INTRODUCTION

The literature provides a great number of studies on the effects of a variety of intrinsic and extrinsic factors on the cognitive abilities of fish and other ectotherms (e.g., Braithwaite, 2006; Brown et al., 2008; Shettleworth, 2010). These studies include effects on various processes, including spatial learning (Laland et al., 2003), which is one of the contemporary topics and basal research areas in fish cognition and behaviour (Pouca & Brown, 2017). It has been revealed that spatial learning in fish depends on sex in blennid fishes (Costa et al., 2011) and in guppy (Lucon-Xiccato & Bisazza, 2017), habitat type in gobies (e.g., White & Brown, 2014) or personality.

RESEARCH ARTICLE

The effect of temperature on the spatial learning rate of zebrafish (Danio rerio)

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Abstract
Temperature has a wide range of effects on the biology and ecology of ectotherms, including fish. However, the literature provides only a few studies assessing its effect on their cognitive abilities. We tested the effect of temperature on the spatial learning rate of zebrafish by comparing the daily rate of change in the number of fish choosing the correct arm first and behavioural performance (i.e., distance travelled, time needed to locate the daily food reward and swimming speed) in two temperatures (21 and 31°C) in 7 successive sessions. The daily rate of change in behavioural performance was expressed as the percentage difference between the value in a given session and the value in the previous session. Two experiments were performed in a T-maze with single feeders in the arms, one empty and the other with a food reward. In each of the experiments, we used 10 naive fish, which were placed in the T-maze individually once a day. The fish were fed between sessions to avoid increasing differences in hunger levels and, in turn, increasing the differences in motivation to find the food between the temperature treatments. The results revealed that the learning rate was greater at the higher temperature, which was apparent in a greater percentage reduction in the time and distance travelled needed to locate the daily food reward between successive sessions at the higher temperature than at the lower temperature. The results show there was a significant effect of temperature on the daily rate of change in the number of fish choosing the correct arm, distance travelled and time needed to locate the daily food reward, which may be attributed to the positive effect of temperature on learning rate, and may indicate the importance of temperature for the cognitive testing of zebrafish.

KEYWORDS

The effect of temperature on the spatial learning rate of zebrafish (Danio rerio)

1 INTRODUCTION

The research provides a great number of studies on the effects of a variety of intrinsic and extrinsic factors on the cognitive abilities of fish and other ectotherms (e.g., Braithwaite, 2006; Brown et al., 2008; Shettleworth, 2010). These studies include effects on various processes, including spatial learning (Laland et al., 2003), which is one of the contemporary topics and basal research areas in fish cognition and behaviour (Pouca & Brown, 2017). It has been revealed that spatial learning in fish depends on sex in blennid fishes (Costa et al., 2011) and in guppy (Lucon-Xiccato & Bisazza, 2017), habitat type in gobies (e.g., White & Brown, 2014) or personality.
type in weakly electric fish (Kareklas et al., 2017) and in brook trout (White et al., 2017). In spite of great interest in the topic, the effect of ambient temperature as a "master extrinsic ecological factor" (Willmer et al., 2009) on the spatial learning rate of fish seems to be understudied.

At the physiological level, elevated temperature increases the kinetic energy of biochemical reactions, which speeds up metabolic processes. This increase in speed is often expressed as the $Q_{10}$ value, which indicates the rate of increase in enzymatic reactions and physiological processes per 10°C increase in temperature (Schmidt-Nielsen, 1979). Within the range of optimal temperatures, this value is usually around 2 (the $Q_{10} = 2$ assumption), suggesting that the rates of most biochemical reactions increase approximately exponentially with increasing temperature (Gliwicz et al., 2018; Gliwicz & Maszczyk, 2016). According to the metabolic theory of ecology, the metabolic rates of individual organisms translate predictably to other rates and processes at both the individual and higher ecological levels (Brown et al., 2004; Gillooly et al., 2001; Pawar et al., 2015).

As learning abilities in ectotherms are affected by temperature-dependent enzymatic reactions and physiological processes (Abel & Lattal, 2001; Wang et al., 2006), it could be expected that they also increase according to the $Q_{10} = 2$ assumption. On the other hand, cognition, including learning, is complex phenomenon, which consists of memory acquisition, consolidation, retention and retrieval (Abel & Lattal, 2001; Inda et al., 2011; Wang et al., 2006). Thus, the kinetic effects of temperature would affect each of these stages to a different extent. Moreover, mechanistic studies on insects (e.g., Abram et al., 2017) and fish (e.g., Gliwicz & Maszczyk, 2016) indicate that the effects of temperature on their behaviour are not purely metabolic. For instance, it is well known that fish swim faster at an elevated temperature (e.g., Gliwicz & Maszczyk, 2016), which would not only be the direct result of increased physiological processes, but also an individual's intentional acceleration. A greater swimming speed would result in a faster encounter of stimuli, and therefore, in an increased spatial learning rate. Therefore, both the kinetic and intentional effects of temperature on cognitive abilities would result in enhanced learning at a rate greater than predicted by the $Q_{10} = 2$ assumption.

The literature provides only a few studies on the effects of temperature on the spatial learning of ectotherms. Most of these studies focus on the effect of temperature on developmental processes at the embryonic or juvenile stages and the associated consequences on the learning abilities of adults. For instance, studies on lizard hatchlings have shown that elevated temperatures during incubation affect performance in behavioural learning tasks in later life (Amiel et al., 2014; Amiel & Shine, 2012; Clark et al., 2014; Krekorian et al., 1968). However, one report indicated that hatchlings from hot-incubated eggs (mean temperature 27°C) were slower learners than hatchlings from cold-incubated eggs (mean temperature 23.2°C; Dayananda & Webb, 2017). Other studies investigated the effects of thermal stress (i.e., the exposure to suboptimal temperatures), before or after contact with a learned stimulus, on spatial learning of fruit fly (Drosophila melanogaster, Zars & Zars, 2006) and rainbow trout (Oncorhynchus mykiss, Colson et al., 2019). For instance, Colson et al. (2019) revealed that thermal stress in female rainbow trout (O. mykiss) triggered the inhibition of locomotor fear-related responses upon exposure to a novel environment, leading to a decrease in the spatial learning abilities of progeny. Some studies have investigated the effect of optimal and suboptimal ambient temperatures during acclimation on the memory consolidation and spatial learning of freshwater fish (Borsook et al., 1978; Brezden et al., 1975; Rahmann et al., 1980). Although the results of some of the aforementioned studies suggest that the effect of elevated temperatures in the optimal range on learning abilities is positive (e.g., Clark et al., 2014; Rahmann et al., 1980) or negative (Dayananda & Webb, 2017), the focus of these studies was rather on other phenomena (on the effect of temperature on optimal conditions during development or on the acclimation rate) rather than on the effect of ambient temperature on the learning rate itself. Furthermore, if elevated temperature improves learning rate, none of these studies tested whether this improvement is lower, equal or greater than predicted by the $Q_{10} = 2$ assumption.

The aim of our study was to test two hypotheses: first, that the spatial learning rate in adult zebrafish (D. rerio) is greater at a higher temperature; and second, that the increase in the learning rate due to temperature is close to the prediction of the $Q_{10} = 2$ assumption. The zebrafish was used as a model organism to study the effects of a variety of intrinsic and extrinsic factors, including temperature, on behavioural performance and cognitive abilities to verify a variety of hypotheses in the field of developmental and molecular biology, and toxicology (Ruzicka et al., 2015).

## 2 | MATERIALS AND METHODS

### 2.1 | The approach

The experiments were conducted in a simple system comprised of a T-maze, as a standard tool for investigating individual spatial learning (e.g., Wenk et al., 1998). The maze had a feeder in each arm, one empty and the other with a food reward. It was placed inside a ZebraCube box (ViewPoint Behaviour Technology®)—a cubicule to control light, sound and vibrations, which allowed video recording of the fish performance. To verify the hypothesis, we conducted four behavioural experiments: (1) at 21°C and with the left side of the T-maze containing the reward, (2) at 21°C and with the right side of the T-maze containing the reward, (3) at 31°C and with the left side of the T-maze containing the reward and (4) at 31°C and with the right side of the T-maze containing the reward. The experimental temperatures were established in such a way that they were sufficiently distant from each other in order to observe the hypothetical differences in learning. They were within the hypothetical optimal temperature range for learning, which was determined based on the optimal temperature range assessed for other physiological performances in zebrafish, including swimming speed (between 16 and 30°C, Wakamatsu et al., 2019), individual growth rate (between 28 and 29°C, Tsang et al., 2020) and fecundity (between 25 and 28°C, Babkiewicz et al., 2019).
Brand et al., 2002). In each of the experiments, we used 10 naive fish. The fish were placed in the maze individually in randomised order once a day over 7 days (7 experimental sessions). Therefore, a total of 280 experimental sessions were conducted (2 replicates [left or right side] × 2 temperatures × 10 fish × 7 experimental sessions, using a total of 40 fish). The side with the location of the food reward was always the same for each individual. Before each of the experiments, the fish were acclimated to the experimental temperature over 14 days. They were fed between experimental sessions to avoid differences in hunger levels, which may have led to differences in motivation to find the food in successive experimental sessions between temperature treatments. In the recordings, we assessed the changes in the number of fish, which chose the arm with food first, changes in distance travelled and time needed to locate a daily food reward in successive experimental sessions, and changes in the swimming speed of the fish.

We evaluated the STRANGEness of our test sample (Webster & Rutz, 2020) at several points: (1) social background: the experimental fish had known social backgrounds including the nature and frequency of their interactions with others, and past opportunities to learn socially from other individuals or their products, as the fish in the experiment participated individually and originated from one cohort, and their rearing history was known since we used a long-established laboratory line; (2) trapability and self-selection: the fish participating in the experiment were not the first ones, which were caught by the fishing net, but they were fished until 5 males and females were obtained for each test (similar in dimensions and weight); (3) rearing history: their rearing history was known, as we used a long-established laboratory fish line; (4) acclimation and habituation: the fish were allowed to acclimate to the new temperature and to habituate themselves to the experimental system and home tanks; (5) we took into account natural changes in their responsiveness (e.g., the experiments were conducted during day); (6) genetic makeup: we used a wild-type AB strain of zebrafish, with males and females participating in equal proportions; however, the different fish line may have behaved a little differently; and (7) experience: the experimental fish had not participated in any other behavioural experiment.

### 2.2 Experimental animals

In each experiment, a new cohort of 10 randomly chosen adult (1 year old) zebrafish was used. The fish were selected to obtain 5 males (33.5 ± 0.5 mm) and 5 females (35.0 ± 1.4 mm). They were of the wild-type AB strain, originating from the Karlsruhe Institute of Technology, Germany, bred at the Zebrafish Core Facility (ZCF) at the International Institute of Molecular and Cell Biology (IIMCB) in Warsaw. They were housed in a group (7 ind. × L⁻¹) in the 6 months prior to the experiment in a 14:10-h L:D photoperiod and placed individually in transparent 2-L tanks for gradual acclimation (1°C increase or decrease every 1.5 days in relation to starting at 27°C) to either 21 or 31°C. The temperature was changed using the air conditioning in the laboratory room and a water heater connected to the water circulating system connecting all the tanks with the fish being temperature acclimatised. They reached their final temperature 7 days before the experiment was started. The tanks were placed adjacent to each other, so the fish were in visual contact with neighbouring individuals. The air and the water were kept at the same temperature. During acclimation and 4 h before the sessions of each day, the fish were fed 50 mg of Gemma Micro 300 (Skretting®) daily, a standard food for adult zebrafish. This amount provides more than 5% of mean body weight and is recommended by the producer as the amount to provide fish only once per day (https://zebrafish.skrettingsusa.com/products/gemma-micro-300). During each session, the fish were offered small amounts (~7.5 mg) of dried and crushed *Daphnia* (Tropical®) placed in floating feeders.

### 2.3 Experimental system

The system (Figure 1a) consisted of a T-maze placed in a water bath inside the ZebraCube box with a camera connected to a computer operating ViewPoint software. The ZebraCube consisted of a dark inner part, a closed box of 45 × 58 × 90 cm of length, width and height, respectively. A video camera was mounted on the ceiling that operated in the infrared range (Sony 1/3 inch CCD monochrome camera DR2-HIBW-CSBOX, 30 fps and an infrared filter). The ZebraCube was illuminated from the ceiling by 8 LED lamps emitting a constant light intensity (10.0 ± 0.5 µmol m⁻² s⁻¹). The ZebraCube floor was a matrix of infrared diodes covered with an infrared permeable cover. This, together with ViewPoint’s application ZebraLab Tracking ver. 3.22.3.9, allowed us to track and visualise the fish’s movements and calculate fish locomotion, speed and distance travelled. The construction of the ZebraCube prevented vibrations and noise from the surroundings disrupting the sessions. The water bath was connected to a 60-L plastic barrel below the aquarium with a thermostat (ADA-REX ZEFIR® water heater) submerged in it. Thermal regulation was accomplished by a combination of cooling with the compartment’s air conditioner set at 0.1°C below the desired temperature and heating with an ADA-REX ZEFIR water heater with a thermoregulatory capability of 0.1°C submerged in the barrel. The experimental water circulated between the water bath and barrel maintaining 21 or 31°C (±0.02°C) in the experimental set-up (Figure 1a). The water was prepared in a second 60-L plastic barrel in the same room, using the same heating and cooling devices. The water bath for the T-maze was made of a transparent Plexiglas® 48 × 48 cm tank, 15 cm in height with a volume of 34.5 L. The T-maze (Figure 1b) was also made of transparent 8 mm Plexiglas®, with its side walls covered with black foil on the outer surface, except for the bottom, to allow the transmission of infrared illumination. The
side arms were shorter (18 cm long) and the starting arm was longer (36 cm), with 2 cm supports mounted beneath at the end of each arm (this allowed the water to circulate around and underneath the T-maze for thermoregulation). Each arm was 8 cm wide and 15 cm high. Floating feeders, made of a white, plastic ring (with diameter = 2.5 cm), were mounted to the end of the left and right arms of the T-maze wall with a suction cup, which prevented the offered food reward from deliquescenting on the water surface (Figure 1c). To get the food reward, the fish had to swim under the feeder in the form of a floating ring to eat a piece of crushed Daphnia floating inside the feeder on the water surface. Since colour-cued learning with a food reward is a more discriminative method than a food reward alone for examining cognitive changes in the zebrafish (Kim et al., 2017), a red or green cue card (8 × 15 cm) was attached to the end of each of the side arms, with the green card always indicating a food reward. The fish could see either a green or red card after swimming out from the starting arm, depending on the side the fish turned towards. Reds and greens elicit distinct differences in learning, are equally preferred over other colours by zebrafish and are good choices for appetitive experiments (Avdesh et al., 2012; Roy et al., 2019). The starting box (5 × 8 cm) was placed at the end of the starting arm, separated from the experimental area with a transparent, Plexiglas® guillotine door, which moved up and down through the use of an attached nylon strip.

2.4 Experimental procedure

In the 7 days before each of the experiments, each group of fish (10 ind.) was acclimated to the handling procedure. Once a day
for 7 days, individual fish were caught with a fish net and transferred from their 2-L individual home tanks to 1-L wider containers (15 x 8 x 10 cm of length, width and height, respectively) for 10 min and returned to their home tank. During this time, the fish were also habituated to dried Daphnia as food, as they were usually offered standard food for adult zebrafish. All 10 fish were allowed to swim together overnight (12 h) inside the T-maze without a food reward just before the experiment started. One day before the experiment, 60 L of experimental water in the barrel was prepared and the temperature was adjusted to either 21 or 31°C. At the commencement of each session, all individuals were transferred from their home tanks into containers. The food reward was added at the beginning of each session into one of the two arms of the T-maze (the one marked with the green card) with an amount of ~7.5 mg (this amount provides 15% of the zebrafish’s average daily food demand). Each single fish was then placed in the starting box of the T-maze for five minutes of acclimation, after which the guillotine door was opened and the fish was allowed to swim out into the experimental area. When the fish left the starting box, video recording and fish locomotion tracking were started. The sessions lasted 10 min. The time of eating the first piece of dried Daphnia was noted. The fish could eat the whole food reward before the end of the experiment. At the end of each session, the video recording was stopped, the fish was returned to the home tank and the T-maze was emptied. The maze was rinsed thoroughly before it was filled again. Then, the next fish was placed in the T-maze and the next session was started. Each temperature treatment was replicated twice (on the left or right side of the T-maze) in random order. Each temperature treatment on the one side was conducted with 10 new, naive fish and with the colour cards and feeders with and without food switched to opposite arms. The side was the same for each subject throughout the seven sessions of each experiment in order to test whether individuals turn the same way and whether this continued in the following days of the experiment.

2.5 | Data analysis

The number of fish choosing the correct arm (the arm with food) first and the performance time (the time in seconds required to find the food) were measured from the video recordings obtained during the experiments and analysed by the ZebraLab Tracking ver. 3.22.3.9 programme (ViewPoint software). The total distance travelled by a fish (in centimetres) and the number of entries into the correct arm required to find the food were estimated with the ZebraLab Tracking ver. 3.22.3.9 programme, based on fish locomotion tracking. The videos were watched again by the naïve scorer to obtain the data for the number of fish choosing the correct arm first. The data for the performance time, the total distance travelled by a fish required to find the food and number of entries into correct arm were counted automatically by a PointView software. The mean swimming speed (in centimetres per second) was calculated based on performance time and distance travelled.

The effect of temperature on fish performance was expressed as the \( Q_{10} \) value, which was calculated according to the formula provided in Schmidt-Nielsen (1979): \( Q_{10} = \left( \frac{R_2}{R_1} \right)^{10/(T_2 - T_1)} \), where \( R_2 \) is the measured reaction rate at temperature \( T_2 \) (where \( T_2 > T_1 \)) and \( R_1 \) is the measured reaction rate at temperature \( T_1 \). Note that the more the performance time and distance travelled decreased in consecutive days, the stronger the temperature effect was. Thus, in the case of these parameters, to avoid obtaining negative values in the \( Q_{10} \) calculations after substituting the data to the original temperature coefficient formula, we modified the formula in such a way that the larger average value of a given parameter was divided by the smaller one, even if it was inverse to the original \( Q_{10} \) formula.

Statistical analyses were conducted using IBM SPSS Statistics ver. 25 with an accepted statistical significance of \( \alpha = .05 \). In order to assess the effects of water temperature on fish performance, we compared the number of fish choosing the correct arm first, swimming speed and distance travelled in the two experimental temperatures (21 and 31°C) in 7 successive experimental sessions. Additionally, in order to assess the effects of water temperature on spatial learning rate, we compared the number of fish choosing the correct arm first in the two experimental temperatures (21 and 31°C) in 7 successive experimental sessions. In the analyses, we used the generalised linear mixed-effects model (GLMM) with repeated measures for 7 sessions and with water temperature as an inter-group factor. Fish ID numbers were included as the only random factor. Fish ID numbers were included as the only random factor. We also analysed whether there was an interaction between the temperature and consecutive session. Due to known sex differences in performances in the species (e.g., Ampatzis & Dermon, 2007; Leris et al., 2013), additional analysis was performed including “sex” as an inter-group factor. The AIC comparisons were run to check which factors should be retained in the model. For distance travelled and swimming speed, we used the gamma distribution with the power link function due to the right skewness of the data. For performance time, we used log transformation to Gaussian and for choice of correct arm first—the binominal distribution with the logit link function.

Moreover, in order to assess the effect of temperature on learning rate, we compared the daily rate of change, expressed as the percentage difference between the value in the given session and the value in the previous session (the latter assumed to be 100%, e.g., the first session was 100% in comparison with the second session, the second session was assumed to be 100% in comparison with the third session), of the swimming speed, distance travelled and time needed to locate the daily food reward in the two experimental temperatures (21 and 31°C) for all of the 7 experimental sessions. To conduct this analysis, we used the generalised linear mixed-effects model (GLMM) with repeated measures for differences between the value in a given session and the value in the previous session, and water temperature as an inter-group factor. Additional analysis was also performed with “sex” as an inter-group factor. The AIC comparisons were run to check which factors should be retained in the model. For the percentage difference between the values for distance travelled, swimming speed and performance time, we used the Gamma distribution with the power link function.
### Results

The AIC value was lower for models without the factor “sex”; therefore, this factor was excluded from another steps of the analyses.

We found that temperature had a significant effect on fish performance: distance travelled, time to find the food reward (performance time), swimming speed and number of fish choosing the correct arm first (Table 1, GLMM with repeated measures). The distance travelled and time to find the food reward were both shorter (on average 19.9 and 12.4 times, respectively; Figures 2 and 3a,c, Table 2), while swimming speed was greater (on average 1.5 times; Figure 3e, Table 2) at higher temperature. On average, until the first capture, fish entered the arm with the food reward more often in the 21°C treatment group than in the 31°C treatment group (6.6 and 1.8 times, respectively). Although the number of fish choosing the correct arm first was, on average, 1.6 times greater at the higher temperature (Figure 3g; Table 2), the difference was not significant (Table 1, GLMM with repeated measures). While the time to find the food reward decreased, the swimming speed and the number of fish choosing the correct arm first increased with consecutive experimental sessions (Table 1 in the main text, detailed analysis in Table A1, GLMM with repeated measures, Figure 3). The effect of consecutive experimental sessions was greater at lower temperature for performance time and swimming speed and was greater at higher temperature for the number of fish choosing the correct arm first (Table 1 in the main text, detailed analysis in Table A1, GLMM with repeated measures, Temp. × Session; Figure 3).

The effect of temperature was significant for the daily rate of change expressed as the percentage difference between the value in the given session and the value in the previous session of distance travelled and time needed to locate the daily food reward (Table 3, GLMM with repeated measures; Figure 3b,f). The effect of consecutive experimental sessions was greater at higher temperature for performance time and swimming speed and increased with consecutive experimental sessions (Table 3 in the main text, detailed analysis in Table A2, GLMM with repeated measures; Figure 3b,f). The effect of consecutive experimental sessions was greater at higher temperature for performance time and swimming speed.
FIGURE 3 The experimental results. The performance time (a and b), distance travelled to find the food reward (c and d), swimming speed (e and f) of the fish and the number of fish choosing the correct arm first (g) at the lower (21°C, grey circles and best fit line) and higher (31°C, black circles and best fit line) temperature shown as the absolute mean values (a, c, e, and g), and as a percentage of the value during the first experimental session (b, d and f). Error bars represent the standard deviation of the data plotted for all fish in each of the two temperatures (and when the food reward was offered on the left and right sides; n = 10 in each experimental group) for both temperature treatments.

| Performance time | Mean of absolute values | Daily rate of change |
|------------------|------------------------|----------------------|
| Distance travelled | 12.4 | 1.6 |
| Swimming speed | 1.5 | 1.3 |
| N fish choosing the correct arm first | 1.6 | 1.6 |

Table 2 The Q_{10} value for performance time, distance travelled, swimming speed and number (N) of fish choosing the correct arm first for the mean of absolute values for all of the 7 experimental sessions and for the daily rate of change expressed as the percentage difference between the value in the given session and the value in the previous session.

| Factor or interaction | Distance travelled | Performance time | Swimming speed |
|-----------------------|-------------------|-----------------|---------------|
| Temperature | 12.14 | .001 | 14.30 | <.001 | 0.24 | .628 |
| Session | 1.98 | .083 | 2.32 | .045 | 2.29 | .046 |
| Temp. × session | 1.84 | .106 | 2.35 | .043 | 2.38 | .040 |

Note: Significant effects are marked in bold.

Table 3 The results of the GLMM with repeated measures of 7 sessions to assess the effect of two factors: (1) temperature treatment (21 or 31°C) and (2) consecutive experimental days and their interaction on the daily rate of change, expressed as the percentage difference between the value in the given session and the value in the previous session, for distance travelled, time needed to locate the daily food reward (performance time) and swimming speed.
speed (Table 3 in the main text, detailed analysis in Table A2, GLMM with repeated measures, Temp. × Session; Figure 3b,f).

4 | DISCUSSION

The most important result obtained in the experiments on individually foraging zebrafish was that the daily rate of change in distance travelled and time needed to locate the daily food reward and the number of fish choosing the correct arm first were greater at the higher temperature (Figure 3b,d,g). The increase in the mean rate was close to 2 for the parameters, 1.6 for the distance travelled, 2.3 for the time and 1.6 for the number of fish choosing the correct arm first, which supports our hypothesis. The positive effect of temperature on the daily rate of change may be due to a higher metabolic rate (Clarke, 2004; Clarke & Johnson, 1999), and, in turn, an increased hunger level and motivation to find the food (e.g., Jobling, 1995) of the fish in the 31°C treatment. However, this explanation seems unlikely, as we fed the fish a standard and relatively high amount of food between foraging sessions. A more likely explanation of the effect of temperature on the measured parameters in successive feeding sessions is that the elevated temperature enhanced the spatial learning rate of the fish. The results are consistent with previous studies concerning acclimation to temperature conditions on the memory consolidation and learning of freshwater fishes (Borsook et al., 1978; Brezden et al., 1975; Rahmann et al., 1980), where they indicated that prolonged exposure to a higher temperature or transfer to a higher temperature results in improved learning.

It should be pointed out that we obtained the opposite effect of temperature on learning rate, expressed as the absolute and percentage difference between the value in the given session and the value in the previous session of distance travelled and time needed to locate the daily food reward. For instance, the distance required to locate the food reward in the T-maze between the first and last experimental sessions expressed in per cent values decreased by 59% in the high temperature treatment and only by 47% in the low one. However, in absolute values, it decreased by 0.98 m in the low temperature treatment and only by 0.16 m in the high one (Figure 3). This discrepancy stems from the fact that, in the high temperature treatment, fish performance metrics in the simple T-maze reached their lowest values, which could not be decreased further in the following days of the experiment, hence the low slope of the graphs for the learning rate at a higher temperature in absolute values. In more demanding conditions, such as a more complicated experimental system or more difficult tasks to solve, it could be expected that the learning rate would be greater at higher temperatures, when it is expressed not only as percentage values, but also as absolute ones.

At least four potential non-exclusive mechanisms could explain the positive effect of temperature on the learning rate of the fish in our study. The first stems from the specificity of the spatial learning tests. With each successive feeding session, fish at the higher temperature treatment started to forage faster and encountered the food reward earlier. Therefore, they had a longer time to learn about the stimuli to the end of the feeding session. This, in turn, could have contributed to the more efficient memorisation of the location of the food reward. This explanation is consistent with results obtained by Angiulli et al. (2020), which showed that a higher temperature reduces anxiety and increases the boldness of zebrafish, which could then spend more time in potentially dangerous areas of the tank. Secondly, the functioning of some senses was improved, thus increasing the learning process. For instance, it was revealed that elevated temperatures influence hearing thresholds and the shape of auditory evoked potentials (Wysocki et al., 2009), as well as increase the speed and improve the accuracy of the retinal response (Gačić et al., 2015) of fish. Thirdly, elevated temperatures influence the processing of received information in the central nervous system, and it is known that a fish’s brain has neurones that are sensitive to temperature (van den Burg et al., 2005). Finally, we cannot exclude that the fish trained in the colder temperature learned equally as the fish trained in the warmer temperature, but the retrieval of memory may be better in the warmer temperature as memory consolidation and re-consolidation are quite different processes (Alberini, 2005; Tronson & Taylor, 2007).

In our experimental design, the fish also most likely learned to associate the green card with a food reward (Fernandes et al., 2016). However, we tested whether the fish turn the same way. In order to be able to distinguish between these two factors with such an experimental design, the side with the food would have to be randomly designated for each fish and each time it was tested, and we would have to check whether the fish still selects the side with the green card, even if it contained no food.

Additionally, the results revealed that higher temperature increased the performance of the fish by reducing the mean time (Figure 3a) and mean distance required to locate the food reward in a T-maze (Figure 3c), and by increasing the mean swimming speed (Figure 3e) in 7 successive experimental sessions. The difference in these parameters was already apparent during the first experimental session and was maintained during subsequent sessions. The Q values for two of the four parameters (time and distance required to locate the feeder and start feeding) far exceeded the value predicted for the effect of temperature on the rates of biochemical reactions and biological processes, being 19.9 times greater for time and 12.4 times greater for distance. The most likely explanation of the magnitude of the effect may be the interactive effects of decreased temperature on physiological processes, all translating into a significant decrease in net energy requirements, and, in turn, in decreased motivation to find the food reward (Stoner & Sturm, 2004; Watz et al., 2014). The possibility that the magnitude of the effect was due to the fact that the elevated temperature enhanced the spread of the food’s odour is less likely. Although the literature on olfaction in zebrafish indicates that this species has a rather sensitive olfaction (e.g., Michel & Lubomudrov, 1995), the behaviour of the fish at the beginning of the experiment (first two days) suggests that the fish did not find the reward on the basis of odour, since they similarly explored the entire experimental area in both experimental temperatures (see Figure 2). Moreover, the potential dispersal of the smell of the food...
was limited by the fact that only a small amount of it was provided in static water for a short period of time in a single location only on the water’s surface (the pieces of dried and crushed Daphnia did not sink). Additionally, the food was distributed over a small surface inside the feeder and the uneaten food was removed each time together with the whole volume of experimental water. The T-maze was rinsed before the next replication was conducted with a new portion of water that did not have contact with the food reward.

The possibility cannot be excluded that such a significant effect could be due to the suboptimal temperature used in the lower temperature treatment (21°C), which might lead to declined foraging efficiency. However, the three arguments suggest that both temperatures used in the experiments were well within the temperature range experienced by both laboratory and wild zebrafish. First, the swimming speed of the fish in the 21°C treatment was only slightly lower compared with the 31°C treatment, suggesting that foraging behaviour was only slightly constrained by the temperature. Second, both temperatures used in the experiments were within the range of temperatures that zebrafish most commonly experience in the wild (Engeszer et al., 2007; Lawrence, 2007; López-Olmeda & Sánchez-Vázquez, 2011; Spence et al., 2008). Finally, they were within the optimal temperature range assessed for other physiological performances in zebrafish, including swimming speed (16 and 30°C, Wakamatsu et al., 2019), individual growth rate (28 and 29°C, Tsang et al., 2020) and fecundity (25 and 28°C, Brand et al., 2002). Moreover, it should be noted that even when the temperature was suboptimal we mitigated its negative effects by acclimating the fish to the experimental temperatures for two weeks prior to the experiments. The two-week acclimation period of the fish before the experiment should also exclude the possibility that the fish tested in the lower temperature were in cold shock (Donaldson et al., 2008).

In conclusion, the results of our study suggest that the higher temperature slightly increased the spatial learning rate of the zebrafish, which was close to the prediction of the \( Q_{10} = 2 \) assumption. Until now, in zebrafish, this type of learning has been demonstrated in many examples and has proved to depend on the strain used and rearing environment (Spence et al., 2011), environmental geometry (Baratti et al., 2020) or population (Roy & Bhat, 2018). These results also indicate that the temperature matters in the cognitive testing of zebrafish, that is testing at 21°C will lead to worse results than testing at 31°C. Even when an elevated temperature improves the learning rate of fish only slightly, it could be expected that this effect would be even lower for its potential invertebrate prey since planktonic animals often have simple brain and cognitive functions compared with fish (e.g., Wiese, 2013). This asymmetry, in turn, would result in increasing the foraging rate of the fish at an elevated temperature (Dell et al., 2014; Öhlund et al., 2015). Our study concerned individual spatial learning abilities to locate a food reward, but this does not exclude the possibility that other learning abilities could also be affected by temperature, such as social or numerical learning abilities.

Since we evaluated point by point the STRANGEness of our test sample (Webster & Rutz, 2020), we conclude that the specific biases that may apply to our study indicate potential limits to the generalisability of our findings. We cannot exclude the possibility that a different fish line may behave a bit differently in our experiment. We tried to maintain fish diversity and sex equality, our fish originated from a long-established laboratory line, and the experiment was conducted in precisely controlled conditions preceded by an appropriate acclimation time for the animals.

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CONFLICT OF INTEREST

We declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The author elects not to share data.

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