OIKOS

Research article

The effect of temperature on fish swimming and schooling is context dependent

Maria Kuruvilla, Anthony Dell, Ashley R. Olson, Jason Knouft, John M. Grady, Jacob Forbes and Andrew M. Berdahl

M. Kuruvilla (https://orcid.org/0000-0003-3522-9107) ✉ (maria75799@gmail.com) and A. M. Berdahl (https://orcid.org/0000-0002-5057-0103), Quantitative Ecology and Resource Management Program, Univ. of Washington, Seattle, WA, USA and School of Aquatic and Fishery Sciences, Univ. of Washington, Seattle, WA, USA. – A. Dell, J. M. Grady and J. Forbes, National Great Rivers Research and Education Center, One Confluence Way, East Alton, IL, USA. – A. R. Olson (https://orcid.org/0000-0002-3753-4482), School of Science, Psychology and Sport, Federation Univ. Australia, Churchill, VIC, Australia. – AD and J. Knouft, Dept of Biology, Saint Louis Univ., St. Louis, MO, USA. JF also at: Dept of Biological Sciences, Southern Illinois Univ. Edwardsville, Edwardsville, IL, USA. AD also at: AD also at:Dept of Biology, Washington University in St. Louis, St. Louis, MO, USA.

Oikos
2022: e09202
doi: 10.1111/oik.09202
Subject Editor: Gregor Kalinkat
Editor-in-Chief: Dries Bonte
Accepted 9 September 2022

Temperature is highly influential on the physiology and behaviour of ectotherms. In fish, temperature affects social interactions such as schooling behaviour, a common defence against predation. However, the effect of temperature on the ability of schooling fish to collectively respond to a predator is unknown. Here we used a loom stimulus to simulate an approaching predator that elicited a fleeing response in schooling fish over a range of water temperatures (9–29°C) and group sizes (1–16 fish). While speed and acceleration always exhibited a positive curvilinear response to temperature, the optimal temperature at which performance peaked was different during the predation threat versus when they were unperturbed. Similarly, group-level metrics were sensitive to temperature immediately after a loom stimulus but showed no response to temperature during unperturbed swimming. The time taken for fish to respond to the loom stimulus was minimal at 20°C. The proportion of fish that startled, during a loom, peaked at 13°C – around the same temperature at which speed, and acceleration was maximum. Taken together, our results suggest that ectothermic fish may be able to compensate for their slower swim speeds at lower temperatures during unperturbed swimming by increasing their sensitivity to startle in response to a predation threat. More generally, we show that in ectotherms the qualitative and quantitative effect of temperature on a behavioural trait may be dependent on the context.

Keywords: behavioral ecology, collective behavior, golden shiners, predation, thermal ecology

Introduction

Ectothermic animals are especially sensitive to changes in external temperatures compared to endotherms because ambient temperature controls physiological rates in ectotherms. Ambient temperature not only affects the growth and development of ectotherms (Brett et al. 1969, Zuo et al. 2012) but also their movement (Payne et al. 2016). Biological responses to temperature are often characterized by thermal performance curves in which

NORDIC SOCIETY OIKOS
www.oikosjournal.org

performance (e.g. metabolic rate, development time, movement) increases gradually with temperature, peaks at some optimum and then decreases rapidly at higher temperatures (Huey and Stevenson 1979, Angilletta Jr and Angilletta 2009, Dell et al. 2011, Clarke 2017). Ectothermic species encounter varying temperatures due to daily and seasonal temperature cycles, the range and predictability of which may be altered due to directional changes in climate (Deutsch et al. 2008).

As ectotherms, many aspects of fish behaviour are influenced by temperature. In the presence of predators, guppies *Poecilia reticulata* increase both the time spent in predator inspection and foraging activities at higher temperatures than at lower temperatures (Weetman et al. 1998). Additionally, there is evidence for behavioural regulation of activity levels as water temperatures rise. For example, the routine swimming speed of juvenile walleye pollock *Gadus chalcogrammus* decreases as temperature increases, but their maximum swimming speed increases as temperature increases (Hurst 2007). This suggests a behavioural rather than physiological regulation of their swimming speed.

Temperature also affects fish social behaviour. Some previous studies have shown that the average nearest neighbor distances increase (fish become more dispersed) with increasing temperature (Hurst 2007, Bartolini et al. 2015, Colchen et al. 2017), but this is not always the case as Weetman et al. (1998) found that average nearest neighbor distance was higher for guppies at 22°C than at 26°C. The contrasting results of these studies suggests either non-monotonic, or taxon-specific, behavioural responses to thermal conditions and merits further investigation.

Schooling is a wide-spread behaviour in fishes that yields a tradeoff of fitness benefits and costs. Schooling helps fish to avoid predation, forage, navigate, find mates more easily and, conserve energy by exploiting hydrodynamic factors and offers protection from predators (Krause et al. 2002, Ioannou 2017, Berdahl et al. 2018, Li et al. 2020, Polyakov et al. 2022). However, schooling can incur costs, including increased intraspecific competition for food (Polyakov et al. 2022) and increased parasites and disease transfer (Walsman et al. 2022). The size of the group can influence the behaviour of individual fish within that group. For instance, Gil and Hein (2017a) found that each fish spends less time feeding when fewer fish are present. However, given the abundance of factors promoting and discouraging grouping listed above, the effect of group size on the behaviour of fish is context specific. Group size and temperature both influence fish behaviour and are biotic and abiotic factors, respectively. Considered together, group size and temperature may create a multi-stressor effect on the behaviour of fish, with unknown interactions between these factors.

Since schooling is one of the primary ways in which many fish species avoid predation (Foster and Treherne 1981, Ioannou 2017), and schooling is ultimately driven by the movement of individual fish, there is a need to understand how temperature affects the ability of prey fish to collectively escape from a predator. Moreover, fish can be preyed upon by endotherms, whose performance may be much more robust to changes in temperature, creating an asymmetry in performance with changing temperatures that shape ecological interactions (Grady et al. 2019). Thus, thermally-mediated changes in ectotherms' individual and collective responses to predation may be critical to their survival and fitness.

We addressed the knowledge gap of how water temperature and group size affect the behaviour of schooling fish both during unperturbed swimming and during response to a potential predation event. We simulated predation events with looming stimuli and quantified fish behaviour via automated tracking of overhead videos. We hypothesized that: 1) following simulated predation events, many responses relevant to escape (e.g. speed, acceleration and proportion of individuals that startle) would exhibit a positive curvilinear response to temperature, indicating an optimal temperature for predation response; 2) during unperturbed swimming, speed and acceleration would follow a similar positive curvilinear response to temperature, but it would be attenuated, since fish would be less likely to push their performance limits in non-life-threatening situations; 3) collective behavioural traits like average nearest neighbor distance exhibit a positive curvilinear response to temperature during unperturbed swimming as well as following simulated predation events; and 4) there would be an interaction between the effect of group size and the effect of temperature on fish behaviour during a predation threat.

We used golden shiners *Notemigonus crysoleucas*, a small cyprinid fish species, as they have been used in other collective behaviour experiments (Katz et al. 2011, Berdahl et al. 2013, Polverino et al. 2013), including those on collective escape responses (Rosenthal et al. 2015). Golden shiners aggregate when faced with risk of predation in the wild (Johannes 1993), which renders them ideal to study collective escape responses. In Missouri, where our fish were sourced, water temperatures in golden shiner habitat ranges from 2 to 25°C and is approximately 15°C when golden shiners spawn (Supporting information). By the peak of winter, water temperatures are as low as 2°C. We chose experimental water temperatures of 9–29°C to reflect the large range of temperatures golden shiners experience and to study how warmer temperatures will affect their ability to escape predators.

Studies of golden shiners in the wild observed single species shoal size from 6 to 110 individuals with a mean of 31 (Krause et al. 1998). While group sizes are highly variable in the wild, they tend to be distributed either exponentially or as a power-law, and in each case the mode of the distribution is at small group sizes (Bonabeau et al. 1999). We chose group sizes of 1, 2, 4, 8 and 16 fish which span a 16-fold range in increments of 2^n, which is thought to be distinguishable by fish (Wong et al. 2007).

Material and methods

Study system

We obtained juvenile golden shiners from Welpman Springs Goldfish Hatchery, in Stover, MO, USA, and transported

them to the laboratory at Saint Louis Univ. in St. Louis, MO, USA. In the laboratory, the fish were housed in approximately equal numbers in 24 aquaria, which we will call housing tanks, with dimensions of 60 × 30 × 40 cm and a maximum volume of 72 L. Fish were fed a uniform commercial fish food (Purina Aquamax Premium Fish Food) daily and leftover food was removed after each feeding session. Approximately a quarter of the water in each housing tank was replaced every day with new dechlorinated water matching the temperature of the housing tank to remove nitrogenous waste. We tested the concentration of nitrogenous waste products (ammonia, nitrite and nitrate) approximately every 48 h using commercial test kits (API brand). Concentrations of ammonia and nitrite were maintained at undetectable levels and the concentration of nitrate was maintained at five ppm or less according to our tests. We also treated each tank with the instructed dose of Seachem 'Prime', a commercially available product designed to neutralise the toxicity of all three forms of nitrogenous waste in aquariums and aquaculture systems. We maintained a pH of 7.2 in each tank and light was provided on a 14L:10D cycle, matching the approximate conditions of the hatchery during the boreal summer. To avoid fish becoming acclimatised to looming stimuli, the housing tanks were covered on all sides by white curtains, which were only drawn for feeding and tank cleaning.

Water temperature in the housing tanks was controlled by aquarium chillers (JBJ 1/10 HP Titanitum Arctica Chiller DBA-075) and for warmer housing tanks also heaters (EHEIM Jager Aquarium Thermostat Heater 100W). Supplemental water circulation and aeration was provided by a small aquarium air pump (100 l h^{-1}) connected to each housing tank. When the fish first arrived, all housing tanks were set to 15°C (the approximate water temperature at the fish hatchery). Water temperature was then increased or decreased by ≤ 3°C per day until the desired final temperature for that housing tank was attained. The final six temperatures ranged from were 9 ± 0.6, 13 ± 0.4, 17 ± 0.4, 21 ± 0.3, 25 ± 0.3 and 29 ± 0.3°C, with four housing tanks per temperature. The maximum temperature was lower than the temperature at which heat stress is observed in golden shiners (34°C) (Chen et al. 2003). All fish had two weeks to acclimate in the lab, of which at least one week was at their final acclimation temperature. Experiments were conducted over two weeks in July–August 2019. The body length (estimated by considering the median of the diagonal of the images associated to each individual (Romero-Ferrero et al. 2019)) of a subset (33) of the fish were measured by *idtracker.ai* for which the mean was 4.03 ± 0.29 cm. See the Supporting information for median body length of fish in each combination of group size and temperature.

Experimental design and procedure

We used a factorial design in which we independently varied water temperature and group size (number of fish). We investigated the five different group sizes at each of the six temperatures (listed above), for a total of 30 different treatments. We performed 10 replicates of each combination of water temperature and group size, for a total of experimental 300 trials. Within each replicate/trial the fish were presented with five evenly spaced replicate loom stimuli (Fig. 1c), such that in total we had response data from 1500 looms.

Trials took place simultaneously in six approximately square (46 × 47 cm), shallow (~ 5 cm water depth) experimental arenas (Fig. 1). The shallow arenas constrained the fish to move in mostly two-dimensions, greatly simplifying our ability to track movement with a single camera from above. Each experimental arena was situated in a larger (110 × 75 × 30 cm) water bath, in which the water was controlled by either 1) a chiller (temperatures < 21°C), 2) a chiller and heater (temperatures > 21°C) or 3) the ambient room temperature (21°C). These experimental arenas were filled with water from the housing tanks prior to the experiments and the temperature in each arena was recorded with aquarium thermometers to ensure that water temperature did not vary more than 1°C.

Each experimental arena was backlit by an infra-red (IR) LED panel and trials were filmed from directly above with an IR light sensitive video camera (Basler acA1300-60-gmNIR). An electronic tablet (YUNTAB, 10.1 inch) was placed beside each arena with the bottom edge of the tablet one inch above the arena and tilted down at an angle of 10–15°. Additional, visible-light cameras (Basler acA1300-60-gmNIR without the IR pass filter) were placed beside the arenas facing the screens to record the loom stimulus. To make the loom stimuli more apparent, the lights in the experimental room were dimmed so that the surface of each experimental arena was approximately 400 lux (measured with a light meter). This 400 lux light level corresponds to sunrise/sunset conditions – times of heavy activity for golden shiners, which are crepuscular.

During the experiments, the electronic tablets displayed a video featuring a series of loom stimuli that had been generated using the R package loomeR (Carey 2019). Each loom stimulus consisted of a black disk increasing in size at an increasing rate that simulated an object (predator) approaching at 500 cm s^{-1}. This method has been used previously (Dill 1974, 1990) and allowed us to control the timing and speed of approach of the 'predator'. The loom video began with a ten-minute pre-looms period that we used to quantify the baseline fish behaviour. This was followed by a series of five looms events each spaced three minutes apart, allowing ample time for the fish to resume normal activity (Gil and Hein 2017b). Each trial lasted for about thirty minutes with recording being stopped five minutes after the final loom (Fig. 1c).

The order of trials was randomised to reduce any influence of the timing of a trial. We randomly assigned group sizes to each of the six experimental arenas. To transfer the fish from the housing tank to the experimental arena, we first filled portable containers with water from the housing tank. We then transferred the desired number of fish from the housing tank to the container with a net. We then transferred the fish along with the water to the experimental arena. Once fish

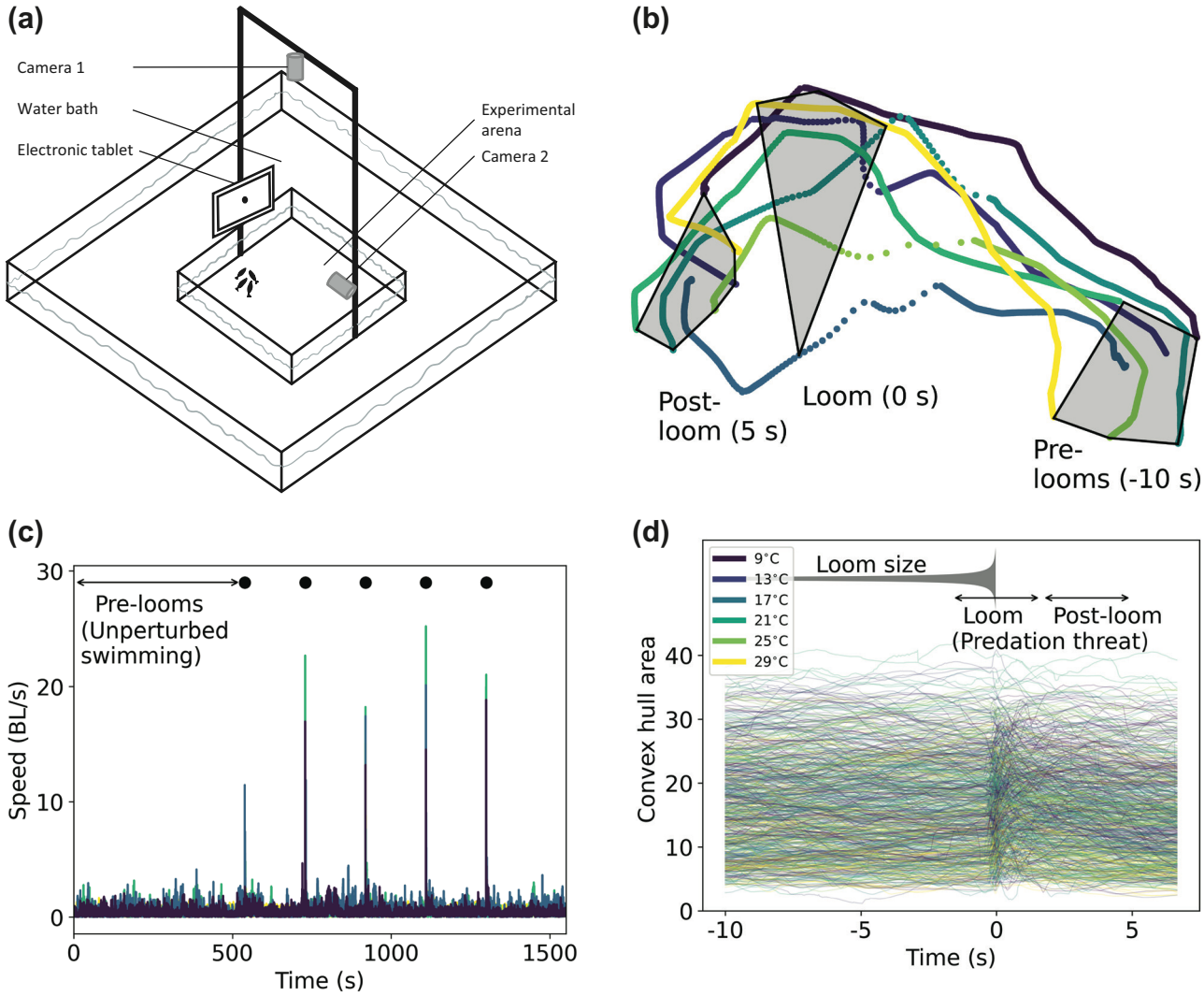

Figure 1. Experimental overview. (a) Schematic of one of our six experimental setups. Each experimental arena was contained in a larger water bath to help with temperature control. Experiments were recorded using a near-IR camera positioned directly above the experimental arena. A loom stimulus was displayed periodically on an electronic tablet located above the surface of the water on one side of the arena, but angled downward. A second camera recorded the tablet so we could synchronize the timing of the loom with the fish tracking. (b) Trajectory of each fish during a single loom event. Each trajectory is constructed with a marker every 0.033 s, so that the distance between the markers is proportional to body speed. The dotted section of each trajectory indicated the fish is moving fast after being startled (either by the loom directly, or by other fish in the arena). The polygons indicate the convex hull area of the group three times during the event (pre-looms, loom, post-loom). (c) An example of a time series of body speed of each fish in the group during one trial, showing startles during each stimulus. We define unperturbed swimming as the pre-looms period which occurs before any loom stimulus. The black circles indicate loom events, with each experimental trial consisting of five loom stimuli separated by three minutes. (d) Each line represents the convex hull area of a school of fish during a single loom event. The time axis is re-scaled for all loom events, so that 0 s coincides with the end of the loom stimulus. 'Loom' is defined as the period from −1.67 s to 1.67 s. 'Post-loom' is defined as period from 1.67 s to 5 s after the end of the loom stimulus. The loom stimulus started growing at frame −10 s and increased in size at a rate that mimicked an object approaching at a constant speed (500 cm s^{-1}).

were in the arenas, the curtains that separate the experimental arena from the rest of the lab were drawn and the fish were allowed to acclimate for 20 min. Following this, the loom video on each tablet was started and we commenced filming, signifying the beginning of one experimental trial. At the end of the trial, we transferred all the fish to a portable container with a net and then transferred them to a different housing tank to keep them separate from fish that have not yet been through an experimental trial. No individual fish was used in the experiment more than once. All experimental trials were conducted between 9:00 and 19:00.

Fish tracking

Overhead video recordings of the experimental trials were converted to pseudo 2D trajectories using *idtracker.ai*, which

allows tracking of large number of unmarked animals using deep neural networks to maintain identities and prevent error propagation after crossovers (Romero-Ferrero et al. 2019). Distance-related metrics, such as speed and acceleration, were scaled by the body length (BL) of the fish (Romero-Ferrero et al. 2019). All the videos used for analyses had a tracking accuracy above 98%, which is calculated by *idtracker.ai* based on the average probability of assigning an identity to the fish. While idtracker.ai performed well with detections, in videos with larger group sizes there were occasional temporary mismatches in identities, which resulted in artificially elevated spikes in velocity and acceleration when idtracker.ai corrected these swapped identities. In order to avoid incorrectly classifying these spikes in kinematic variables as real fish movement, we created a filter that removed all frames in which the speed or acceleration were above a certain threshold. Since mismatched identities do not occur in videos with a single fish, the threshold was determined by the maximum speed (30 BL s^{-1}) and maximum acceleration (3338 BL s^{-2}) in all the single fish videos. The sensitivity of our models to these thresholds were determined in all our analyses. 75 videos were excluded from analyses due to tracking failures (n = 38), fish/debris in water bath (n = 19), dropped frames and corrupted video files (n = 10) or fish jumping from the experimental arena (n = 8). We were left with 225 videos with approximately 113 h of recording in total. See Table 1 for the total number of trials for each combination of group size and temperature.

Behavioural metrics

We calculated all behavioural parameters using the trajectory data output from *idtracker.ai* and the trajectorytools package in Python (Romero-Ferrero et al. 2019). We examined the relationship between temperature and various behavioural metrics during two different types of movement. The first type of movement was unperturbed swimming, for which we used the data from the ten-minute period before any loom stimulus was presented to the fish, also known as the pre-looms period (Fig. 1c). The second type of movement was the predation threat escape, which we further divided into two phases. The first phase was during the loom stimulus (−1.67 to 1.67 s) and the second phase was after the loom stimulus or post-loom (1.67–5 s) (Fig. 1b). In both the unperturbed swimming and predation threat escape, we measured several individual movement and collective spatial metrics that we list below.

Table 1. Total number of trials for each combination of group size and temperature.

	Number of trials				
	Group size				
Temperature	1	2	4	8	16
29	8	9	8	7	7
25	7	6	6	6	9
21	6	8	6	6	6
17	4	9	8	9	9
13	6	8	9	7	7
9	7	8	10	10	9

We explored how the 99th percentile of speed and acceleration changes as a function of temperature. We also calculated the mean and median speed and acceleration of all fish over all frames, but found that the 99th percentile better captured performance limits (Gannon et al. 2014), without being sensitive to thresholds (30 BL s^{-1}, 3338 BL s^{-2} respectively) that we used to eliminate tracking errors. Since this was an estimate of the maximum speed/acceleration, henceforth, we refer to the 99th percentile of speed/acceleration as the maximum speed/acceleration. Maximum speed is a measure of swimming performance that determines how fast the fish can get away from the predator, but how quickly a fish can accelerate to that speed also influences the outcome of the interaction (Walker et al. 2005). We also examined the role of temperature on several collective behaviour metrics of a school during both unperturbed swimming and predation threat escape. The first collective behaviour trait, local polarization, is a measure of alignment of swimming direction between the focal individual and its nearest neighbor ranging continuously from 0 (when fish are swimming in perpendicular directions) to 1 (when fish are swimming in parallel directions). Secondly, we considered the average distance between the focal individual and its nearest neighbor, known as average nearest neighbor distance (ANND). Finally, we quantified the convex hull area, which is the area of the smallest polygon enclosing all the fish in the group. Convex hull area and ANND are complementary measures of the spatial distribution of fish. For example, if fish are distributed in several clusters around the experimental arena, ANND will be small but the convex hull area will be large. In addition to the parameters listed above, we measured two parameters specific to the predation threat escape behaviour. Since the time taken to respond to a threat is an important factor in escaping predators, we measured latency of response, which we defined as the time between the end of the loom stimulus and the first fish in the experimental arena to exhibit an escape response. Fish often startled before the end of the loom, so this metric had potentially negative values. The probability of responding to a predation threat is also an important factor that determines survival, and so we calculated the 'startle probability' which we defined as the proportion of individuals in the group that startled during the loom. Latency and probability to startle are both important but complementary parameters that determine the outcome of predator evasion. For example, if a fish has a high probability of responding to predation threats but takes a long time to respond, the chances of evading a predator are low. For both latency and startle probability, we defined a startle response to occur when an individual's speed exceeded a threshold of 10 BL s^{-1}. We examined the sensitivity of our results to the startle threshold (10 BL s^{-1}), speed threshold (30 BL s^{-1}) and acceleration threshold (3338 BL s^{-2}). However, our results were largely insensitive to the specific value of these thresholds. Sensitivity analysis can be found in the Supporting information.

Since in each trial the unperturbed swimming occurred before the predation threat (Fig. 1c) the differences between

unperturbed swimming and predation threat escape are potentially confounded with time. However, we examined the effect of temperature on all the behavioural metrics immediately before each loom stimulus (−11.67 to −10 s) and found the same results as the effect of temperature on unperturbed swimming. Thus, we used the period before any loom stimulus as the unperturbed swimming phase, since it had more data and loom number was not a significant predictor in these models.

Statistical methods

We used linear models (LMs) (www.r-project.org) to investigate the effect of temperature, the effect of group size and the interaction between temperature and group size on each behavioural metric described in section (d), during both unperturbed swimming and behaviour following a predation threat stimulus. Since performance traits in ectotherms generally show a curvilinear response to temperature (Huey and Stevenson 1979, Angilletta Jr. and Angilletta 2009, Dell et al. 2011, Clarke 2017), we also included a nonlinear temperature term (temperature^2) as a covariate in all the statistical models.

Unperturbed swimming

The general form of the equation we used for all behavioural metrics during unperturbed swimming is given in Eq. 1. We examined the residuals for heteroscedasticity and nonlinearity (<www.r-project.org>, Gelman and Su 2020) and we transformed the response variable when appropriate (Venables and Ripley 2002) as shown in Table 2. We also log transformed the group size variable in all the models. In addition to investigating the effect of temperature and group size on unperturbed swimming behaviour, we also examined whether group size mediates the way temperature affects behaviour by testing a model with an additional interaction term between temperature and group size (Eq. 2). We used a likelihood ratio test (LRT) (Zeileis and Hothorn 2002) to determine if the inclusion of the interaction term improved the fit of our model. If the p-value of the LRT was < 0.05, we used the more complicated model with the interaction term to make predictions for that behavioural metric and to test for the significance of each of the parameters. We examined normality using Q-Q plots (Wilk and Gnanadesikan 1968). For all the models used on unperturbed swimming, the errors were normally distributed. We used these models to make predictions with 95% confidence intervals to visualize the relationship of the behavioural metrics with temperature.

$$\begin{aligned}\text{Behaviour}_i = {} & \beta_0 + \beta_1 \text{Temperature}_i + \beta_2 \text{Temperature}_i^2 \\ & + \beta_3 \log_2(\text{Group size}_i) + \varepsilon_i\end{aligned} \tag{1}$$

$$\begin{aligned}\text{Behaviour}_i = {} & \beta_0 + \beta_1 \text{Temperature}_i + \beta_2 \text{Temperature}_i^2 \\ & + \beta_3 \log_2(\text{Group size}_i) + \beta_4 \text{Temperature}_i \\ & \times \log_2 \text{Group size}_i + \varepsilon_i\end{aligned} \tag{2}$$

Predation threat

For behaviour following a predation threat stimulus, in addition to temperature and group size, we investigated the effect of loom number on different behavioural metrics (Eq. 3). We examined normality using Q-Q plots (Wilk and Gnanadesikan 1968). All the errors were normally distributed for all behavioural metrics except for proportion of individuals that startled. Since the data for proportion of individuals that startled were proportions (between 0 and 1), we used generalized linear model (GLM) with binomial distribution and logit link function (logistic regression). We used Pearson's X^2 for goodness of fit to test whether the GLM was correctly specified (Pearson 1900). We used residual plots to examine for heteroscedasticity and nonlinearity for all the models except for logistic regression where we used binned residual plots (<www.r-project.org>, Gelman and Su 2020). We transformed the response variable when appropriate (Venables and Ripley 2002) as shown in Table 2. Since latency was the only behavioural metric for which we could easily determine errors caused due to tracking by watching the videos, we investigated influential points with Cook's distance (Cook 1977, Faraway 2016). We removed four influential points after the raw videos proved that they were affected by tracking errors. Similar to unperturbed swimming, we also included the interaction term between group size and temperature and used the more complicated model with the interaction term for predictions and significance of parameters, if the value of the LRT was < 0.05. For maximum acceleration during the loom, we used Eq. 4 since that model performed better than the model without the interaction term. We used Eq. 4 to make predictions with 95% confidence intervals to visualize the relationship of these two behavioural metrics with temperature and to test for the significance of each parameter. For all other behavioural metrics under predation threat, we used Eq. 3 to make predictions and test for significance. The R^2 value for each model and the significance of each predictor variable is shown in Table 2 (<www.r-project.org>, Zhang 2021).

$$\begin{aligned}\text{Behaviour}_i = {} & \beta_0 + \beta_1 \text{Temperature}_i + \beta_2 \text{Temperature}_i^2 \\ & + \beta_3 \log_2(\text{Group size}_i) + \beta_4 \text{Loom}_i + \varepsilon_i\end{aligned} \tag{3}$$

$$\begin{aligned}\text{Behaviour}_i = {} & \beta_0 + \beta_1 \text{Temperature}_i + \beta_2 \text{Temperature}_i^2 \\ & + \beta_3 \log_2(\text{Group size}_i) + \beta_4 \text{Loom}_i \\ & + \beta_5 \text{Temperature}_i \times \log_2\left(\text{Group size}_i\right) + \varepsilon_i\end{aligned} \tag{4}$$

We also tried an alternate statistical modeling approach where we used different candidate models for each behavioral metric and chose the best fitting model using AIC (Supporting information). Since there were minimal differences between the results of both modeling approaches, we ultimately used the results of the simpler approach with the same model for all behavioural metrics.

Table 2. Summary of model results for each behavioral metric. The significance level of each of the variables is given beside each coefficient such that ***, ** and * corresponds to < 0.001, < 0.01 and < 0.05 respectively. The last column specifies the temperature at which the response variable (x) is maximum or minimum (within the range of temperatures used in the experiment) according to model predictions. A range is given when the maximum or minimum varies with group size.

Response variable (x)	Transformation/link function	Intercept	Temperature	Temperature^2	Group size	Loom	Temperature × Group size	R^2	Min/max temperature (°C)
Unperturbed swimming									
Maximum speed	$\log(x+1)$	0.781***	0.034*	−0.001	−0.001	–	–	0.128	28
Maximum acceleration	$\log(x+1)$	4.131***	0.026	−0.001	−0.045***	–	–	0.105	26
ANND	$\log(x)$	0.770***	0.023	−0.001	−0.567***	–	–	0.764	–
Convex hull area	$\sqrt{x}$	1.606***	0.037	−0.002	0.706***	–	–	0.574	–
Polarization	$\sqrt{x}$	0.551***	−0.003	0	0.002	–	–	0.011	–
Predation threat									
Maximum speed	$\sqrt{x}$	2.358***	0.05*	−0.002**	0.231***	−0.078***	–	0.174	12
Maximum acceleration	$\log(x+1)$	4.577***	0.082***	−0.003***	0.236***	−0.056***	−0.004*	0.185	13-16
Latency	1	0.059	−0.025***	0.001***	−0.035***	0.01	–	0.064	20
Proportion of individuals startling	logit link	−0.881	0.124*	−0.005**	0.064	−0.105*	–	0.039	13
ANND	$\log(x)$	1.508***	−0.072**	0.001	−0.630***	0.001	0.007*	0.413	–
Convex hull area	$\sqrt{x}$	2.502***	−0.049*	0.001	0.684***	0.009	–	0.418	–
Polarization	$\sqrt{x}$	0.842***	−0.019**	0.001**	−0.034***	−0.013*	–	0.043	19

Results

Unperturbed swimming

Individual behaviour

Temperature was the only significant predictor of maximum speed during the unperturbed swimming phase (Table 2). The maximum swimming speed had a positive curvilinear relationship with temperature, with the peak speed occurring at 28°C (Fig. 2a, Table 2). In contrast, only group size was a significant predictor of maximum acceleration during the unperturbed swimming phase. Although the effect was not significant, the maximum acceleration had a positive curvilinear relationship with temperature with the peak approximately 26°C (Fig. 2a, Table 2). While the same relationship was observed for all group sizes, the intercept decreased for larger group sizes (Table 2). The mean and median of acceleration also exhibited a positive curvilinear relationship with temperature (Supporting information).

Collective behaviour

We did not observe a significant effect of temperature on average nearest neighbor distance, convex hull area or polarization during the unperturbed swimming portion of the trials (Table 2).

Predation threat

Individual behaviour

Temperature, temperature^2, group size and loom were all significant predictors of maximum speed during the predation threat (Table 2). The maximum speed during the loom stimulus had a positive curvilinear relationship with temperature with the maximum at approximately 12°C (Fig. 3a, Table 2). While the same relationship was observed for all group sizes and looms, the intercept increased with higher group sizes and decreased with subsequent looms (Table 2). The optimal temperature at which speed was maximum during an escape response (12°C) was much lower than the temperature at which speed peaks during the unperturbed swimming phase (28°C) (Fig. 2a, Table 2). In addition to temperature, temperature^2, group size and loom, the interaction between temperature and group size was also a significant predictor of maximum acceleration during the predation threat (Table 2). Maximum acceleration had a positive curvilinear relationship with temperature and reached a maximum at 13–16°C (Fig. 3b), which was 10–13°C lower than the optimal temperature during unperturbed swimming (Fig. 2b, Table 2). As group size decreased, the optimal temperature at which maximum acceleration peaks during predation threat increased (Fig. 3b). For group size = 1, the optimal temperature was approximately 16°C, which was still 10°C lower than the optimal temperature during unperturbed swimming (Fig. 2b). While the same relationship between acceleration, temperature and group size was observed for every loom, the intercept decreased with subsequent looms (Table 2).

As shown in Table 2 temperature, temperature^2 and group size were significant predictors of latency. Latency exhibited a negative curvilinear relationship with temperature and had a minimum at approximately 20°C. Although we observed the same relationship with temperature for all group sizes, the intercept decreased with increased group size (Table 2). temperature, temperature^2 and loom were significant predictors of the proportion of individuals startling during the loom stimulus (Table 2). While the proportion of individuals startling had a positive curvilinear relationship with temperature with a maximum at approximately 13°C (though this exact value varied slightly with the startle threshold as shown in the Supporting information), we did not observe an effect of group size (Fig. 3d, Table 2). While the same relationship held for all loom stimuli, the intercept again decreased for subsequent looms, suggesting an acclimation to the loom stimuli or learning (Table 2).

Collective behaviour

During the post-loom period, the average distance between individuals and their nearest neighbor decreased as group

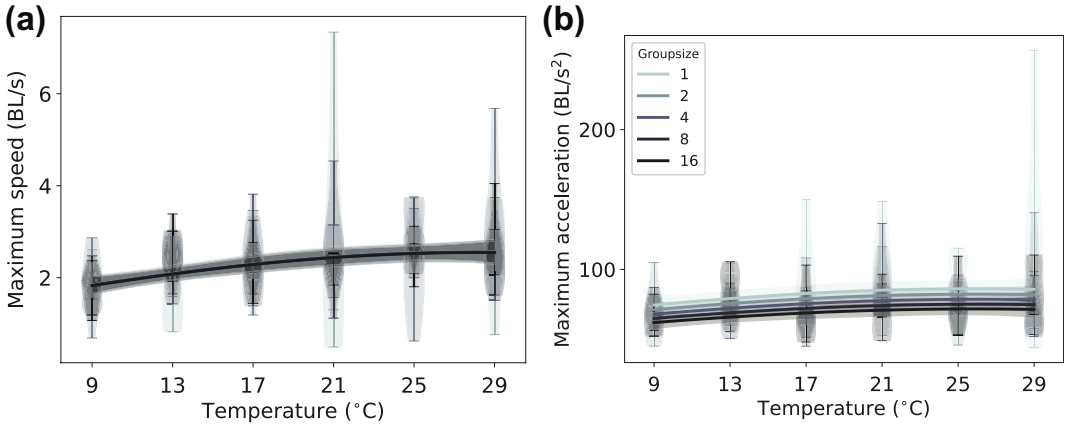

Figure 2. Individual behaviour during unperturbed swimming. (a) Maximum speed and (b) maximum acceleration had a positive curvilinear relationship with temperature but with the peak towards the highest temperatures in this range. The same legend holds for both figures.

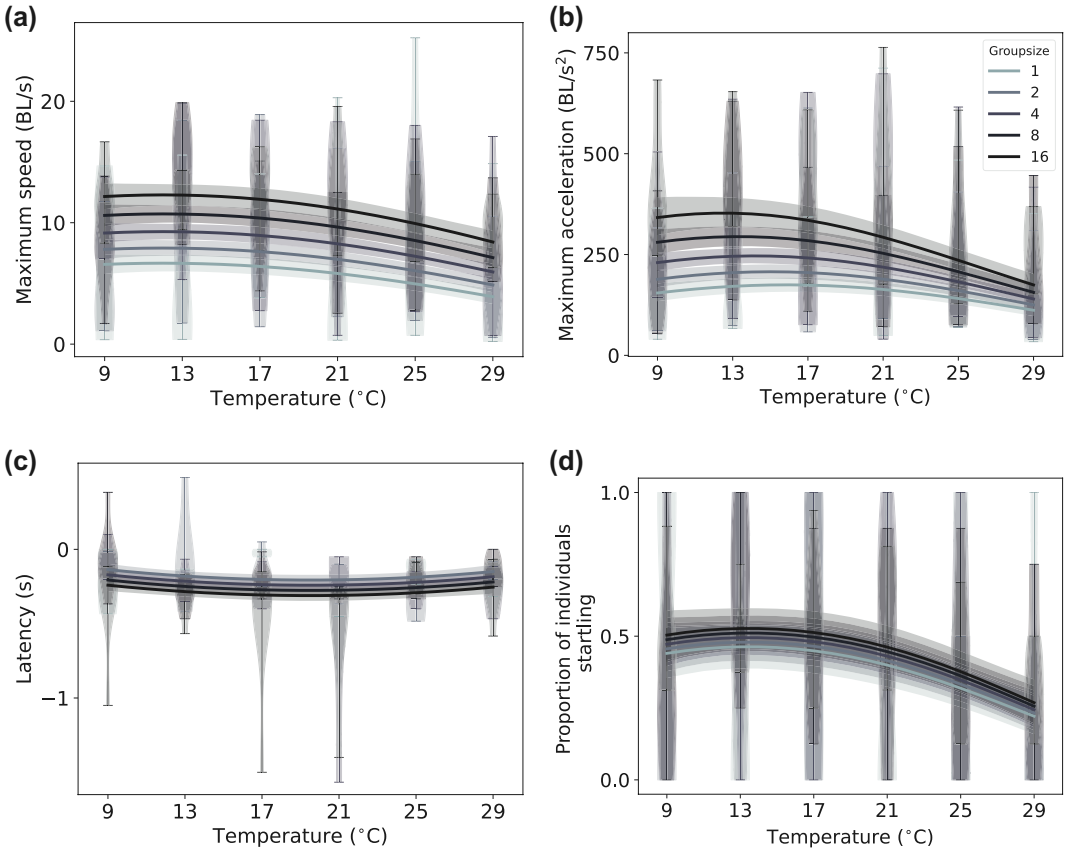

Figure 3. Individual behaviour during the predation threat. (a) Maximum speed and (b) maximum acceleration during the loom stimulus exhibited a positive curvilinear relationship with temperature. However, the value of that optimal temperature at which acceleration peaked varied with group size. (c) Latency (reaction time in seconds after end of loom) also exhibited a minimum at an ostensibly optimal temperature. (d) The proportion of individuals that startled peaked at an optimal temperature. The same legend holds for all four figures.

size increased and as temperature increased (Table 2). The interaction effect of group size and temperature was also significant and there was a reduced effect of temperature on ANND in larger group sizes (Fig. 4a). We also found that the convex hull area decreased monotonically as temperature increased (Fig. 4b). While the same relationship with temperature was observed for all group sizes, the convex hull area increased with increased group sizes (Table 2). Although the model with local polarization explained less than 5% of the variance in our data, we found that temperature, temperature^2, group size and loom were significant predictors of local polarization (Table 2). Local polarization was minimum at 19°C (Fig. 4c, Table 2) for all group sizes and loom stimuli, although the intercept decreased with increased group sizes and subsequent looms (Table 2).

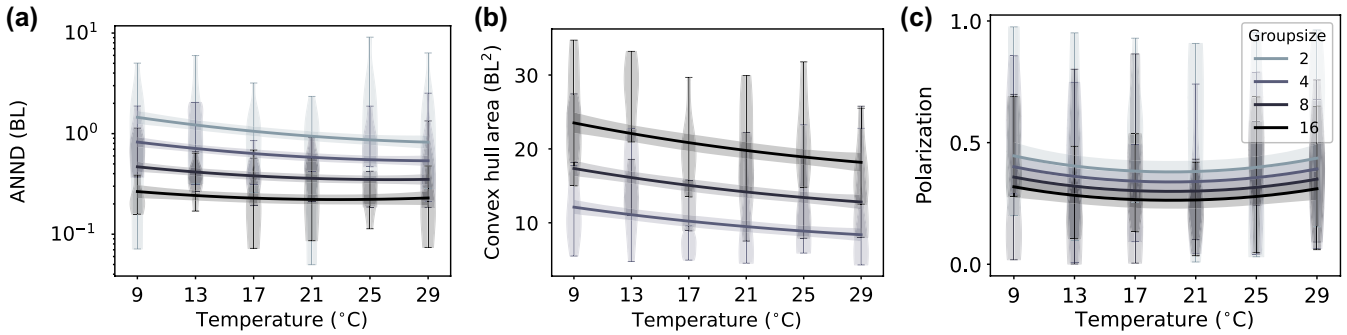

Figure 4. Collective behavior during the predation threat. (a) Average nearest neighbor distance during post-loom phase decreased with increasing temperature. The effect of temperature was less pronounced for larger group sizes as shown by the black curve for group size 16. (b) Convex hull area of the group during post-loom phase decreased with temperature. (c) Local polarization of the group following a predation stimulus has a minimum at 19°C. The same legend holds for all three figures, but convex hull area was calculated only for group size 4, 8 and 16.

Discussion

Context dependence

While the importance of water temperature to the performance of freshwater ectotherms has long been recognized, ongoing and directional changes in climate have elevated the importance of understanding these relationships due to the likely novel thermal conditions that will be experienced by freshwater taxa in the coming decades (Whitney et al. 2016, Knouft and Ficklin 2017). In our experiments, temperature had a context-dependent effect on the movement of individual fish and the collective dynamics of schools. We found support for our first hypothesis that traits relevant to escape would exhibit a positive curvilinear response to temperature. However, we found that the optimal temperature to escape from their predators – the temperature at which speed and acceleration were maximized – was different from their optimal temperature during unperturbed swimming.

During unperturbed swimming, individual speed and acceleration both increased with temperature, reaching a maximum only at and beyond 25°C, respectively. This pattern is consistent with the fact that biochemical reaction rates, and thus metabolic rates increase exponentially with temperature in ectotherms (Brown et al. 2004). However, the increase was far lower than predicted by the metabolic theory of ecology, pointing to the considerable variance that accompanies macro ecological patterns. This was in support of our second hypothesis that the effect of temperature on unperturbed swimming would be attenuated since fish would be less likely to push their performance limits in non-life-threatening situations. In contrast to unperturbed swimming, during a predation threat, individual speed increased with temperature up to 12°C for speed and acceleration increased with temperature up to 13–16°C, after which these response variables then decreased as temperature continued to rise. Most studies have examined the influence of external factors on only unperturbed swimming at the individual and collective level (Herbert-Read et al. 2017, Ginnaw et al. 2020). In this study, however, we compared the unperturbed swimming and escape response. We found an asymmetry in the effect of temperature between these two behaviours, demonstrating that the effect of temperature on one measure of behaviour may not extend to other behaviours and rates.

Behavioural regulation

Our results are consistent with findings from other species that activity levels can be regulated by behaviour as temperature changes (O'Steen and Bennett 2003). First, since temperature affects maximum speed and acceleration differently depending on whether the fish are trying to evade a predator or not, the fish may be behaviourally regulating their maximum speed or acceleration as opposed to a physiological change caused due to external temperature. Second, the proportion of fish that startle during predation also peaks at 13°C, around the same temperature at which maximum speed and acceleration of the fish peaks. Speed, acceleration and probability to startle are also highly correlated during predation threat (Supporting information) indicating that maximum speed and acceleration might be driven by the probability to startle. For example, the maximum speed and maximum acceleration are more likely to be higher when 50% of the fish are startling than if 25% of the fish are startling. If the trends in speed and acceleration during predation are being driven by the fish that startle, then the fish may be compensating for their inability to swim faster at lower temperatures by being more responsive to potential threats at lower temperatures. However, there might be physiological effects of temperature, such as lower metabolic rate, that are influencing their swimming speeds below 13°C.

Collective behaviour

In contrast to previous results (Bartolini et al. 2015, Colchen et al. 2017), we did not observe a significant effect of temperature on average nearest neighbor distance, convex hull area or polarization during the unperturbed swimming. While we did observe a significant effect of temperature on average nearest neighbor distance during post-loom phase, the effect of temperature was monotonic and opposite to that found by Bartolini et al. (2015) and Colchen et al. (2017). Polarization had a negative curvilinear response to temperature following simulated predation events. The effect of temperature on all the collective behaviour traits contrasted with our third hypothesis that the traits would exhibit a positive curvilinear response to temperature during unperturbed swimming as well as following simulated predation events. We also found that ANND and convex hull area decreases with increasing temperatures after each loom stimulus. These results add to the existing literature about how temperature affects social behaviour, but also provide insight on the asymmetry in the effect of temperature on social behaviour in the context of predation.

Multistress responses

The presence, decisions and performance of other animals can act as social information to guide the behaviour of the focal individual (Doligez et al. 2010). For species that use social information, the effect of temperature on behaviour might not be simple extensions of the effect of temperature on individuals. Maximum acceleration had an optimal temperature that shifted to a lower temperature for larger group sizes (Fig. 3b). This supports our fourth hypothesis that there would be an interaction between the effect of group size and the effect of temperature on fish behaviour during a predation threat. There is growing awareness about how multiple stressors can have an interactive effect on animals (Darling and Côté 2008) and our study provides empirical data for the ecology of multistress responses in fish.

Dampened response to temperature

Temperature was a significant parameter in most of the models we fit to the data, however temperature explained only a modest percentage of the variance. This dampened response to temperature could be due to the large range of temperatures throughout the year that golden shiners experience in the Missouri region (Jones et al. 2011, Knouft et al. 2021). Despite this large range in temperature tolerance we still found a significant effect of temperature, suggesting that temperature could have a stronger effect on fish species with tolerance to a smaller range of temperature. Another possible reason why we did not observe a stronger effect of temperature is that we gave the fish at least one week to acclimatize to the respective temperatures. Beitinger and Bennett (2000) showed that acclimation can effectively double thermal tolerance for some species and acclimation played a larger role at lower temperatures than higher thermal tolerance temperatures. This suggests that future research should incorporate a wider range of temperatures range and also test the effects of more abrupt changes in temperature.

Laboratory conditions

In this study, like in many collective behaviour studies, fish were restricted to mostly swimming in one plane (shallow water) to ensure easy detection and tracking of individuals and easier analysis of the data. Since many animal groups move in three-dimensional space, more studies have extended the models to three dimensions. These studies have shown that many of the qualitative results obtained in 2D also hold in 3D (Giardina 2008). Additionally, golden shiners are often found in the littoral zone of shallow lakes (Krause et al. 1996). Hence, while swimming in shallow water is not uncommon for this species, it could change their schooling dynamics. In this study, we found that the optimal temperature for golden shiners to escape from predators is different from their optimal temperature during unperturbed swimming. Future research should test this result in wild conditions.

Rate asymmetry

Unperturbed swimming and predator escape responses both play a major role in determining the strength and outcome of consumer–resource interactions. Unlike previous work examining the effects of thermal asymmetries on different ecological rates (Dell et al. 2014, Amarasekare 2015), our study reveals evidence of rate asymmetry within the same species. Differences in thermal sensitivity of different behaviours of the same species can affect ecological dynamics and food web structure. If fish are indeed able to compensate for lower speeds at lower temperatures, this could indicate more resistance to temperature changes and a more stable food web structure (Vallina and Le Quéré 2011). On the other hand, increasing global temperatures are likely to push prey fish towards a temperature at which their speed and acceleration are less than optimal when they are escaping from predators. Our results suggest that these changes may be more nuanced than macro metabolic models, reflecting the variable role temperature plays across traits and social structures.

Acknowledgement – We thank Molly A. Roberts for her edits to the manuscript.
Funding – This work was supported in part through an internship to JF provided by the National Great Rivers Research and Center through grant no. NGRREC-IP2019-18. AMB was supported by the H. Mason Keeler Endowed Professorship in Sports Fisheries Management. JMG and AID were funded by the NSF Rules of Life award DEB-1838346.

Author contributions

Maria Kuruvilla: Data curation (lead); Formal analysis (lead); Methodology (equal); Validation (lead); Visualization (lead); Writing – original draft (lead); Writing – review and editing (equal). **Anthony Dell**: Conceptualization (equal); Data curation (supporting); Funding acquisition (supporting); Methodology (equal); Project administration (lead); Resources (equal); Software (lead); Writing – review and editing (equal). **Ashley R. Olson**: Formal analysis (supporting); Methodology (equal); Writing – review and editing (equal). **Jason Knouft**: Resources (lead); Supervision (equal); Writing – review and editing (equal). **John M. Grady**: Methodology (supporting); Writing – review and editing (equal). **Jacob Forbes**: Methodology (supporting). **Andrew M. Berdahl**: Conceptualization (equal); Funding acquisition (lead); Methodology (supporting); Resources (equal); Writing – original draft (supporting); Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.h44j0zpmp> (Kuruvilla et al. 2022). The code used for the analysis has been uploaded at <https://github.com/maria-kuruvilla/stats> and <https://github.com/maria-kuruvilla/temp_collective_new>.

Supporting information

The Supporting information associated with this article is available with the online version.

References

Amarasekare, P. 2015. Effects of temperature on consumer–resource interactions. – J. Anim. Ecol. 84: 665–679.
Angilletta Jr., M. J. and Angilletta, M. J. 2009. Thermal adaptation: a theoretical and empirical synthesis. – Oxford Univ. Press.
Bartolini, T. et al. 2015. Temperature influences sociality and activity of freshwater fish. – Environ. Biol. Fish. 98: 825–832.
Beitinger, T. L. and Bennett, W. A. 2000. Quantification of the role of acclimation temperature in temperature tolerance of fishes. – Environ. Biol. Fish. 58: 277–288.

Berdahl, A. et al. 2013. Emergent sensing of complex environments by mobile animal groups. – Science 339: 574–576.
Berdahl, A. M. et al. 2018. Collective animal navigation and migratory culture: from theoretical models to empirical evidence. – Phil. Trans. R. Soc. B 373: 20170009.
Bonabeau, E. et al. 1999. Scaling in animal group-size distributions. – Proc. Natl Acad. Sci. USA 96: 4472–4477.
Brett, J. et al. 1969. Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. – J. Fish. Board Can. 26: 2363–2394.
Brown, J. H. et al. 2004. Toward a metabolic theory of ecology. – Ecology 85: 1771–1789.
Carey, N. 2019. nicholascarey/loomeR: looping, custom frame numbers and filenames! – Zenodo, <https://doi.org/10.5281/zenodo.3279092>.
Chen, R. et al. 2003. Alternative complement activity and resistance to heat stress in golden shiners *Notemigonus crysoleucas* are increased by dietary vitamin C levels in excess of requirements for prevention of deficiency signs. – J. Nutr. 133: 2281–2286.
Clarke, A. 2017. Principles of thermal ecology: temperature, energy and life. – Oxford Univ. Press.
Colchen, T. et al. 2017. Temperature modifies activity, inter-individual relationships and group structure in a fish. – Curr. Zool. 63: 175–183.
Cook, R. D. 1977. Detection of influential observation in linear regression. – Technometrics 19: 15–18.
Darling, E. S. and Côté, I. M. 2008. Quantifying the evidence for ecological synergies. – Ecol. Lett. 11: 1278–1286.
Dell, A. I. et al. 2011. Systematic variation in the temperature dependence of physiological and ecological traits. – Proc. Natl Acad. Sci. USA 108: 10591–10596.
Dell, A. I. et al. 2014. Temperature dependence of trophic interactions are driven by asymmetry of species responses and foraging strategy. – J. Anim. Ecol. 83: 70–84.
Deutsch, C. A. et al. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. – Proc. Natl Acad. Sci. USA 105: 6668–6672.
Dill, L. M. 1974. The escape response of the zebra danio *Brachydanio rerio* I. The stimulus for escape. – Anim. Behav. 22: 711–722.
Dill, L. M. 1990. Distance-to-cover and the escape decisions of an African cichlid fish, *Melanochromis chipokae*. – Environ. Biol. Fish. 27: 147–152.
Doligez, B. et al. 2010. Social information use. – Encycl. Anim. Behav. 3: 242–250.
Faraway, J. 2016. faraway: functions and datasets for books by Julian Faraway. – R package ver. 1.0.7, <https://CRAN.R-project.org/package=faraway>.
Foster, W. and Treherne, J. 1981. Evidence for the dilution effect in the selfish herd from fish predation on a marine insect. – Nature 293: 466–467.
Gannon, R. et al. 2014. Thermal limitation of performance and biogeography in a free-ranging ectotherm: insights from accelerometry. – J. Exp. Biol. 217: 3033–3037.
Gelman, A. and Su, Y. S. 2020. Arm: data analysis using regression and multilevel/hierarchical models. – R package ver. 1.11-2, <https://CRAN.R-project.org/package=arm>.
Giardina, I. 2008. Collective behavior in animal groups: theoretical models and empirical studies. – HFSP J. 2: 205–219.
Gil, M. A. and Hein, A. M. 2017. Social interactions among grazing reef fish drive material flux in a coral reef ecosystem. – Proc. Natl Acad. Sci. USA 114: 4703–4708.
Ginnaw, G. M. et al. 2020. Effects of multiple stressors on fish shoal collective motion are independent and vary with shoaling metric. – Anim. Behav. 168: 7–17.
Grady, J. M. et al. 2019. Metabolic asymmetry and the global diversity of marine predators. – Science 363: eaat4220.
Herbert-Read, J. E. et al. 2017. Anthropogenic noise pollution from piledriving disrupts the structure and dynamics of fish shoals. – Proc. Natl Acad. Sci. USA 284: 20171627.
Huey, R. B. and Stevenson, R. 1979. Integrating thermal physiology and ecology of ectotherms: a discussion of approaches. – Am. Zool. 19: 357–366.
Hurst, T. P. 2007. Thermal effects on behavior of juvenile walleye pollock *Theragra chalcogramma*: implications for energetics and food web models. – Can. J. Fish. Aquat. Sci. 64: 449–457.
Ioannou, C. 2017. Grouping and predation. – In: Shackelford, T. K. and Weekes-Shackelford, V. A. (eds), Encyclopedia of evolutionary psychological science. Springer, p. 1–6.
Johannes, M. R. 1993. Prey aggregation is correlated with increased predation pressure in lake fish communities. – Can. J. Fish. Aquat. Sci. 50: 66–73.
Jones, J. R. et al. 2011. Temperature and oxygen in Missouri reservoirs. – Lake Reservoir Manage. 27: 173–182.
Katz, Y. et al. 2011. Inferring the structure and dynamics of interactions in schooling fish. – Proc. Natl Acad. Sci. USA 108: 18720–18725.
Knouft, J. H. and Ficklin, D. L. 2017. The potential impacts of climate change on biodiversity in flowing freshwater systems. – Annu. Rev. Ecol. Evol. Syst. 48: 111–133.
Knouft, J. H. et al. 2021. Forested riparian buffers as climate adaptation tools for management of riverine flow and thermal regimes: a case study in the Meramec River Basin. – Sustainability 13: 1877.
Krause, J. et al. 1996. Phenotypic variability within and between fish shoals. – Ecology 77: 1586–1591.
Krause, J. et al. 1998. Body length variation within multi-species fish shoals: the effects of shoal size and number of species. – Oecologia 114: 67–72.
Krause, J. et al. 2000. Fish shoal composition: mechanisms and constraints. – Proc. R. Soc. B 267: 2011–2017.
Krause, J. et al. 2002. Living in groups. – Oxford Univ. Press.
Kuruvilla, M. et al. 2022. Data from: The effect of temperature on fish swimming and schooling is context dependent. – Dryad Digital Repository, <https://doi.org/10.5061/dryad.h44j0zpmp>.
Li, L. et al. 2020. Vortex phase matching as a strategy for schooling in robots and in fish. – Nat. Commun. 11: 5408.
O'Steen, S. and Bennett, A. F. 2003. Thermal acclimation effects differ between voluntary, maximum and critical swimming velocities in two cyprinid fishes. – Physiol. Biochem. Zool. 76: 484–496.
Payne, N. L. et al. 2016. Temperature dependence of fish performance in the wild: links with species biogeography and physiological thermal tolerance. – Funct. Ecol. 30: 903–912.
Pearson, K. X. 1900. On the criterion that a given system of deviations from the probable in the case of a correlated system of variables is such that it can be reasonably supposed to have arisen from random sampling. – Lond. Edinb. Dublin Phil. Mag. J. Sci. 50: 157–175.
Polverino, G. et al. 2013. Fish and robots swimming together in a water tunnel: robot color and tail-beat frequency influence fish behavior. – PLoS One 8: e77589.

Polyakov, A. Y. et al. 2022. Group size affects predation risk and foraging success in Pacific salmon at sea. – Sci. Adv. 8: eabm7548.
Romero-Ferrero, F. et al. 2019. Idtracker.ai: tracking all individuals in small or large collectives of unmarked animals. – Nat. Methods 16: 179–182.
Rosenthal, S. B. et al. 2015. Revealing the hidden networks of interaction in mobile animal groups allows prediction of complex behavioral contagion. – Proc. Natl Acad. Sci. USA 112: 4690–4695.
Vallina, S. M. and Le Quéré, C. 2011. Stability of complex food webs: resilience, resistance and the average interaction strength. – J. Theor. Biol. 272: 160–173.
Venables, W. N. and Ripley, B. D. 2002. Modern applied statistics with S, 4th edn. – Springer, <www.stats.ox.ac.uk/pub/MASS4/>.
Walker, J. et al. 2005. Do faster starts increase the probability of evading predators? – Funct. Ecol. 19: 808–815.
Walsman, J. C. et al. 2022. Shoaling guppies evade predation but have deadlier parasites. – Nat. Ecol. Evol. 6: 945–954.
Weetman, D. et al. 1998. Effects of temperature on anti-predator behaviour in the guppy, *Poecilia reticulata*. – Anim. Behav. 55: 1361–1372.
Whitney, J. E. et al. 2016. Physiological basis of climate change impacts on North American inland fishes. – Fisheries 41: 332–345.
Wilk, M. B. and Gnanadesikan, R. 1968. Probability plotting methods for the analysis for the analysis of data. – Biometrika 55: 1–17.
Wong, B. et al. 2007. Shoaling decisions in female swordtails: how do fish gauge group size? – Behaviour 144: 1333–1346.
Zeileis, A. and Hothorn, T. 2002. Diagnostic checking in regression relationships. – R News 2: 7–10. <https://CRAN.R-project.org/doc/Rnews/>.
Zhang, D. 2021. rsq: R-squared and related measures. – R package ver. 2.2, <https://CRAN.R-project.org/package=rsq>.
Zuo, W. et al. 2012. A general model for effects of temperature on ectotherm ontogenetic growth and development. – Proc. R. Soc. B 279: 1840–1846.