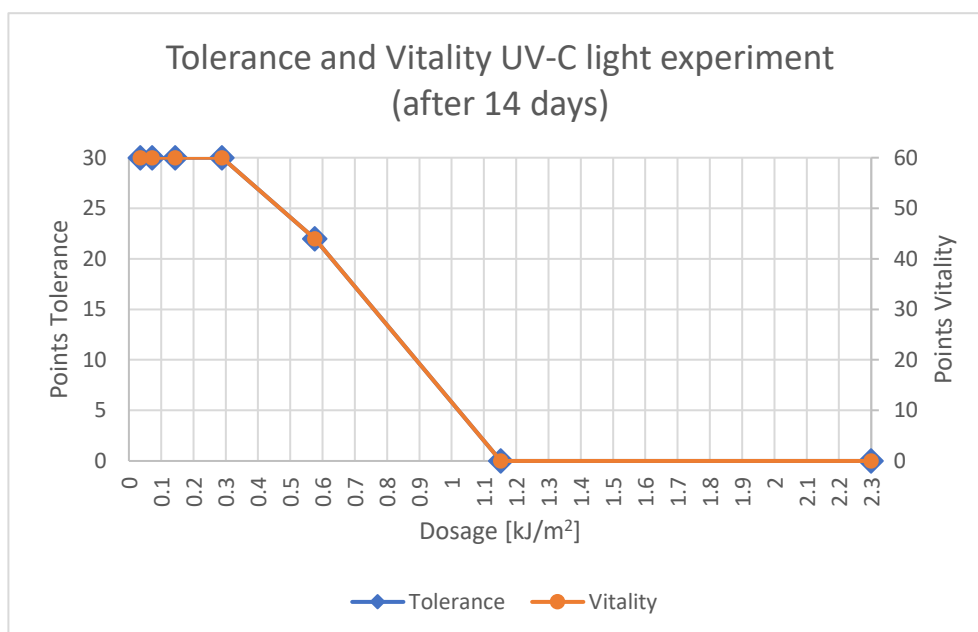


Diagram 1: Tolerance and Vitality 14 days after irradiation

4.2 Results pH experiment

The behavior of *Hypsibius exemplaris* in different pH values changed as expected in the higher and lower pH ranges. Deaths and one of the cryptobiosis forms were visible. The reason why the pH value 2.5 is not represented is that at first only the spectrum from pH 3 to 13 had been covered, but then there was interest to investigate the pH value 2 due to the strongly corrosive effect. pH 2 is still represented in the work as a data point due to the widely differing results.

The tardigrades have not been able to withstand pH 2. Although the animals were very active in the solution at first, they died after five minutes. Interestingly, the outer membrane or cuticle seemed to dissolve and pull "threads".

In pH 3, the tardigrades struggled initially. For more than 10 minutes they became less and less active and some finally switched to a resistance stage. After 15 minutes more and more of the tardigrades became resistant until finally after 30 minutes each of the tardigrades was in a state of cryptobiosis. Osmobiosis was probably used here because the tardigrades took on the typical barrel shape and remained motionless in the water. After 60 minutes in pH 3, the solution was removed and tap water was added (pH 7 to 8). Only 15 minutes later, the tardigrades were able to recover from their cryptobiosis.

The tardigrades were far more resistant to pH 3.5 in comparison to pH 3. Although the movements of some tardigrades took up to 30 minutes to take effect, and from these 30 minutes onwards four of them could hardly move. No tardigrade reached a resistance stage.

The behavior in pH-value 4 and 4.5 was very similar. After 30 minutes in both solutions did only one tardigrade move more slowly. Up to a response time of 60 minutes, one more tardigrade with slightly slower movement was observed in each of the two experiments. In pH 4.5 one of the two slowed tardigrades hardly moved its legs at 60 minutes.

In pH 5 one tardigrade became overall less active after 60 minutes, all others showed no change in their activity in this solution. The tardigrades were not affected in the pH values 6, 7, 8, 9 and 10. During 60 minutes in the solutions mentioned, no unusual reaction of the test animals occurred. They behaved the same way as in their previous environment of pH \approx 8.

From pH 11 the results get interesting again, because after 15 minutes a tardigrade showed a lower activity over the rest of the time. The next major change in behavior was in pH 11.5. After 5 minutes in the solution, around half of the tardigrades in the three series of measurements became less active and the rest could hardly move. After 15 minutes the number of barely moving tardigrades increased to two thirds and stagnated until the end of the experiment.

At pH 12 however, *Hypsibius exemplaris* was no longer able to withstand the high pH values. All tardigrades were active immediately after the addition of the solution, but all except for one died after 5 minutes. The last surviving tardigrade died after 10 minutes. The dead tardigrades lay stretched and somewhat swollen at the bottom of the Petri dish. In addition, they were no longer transparent and glassy looking, but their entire interior turned milky-white, so that the stomach contents were not visible anymore.

Almost the same outcome took place in pH 12.5, but with the exception that almost immediately after addition of the solution a total of 2 *Hypsibius exemplaris* died and the rest hardly moved. The rest was found lifeless after 5 minutes without exception.

pH 13, was even more harmful to the tardigrades. A few moments after the addition of the solution, about one third of all tardigrades died and the rest were hardly active. After 5 minutes the remaining tardigrades were dead as well.

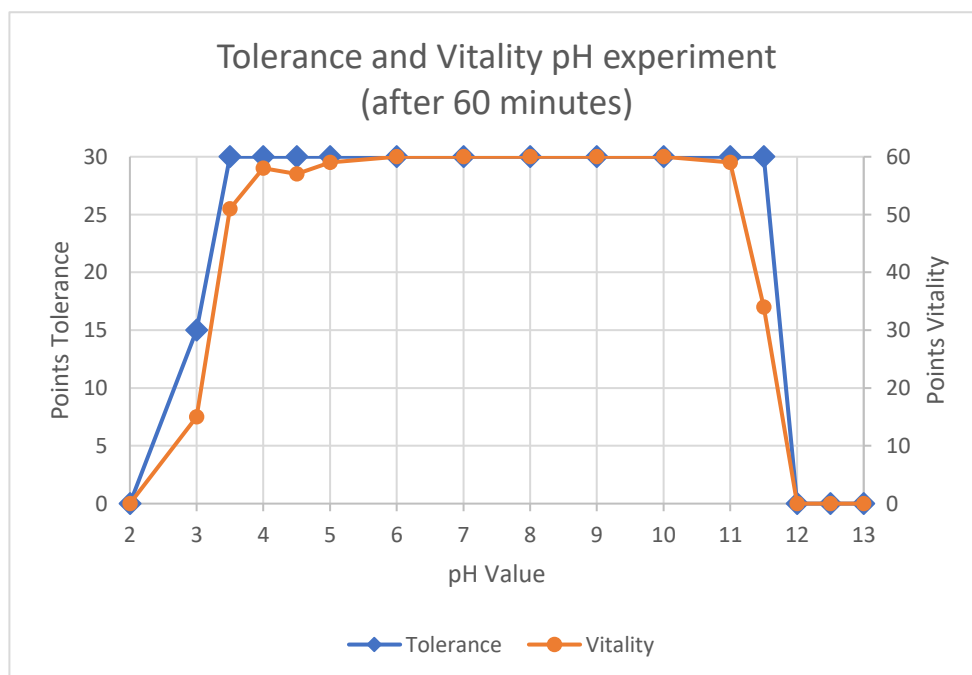


Diagram 2: Tolerance and Vitality after 60 minutes in solution

5 Discussion

5.1 UV-C light experiment

A closer look at the collected data reveals that 64 minutes or a dose of 2.304 kJ/m² was the upper limit in this experiment. None of the examined tardigrades could withstand this dose, since they were no longer alive immediately after the irradiation was completed. In the protocol of the irradiation time of 32 minutes a surprising observation can be seen. After 15 minutes 3 tardigrades were dead in the first measurement series, but after 45 minutes only 2. This paradox can be explained as follows, it is always possible that during observation of activity levels, an experimental animal may not have moved at this moment or had signs of a death situation. So, in the end it is possible that it was an erroneous observation, thus the mentioned contradiction arose. If we continue to look at the data at the UV-C dosage of 1.152 kJ/m², it is notable that *Hypsibius exemplaris* is already more resistant, but all specimens died after about 24 hours. Accordingly, UV-C light causes persistent damage, which can lead to death with sufficient severity, even after the irradiation has stopped. One hypothesis would be that UV-C light damages the DNA due to its high energy content and can therefore lead to death even after irradiation. The paper "Analysis of DNA Repair and Protection in the Tardigrade *Ramazzotius varieornatus* and *Hypsibius dujardini* after Exposure to UVC Radiation" by Daiki D. Horikawa et al.¹⁶ describes that in DNA exposed to UV-C light two adjacent thymine bases can combine to form a thymine dimer.¹⁷ Thymine dimers block DNA replication and mutation. To separate thymine dimers from each other, photoreactivation or normal DNA repair (excision repair) is required.¹⁸

However, the tardigrades in my experiment were not able to use photoreactivation since they were not exposed to sunlight during the experiment. This shows how advanced their DNA repair is on its own. Another possible reason why *Hypsibius exemplaris* suffered persistent damage is the destruction of vital proteins (such as transport proteins) or internal organs by high-energy rays.

At a dosage of 0.576 kJ/m² *Hypsibius exemplaris* seems to be much more resistant, since no animals died initially and over the remaining 14 days only about one third did not survive.

¹⁶ Horikawa, Daiki D. et al., (2013) *Analysis of DNA Repair and Protection in the Tardigrade *Ramazzotius varieornatus* and *Hypsibius dujardini* after Exposure to UVC Radiation*

¹⁷ Horikawa, Daiki D. et al., (2013) *Analysis of DNA Repair and Protection in the Tardigrade *Ramazzotius varieornatus* and *Hypsibius dujardini* after Exposure to UVC Radiation*, p. 1

¹⁸ Spektrum.de, (2018) *Thymin Dimere*

In addition, the activity levels do not seem to change after approximately 3 days. In the 8 minutes or 0.288 kJ/m^2 range the tardigrades are not affected, all experimental animals survived here. If we compare the dosage of 1.152 kJ/m^2 and 0.576 kJ/m^2 , the LD50 for UV-C light irradiation seems to be between these values. Probably closer to the point of 0.576 kJ/m^2 .

What can also be deduced from the work of Daiki D. Horikawa et al. is that terrestrial tardigrades appear to be more resistant to UV-C light than those found in water. According to their measurements, this seems to be due to the more effective DNA repair of terrestrial tardigrades.¹⁹ My hypothesis is that tardigrades are less exposed to harmful UV rays due to the light filtering function of water, so that they do not have to rely so much on their DNA repair. Terrestrial tardigrades are not necessarily protected from the harmful rays by thick layers of water. Therefore, the terrestrial tardigrades need a more efficient DNA repair. However, we should not link this to an adaptation to UV-C light here, but to UV radiation and its damage in general. It refers to UV-A and UV-B rays, which naturally hit the earth, compared to UV-C, which is filtered out by the ozone layer. So why are some tardigrades still resistant to UV-C light? UV-A, -B and -C belong to the same kind of rays, with slightly different wavelengths, with UV-A having the lowest energy and UV-C the highest energy of the three rays. The tardigrades are therefore exposed to UV-A and UV-B and adapt to the resulting damage. If this is large enough and the DNA repair adapted, it should be possible for them to withstand the damage of UV-C light for a shorter time, since this light is more energetic and therefore more harmful.

Interestingly, not a single tardigrade resorted to a resistance stage. One possible reason would be that no resistance stage can withstand UV-C light. This means that the highly energetic rays easily pass through the cuticle and (depending on the cryptobiosis form) built up substances. To find out, one would have to test *Hypsibius exemplaris* for any resistance stages to UV-C light, but this is not within the scope of this work. Another reason could be the absence of receptors which perceive UV-C light as a stimulus and signal *Hypsibius exemplaris* that harmful radiation is present, since such a receptor would hardly be useful in nature, due to the ozone layer that filters out most of the UV-C light anyway.

¹⁹ Horikawa, Daiki D. et al., (2013) *Analysis of DNA Repair and Protection in the Tardigrade Ramazzotius varieornatus and Hypsibius dujardini after Exposure to UVC Radiation*, p. 9

5.2 pH experiment

Tardigrades are naturally exposed to acidic and alkaline environments. In this experiment, the tardigrades withstood most pH values, but were considerably affected by highly acidic and highly alkaline solutions. Lethal solutions were pH 2, pH 12, pH 12.5 and pH 13. In the solutions mentioned, not a single test animal survived in the observed exposure time of a maximum of 60 minutes. It is notable that death in an acidic environment differs from death in an alkaline environment. As soon as an animal was exposed to pH 2, it died in the solution after about 5 minutes. The body of the tardigrades seemed to dissolve somehow. Not only that, but it dissolved the outermost layer and pulled "threads". These transparent "threads" hung at one end on the animal itself, and they became almost longer than the body. A conclusive explanation of what exactly loosens could not be found. I suspect that it is the cuticle, since it is the outermost layer and when the bodies of the tardigrades dissolved, they still had all the characteristic features and forms.

This was quite different in comparison to pH 12 to 13, where nothing dissolved, but the animals stretched and became swollen. In addition to swelling and stretching of the body, the tardigrades sank to the bottom of the Petri dish. These are all characteristics of tardigrades which have generally died due to various factors. Interestingly, there was another unexpected feature in the body of the deceased animals. The body was no longer transparent, but had a milky white color. Internal structures like the stomach contents could not be recognized. In order to explain this color change, it is necessary to know which structures can be destroyed in such a way. Since the animal did not show any changes from the outside, it must be inner tissue. I suspect it is the muscle tissue of the tardigrades. Not only do the muscles cover most of the body, but muscles consist of proteins. Proteins are known to denature, and this can lead to the milky or white color. Of course, this is only a hypothesis and I could not prove that it was muscle tissue, but I started a small side experiment to verify my statement. Egg whites are proteins too, so I took an ordinary hen's egg, separated egg white from egg yolk and put a small part of the egg white on a petri dish. Now the alkaline solution was added. For this I used the test solution with a pH value of 13. After five minutes I could observe how the protein denatured and also became milky-white and solidified. The result supports my hypothesis that the lethal alkaline solutions entered the body of the tardigrades and triggered a reaction with the muscle tissue. Thus, the cause of death is most probably the denaturation of the muscle tissue.

Another mentionable aspect of the experiment was that *Hypsibius exemplaris* actually used a form of cryptobiosis. Only in pH 3 such an endurance stage could be observed. After 30 minutes, all experimental animals resorted to this stage. The shape was similar to a cylinder, the legs were retracted, the head hardly recognizable and the body size was significantly smaller than before the addition of the acid solution. All these observations speak for the barrel shape of osmobiosis as well as the present H_3O^+ and OH^- ions. Since osmobiosis is only used as soon as the ion concentration is no longer favorable and cells can be destroyed, it is advantageous for the tardigrades to perform osmobiosis in acidic and alkaline solutions. Thus, almost all the water flows out of the animals and at the now open critical binding sites, carbohydrates are added.

At pH 3.5 the tardigrades no longer used any kind of cryptobiosis. Although some test animals were affected, there seemed to be no need to protect themselves. From pH 4 to 11 *Hypsibius exemplaris* does not seem to struggle. From pH 11.5 the situation worsens again, but is not yet lethal. An LD50 could not be determined since it lies between pH 11.5 and pH 12 for alkaline solutions and this range was not investigated further. For acid solutions the LD50 probably lies between pH 2 and pH 3.

According to all the findings, it is also notable that although a form of cryptobiosis was to be seen in an acidic environment, it was not used in an alkaline one. My hypothesis is that *Hypsibius exemplaris* probably has receptors for acid solutions and for H_3O^+ ions and that these receptors transmit signals to the nervous system. They signal that there is a change in the ion concentration and that osmobiosis has to be performed. The reason why alkaline solutions do not cause irritation is probably due to lack of receptors for them. If we consider the natural conditions, it seems logical that receptors for acidic liquids are present, since more acidic waters can be found in nature and hardly any alkaline ones due to the more frequent water acidification.²⁰ Acidic waters can be waters with a high degree of minerals or volcanic lakes. They are much more common than alkaline waters. Soda lakes with their high pH value and high salt content belong to alkaline waters.²¹

²⁰ Spektrum.de, (2018) *Gewässerversauerung*

²¹ Wikipedia, (2018) *Natronsee*

It is also noteworthy that cryptobiosis was only used in a small range of acid measurements. The threshold up to LD50 and the threshold for cryptobiosis where it is no longer needed when the pH value rises are not far apart. Almost the same applies to the alkaline range with the difference that the activity drops abruptly from pH 11 to 11.5 and the lethal pH value is close by.

The tolerance and vitality diagram reveals that *Hypsibius exemplaris* does not do well in a highly acidic (pH 3) and highly alkaline environment (starting at pH 11.5). Both graphs are almost identical, only the vitality graph is slightly lower than the tolerance graph at pH values 3, 3.5, 4, 4.5, 5, 11 and 11.5. Thus, the activity of *Hypsibius exemplaris* is lower at the mentioned levels, despite the highest possible tolerance.

Regarding the tolerance curve, it can be said that the tolerance range lies within pH 2 and pH 12. The minimum is at pH 3 and the maximum at pH 12. The optimum lies between pH 3.5 and 11.5 if only the tolerance curve is considered. If the vitality curve is considered as well, the optimum is between pH 6 and 10. No statements can be made about pessimum or ecological potency in this experiment, since reproduction was not included as a factor.

5.3 The global aspect

The results of both experiments show that *Hypsibius exemplaris* withstands a wide range of environmental influences. Especially the pH experiment reveals *Hypsibius exemplaris*' capability of inhabiting almost every water body in our world without relying on cryptobiosis, as long as there is enough food for them. In case of facing rapid adverse changes, this species uses cryptobiosis to outlast those changes until the environment shows more favorable conditions. My work provides data on the adaptability of *Hypsibius exemplaris* to extreme environments and contributes to extending the existing knowledge on their adaptive skills. How exactly tardigrades adapt this fast to changing environments is still largely unclear, but those extremes may someday become common conditions with adaptation being the only key to survival. Understanding one of the toughest animals found in aquatic ecosystems may contribute to finding solutions to upcoming problems and to protecting the vast and fascinating world living underwater.

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