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Do fish prefer to associate with conspecifics with similar metabolic rates?

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Abstract

Living in groups is common across animal taxa as associating with conspecifics can create a lot of benefits for individual group members. It can aid in predator detection and avoidance, foraging and reproductive success. However, the individuals which make up the group can influence how these benefits are distributed between the group members. Previous studies have shown preference to associate with morphologically and behaviourally similar conspecifics, but it is less understood how physiological traits affect group assortment.

In this study I was interested in investigating if metabolic rate influences the choice of group mates. When presented with a choice between two groups (high or low SMR) of conspecifics all fish, regardless of their own phenotype, preferred to associate with fish of high SMR. Fish with higher SMR were also found to have a higher average velocity during the trials. Higher activity can indicate higher fitness and foraging capabilities. This could help explain why fish with lower metabolism also showed a preference to associate with fish with higher SMR despite risk of being outcompeted. Fish were also tested in an open field trial where they were allowed to swim freely in groups of different compositions based on SMR phenotypes (homogenous high SMR, homogenous low SMR and heterogeneous mixed SMR). No difference was found in the number of associations among individuals, both between groups and within the heterogeneous mixed groups. My findings could indicate that other traits rather than metabolic rate has a stronger effect on within school sorting.

Table of Contents

Acknowledgements	4
1 Introduction	5
2 Methodology	8
2.1 Animals	8
2.2 Measuring metabolic traits	8
2.3 Assortment trial	10
2.3.1 Video analysis	11
2.3.2 Statistical analysis - Assortment trial	12
2.4 Open field trial	13
2.4.1 Photo analysis	14
2.4.2 Statistical analysis - Open field trial	15
3 Results	16
3.1 Preference	17
3.2 Velocity	20
3.3 Open field - Between groups	22
3.4 Open field - Within groups	23
4 Discussion	27
4.1 Preference	27
4.2 Velocity	28
4.3 Open field - Between groups	29
4.4 Open field - Within groups	29
4.5 Conclusions	30
6 References	32

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1 Introduction

Living in social groups is common across animal taxa, be it flocks of birds, swarms of insects, or schools of fish (Krause and Ruxton, 2002; Wright et al., 2006; Couzin, 2008). There have been many attempts at defining what constitutes a “group”. Definitions range from Wilson (1975) stating that a group is “any set of organisms, belonging to the same species, that remain together for a period of time interacting with one another”, to Lee (1994) suggesting that “when two or more animals live together they constitute a social unit”. However, as also emphasised by Krause and Ruxton (2002), these definitions quickly become ambiguous when considering the large range of organisms that aggregate in groups, and the vast diversity of life histories and behaviours behind this phenomenon.

Nonetheless, the reasoning behind animals living in groups is that associating with conspecifics creates benefits for the individual group members. It can aid in predator avoidance by making it difficult for predators to single out individuals within the group. It also allows each individual to spend less time being vigilant and looking out for predators (Krakauer 1995; Lima 1995). This, in turn, means that time can be more efficiently spent foraging for food. Foraging in a group also allows for higher efficiency at finding food as well as ability to protect food patches or items against other groups or solitary competitors (Ranta, Rita and Lindstrom, 1993; Beauchamp, 2014b). Living in groups also makes it easier to find mates and thus reproduce. Any energy that would have been spent searching for mates can instead be spent on, for example, egg production in females (Strodl and Schausberger, 2013) and aggression or territoriality in males. In some social communities, such as packs of wild dogs (Carbone, Du Toit and Gordon, 1997), the rearing and care of the young is also shared among individuals, thus reducing the burden on the mother. Moving in groups can also have hydro/aerodynamic benefits. This is best displayed by fish schools and birds flying in formation, however even humans utilise the same principles in bicycle pelotons. Individuals holding positions further back in the school or flock can benefit from the vortices created by the conspecifics further forward (Liao et al., 2003; Weimerskirch et al., 2001). This allows for reduced energy consumption while still being able to keep pace with the rest of the group.

While living in groups brings many benefits, there are also draw-backs. The main trade-off for associating with conspecifics is competition. Competition between individuals can manifest in different ways, such as access to mates, lowering the chances of reproductive success, access to shelter and space, as well as food availability and quality (Krause and Ruxton, 2002). There is also a higher risk of contracting diseases and parasite infections when living in close proximity to conspecifics (Côté and Poulin, 1995). The degree of competition, however, can vary heavily with social structure and group size, as well as with the environment and behaviour of the species. Group social structure is important as it influences energy acquisition and expenditure of the group members. Hierarchical communities are very common and rest on the principle that some individuals are dominant over others. Well known examples of animals living in hierarchical societies include primates such as chimpanzees and baboons, but hierarchical systems can also be found among birds and fish (Alexander, 1974; Dall, 2004; Reid, Armstrong and Metcalfe, 2012). Dominance comes with a number of advantages, such as better access to food and higher likelihood of reproduction. However, dominance can also come at a cost as individuals often engage in antagonistic behaviours when asserting and attempting to maintain dominance, thus resulting in higher energy expenditure (Briffa and Sneddon, 2007; Killen et al., 2014). Group size can also play an important role in competition. Larger groups require more space and food and thus increase the risk of potential competition for these resources. While larger groups can benefit from easier predator detection, increased group size can also have the reverse effect by making the group more conspicuous to predators. The group as a whole thus face a higher predatory pressure, albeit divided over a larger number of group members.

The individuals making up the group also influence social dynamics and thus the structure of the group. It is well established that there exists consistent inter-individual behavioural differences within animal groups (Rupia *et al.* 2016; Jolles *et al.* 2017). These differences are often referred to as animal personalities and concern a number of different traits such as boldness and aggression (Sih, Bell and Johnson, 2004; Frost et al., 2007; White, Meekan and McCormick, 2015). Boldness and aggression are closely linked and have also been related to physiological traits of the animal, such as metabolic rate. Bolder individuals are generally more

aggressive and prone to risk taking behaviour while foraging, which could be driven by a higher nutritional need resulting from an intrinsically higher metabolic rate (Killen et al., 2016). Additionally, these animals are likely to be the dominant individuals of the group as dominance is another trait that has been positively linked with metabolic rate (Metcalf, Van Leeuwen and Killen, 2016).

Differences in behavioural and physiological traits can also regulate an individual's spatial position within the group. This in turn can create sub-groups within a larger group, with neighbouring animals being more similar than individuals further apart (Killen et al., 2017). For animals living in groups where synchronous movement is important (e.g. for predator avoidance), such as birds or fish, it is crucial to be able to perceive and match the movement of neighbouring individuals. In situations like this, inherent differences in both physiology and behaviour can play an important role in the survival of the individual, which emphasises the importance of group composition.

To avoid “the oddity effect” of being singled out among the crowds, it is common for animals to aggregate in groups of morphologically similar conspecifics (Krakauer, 1995), however there can also be other underlying reasons for assortment of individuals among groups. Mechanisms underlying assortment can be loosely divided into passive or active assortment (Killen et al., 2017). Passive assortment can occur when individuals aggregate in a suitable environment or habitat for their phenotype, such as temperature or oxygen concentration (Croft et al., 2003) and thus forming a group. Another way that passive assortment can occur is if a number of individuals become a group due to similar performance capacities. This can, for example, occur during predation events: individuals of different phenotypes can be separated during the predation event and end up forming separate schools in the aftermath (Hoare et al., 2000). Active assortment occurs when individuals make active choices of group mates. Previous studies have shown association preferences for conspecifics of similar size, body shape, and colouration, which increase visual homogeneity (Hoare et al., 2000; Hemelrijk and Kunz, 2005; Croft et al., 2009). However, it is less understood how individual physiological traits affect group assortment. Due to the close relationships among

morphology, physiology, and behaviour, it is very likely that individuals group together non-randomly.

In this study I was interested in investigating if metabolic rate influences the choice of group mates. Using fish schools, two experiments were designed: one where individual fish were allowed to make active choices between separate groups of conspecifics with different metabolic rate phenotypes (high vs. low metabolic rate), and another where a group of fish with known metabolic rate phenotypes were roaming together freely and allowed passive within-group assortment. My main questions were: (1) do individual fish prefer to assort with conspecifics with similar metabolic rates as themselves, and (2) do preferences change with the metabolic rate composition of the group? I hypothesised that all fish, regardless of their metabolic rate phenotype, would prefer to associate with fish with lower metabolic rates as these fish theoretically would be poorer competitors.

2 Methodology

2.1 Animals

Common minnows (*Phoxinus phoxinus*) were caught using large dip-nets in the river Kelvin, Glasgow, Scotland in June 2016 and transferred to the aquarium facilities in the Graham Kerr building at the University of Glasgow. Fish were left to acclimate in the aquarium over a period of six months before having any procedure performed. Fish were kept in 4 stock tanks (100 x 40 x 30 cm) with approximately 45 individuals per tank. The tanks were supplied with re-circulating, UV treated, freshwater and kept on a 12 h light: 12 h dark photoperiod. The water temperature in the aquarium facilities was kept between 13 and 14°C. The fish were fed once a day with defrosted bloodworm or aquarium flake food.

2.2 Measuring Metabolic Traits

To determine the metabolic phenotype of individual minnows I used intermittent-closed respirometry to measure the oxygen uptake rate of the fish as a proxy for their metabolic rate (Steffensen, 1989; Clark, Sandblom and Jutfelt, 2013). The experimental setup consisted of 95 ml glass respirometry chambers placed in an

aerated and temperature-regulated rectangular water bath. Temperature was kept at 13°C to reflect the holding conditions. Oxygen content of the water in the respirometry chambers was recorded every two seconds using a 4-channel fibre-optic oxygen meter with associated oxygen sensors and software (FireStingO₂; PyroScience GmbH, Aachen, Germany). A total of 180 minnows were measured for their standard metabolic rate (SMR) as well as their maximum aerobic metabolic rate (MMR). Only the SMR data were used as the trait of interest in the present study, while the MMR data were used as part of a different project carried out by another student.

On experimental days, fish were haphazardly caught and removed from their holding tanks using dip nets. Once caught, the fish were transferred to the lab where they were placed one by one into a large circular bucket (40 cm diameter) with a water depth of 10 cm. The fish were then manually chased to exhaustion by the experimenter for 2 min after which they were immediately transferred to the respirometry chambers (Killen, 2014). The set up consisted of 16 chambers allowing for a maximum number of 16 fish to be measured per day (overnight). MMR measurements occurred during the first 8-10 min after being placed in the chambers following chasing. The protocol for MMR measurements assumes that the maximum oxygen uptake rate occurs during recovery from partly anaerobic exercise, in this case being chased, which there generally is good support for (Norin and Clark, 2016; Killen, Norin and Halsey, 2016). All chambers were connected to an automated flush pump controlled by a timer. The pump was left off for a period of 8 min during which the oxygen uptake rate was measured. Each 8min period was followed by a 5 min flush period where the automated pump would turn on and allow fully aerated water to enter the chamber. To allow for water mixing within the chambers during the closed period, each chamber was also connected to a peristaltic pump (Killen, 2014; Killen, Nati and Suski, 2015). To quantify the SMR, the fish were left in the respirometry chambers overnight for approximately 20 h. An opaque plastic cover was placed over the respirometry setup to shield the fish from direct light and disturbance.

The following morning, the fish were removed from the respirometry chambers and lightly anaesthetised using benzocaine (0.1 gL⁻¹) before being tagged for individual

identification using Visible Implant Elastomer (VIE) tags (Northwest Marine Technology Inc., Shaw Island, USA). Each fish was tagged with four tags using combinations of four colours (red, green, yellow, and orange). A tagging scheme was generated using SalaMarker code generator (MacNeil, Dharmarajan and Williams, 2011). After being tagged the fish were also measured for standard length (mm), total length (mm), as well as mass (g) before being placed back into their holding tanks.

Once the respirometry measurements and the VIE tagging had been completed, Lab Chart 7 (LabChart v. 7.3.7; ADInstruments, Dunedin, New Zealand) was used to carry out the analysis of the respirometry data. MMR and SMR were calculated as rates of oxygen uptake ($\text{mg O}_2 \text{ h}^{-1}$) by first multiplying the linear slopes for the decline in oxygen concentration over time as the fish were respiring ($\text{mg O}_2 \text{ L}^{-1} \text{ s}^{-1}$) with the volume of the respirometry chamber (L) minus the volume of the fish (assuming a density of 1 g mL^{-1}) and then by 3600 s h^{-1} .

Upon completion of the metabolic rate analyses, SMR was chosen as the basis for the behavioural experiments as the behavioural trials. This decision was made as a higher SMR is an indicator for higher resource requirements which in turn has been linked to a lot of different behaviours that could influence competitive ability (e.g. aggression and boldness) (Biro and Stamps, 2010; Killen et al., 2017). To normalise the data, both metabolic rate and body mass data were \log_{10} transformed. To account for variation in body mass, an adjusted SMR value was calculated by adding the residuals from the predicted relationship between metabolic rate and body mass to the metabolic rate predicted for a fish with the mean body mass (Auer et al., 2015b; Cutts, Metcalfe and Taylor, 2002). Based on the adjusted SMR, 40 fish with the highest SMR as well as 40 fish with the lowest SMR (80 individuals in total) were identified. All fish were then relocated and split between four of the holding tanks ($100 \times 40 \times 30 \text{ cm}$), with each tank containing 10 fish from both the high and low SMR group along with 20 other fish with intermediate SMR.

2.3 Assortment Trials

For the assortment trials a test tank ($60 \times 30 \times 26 \text{ cm}$) was divided into three sections using plexi-glass plates: a middle compartment ($34 \times 30 \times 26 \text{ cm}$) flanked by two smaller end compartments ($13 \times 30 \times 26 \text{ cm}$) (Figure 1) (Ward, Hart and

Krause, 2004). To allow for olfactory cue exchange between the sections, holes were drilled in the divider plates. The water level in the tank was kept at 12 cm to prevent any of the fish from attempting to escape the tank and to reduce the three-dimensional space the fish could potentially occupy. Between each trial, a small water change was made by draining the water level by 2 cm (approximately 17% of total water volume) to maintain the water temperature as well as add fully aerated water. The added water was circulated through a chiller unit and aerated with an air stone. The water temperature was kept at 13-14°C to mimic the conditions of the holding tanks. Sheets of Styrofoam were attached to the outside of the experimental tank to maintain the temperature as well as reduce glare from the glass. To minimise light and movement disturbances the entire experimental area was shielded using thick curtains.

For each trial, four fish with either high or low SMR from the same holding tank were placed in either end compartment (i.e. four high-metabolic-rate fish at one end and four low-metabolic-rate fish at the other). Fish in both end compartments were size matched within 3 mm. For some trials ($n = 6$), it was not possible to obtain four size matched fish per side and so only three fish were placed in each of the end compartments to act as stimulus. Which end compartment contained the high vs. low SMR fish was randomly determined to prevent observer bias. Fish were then left to settle for 10 min. One fish of either high or low SMR was then placed within a circular glass cylinder (12 cm in diameter x 30 cm tall) in the centre of the middle compartment. After allowing the focal fish to settle for 5 min, video recording using a Logitech HD web cam and iSpy recording software was initiated before the cylinder was raised, releasing the focal fish from its confinement. Video recording continued for 30 min for each trial. After completing video trials for all 80 individual fish the whole procedure was repeated a second time to assess repeatability of the results.

2.3.1 Video analysis

Once both runs of the video trials had been completed, EthoVisionXT 10 (Noldus, Spink and Tegelenbosch, 2001) was used to carry out the video analyses. Using the EthoVisionXT 10 software, zones were drawn in the middle compartment adjacent to the stimulus fish at either end (Figure 1). The width of each zone was 6.6 cm. This number was based on the mean total length of all the fish in the trial (6.63

cm). For each trial video, the focal fish in the middle compartment was tracked and the cumulative time (s) spent within each of the zones was recorded. From this, the amount of time spent with low or high SMR stimulus fish, respectively, could be calculated and compared between individuals. The mean velocity of the focal fish was also calculated. During the video trials the EthoVisionXT 10 software tracked the focal fish velocity within the arena (Figure 1) throughout the trial. To get an accurate mean velocity of when the fish was swimming, a movement threshold of 0.5 cm s^{-1} was set to filter out any time the fish was resting on the bottom. If any videos were shorter than 30 min the velocity values were recalculated based on a 30 minute trial to make the means comparable.

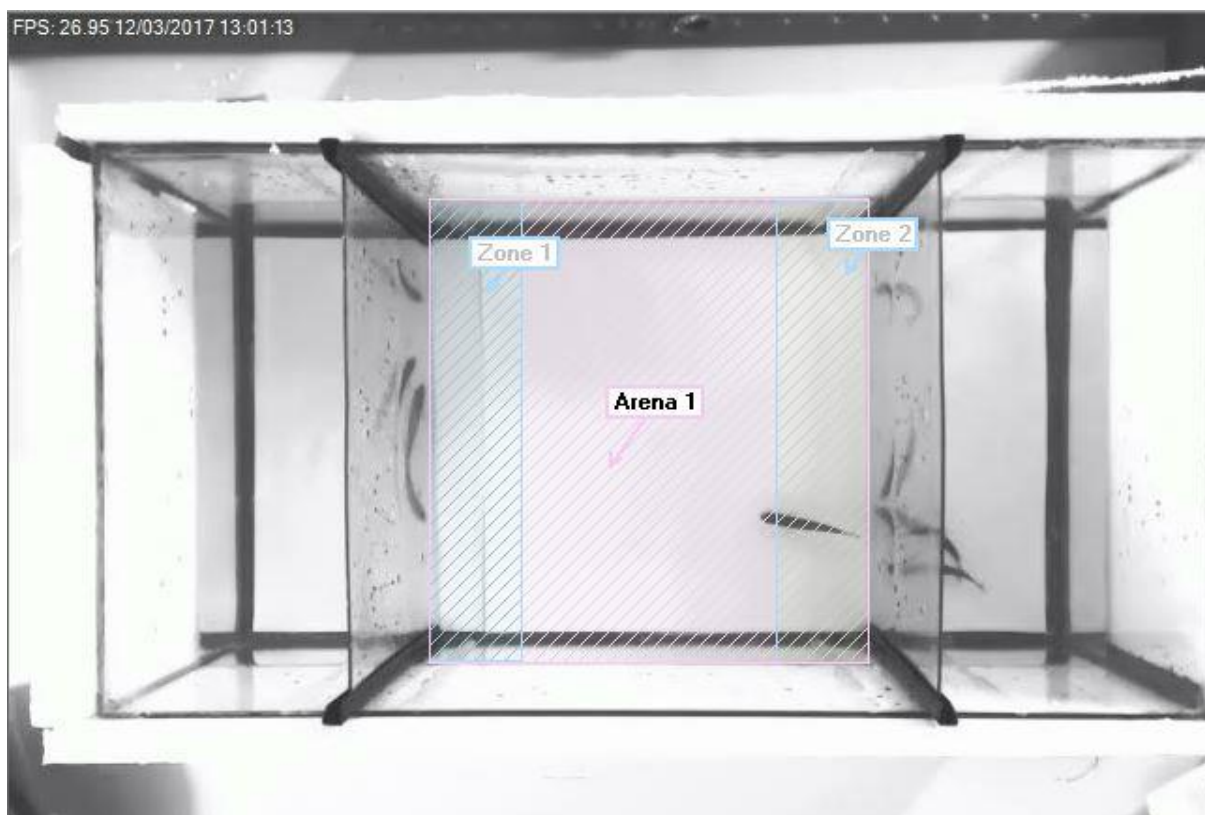


Figure 1: Image of the assortment trial tank with three compartments as well as zone division of the middle arena.

2.3.2 Statistical analysis - Assortment trial

For the statistical analysis, RStudio statistical software was used (RStudioTeam, 2016). A linear mixed effects model was used to model the duration in log time (s) spent in either zone, with log time being the response and stimulus group SMR

(high or low) being the explanatory variable. Individual fish ID was included in the model as a random factor to account for the two rounds of trials conducted. The SMR and mass of the focal fish was initially included in the model as explanatory variables but were later removed from the final model as they were not significant. A linear mixed effects model was also used to investigate focal fish velocity. In this model the response was the log adjusted velocity with log SMR and log mass of the focal fish as explanatory variables. Residual-fit plots were used to verify the normality, linearity, and homoscedasticity of the models.

2.4 Open Field Trials

In the open field experiment a large circular basin (110 cm diameter x 30 cm height) was used as an arena (Figure 2). Above the basin, a Canon EOS 6D camera was positioned to make sure the entire arena was within view. The bottom of the basin was covered in white plastic to make the fish stand out more to the camera and make the analysis easier, as well as make the environment as uniform as possible. An LED light strip was attached to the sides of the basin around the circumference as a light source. An opaque white cylinder was placed in the middle of the arena to promote exploratory swimming. The water in the basin was kept at 15°C as this was the temperature of the aquarium holding tanks at the time of this experiment. The water level was kept at 10 cm to prevent any fish from jumping out of the basin during the trials. The basin along with the camera was covered by black plastic to prevent any light penetration as well limit disturbance. Three different group compositions of fish were tested during the experiment: homogenous low SMR, homogenous high SMR as well as heterogeneous mixed SMR. The heterogeneous mixed groups consisted of three fish from each SMR category (high, intermediate, and low). Due to multiple data sets being collected during this experiment, fish with intermediate SMR were included in the heterogeneous groups. During each trial, nine fish within the desired SMR bracket were randomly selected from one of the four holding tanks. Before being placed in the basin, each fish was fitted with a brightly coloured felt tag approximately 4 x 4 mm in size (Figure 2, Figure 3). The tags were attached using a small harness made out of fishing line which was attached just in front of the dorsal fin. The felt and fishing line used for the tags was light weight to avoid impairing the fish's balance, limit any hindrance when swimming as well as not to cause any harm. These extra tags

had to be used as identification due to it being impossible to distinguish the VIE tags from the video recordings. Upon being placed in the basin the fish were left to acclimate for 30 min time before commencing the video trial. Each recording lasted for 30 min.

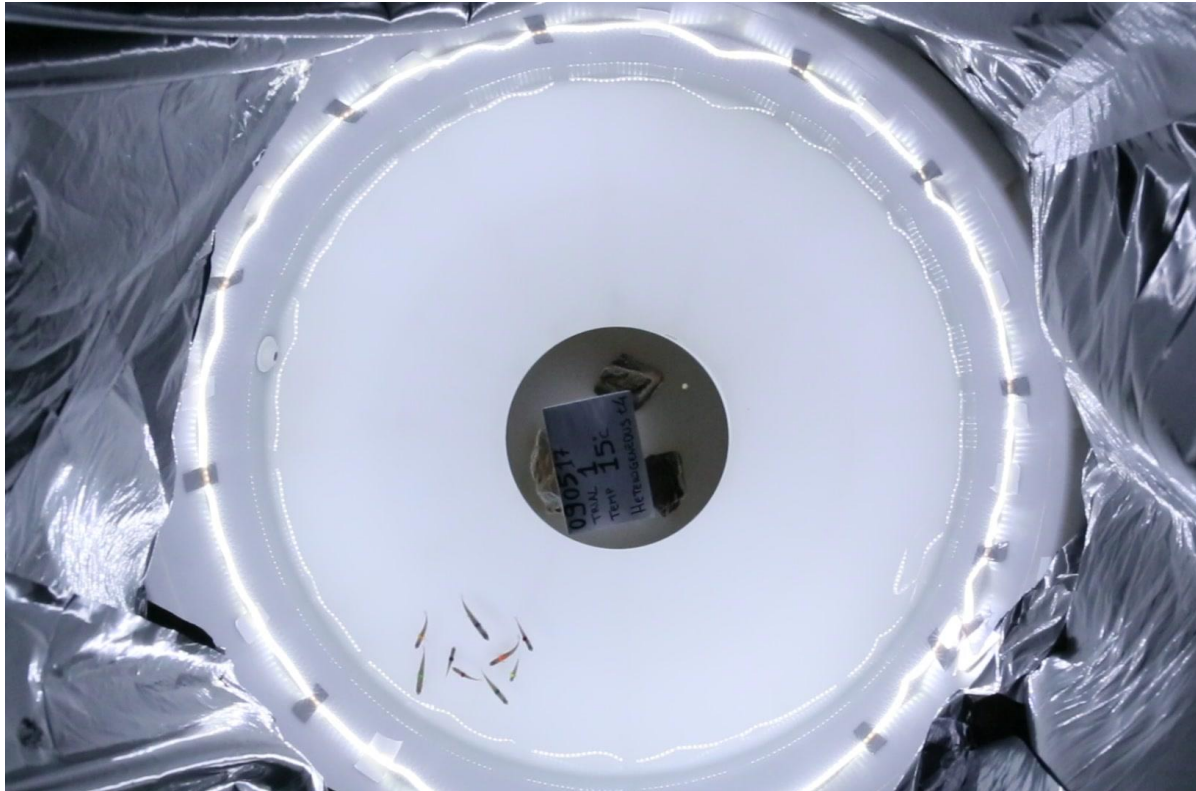


Figure 2: Camera view of the open field set up. All nine fish are clearly visible and identifiable because of the coloured tags.

2.4.1 Photo analysis

From each 30 min video trial, still photos were sampled every 2 min resulting in 16 photos per video. Using ImageJ software (Rasband, 2016) the distance between all fish was measured in each photo. Fish were determined to be associated (linked) with each other if the distance between them was within one average body length (6.63 cm) (Figure 3). Measurements were made from the fish's centre of mass (roughly the location of the tags) to standardise procedure as well as to account for differences in fish size and length. This was determined to be the best method for this experiment rather than the minimum distance as measurements done e.g. snout to snout versus tail to tail would not be comparable in terms of association. If two fish were within one body length of each other they were given a score of 1. If fish were further apart they were given a score of 0. This is in accordance with

social network theory which uses as binary system of nodes (representing the individuals) and edges (associations or links between individuals) (Croft, James and Krause, 2008; Liu et al., 2017). Fish were assigned scores in all 16 photos making the maximum association score for each video 16.

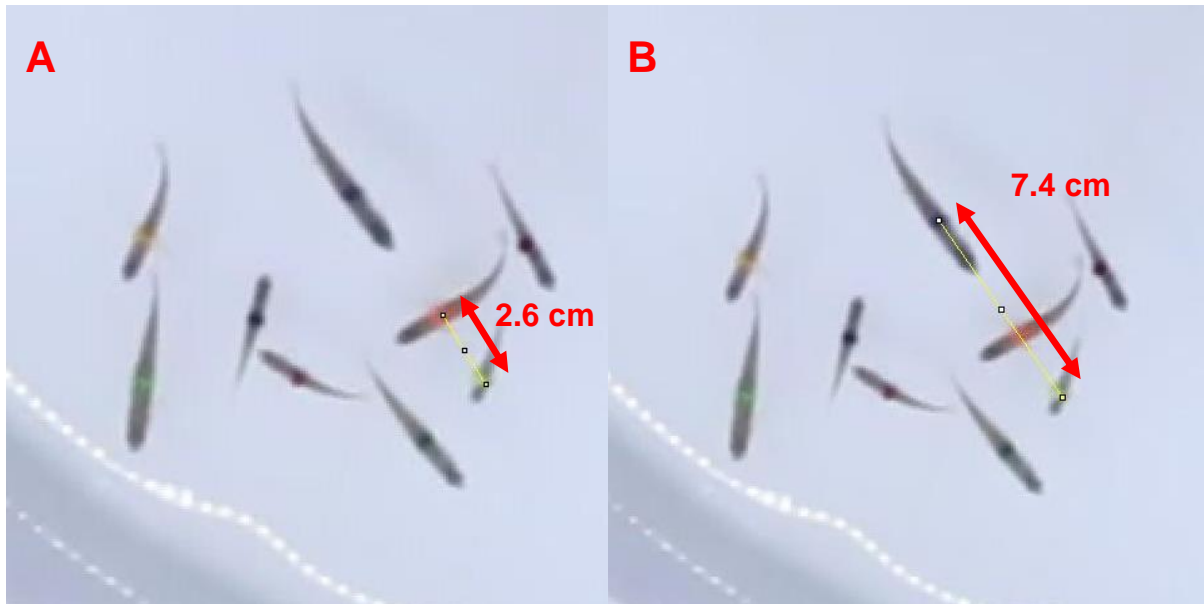


Figure 3: Example of photo analysis. Distance from the yellow fish to the orange fish is 2.6 cm (A) while distance from the yellow fish to the purple fish is 7.4 cm (B). Since 7.4 cm is longer than one average body length (6.63 cm), the yellow fish was determined to be associated with the orange fish (a score of 1) but not with the purple fish (a score of 0).

2.4.2 Statistical analysis - Open field trial

For these analyses, a linear mixed effects model (RStudioTeam, 2016) was used to model the number of links per individual (response variable) against group composition (homogenous high SMR, homogenous low SMR, or heterogeneous) and individual SMR (explanatory variables). An interaction between group composition and individual SMR was also included along with fish ID as a random effect. Model assumptions were verified using the residual-fit plots.

The heterogeneous groups were tested further to investigate whether there were different numbers of links between the fish from different SMR groups. To do this, one sample t-tests were carried out comparing the observed mean number of links to the expected mean number of links. The expected number of links were calculated using the total number of links divided between the other fish in the

school based on the assumption that each fish interacted equally with all school mates.

3 Results

180 minnows had their SMR measured using respirometry. Fish mass and SMR were log₁₀ transformed and when plotted against each other displayed a positively correlated relationship (Figure 4) i.e. fishes with higher mass have a higher SMR.

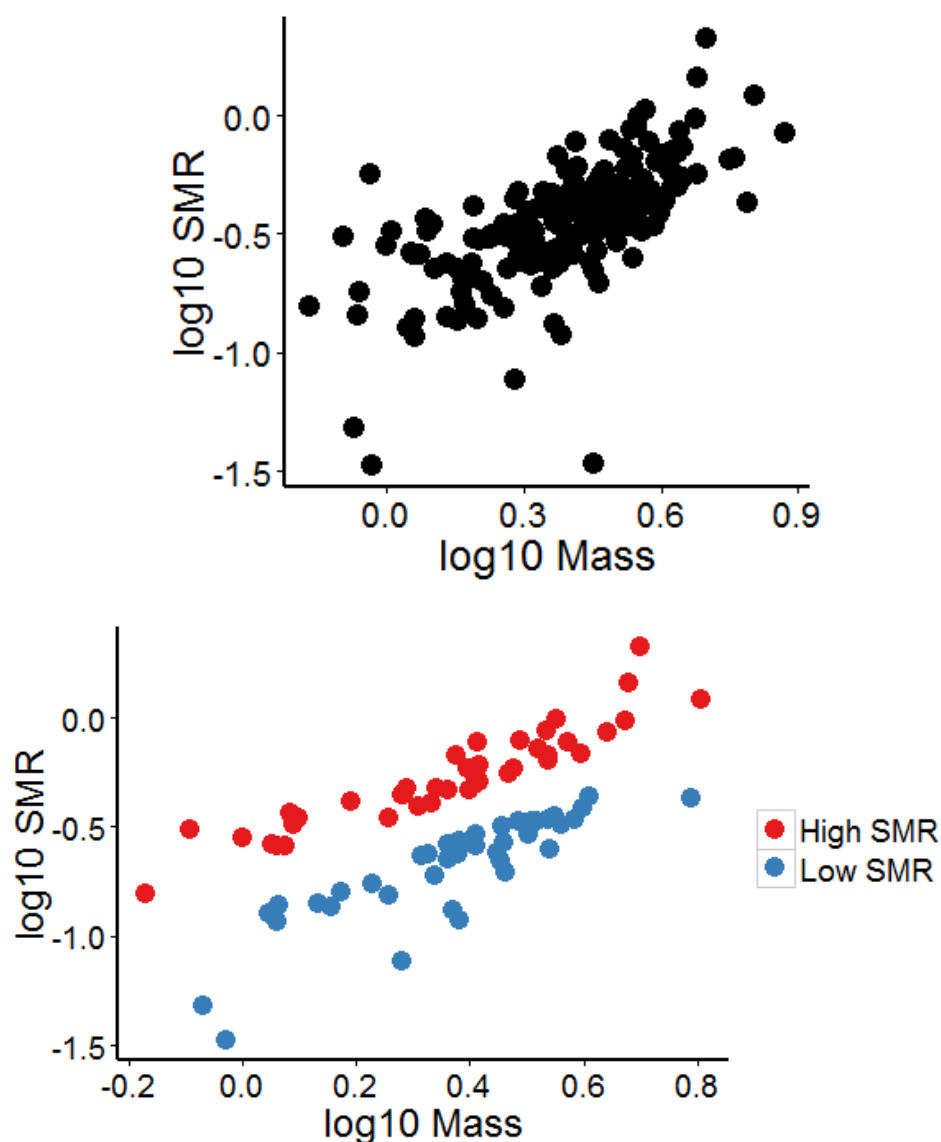


Figure 4: Two graphs displaying the relationship between the logged normalised mass and logged normalised SMR. The top graph includes all 180 individuals measured using respirometry. The bottom graph shows only the 80 individuals (40 low SMR and 40 high SMR) used for the behavioural trials.

3.1 Preference

For the assortment trials, a total of 160 videos were collected, two per individual fish. Fish showed a preference to associate with the high SMR stimulus school, independent of focal fish SMR ($p_{\text{Stimulus school (Low SMR)}} = 0.0144$) (Table 1, Figure 5). On average, 757 seconds were spent with the high SMR fish in comparison to 669 seconds spent with the low SMR fish. Figure 8 shows examples of four heat maps generated from the EthoVision software during analysis. The heat maps indicate where in the experimental arena the fish spent most of their time during the trials.

Table 1: Model (GLMM) summary for the effect of stimulus school on duration (s) spent in either zone.

Term	Estimate	Standard error	Degrees of Freedom	t-value	p-value
Intercept	2.84244	0.01957	318	145.25	<2e-16
Stimulus school (Low SMR)	-0.06808	0.02767	318	-2.46	0.0144

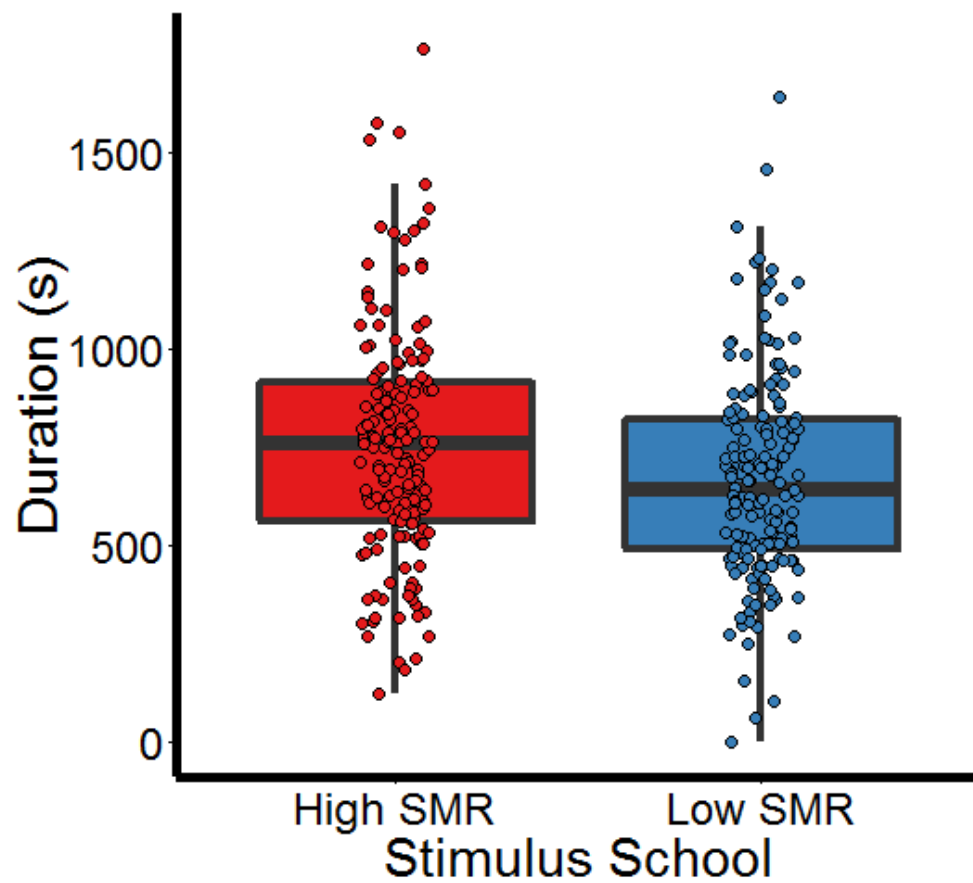


Figure 5: Graph displaying the duration (s) spent with stimulus fish of either high or low SMR. Boxes and lines shows the quartiles. Boxes represents 50% of the data points. The horizontal lines represents the means. This graph includes all fish, regardless of individual focal fish SMR.

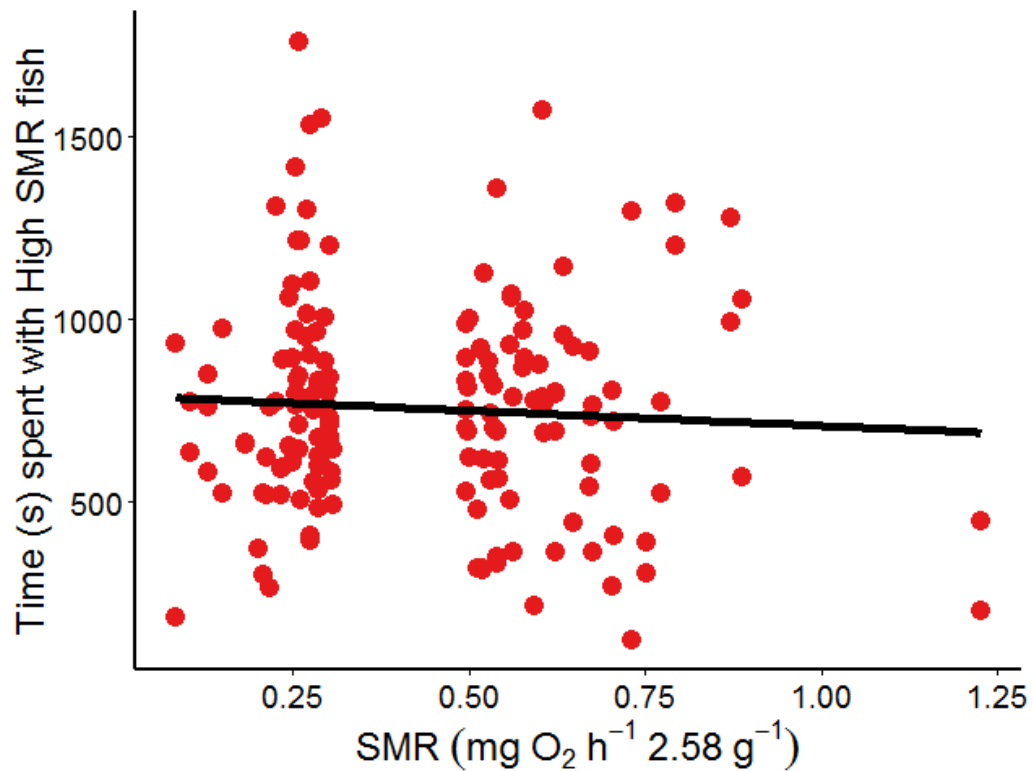


Figure 6: Scatterplot displaying the time (s) spent with stimulus fish with high SMR against individual focal fish SMR. SMR has been adjusted for body size.

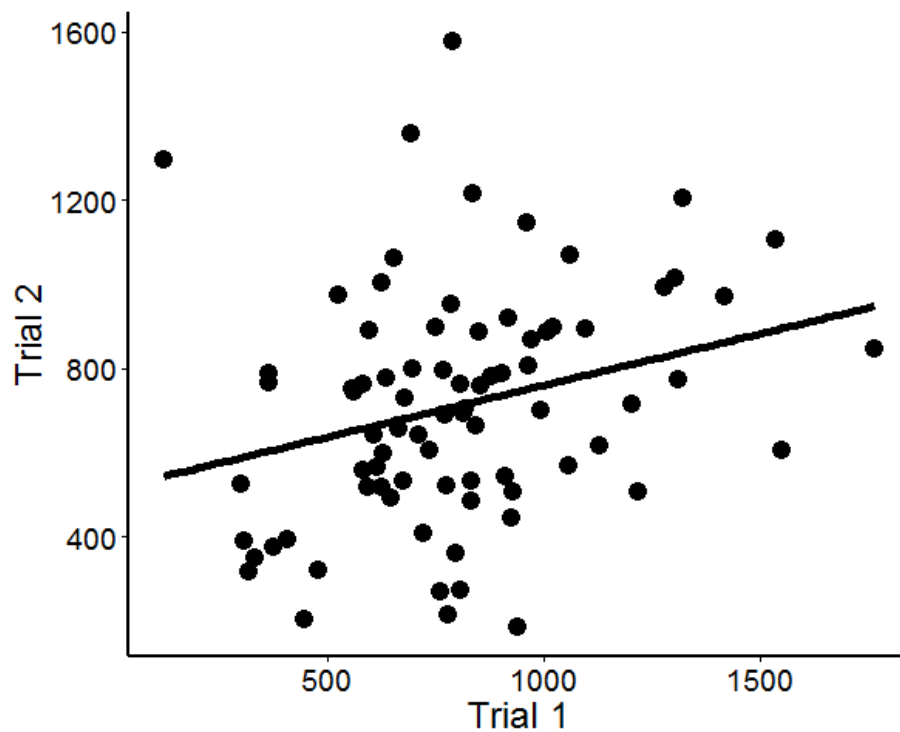


Figure 7: Scatterplot displaying the duration (s) spent with high SMR fish in Trial 1 against duration (s) in Trial 2. The regression line represents the level of repeatability between the trials.

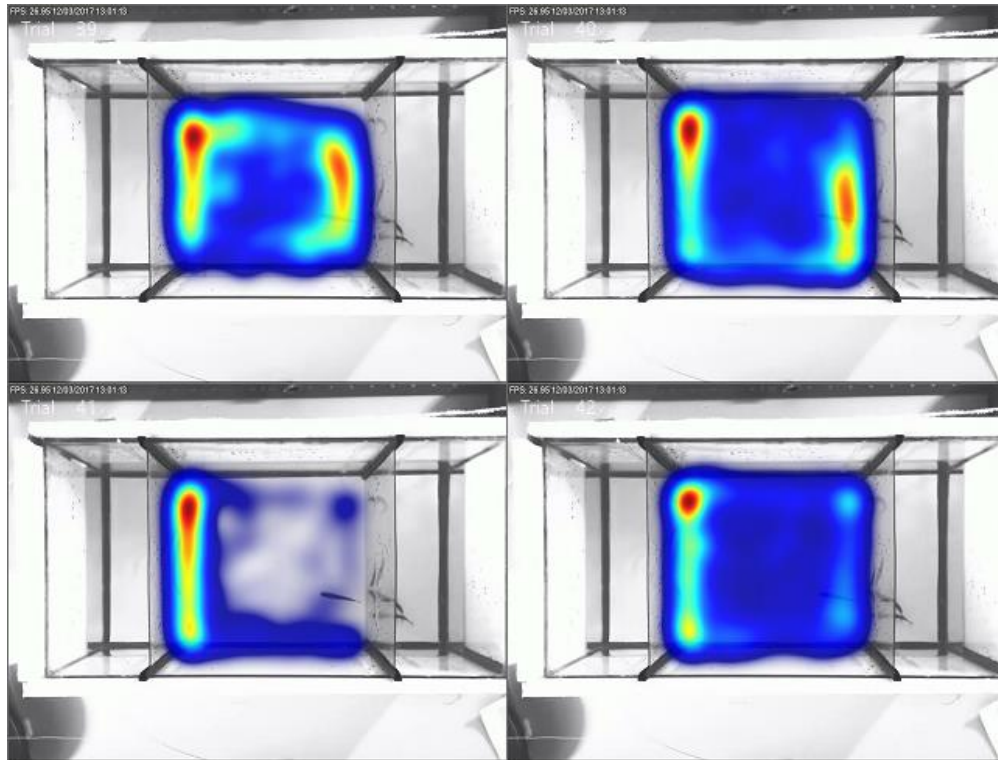


Figure 8: Example heat-maps visualising where four fish spent most of their time during the assortment trials. For these trials fish with high SMR were placed on the left side. Warmer colours indicate more time spent in that area. Blue or transparent means the fish spent very little or no time at all in these areas. While the top two individuals appears to spend time with both stimulus shoals, the fish on the bottom have more of a preference for the fish with high SMR.

3.2 Velocity

The velocity of the focal fish during the trials was also investigated. The average velocity of the focal fish was positively correlated with individual SMR. Focal fish SMR was found to have a statistically significant influence on focal fish velocity ($p_{\text{Log10 SMR}} = 0.0343$) (Table 2) as fish with higher SMR would on average swim faster during the trials. The relationship is displayed in the scatterplot in Figure 9.

Table 2: Model (GLMM) summary for the effect of log10 SMR and log10 Mass on focal fish average velocity (cm s^{-1}).

Term	Estimate	Standard error	Degrees of Freedom	t-value	p-value
Intercept	0.58690	0.04107	77	14.290	<2e-16
Log10 SMR	0.09289	0.04312	77	2.154	0.0343
Log10 Mass	0.07703	0.06359	77	1.211	0.2295

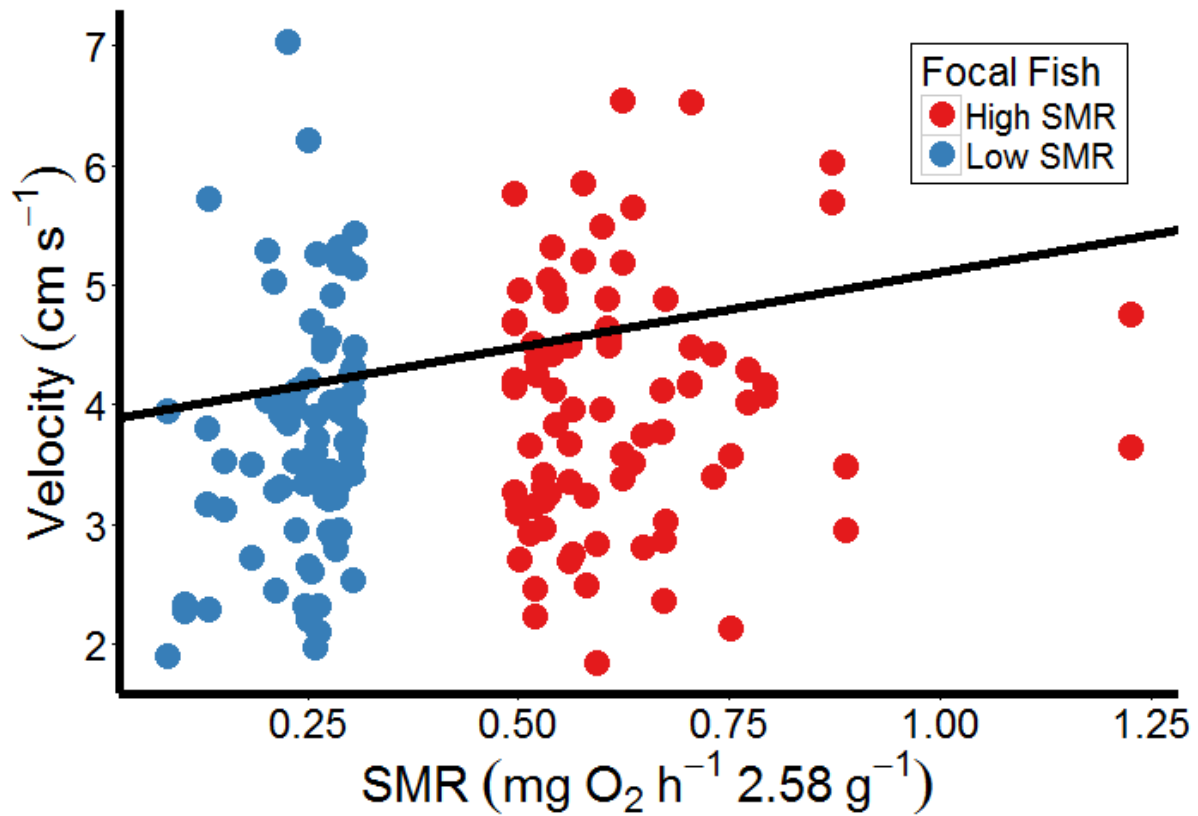


Figure 9: Graph displaying the average velocity (cm s^{-1}) of the focal fish, when moving, against the individual SMR adjusted for body size.

3.3 Open Field - Between Groups

A total of 13 videos were analysed for the open field experiment: four groups of homogenous high SMR, four groups of homogenous low SMR, and 5 groups of heterogeneous composition. However, only three of the videos of the homogenous high SMR groups were useful for data analysis. This was due to the lighting being insufficient to be able to identify individual fish in one of the trials. Neither log10 SMR ($p_{\text{Log10 SMR}} = 0.8747$), log10 Mass ($p_{\text{Log10 Mass}} = 0.3624$), or group composition ($p_{\text{Group: Homogenous High}} = 0.1424$, $p_{\text{Group: Homogenous Low}} = 0.3209$) were found to be significant in terms of the number of links. There was also no significant interaction found between log10 SMR and group composition ($p_{\text{Log10 SMR*Group: Homogenous High}} = 0.0773$, $p_{\text{Log10 SMR*Group: Homogenous Low}} = 0.1809$) (Table 3, Figure 10).

Table 3: Model (GLMM) summary for the effect of log10 SMR, log10 Mass, group composition and an interaction between group composition and log10 SMR on the total number of links per individual fish.

Term	Estimate	Standard error	Degrees of Freedom	t-value	p-value
Intercept	72.847	9.087	87.140	8.017	4.53e-12
Log10 Mass	-12.234	13.362	87.750	-0.916	0.3624
Log10 SMR	1.567	9.909	91.620	0.158	0.8747
Group: Homogeneous High	8.149	5.481	49.560	1.487	0.1434
Group: Homogenous Low	8.994	8.998	71.160	1.000	0.3209
Log10 SMR * Group: Homogenous High	26.937	14.981	59.210	1.798	0.0773
Log10 SMR * Group: Homogenous Low	16.245	11.976	50.920	1.356	0.1809

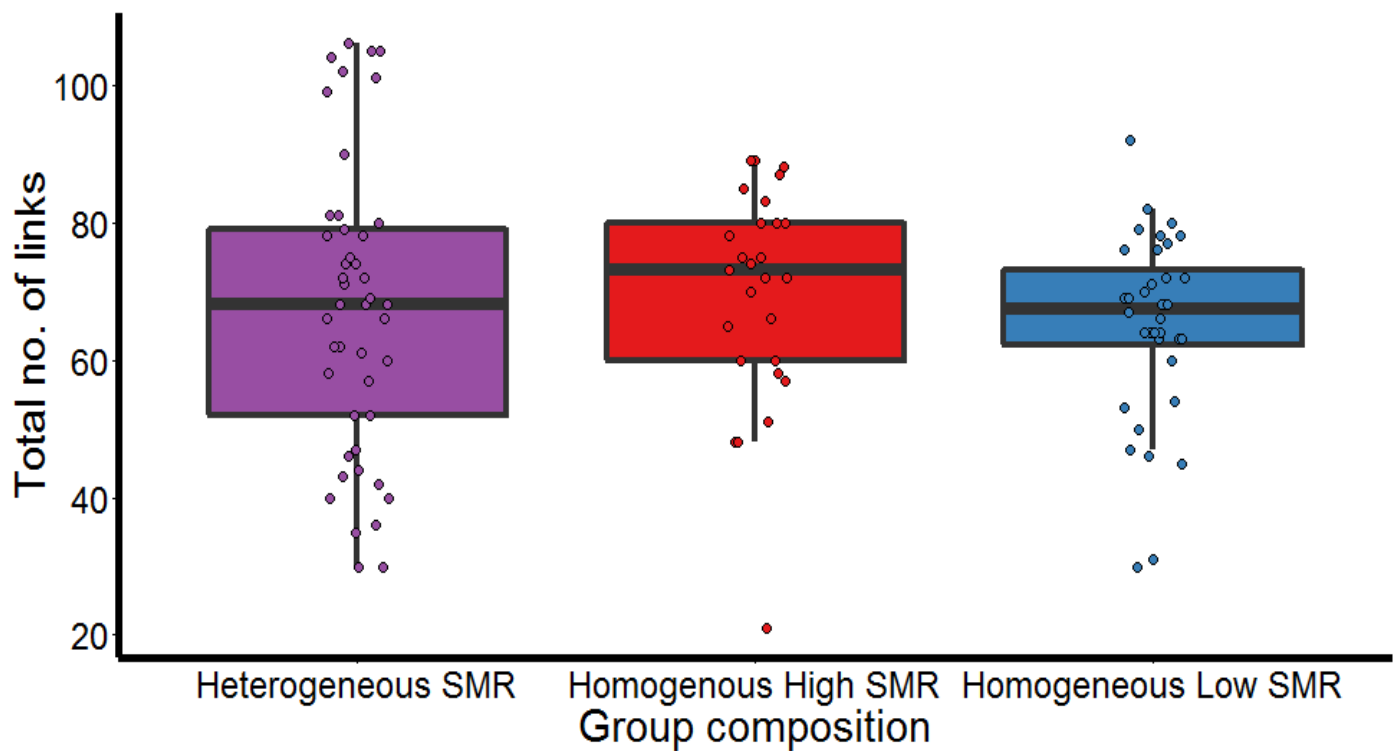


Figure 10: Graph displaying the total number of number of links per individual fish per trial against the SMR based group composition.

3.4 Open Field - Within Groups

Further analysis was carried out using the five heterogeneous mixed groups. One sample t-tests were used to evaluate if the number of links measured between individuals of different SMR phenotype differed from the expected number of links if all fish associated equally. The t-tests showed no significant difference between the true mean and the expected mean ($p_{\text{Links to High SMR fish}} = 0.2004$, $p_{\text{Links to Intermediate SMR fish}} = 0.1287$, $p_{\text{Links to Low SMR fish}} = 0.363$) (Table 4).

Table 5 shows a summary of the linear mixed model used to investigate the influence of \log_{10} SMR and \log_{10} Mass on the total number of links in the heterogeneous trials. Neither was found to be significant ($p_{\text{Log10 Mass}} = 0.294$, $p_{\text{Log10 SMR}} = 0.826$). The graph in Figure 11 shows the total number of links against SMR phenotype. Netdraw software (Borgatti, 2002) was used to visualise the social networks from the trials (Figure 12).

Table 4: One sample t-tests performed to investigate if the true mean of the number of links to the fish of the different SMR phenotypes in the heterogeneous mixed groups differed from the expected means.

Term	Estimate	Standard error	t-value	p-value
Intercept	75.529	11.726	6.441	9.21e-08
Log10 Mass	-19.109	18.001	-1.062	0.294
Log10 SMR	2.723	12.340	0.221	0.826

Table 5: Model (GLM) summary for the effect of log10 SMR and log10 Mass on the total number of links per individual fish in the heterogeneous mixed open field trials.

Term	Degrees of Freedom	True mean	Expected mean	t-value	p-value
Links to High SMR fish	44	23.57	22.43	0.8483	0.2004
Links to Intermediate SMR fish	44	23.93	22.38	1.1474	0.1287
Links to Low SMR fish	44	23	22.5	0.3526	0.363

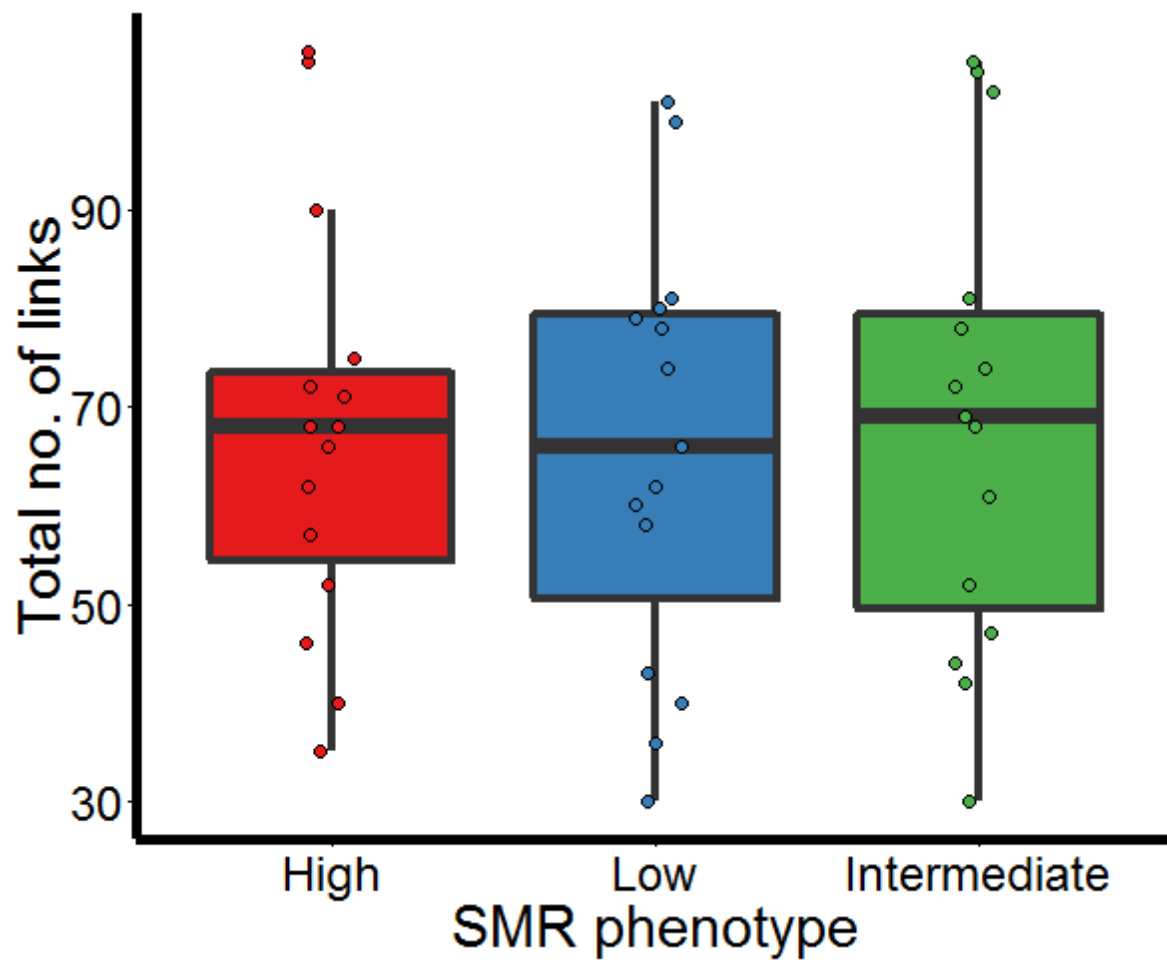


Figure 11: Graph displaying the total number of links per individual fish per trial against the SMR phenotype for the five heterogeneous mixed groups during the open field trial.

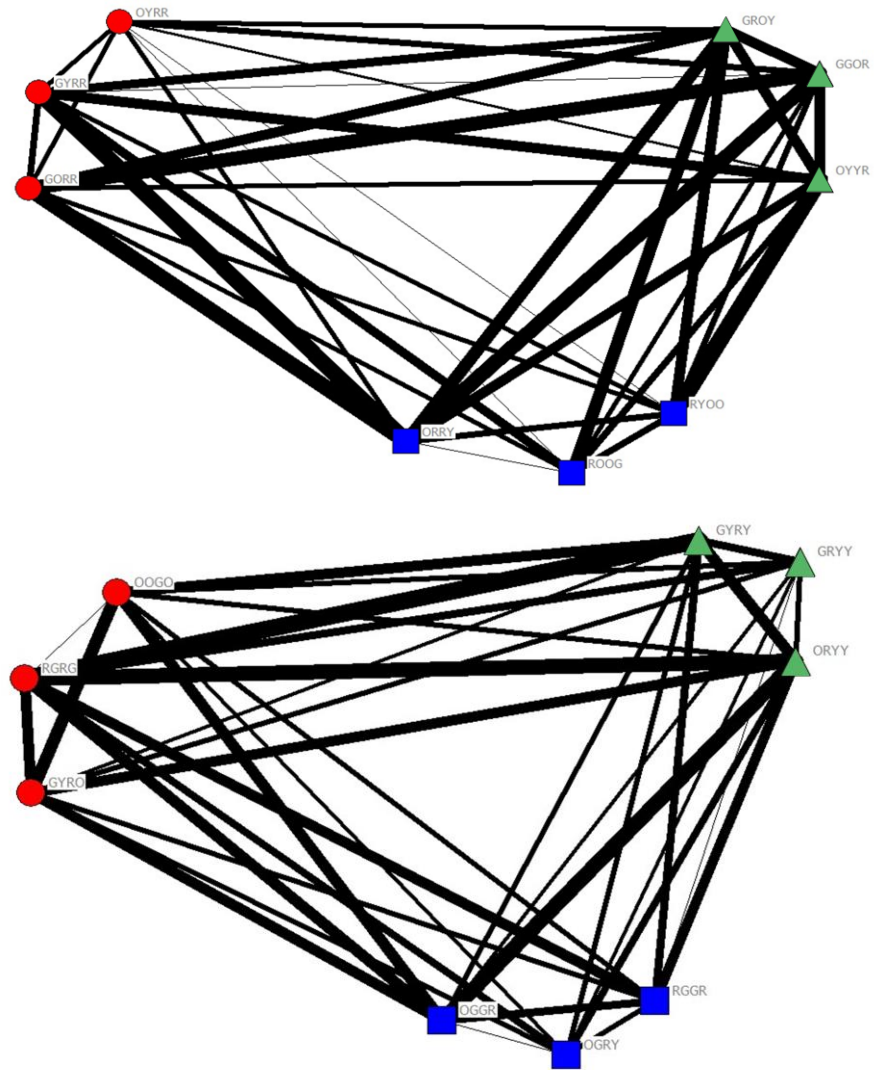


Figure 12: Examples of social networks created from two of the open field trials using the heterogeneous mixed groups. A thicker line indicates a higher number of links between individuals. Fish with high SMR are displayed in red, fish with low SMR in blue, and fish with intermediate SMR in green. The letter combination next to the symbols shows the VIE identification code for each fish (e.g. OYRR = Orange Yellow Red Red).

4 Discussion

4.1 Preference

My initial hypothesis was that all fish, regardless of individual SMR, would prefer to associate with fish of low SMR. I theorised that focal fish of low SMR would prefer to associate with fish of similar metabolic phenotype as this would reduce competition. Likewise I thought that focal fish with high SMR would also prefer the low SMR stimulus fish as they would potentially be easier to outcompete. This hypothesis was in line with results from the study by Metcalfe and Thomson (1995) which suggests that fish prefer to school with poorer competitors. However these are not the results I observed.

Instead, I found that all focal fish showed a preference to associate with the stimulus fish with high SMR - the opposite of my hypothesis. Fish on average spent 88 seconds longer with fish of high SMR during the trials (Figure 5). Additionally, the spread of the data is higher for the high SMR stimulus fish (Figure 5). My results are consistent with the findings of Harcourt et al. (2009) who conducted a very similar study using stickleback. The focus of their study was to let individual fish choose between stimulus schools of bold or shy fish. Like in my study, all fish showed a preference to associate with bolder fish independently of their own behavioural phenotype (bold or shy).

As previously stated, having a higher SMR has been correlated with being more bold. One of the reasons behind this preference could be that fish with higher SMR (which have a higher energetic demand) need to be better at foraging to sustain themselves (Auer et al., 2015a). Being bolder while foraging also leads to higher foraging success as well as being able to take better advantage of a situation where food is abundant (Dyer et al., 2009; Auer et al., 2015a). With higher amounts of food available, it would allow fish with lower SMR to feed, even if they are outcompeted for the majority of the food items. While foraging, bolder fish with higher SMR are also more likely to occupy positions towards the front of the school (Ward et al., 2004). These positions would give them access to the food first, but also makes them more likely to get exposed to predators. Shyer fish with lower SMR could then stay further behind while feeding which would increase their chance of survival during a predation even.

My findings could also relate to social preference. Fish are able to distinguish between unfamiliar and familiar individuals (Griffiths, 1997; Killen et al., 2014) and could therefore use familiarity as a basis for how to interact with certain individuals. Griffiths and Magurran (1997) found that wild female guppies preferred to associate with familiar individuals over unfamiliar ones.

After being separated into SMR phenotype group, all the fish that were used together in the same trials during my experiments were housed together in the same tanks to try account for varying familiarity. Magurran et al. (1994) showed that guppies that were housed together for two months under lab conditions were able to tell apart familiar fish from unfamiliar individuals. While the fish used in the trials were kept together for approximately the same time period between the respirometry measurements and the start of the assortment trials, there is no way to account for previous familiarity to tank mates from the point of them entering the aquarium facilities in June 2016 until January 2017.

4.2 Velocity

The activity of the stimulus schools during the experimental trials could also have affected the preference of the focal fish. Higher activity may be associated with a higher SMR as it would allow more energy for movement (Killen et al., 2012a). Due to the experimental setup it was not possible to quantify the activity of the stimulus schools, however when looking into the velocity of the focal fish during the trials I found that fish with higher SMR did have a higher average velocity compared to fish with lower SMR (Table 2, Figure 9). Activity could also indicate increased swimming performance (Marras et al., 2013). Individuals will need the capability to keep up with the rest of the school while foraging but also when avoiding predators. This could be one reason behind the preference to associate with high SMR conspecifics. Focal fish with high SMR would join a group of fish of similar activity levels, while fish with lower SMR could position themselves behind faster individuals and take advantage of the hydrodynamic effects created by the individuals in front (Liao et al., 2003; Stewert et al., 2016). Joining a group of faster individuals could also lead to a training effect whereby slower fish eventually become more “fit” because they are driven by their incentive to remain with the group (Sinclair et al., 2014).

4.3 Open field - Between groups

The open field trials allowed for both active and passive assortment to potentially occur. Fish could choose to interact with certain individuals and they could also end up passively assorting according to phenotype. Based on the results from the assortment trials, I predicted similar trends for the association preferences of fish with higher SMR in the open field trials. However, I found no support for this (Table 3). As individuals with higher SMR are also assumed to be bolder and more prone to explore new environments (Dall, 2004), I hypothesised that the group consisting of homogenous high SMR fish would be more loosely associated which would result in fewer links between fish. Respectively, fish in the homogenous low SMR groups were expected to be shyer and therefore stick closer together and have a higher number of links. None of these hypotheses turned out to be correct (Figure 10). While the homogenous high SMR group has a slightly larger spread in the number of links, the means of the homogenous high and low SMR groups are very similar. There was also no significant difference in the total number of links looking at the heterogeneous mixed group, despite heterogeneous group having a much higher variability in the number of links compared to the homogenous groups (Figure 10). This might be a result of the slightly uneven number of trials for each group. One video of the homogenous high SMR groups was not included in the data set as the lighting failed during the recording, making it impossible to distinguish between the individual fish. A total of 5 videos were also collected for the heterogeneous group compared to four for the homogenous groups. This was due to there being enough fish in one tank to carry out a second trial without having any individual fish overlap between trials.

As with the preference trial, these results might also have been influenced by social preference. Again groups were composed of fish from the same stock tank, however they will have been tested with other individuals compared to the assortment trial, especially fish in the heterogeneous group since the intermediate fish were not included in this trial.

4.4 Open field - Within groups

Similarly to the number of links between groups, I also expected to see a difference in the number of links between individuals with different SMR phenotype within the heterogeneous groups, however as can be seen in Table 4

and Table 5, no statistically significant difference was found. Figure 11 and 12 also support the lack for statistical difference. The spread of data points in Figure 11 are really similar across the different phenotypes and the means are also very similar. In the social networks in Figure 12 there is a slight difference in line thickness indicating a higher number of links between certain individuals, however the difference is not big enough to be statistically significant. These results are based on a quite low number of trials ($n = 5$) which could explain why no difference in the number of links was observed. Future work could include increasing the sample size (number of trials) as well as looking into if the number of links changes with group size by increasing the number of fish in each trial as this could potentially reveal stronger preferences for certain individuals with specific phenotypes.

As we found no significant preference for any metabolic phenotype during the open field trials this could indicate that metabolic rate might not be a very strong driver for within group assortment. How often two individuals associate can have implications on school cohesion and influence how information is transmitted between individuals in the group. How information is passed on can be especially important during predation events and can therefore influence natural selection (Beauchamp, 2014a). This highlights the importance of continued research within the area to attempt to disentangle which traits has the highest influence in school assortment.

4.5 Conclusions

This study found that when presented with a choice of associating with conspecifics of either high or low SMR, all fish (disregarding their own SMR) preferred to associate with conspecifics with high SMR. These results were the opposite of the initially proposed hypothesis which suggested that all fish would prefer to associate with fish of low SMR because these would be poorer competitors. The preference for associating with fish with high SMR can be attributed to the fact that fish with higher SMR usually are bolder and more prone to risk taking, which theoretically makes them more efficient foragers. Fish with higher SMR were also found to be more active during the trial which is in line with current literature. Higher activity levels can act as an indicator of higher SMR which could be one of the reasons explaining the preference to associate with high

SMR fish. Fish of lower SMR can also benefit from joining groups of fish with higher SMR as they can take advantage of hydrodynamic effects while swimming behind faster individuals. However, when tested in an open field arena no significant preference for a specific SMR phenotype, both between and within groups, was found. These conflicting results highlights the importance of study in this field to try to disentangle the underlying causes for group formation and preference as this can have knock on effects on school cohesion and information transfer.

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