Influence of plastic on the warming of sand

Mia Bacalja, Andrea Brzica, Tia Gebauer, Jakov Lušo
Mihaela Marceljak Ilić
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Summary

The aim of this research is to discover if microplastics in and on the sand impact its temperature and to apply that to the theoretical ratio of newborn male and female turtles (*Trachemysscripta elegans* (Wied-Neuwied, 1839) which gender is determined by the sand temperature in which the eggs are incubated. The project was conducted during a two-week period during which the temperature changes on 3 different sand bases were measured: plain sand, sand with a microplastic mixture and sand with a microplastic mixture and plastic pieces on top of it. The sand was heated using bulbs with IR and UV radiation and the temperature was kept between 28,4°C and 28,8°C, a temperature interval in which only male individuals of *Trachemysscripta elegans* hatch, using thermometers that were buried in the sand. The average temperature of sand with microplastics in and on it was 0,5°C higher than the average temperature of plain sand. That rise in temperature passes the temperature interval in which only male individuals are hatched and moves into the interval in which male and female individuals are born in the same ratio.

Introduction

It is noticeable that plastic is appearing in nature more often whether coming from plastic bottles or little microplastic particles that surround us. Despite an everyday rise in plastic pollution, the impacts of microplastic pollution are not entirely known. Considering that many animals lay their eggs in the sand for incubation and since the determination of the sexes in some animal species is temperature-dependent, we have decided to research how plastic in and on sand impacts its temperature.

If plastic does impact the temperature of the sand which has already been seen in some research (Lavers J. L. et al., 2021) we can conclude that plastic pollution is one of the factors that contribute to the warming effect of climate change and with that also having a negative effect on animals and plants whose life cycle is connected to sand. For instance, turtles are animals whose reproduction is tied to sand. Namely, many species of turtles have temperature-dependent sex determination which means that the temperature at which the laid eggs are incubated will determine the sex of the individual. Therefore the changes in the normal temperatures that these species are adjusted to will have a significant impact on their biological processes and the development of those species in areas polluted by plastic.

For this research to be relevant it was decided that the temperature of sand would be regulated in a biologically significant temperature interval. In this research aimed temperature interval was between 28,4°C and 28,8°C. That interval was chosen because in nature at temperatures below 29°C exclusively male individuals of the red-eared slider turtle *Trachemys scripta elegans* are hatched, while at temperatures between 29°C and 29,5°C the ratio of hatched individuals is 1:1. Increasing the temperature over 29,5°C more and more female individuals of the red-eared slider turtle will be hatched as shown by research (Bull J. et al., 1982; Crews D. et al., 1990; Ewert M.A. et al., 1991; Wibbels T. et al., 1994).

In addition to measuring the temperature of sand, using GLOBE pedological protocols, pH and infiltration measurements were conducted for all three groups of sand substrates used in this research. The stated measurements were done to establish the impact of microplastic on the pH value of sand and how the sand retains water considering that studies (Guo Z. et al., 2022) show that soil containing microplastic becomes more porous and rinses off easier and has a lower pH value (Gharahi N., 2022). Such a phenomenon can have a great impact on the wildlife dependent on sand because if the soil becomes more porous it rinses out during heavy rain and changes the habitat of many plant and animal species.

Research questions and hypotheses

Our research questions were:

1) How does the temperature of sand change depending on the presence of plastic on/in it?
2) How does the presence of microplastic in/on sand impact pH value and infiltration?

Based on the stated research questions, the following hypotheses were set:

1) The temperature of the sand with microplastic will be higher than the temperature of sand without any microplastic while being exposed to the same amount and power of IR and UV radiation.

For the substrate with plastic on the surface, it is expected that the plastic cover will have a separate impact on the temperature of the sand. Research shows that plastic waste impacts the temperature of sediments in a way that changes the input/output heating power, apropos the absorption of IR radiation. That mechanism increases temperature amplitudes and shifts the daily rhythm of sand. As the plastic fragment absorbs a certain amount of heat, it is presumed that upon its release the sand will warm up more. Considering that plastic is a well-known isolator, the presumption is that the plastic cover will prevent heat from penetrating the surface and reduce the impact of plastic heating in the measured depth. It has been shown that the temperature of sand stabilises at greater depths and in cases where plastic covers 100% of the surface that effect is disturbed and the minimum and maximum temperatures of the substrate fluctuate more (Lavers J. et al., 2021).

The tempšerature interval between 28,4 °C and 28,8 °C was chosen because it was planned to include turtle eggs in the research. In that way, it would be possible to check how microplastic impacts the determination of sex. In the control group, only male individuals would be hatched while in the terrarium with microplastic males and females would be hatched. Unfortunately, eggs were not available so the obtained data was connected to and interpreted through the information in the literature.

2) The pH value of the control group will be higher compared to the pH values of sand with microplastics and sand with microplastic and plastic on the surface, while it is presumed that the control group will have the lowest infiltration.

The stated is hypothesised because plastic usually contains various dyes and chemicals that lower the pH during their decomposition (Gharahi N., 2022). It is hypothesised that sand with microplastic and a plastic cover will have the highest infiltration, then sand with microplastic and then the regular sand in the control group. The stated is presumed because research shows that microplastic impacts the infiltration of sand by making it more porous (Guo Z. et al., 2022).

Research methods

An experimental setup was designed so that it was possible to test the impact of microplastic on the heating of sand in controlled environments. In this study, three different substrates were used. Because of the precision of the used instruments the upper limit was sat at 28,8°C (instead of 29°C) so that it would be ensured that the control groups would be kept in the interval in which female individuals would not hatch. In the end, statistical analysis was conducted with the measured temperature values.

Research time

The total time of research was 14 days. During the first five days, the system of bulbs and thermometers was calibrated so that the same temperature was achieved in each of the six glass containers (dimensions 23x14x16cm). After all of the containers were heated to the same temperature, the containers were watched for two more days to make sure that all of them evenly sustained the temperature (7 days of calibration). On the eleventh day, three experimental substrates were placed in the six containers.

Calibration

For the purpose of precise regulation of the temperature of sand inside of the experimental setup, above all glass containers terrarium carriers JBL TEMPSET BASIC were placed at the same height. D3 UV Heat Spot bulbs with an output power of 80W were mounted on the terrarium carriers. In all of the glass containers stick thermometers (Vernier) were buried at the depth of 10 cm and connected to the LabQuest software. The software was used to collect and store data of sand temperatures. Before the experiment began it was established that the lamps do not equally heat even plain sand at the same height so it was necessary to adjust the setup by changing the height of the terrarium carriers. The defined procedure of calibration was conducted above glass containers filled exclusively with plain sand (no microplastic or plastic cover) as shown in picture 1. Calibration was directed by burying thermometers in glass containers loaded with plain sand and the height of lamps above the glass containers until a system in which, during the calibration time (7 days), the temperature oscillations were the same. During calibration, the layout of the containers was changed as was the amount of space between them until a system in which all substrates are heated equally was set up (Picture 2.). By this method, it was ensured that all terrariums were absorbing the same amount of heat.

Picture 1. Experimental setup during the process of system calibration to the same input of heat

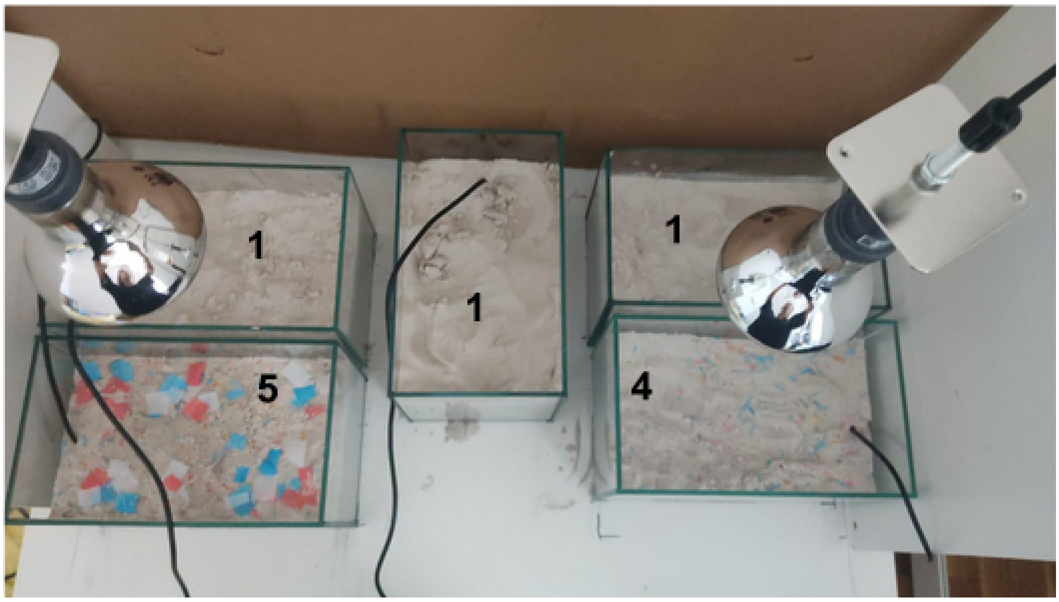
The system was then put into circulation and the thermometers and bulbs kept the temperature of the sand in the control substrates in the desired interval between 28,4°C and 28,8°C. In the experimental setup used in this research the temperature interval of sand was strictly controlled using the previously described thermometers (buried at a depth of 10 cm) in a way that if the temperature in the control glass container lowered to 28,4°C, the bulbs above all containers would turn up and stay turned on until the temperature in the control container did not reach 28,8°C.

Group set-up

The experiment used five containers which were divided into 3 systems (heaters). In each system, one of the containers was a control. The first system was made up of a substrate with microplastics in and on the sand and a control substrate and the second one substrate with microplastics in the sand and a control substrate. It was crucial to have a control group for each of the experimental containers so that the temperature interval could be observed relative to the control group since it was established during calibration that the lamps do not heat equally. The third system contained only one control group under a separate heater which was used primarily for easier control and extra regulation of the temperature but also for controlling moisture of sand.

Creating the substrates

Each of the containers contained 4 kilograms of plain terrarium sand Flamingo brand. It is a natural, non-toxic sand for terrariums which does not contain dyes and other chemical additives. Also as stated by the specifications it does not contain organic matter or pathogens. Overall three control containers (label 1, Picture 2.) which were filled with the specified sand were used. In the fourth container (label 4, Picture 2.) along with the 4 kilograms of sand 20 grams of microplastic were added. The microplastic was made by mechanically breaking plastic straws and cups of different colours using a kitchen blender and then added to the sand of the fourth glass container using a spoon. The fifth container (label 5, Picture 2.) had the same composition as the substrate in container 4, however to this container among the plain sand and 20 g of mixed-in microplastic a plastic cover in the shape of thin plastic sheets, which was made by cutting them out of colourful plastic cups, was added. They were laid on top of the sand and covered 30% of the surface, so that this container had microplastic in the substrate (20g), but also plastic on the surface.



Picture 2. Experimental setup during the research (label 1- control group, label 4- the group with microplastic in the sand, label 5- the group with microplastics in the sand and plastic on the surface)

After calibrating the system (7 days) measurements were taken in all three systems i.e. in five glass containers. Temperature sensors recorded the temperature of sand every 30 minutes on the LabQuest software.

To simulate the natural conditions it was necessary to dampen the sand. To every group of sand, 600 mL of water was added by spraying so that it would evenly distribute through the whole volume of the sand. The moisture of sand was measured using a hygrometer and kept at 50%. Because of the heating of the sand water evaporated and it was needed to spray the sand with water daily. so that the moisture would stay at the same value. 10 mL of distilled water was additionally sprayed every day.

Picture 3. Experimental setup for measuring the infiltration of sand

After finishing the experiment, using the GLOBE protocols for soil analysis (Program GLOBE-Hrvatska, 2021) infiltration of soil i.e. the sand used in the experiment in all three substrates. To measure infiltration *LearningActivity* protocol *„JustPassingThrough“* (GLOBE.gov, 2014) was used because of the inability to carry out the measurements using the GLOBE *Soil Infiltration* protocol. Using the *„JustPassingThrough“* protocol infiltration is measured by assembling a plastic bottle as shown in Picture 3. and adding sand and water in the volume ratio of 2:1. After the water stopped dripping, the wet sand was weighted and from the measured mass, the mass of the dry sand, which was measured before adding water, was subtracted, so that the mass of water that the sand absorbed could be calculated. Using the calculated mass of water and the density of water the volume of absorbed water was calculated by which the infiltration was measured. The pH value was also measured for all three groups after the experiment.

Results

Analysis of the results of this experiment is based on the temperature data measured for all three substrate groups in the span of a week.

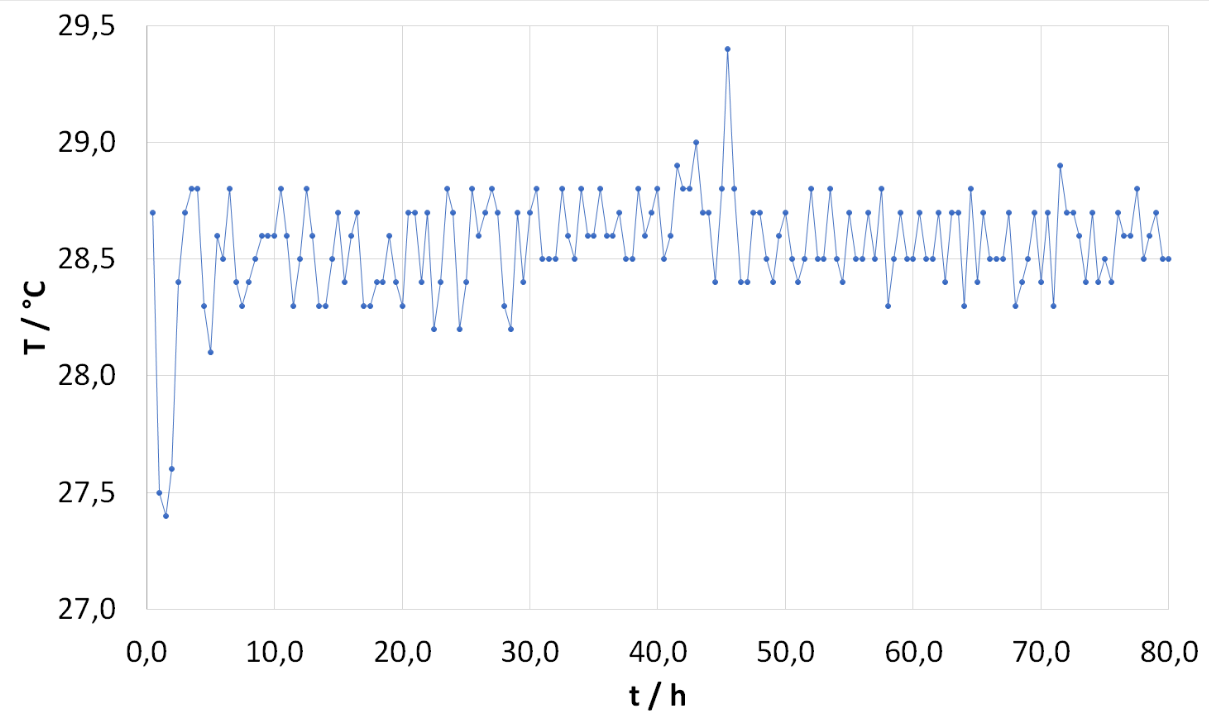


Figure 4. Sand temperature in control groups during experimental week period

Figure 4. shows how the temperature of sand of the control group changed during a time period of a week. The average temperature of sand in the control group was 28,6°C. The standard deviation is ±0,23°C. The maximum measured value was 29.4°C.

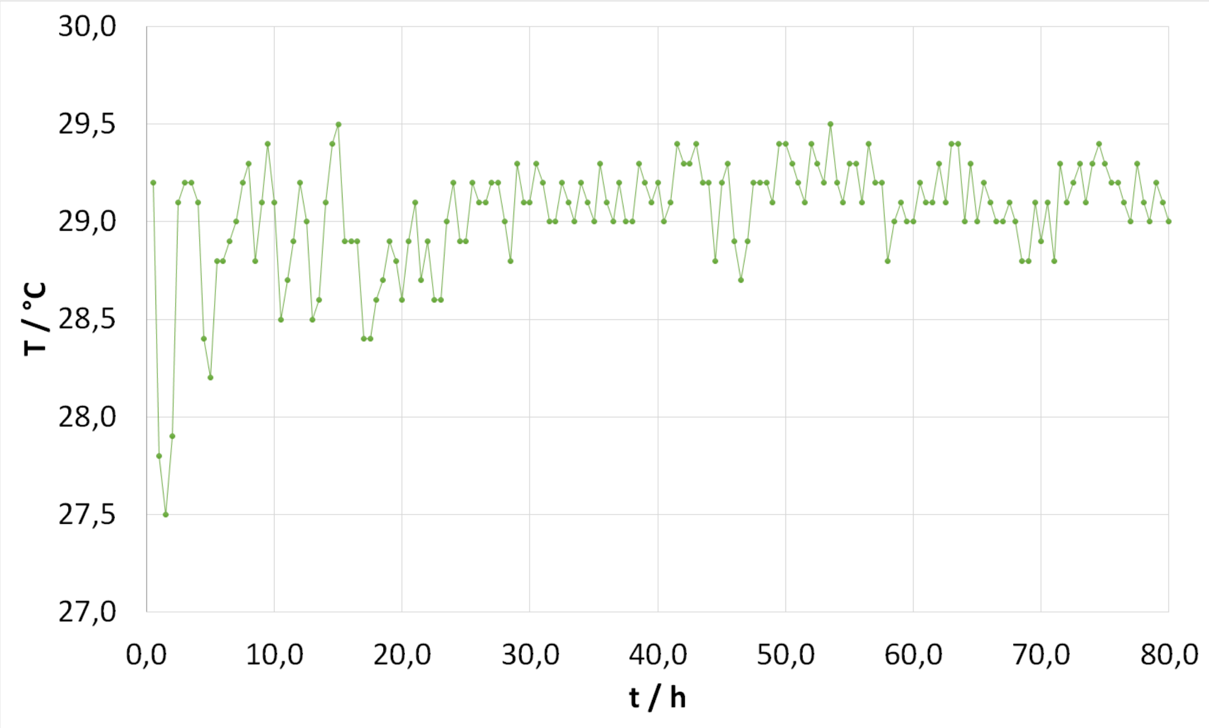


Figure 5. Sand temperature with microplastic mixture during the experimental week period

Average temperature of sand with microplastic was 29,1°C, with a standard deviation of ±

0,27°C. The maximum measured temperature was 29,5°C (Figure 5).

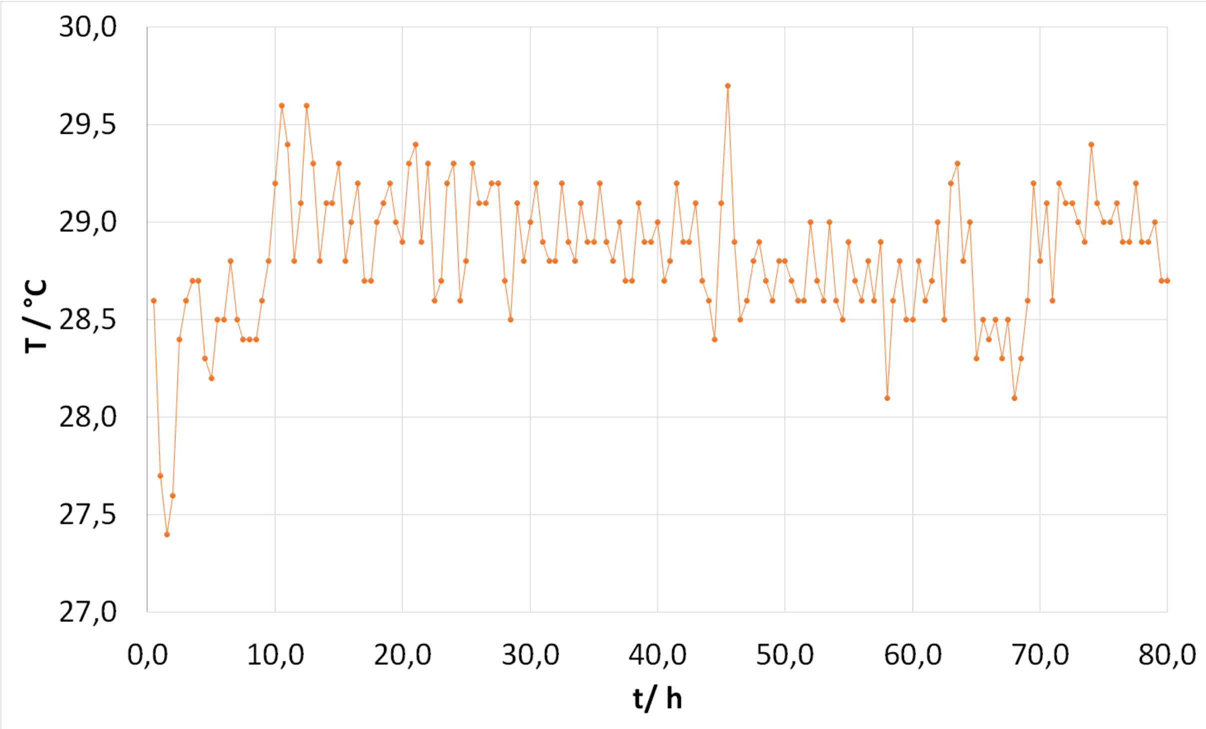


Figure 6. Soil temperature of sand with the microplastic mixture and plastic pieces on top of it mixture during the experimental week period

Figure 6. shows the temperature dependence of sand filled with microplastic and plastic sheets on the surface during a span of a week. The average temperature of the sand was 28,8°C while the standard deviation was ±0,33°C. The maximum measured temperature was 29,7°C.

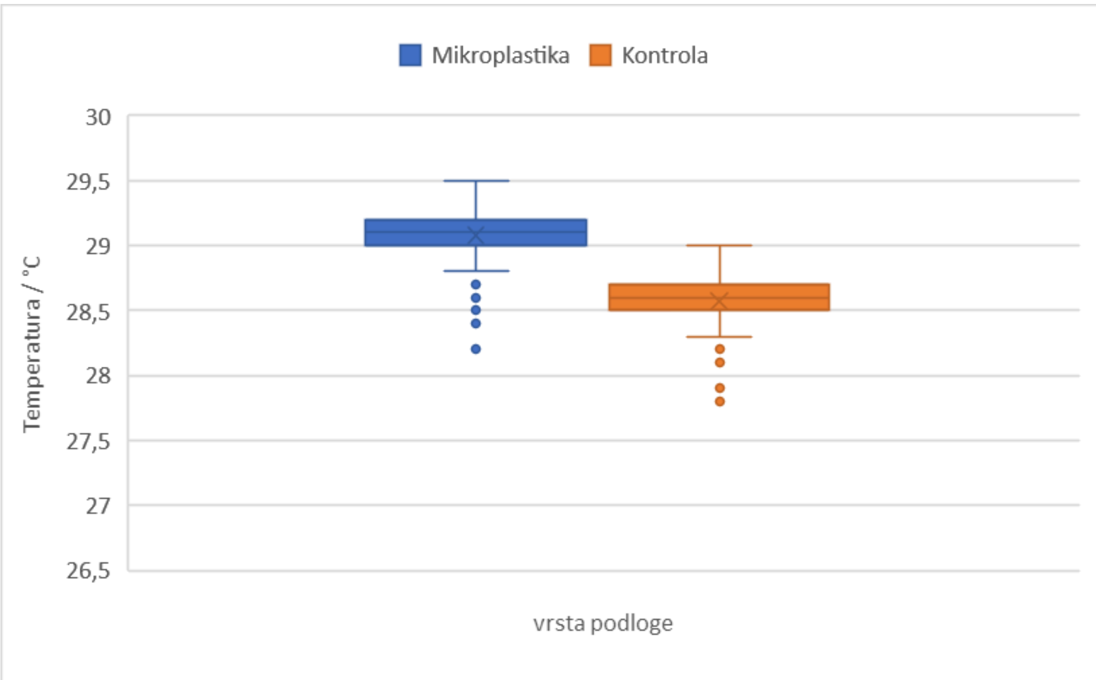


Figure 7. Comparison of sand temperature with microplastic mixture and control group; the box shows the standard deviations, the horizontal line presents the average value, and the vertical line the minimum and maximum values, with a dot display of extreme values.

Figure 7. shows a diagram which allows an understanding of the difference between the temperature of sand with microplastic and the control group. A significant difference is visible in the temperature of the control group compared to the sand with microplastic.

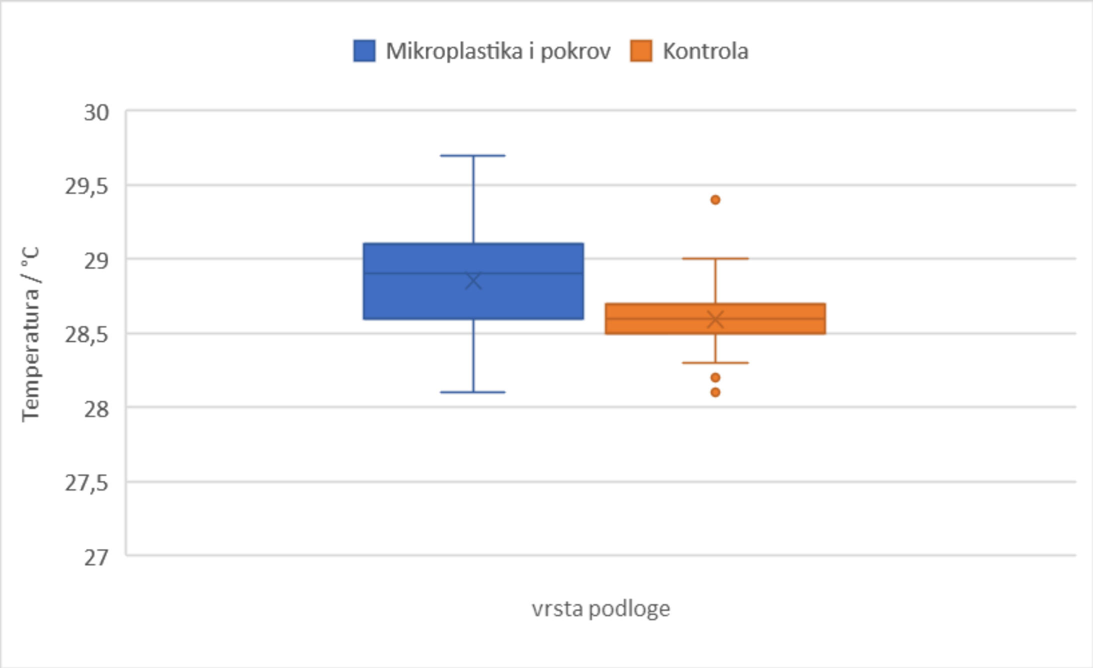


Figure 8. Comparison for soil temperature of sand with microplastic within and microplastic pieces on top and control group; the box shows the standard deviations, the horizontal line presents average value, and the vertical line the minimum and maximum values, with a dot display of extreme values.

Figure 8. shows a diagram that allows an understanding of the difference between the temperature of sand with microplastic and plastic sheets on the surface and the control group. A significant difference is visible in the temperature of the control group compared to the sand with microplastic and plastic sheets on the surface.

The statistical t-test of comparison of the temperatures in the sand with microplastic and the control group and sand with microplastic and plastic on the surface and its control group shows that the difference in temperatures is statistically significant. For comparison, the temperature of sand with microplastic and the control t-value is 24,68329 with a reliability of $p<0,00001$, while the temperature of sand with microplastic and plastic on the surface and its control value t is 10,42417 with a reliability of $p<0,00001$.

Table 1. Average results for pH and infiltration

	sand	sand with microplastic	sand with microplastic and plastic on the surface
pH value	7,0	6,8	7,0
infiltration	0,40	0,40	0,46

In Table 1. it is visible that the pH value is lower for the substrate with microplastic and an increase of infiltration value for the substrate that contains microplastic and plastic on the surface.

Discussion and conclusion

Based on the obtained results, it is concluded that the presence of microplastics in sand and plastic on the sand's surface causes an increase in sand's temperature by more than 0,5°C. Although the resulting difference may not seem large, it can have a significant biological impact. Considering the case of the species *Trachemys scripta elegans,* which lays eggs in the sand at a depth of 10 cm, at a temperature below 29°C they hatch only male individuals, with the increase of temperature to 29,5°C male to female individuals ratio is close to 1:1, and as the temperature more increases the proportion of female individuals increases until it reaches 100%. According to obtained data which shows that the presence of microplastic increases sand temperature by more than 0,5°C, eggs incubated in control groups would produce 100% male newborns, while groups with microplastic in sand and microplastic in and plastic on sand surface would produce newborns in 1:1 male to female ratio, or even higher in favour of females. With the continued increase of temperature, the ratio would be shifted more and more in favour of female newborns, until it reaches 100% of female newborns. In nature male to female ratio varies depending on conditions in their environment; in warmer areas proportion of females will be higher while In colder areas proportion of males will be higher. That increase in temperature can, with the decrease in the proportion of male individuals, disrupt the natural balance of the sex ratio of red-eared sliders. Although it is an invasive species in Croatia, this effect can be applicable to all species which have sex determination depending on the temperature (which a lot of turtle species have). Disturbing a natural balance of sex ratio has a big impact on the mentioned species and all of their ecosystems.

There weren't big differences in pH value and infiltration of sand between control groups and sand with microplastic. Research (Medyńska-Juraszek A., 2022; Gharahi N., 2022) points out that the presence of microplastic in soil decreases its pH value because during UV and IR radiation plastic releases organic carbon compounds which can acidify substrate (Dissanayake P. D.et al., 2022.). The results of this research match with the results in the literature because it is measured that in the sand with microplastic pH value is lower than the pH of the control group with normal sand. The results of sand infiltration are also close to equal, but sand with microplastic plus plastic on its surface is better at keeping water than sand with only microplastic and normal sand. What we have recorded is the opposite of what research in literature says (Guo Z. et al., 2022), but the reason for that is most likely because of big pieces of plastic in the plastic cover on the sand's surface which then kept water from flowing through the sand. We can conclude that microplastic affects the pH of sand making it more acidic, and in this research, it was shown how the presence of microplastic and plastic cover can increase water permeability in sand.

The biggest methodological limitation we had was the lamps we used for warming sand which didn't have equal heating intensity, and because of that calibration was longer and before adding plastic we needed to modify lamp distance from sand to ensure equal heating and to exclude difference in heating to be interpreted as plastic influence. Keeping control groups in a certain interval 28,4°C - 28,8°C was also difficult because of the lamp's late reaction time. After the lamps turn on, it requires some time to actually start heating, and after they turn off they continue to heat until they cool down. So because of that, turning the lamps on and off needs to be modified for the temperature interval to be as close to the wanted interval. After spraying water in a group with plastic cover on sand drops of water were kept on the surface. It would be better to have another way of adding water so this problem wouldn't happen and then it wouldn't negatively affect the amount of moisture in sand and the temperature of plastic cover, that is, the impact of its thermal capacity on temperature. For a better and more accurate conclusion about the impact of temperature on turtle sex, it would be ideal to have turtle eggs and more replicas of glass substrates.

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Badges:

I AM AN ENGINEER

We believe we deserve the I AM AN ENGINEER badge because while setting up our experimental setup we came across many difficulties with our equipment which required us to think outside of the box on how we could fix those problems and arrange our setup that all containers had the same conditions. In the end, we are proud to say that we found solutions to those problems and successfully carried out our experiment.

I AM A DATA SCIENTIST

We believe we deserve the I AM A DATA SCIENTIST badge because while analysing our results we used many different statistical tests and different ways of analysis to make sure that our data is relevant and applicable. Some of those analysis includes the t-test to ensure that our data is statistically significant and the box and whiskers graph that allowed us to easily show the impact of the collected data.

I MAKE AN IMPACT

We believe we deserve the I MAKE AN IMPACT badge because the data collected during our research has a real-world impact and clearly shows how plastic pollution everywhere in the world can impact the ecosystem in even small amounts. In addition to that we have shown how little changes in temperature that may not seem significant to humans are dangerous for ecosystems of the world because wildlife is more sensitive to it than we think.