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Consistency of fish-shoal social network structure under laboratory conditions

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We investigated the consistency of association network structure for groups of sticklebacks *Gasterosteus aculeatus*. Each group was observed twice and we varied the duration between observations and the size of the experimental arena that they were observed in. At the dyad level, we found positive correlations between dyad interaction frequencies across observations. At the group level we found variation in four network metrics between observations, but only in treatments where the duration between observations was short. Specifically, fish formed more and smaller groups in the second observation in this treatment. Fish were also organized into more subunits in the larger arenas. Finally, we saw positive correlations between some group network metrics across observations suggesting relative consistency at the group level. There are several processes that might drive these interaction patterns. Our findings have implications for experimental design and the comparison and integration of findings of experiments from different studies carried out under different conditions.

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Key words: assortment; group; shoaling; social behaviour; social information; social organization.

INTRODUCTION

Group living in one form or another is extensive among animals, shaping and shaped by a range of ecological and evolutionary processes (Krause & Ruxton, 2002; Ward & Webster, 2016). The nature and distribution of interactions between group living animals and the consequences of these, can be complex. Social network analysis encompasses statistical approaches designed to aid in quantifying such behaviour and has proved invaluable in recent years in enabling researchers to describe, model and predict the outcomes of interactions between group members (Wey *et al.,* 2008; Whitehead, 2008; Croft *et al.*, 2009*a*; Pinter-Wollman *et al.,* 2013; Krause et al., 2014).

Social network analyses have been used to investigate interactions ranging from courtship and mating patterns (McGregor, 2005) to the distribution of potentially cooperative interactions (Croft *et al.,* 2006) and the social consequences of personality variation (Pike *et al.,* 2008; Croft *et al.*, 2009*b*; Krause *et al.,* 2010; Aplin *et al.,* 2013; Wilson *et al.,* 2013). Such approaches have also been used to study diffusions, such as the transmission of parasites and diseases through populations (Cross *et al.,* 2004;

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Hamede *et al.,* 2009; Weber *et al.,* 2013), potentially allowing researchers to predict the types of interaction dynamics that might lead to rapid or sustained outbreaks. Similar approaches have been used to study the transmission and spread of information (Atton *et al.,* 2012, 2014; Webster *et al.,* 2013; Boogert *et al.,* 2014; Farine *et al.,* 2015; Wilson *et al.,* 2015; Firth *et al.,* 2016), enabling the identification of directed social learning and providing insight into the development of local traditions and cultures (Allen *et al.,* 2013; Aplin *et al.,* 2015).

The ultimate aim of many research projects utilizing social network analysis is to gain an understanding of the nature and distribution of the social interactions that take place under natural conditions and populations living in the wild and there have been many advances to this end (Lusseau, 2003; Croft *et al.*, 2004*a*; Wolf *et al.,* 2007; Farine *et al.,* 2012; Allen *et al.,* 2013; Aplin *et al.,* 2013, 2015). On the other hand, laboratory experiments can be valuable too, because they allow for well controlled manipulations to be performed and also because they allow for replication, something that is not always possible when studying wild populations in the field.

While a number of studies have investigated factors influencing social network structure under laboratory conditions, little is known about the extent to which measures of network structure are repeatable for groups of animals. Consistency can be considered at two levels. At the level of the dyad, we may ask who interacts with whom and whether individuals interact in similar ways over multiple observation periods. Repeated patterns of interaction may be expected between dyads with particular affiliative bonds, such as between members of mated pairs or parents and offspring, but may also be expected between phenotypically similar individuals, or individuals with similar travelling speeds or habitat preferences (Krause & Ruxton, 2002; Ward & Webster, 2016). At the level of the group it would be informative to determine whether particular conditions consistently give rise to groups with similar network characteristics. Such information can potentially guide researchers in designing experiments, applying the findings of studies to different groups and in making predictions about the responses of populations to changing environmental conditions.

To address these questions we quantified various social network metrics in shoals of threespine sticklebacks *Gasterosteus aculeatus* L. 1758. This species is a well-established model organism in behavioural ecology (von Hippel, 2010) and has previously been used to study social networks under laboratory conditions (Atton *et al.,* 2012, 2014; Webster *et al.,* 2013). Groups were observed on two occasions each and using a fully factorial experimental design we varied the size of the arena in which they were observed and the length of time between the two observations. We quantified both pairwise interactions and group level metrics. The latter included the total proportion of pairwise interactions observed and whether these were evenly distributed or skewed, leading to the formation of cliques. We also recorded the number of smaller units into which the shoals split and the size of the largest of these.

We predicted that within groups we would see positive correlations between the strengths of pairwise associations between the two observation trials. This is reasonable since active choice and passive factors such as swimming speed and fine-scale habitat preferences are known to play a significant role in shaping fish-shoal composition, such that shoals are frequently found to be sorted by a range of phenotypic factors, rather than randomly structured (Krause *et al.,* 2000; Hoare *et al.,* 2000*a*, *b*). We also predicted that arena size would have the greatest effect upon group level metrics, given that the larger arena permitted groups to disperse further and reduced the likelihood

of smaller units reencountering one another, leading to more subunits forming and persisting, with fewer overall interactions and the occurrence of cliquey interactions.

METHODS

SUBJECTS

Gasterosteus aculeatus were collected from the Kinnessburn stream in St Andrews, U.K. (56° 20′ 5·70″ N; 2° 47′ 14·95″ W) during October 2014 (main experiment) and again in October 2016 (time-of-day effects experiment) and held in 90 l aquaria in groups of *c*. 50 fish each. The aquaria contained coarse sand and artificial plants and were fitted with an external filter. They were maintained at 8° C with a 12:12 dark: light photoperiod for the duration of the experiment. Fish were fed daily with frozen bloodworms. Experiments were conducted between December 2014 and February 2015 (main experiment) and in January 2017 (time-of-day effects experiment).

Fish were organized into experimental groups the day before observations began. We tested 40 such groups. In the main experiment, we quantified social network structure for each of 20 groups on two occasions, according to a fully factorial experimental design in which we varied the size of the arena in which they were tested (small or large) and the amount of time between the two observations (5 or 48 h), testing five groups in each of the four treatment combinations, as described below. We tested a further 20 groups in the time-of-day experiment, five in each of the arena size and morning–afternoon treatments, also described below. Each replicate group consisted of eight adult fish measuring 35–40 mm in standard length (L_S). All eight fish were taken from the same holding tank to control for familiarity, ensuring as far as possible that all fish within each replicate group were equally familiar with one another, since familiarity has been shown to have a weak effect on social network structure in this species (Atton *et al.,* 2014). Fish showing signs of being in reproductive state were not used, as this has been shown to affect social behaviour in other stickleback species (Webster & Laland, 2011). Fish were unsexed and groups were presumed to contain both non-reproductive males and females. To enable us to recognize individual fish, each was fitted with a unique, non-invasive tag on the first dorsal spine. These consisted of 5 mm coloured plastic discs. These do not affect shoaling preference or behaviour in this species (Webster & Laland, 2009). Reds and oranges, colours associated with male reproductive colouration, were not used. Each group was housed in its own 30 l aquarium with coarse sand and an internal filter.

EXPERIMENTAL ARENAS

Two arena sizes were used; these consisted of plastic pools lined with white vinyl laminate sheets. The larger arena measured 152 cm in diameter (*c*. 18 100 cm^2) and the smaller one 91·5 cm diameter (6600 cm^2). Both were filled with water to a depth of 10 cm. To provide structure, inverted white paper cups were added to the arenas. These were fitted with white lids and filled with aquarium sand to hold them on the arena floor. The cup diameter was 8 cm at the top (the base of the inverted cup in this experiment) tapering to 5 cm and 12 cm tall. These were arranged in a regular pattern, with eight cups in the small arena and 20 in the large arena, a density of 0·001 cups cm^{-2}. The arenas were placed within a shelter constructed from white corrugated plastic measuring 240 × 290 cm and 190 cm tall. This prevented disturbance of the fish during the observations and helped to ensure even lighting by reflecting light from wall-mounted LED banks from the ceiling into the arenas. Experiments were recorded using high definition webcams (Logitech C920; www.logitech.com) mounted above the arenas.

EXPERIMENTAL PROCEDURE

For each observation, the group of fish to be tested was taken from its holding aquarium and introduced to the arena, close to the edge. They were allowed to settle and move throughout the

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arena for 12 min before the observations began. These lasted for a further 138 min, during which time the entire arena was filmed. Each group was filmed in the arena on two occasions, separated by either 5 or 48 h. Following the first observation the fish were removed and returned to their holding aquarium. At the end of the second observation the tags were removed from the fish and they were transferred to a separate housing aquarium, playing no further role in the study. Groups in the 5 h inter-trial period treatment were observed on the morning and afternoon on the same day, with the first trial taking place at 0900 hours. Groups in the 48 h inter-trial period treatment were tested at either 0900 or 1500 hours for both of their observations.

From the videos, the location of each fish was recorded every 6 min using the tracking program LoggerPro (Vernier Software and Technology; www.vernier.com). Fish were considered to be associating if the distance between them was <7·5 cm (corresponding to approximately two standard body lengths; L_B). A gambit-of-the-group approach was used (Croft *et al.,* 2008), with all members of a group connected to at least one other member by <7·5 cm considered to be associating. Pairwise association data were used to construct association matrices describing the frequency of associations of all members of each group. From this, the network metrics described below were determined and compared between groups. Blinded methods were not used.

CONTROLLING FOR TIME-OF-DAY EFFECTS

Because the 5 h inter-trial duration group were tested twice on the same day, in the morning and afternoon, any differences in their behaviour may have reflected time-of-day effects rather than have arisen in response to being tested twice in the arena. Time-of-day effects may be due to circadian or diel rhythms (Reebs, 2002), learned behaviour (*e.g.* food anticipatory behaviour; Leblond & Reebs, 2006) or may have been due to other underlying mechanisms. In order to control for such effects, 20 further groups were established, as described above. These were tested in the morning (commencing 0900 hours) or afternoon (1600 hours), in the small or large arena, according to a factorial experimental design ($n = 5$ groups per treatment combination). Here, each group was only tested once. The test procedure was otherwise identical.

STATISTICAL ANALYSES

Pairwise interactions

Association matrices were produced for each observation. The association matrices were compared for each pair of observations within each of the treatment combinations using Mantel permutation tests with 1000 iterations. The Mantel tests generated a Pearson correlation coefficient for each pair of observations. For each treatment combination the Pearson correlation coefficients were meta-analysed using Stouffer's weighted z-method (Whitlock, 2005).

Network metrics

Using the association data we calculated separate network metrics. These were: network density, the number of pairwise interactions observed divided by the total number of possible pairwise interactions. Network differentiation, a measure of the evenness of the distribution of the total pairwise interactions between individuals within an association matrix (Edenbrow *et al.,* 2011), was derived from the co-efficient of variation (S.D. of observed interactions for a given pair within the group−mean number of interactions per pair for the group). A greater network differentiation score suggests more variation in the extent to which individuals associate with one another. Number of elements, described the number of subunits (lone individuals or groups of individuals) that were separated from other subunits by >2 L_B. Size of largest element, referred to the number of fish seen in the largest subunit. Network density and differentiation were calculated from the association matrices compiled for each observation. For the number of elements and the size of the largest element we used mean values (determined from the number of elements and size of the largest element recorded at each 6 min sampling interval) for each group. These were analysed using

repeated measures general linear models (GLM), comparing data for the first and second observation, with arena size (small or large) and duration between observations (5 or 48 h) included as categorical covariates. A separate GLM was performed for each of the four metrics.

Time-of-day effects

We first used GLMs to compare the four metrics between the groups tested in the morning and afternoon, including arena size, time of testing and the interaction between these as effects. We then compared these to the metric scores obtained for the first and then the second trials in the 5 h inter-trial period treatment groups. This allowed us to determine whether any change in any of the metrics seen in 5 h inter-trial groups was due to a time-of-day effect on behaviour that may have been independent of the testing regime. Here we used GLMs with time (morning, afternoon and first or second trial), arena size and the interaction between these as factors. These data were used in multiple analyses. We did not perform any correction for multiple testing here (*e.g.* Bonferroni correction), since these have been criticized for being overly conservative when the number of comparisons is low (Moran, 2003).

Within-group consistency

We looked for consistency in each of the four metrics, network density and differentiation, number of elements and size of largest element, comparing the scores obtained for the first and second observations in each of the four treatment combinations using Spearman's rank correlations. Ranked data were used because of the changes in metrics seen between the first and second observations in some treatments.

RESULTS

PAIRWISE INTERACTIONS

Pairwise association strengths were positively correlated between the first and second observation for the majority of groups across the four treatment combinations (Fig. 1). Meta-analysis using Stouffer's weighted z-method identified positive correlations in each of these (small arena, 5 h: $n = 5$, $P < 0{\cdot}001$; small arena, 48 h: $n = 5$, $P < 0{\cdot}001$; large arena, 5 h: $n = 5$, $P < 0{\cdot}001$; large arena, 48 h: $n = 5$, $P < 0{\cdot}005$).

NETWORK METRICS

For all four network metrics we saw variation between the first and second observations, with these differences largely being driven by changes in behaviour in the shorter inter-observation duration treatments (Fig. 2). In general, fish in these treatments interacted less frequently in the second observation than in the first, engaging in fewer pairwise interactions and forming more and smaller subunits. For network density an effect of arena size was seen too, with density being lower in the smaller arenas. Weaker effects of arena size were seen upon the number of separate elements and the size of the largest element as well; there were fewer elements in the smaller arenas, with more fish in the largest unit. Test statistics are presented in Table I.

TIME-OF-DAY EFFECTS

We saw no differences in any of the four network metrics between groups of fish tested in the morning or afternoon (Table II). We then compared these metrics between

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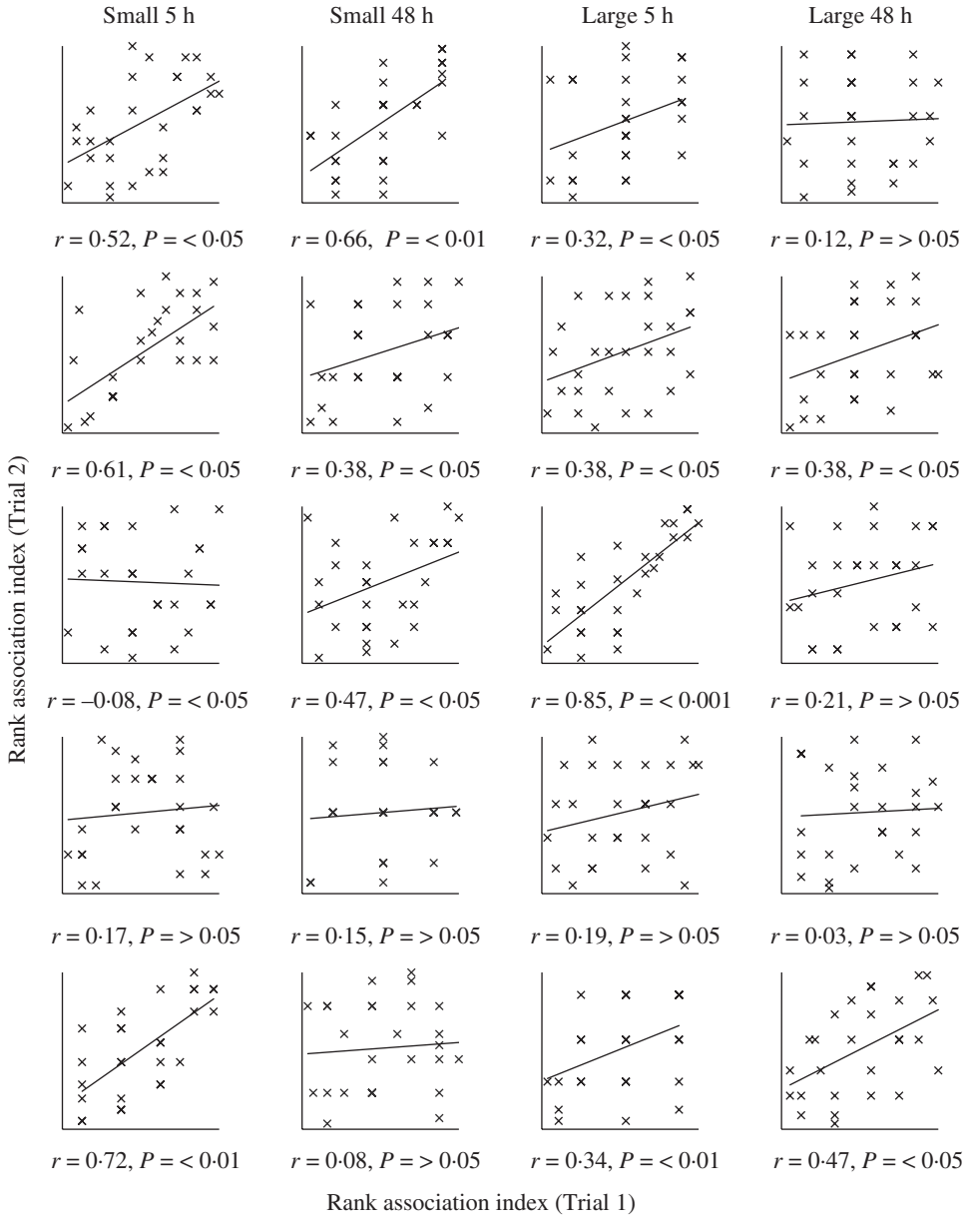

FIG. 1. Scatter plots showing the relationships between the association strength rank of each pairwise interaction for each replicate group of fish in the four treatment combinations. The four experimental treatments (small pool 5 h, interval between tests; small pool 48 h interval; large pool 5 h interval; large pool 48 h interval) are arranged by column, with each column displaying a plot for each of the five replicate groups, such that within a column each row represents one replicate group. Within plots each point (X) represents a dyad. Test statistics show Pearson's *r* correlation coefficients and *P*-values obtained using Mantel tests. There were 8 fish in each group, for a total of 28 association dyads. In some groups multiple dyads had the same association ranks in both trials resulting in fewer than 28 data points being depicted in some plots.

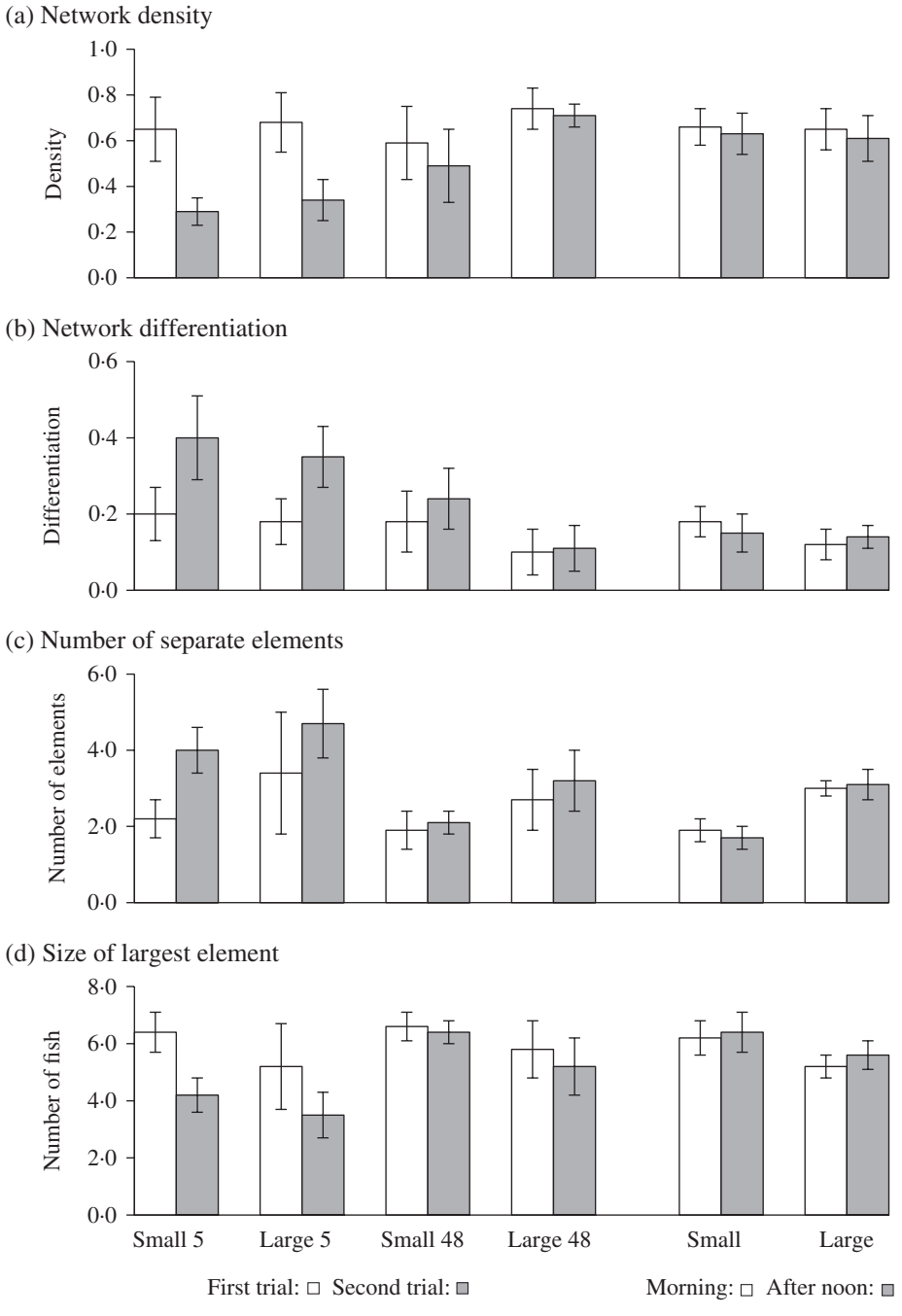

FIG. 2. The mean (±95% C.I.) score per group for each of four network metrics. Groups were either tested twice in small or large arenas at 5 or 48 h intervals (main experiment), or only once in the morning or afternoon in the time-of-day effects experiment.

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TABLE I. Output from repeated measures general linear models investigating the effects of arena size and inter-trial duration on four different network metrics with *Gasterosteus aculeatus*

		d.f.	*F*	*P*
Density				
	Within subjects effects			
	Trials	1	25·56	<0·001
	Trials*Arena size	1	0·01	>0·05
	Trials*Inter-trial duration	1	11·15	<0·01
	Trials*Arena size* Inter-trial duration	1	0·99	>0·05
	Error	16		
	Between subjects effects			
	Arena size	1	5·12	<0·05
	Inter-trial duration	1	7·48	<0·05
	Arena size* Inter-trial duration	1	0·09	>0·05
	Error	16		
Differentiation				
	Within subjects effects			
	Trials	1	8·87	<0·01
	Trials*Arena size	1	0·11	>0·05
	Trials*Inter-trial duration	1	3·59	>0·05
	Trials*Arena size* Inter-trial duration	1	0·26	>0·05
	Error	16		
	Between subjects effects			
	Arena size	1	2·86	>0·05
	Inter-trial duration	1	5·85	<0·05
	Arena size* Inter-trial duration	1	0·23	>0·05
	Error	16		
Separate elements				
	Within subjects effects			
	Trials	1	34·19	<0·001
	Trials*Arena size	1	0·11	>0·05
	Trials*Inter-trial duration	1	14·28	<0·01
	Trials*Arena size* Inter-trial duration	1	1·57	>0·05
	Error	16		
	Between subjects effects			
	Arena size	1	4·32	>0·05
	Inter-trial duration	1	5·99	<0·05
	Arena size* Inter-trial duration	1	0·01	>0·05
	Error	16		
Largest element				
	Within subjects effects			
	Trials	1	83·87	<0·001
	Trials*Arena size	1	0·09	>0·05
	Trials*Inter-trial duration	1	35·89	<0·001
	Trials*Arena size* Inter-trial duration	1	2·71	>0·05
	Error	16		
	Between subjects effects			
	Arena size	1	4·12	>0·05
	Inter-trial duration	1	5·91	<0·05
	Arena size* Inter-trial duration	1	0·96	>0·05
	Error	16		

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groups tested in the morning and afternoon and those of the main experiment groups tested twice in the short inter-observation period treatment of the main experiment, comparing them to their first and second test metrics. We saw no differences in any of these metrics obtained from their first test of the main experiment groups. We did however see differences for all four metrics recorded from the second test of the main experimental groups: density was higher and differentiation was lower in the groups tested for the second time compared with those tested only once in the morning or the afternoon. Similarly, the fish were organized into fewer and smaller elements in the groups tested for the second time (there were also effects of arena size for these metrics, Fig. 2 and Table II).

These findings imply that the change in behaviour of the fish in the second test compared to the first and the resulting changes in observed network metrics, resulted from repeated exposure to the test arena and did not reflect time-of-day effects upon behaviour.

WITHIN-GROUP CONSISTENCY

We saw significant positive correlations between the first and second observations for the size of the largest element in all treatment combinations, while other metrics were also significantly correlated in some treatment groups (Table III).

DISCUSSION

In this study we investigated the consistency of dyadic and shoal-level interactions in small, replicated laboratory groups of *G. aculeatus*. Considering first dyadic interactions, between the two observation periods we saw positive correlations between pairwise association strengths for most of the groups. While the strength of these correlations was variable, they do suggest that to a lesser or greater degree some individual fish tended to associate with the same group mates across both trials. Non-random assortment can occur through a number of different mechanisms (Hoare *et al.,* 2000*a*, *b*; Krause *et al.,* 2000). Animals may associate through active preference; shoaling fishes have been shown to form associations based upon a range of factors including body size (Ward & Krause, 2001; Croft *et al.,* 2009*a*), relatedness (Frommen & Bakker, 2004; Frommen *et al.,* 2007; Piyapong *et al.,* 2011), familiarity (Griffiths & Magurran, 1997; Croft *et al.*, 2004*b*; Frommen & Bakker, 2004; Ward *et al.,* 2009), chemical cues derived from similar diet or habitat use patterns (Ward *et al.,* 2004, 2005, 2007, 2009; Webster *et al.,* 2007, 2008*a*, *b*; Kleinhappel *et al.,* 2014, 2016) and competitive ability (Metcalfe & Thomson, 1995). Assortment may also arise passively through shared habitat preference or site fidelity (Croft *et al.,* 2003; Webster *et al.,* 2011; Ward *et al.,* 2013), similar swimming speeds (Krause *et al.,* 2005) or similar patterns of activity, risk aversion or cover use linked to personality traits (Pike *et al.,* 2008; Croft *et al.,* 2009*b*). The processes or mechanisms behind the positively correlated association patterns seen in this study are not clear; many of the above factors known to affect group composition, such as size, familiarity and habitat and diet use background were held constant as far as possible in our study and are therefore unlikely to be responsible. Personality traits have been shown to play a role in generating assortment in similar studies

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TABLE II. Output from general linear models investigating the effects of time of testing (morning or afternoon) and arena size for four different network metrics, and comparing these behaviours in *Gasterosteus aculeatus* tested in the morning or afternoon against those tested in the first and second trial in the repeated measures experiment

		d.f.	*F*	*P*
Density				
	Time of testing: AM *versus* PM			
	Arena Size	1	0·04	>0·05
	Time	1	0·32	>0·05
	Arena size* Time	1	0·01	>0·05
	Error	16		
	Time of testing and first test of repeated measure			
	Arena Size	1	0·19	>0·05
	Time	2	0·30	>0·05
	Arena size* Time	2	0·55	>0·05
	Error	34		
	Time of testing and second test of repeated measure			
	Arena Size	1	0·42	>0·05
	Time	2	5·73	<0·01
	Arena size* Time	2	0·88	>0·05
	Error	34		
Differentiation				
	Time of testing: AM *versus* PM			
	Arena Size	1	1·64	>0·05
	Time	1	0·05	>0·05
	Arena size* Time	1	1·04	>0·05
	Error	16		
	Time of testing and first test of repeated measure			
	Arena Size	1	2·27	>0·05
	Time	2	1·25	>0·05
	Arena size* Time	2	0·93	>0·05
	Error	34		
	Time of testing and second test of repeated measure			
	Arena Size	1	1·94	>0·05
	Time	2	5·67	<0·01
	Arena size* Time	2	0·88	>0·05
	Error	34		
Separate elements				
	Time of testing: AM *versus* PM			
	Arena Size	1	41·9	<0·001
	Time	1	0·14	>0·05
	Arena size* Time	1	0·50	>0·05
	Error	16		
	Time of testing and first test of repeated measure			
	Arena Size	1	13·74	<0·001
	Time	2	0·14	>0·05
	Arena size* Time	2	0·11	>0·05
	Error	34		
	Time of testing and second test of repeated measure			
	Arena Size	1	11·84	<0·01
	Time	2	6·59	<0·01

TABLE II. Continued

		d.f.	*F*	*P*
	Arena size* Time	2	0·17	>0·05
	Error	34		
Largest element				
	Time of testing: AM *versus* PM			
	Arena Size	1	8·14	<0·05
	Time	1	1·02	>0·05
	Arena size* Time	1	0·08	>0·05
	Error	16		
	Time of testing and first test of repeated measure			
	Arena Size	1	2·12	>0·05
	Time	2	> 0·05	>0·05
	Arena size* Time	2	0·26	>0·05
	Error	34		
	Time of testing and second test of repeated measure			
	Arena Size	1	6·37	<0·05
	Time	2	4·77	<0·05
	Arena size* Time	2	0·17	>0·05
	Error	34		

(Pike *et al.,* 2008) and may have been involved here too. Should similar association patterns be expected in species that form free-entry groups under natural conditions? On the one hand, greater opportunity to disperse might limit the likelihood of individuals re-encountering one another after splitting, though this effect may be countered somewhat if they exhibit site fidelity. Being part of a larger population and immigration by individuals from other areas might also mitigate against repeated encounters, by providing a greater number of opportunities for interaction and a greater pool of potential group mates to select between. On the other hand, the greater heterogeneity often associated with natural habitats might actually facilitate repeated interactions, if sheltered areas or feeding grounds are patchily distributed. The net outcome of these and other factors upon association patterns between individuals in unclear and further work in this area is necessary.

At the level of the group, the four network metrics that we quantified in this study (density, differentiation, mean number of separate elements and the mean size of the largest element) all varied over the two observation periods, a pattern that was driven by an interaction with the inter-observation duration. Arena size in contrast, which had a limited effect upon network metrics, did not affect changes in metrics recorded between the first and second observation periods. Specifically, we saw that only the shorter inter-observation treatment was associated with changes in these metrics. In the second observation trial the fish formed more subunits than they did in the first, with correspondingly fewer fish in the largest element, as well as lower density (indicating fewer pairwise associations) and increasing network differentiation. This final finding indicates that these associations were spread less evenly between dyads, suggesting more clique-like networks were formed in the second observation trial. Why this effect was seen in the shorter, but not in the longer inter-observation duration treatment is

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TABLE III. Spearman correlation co-efficients for two measures of each of four different network metrics using *Gasterosteus aculeatus*

	n	*r*	*P*
Small arena, 5 h inter-trial duration			
Network Density	5	0·70	>0·05
Network differentiation	5	0·60	>0·05
Number of separate elements	5	0·67	>0·05
Size of largest element	5	1·00	<0·001
Small arena, 48 h inter-trial duration			
Network Density	5	0·56	>0·05
Network differentiation	5	0·20	>0·05
Number of separate elements	5	0·90	<0·05
Size of largest element	5	0·97	<0·01
Large arena, 5 h inter-trial duration			
Network Density	5	0·60	>0·05
Network differentiation	5	1·00	<0·001
Number of separate elements	5	0·70	>0·05
Size of largest element	5	1·00	<0·001
Large arena, 48 h inter-trial duration			
Network Density	5	1·00	<0·001
Network differentiation	5	0·70	>0·05
Number of separate elements	5	0·70	>0·05
Size of largest element	5	1·00	<0·001

unclear. One possibility is that the fish were able to remember their recent experience of the arena and were less fearful or stressed during the second observation period, forming more and smaller groups in response. They may have behaved similarly in both observation periods of the longer duration treatment because they could not recall their experience over this longer time period. This line of speculation could be tested by observing fish in one arena type and then testing them again after a short period in either the same arena or in a different one. If familiarity with the arena lies behind their change in behaviour then we would expect to see them form smaller groups during the second observation period in the original arena configuration, but not in the altered one. While not explicitly investigating network characteristics, Ward (2012) found that shoals of mosquitofish *Gambusia holbrooki* Girard 1859 became less exploratory over time, suggesting that familiarity with the experimental arena can indeed lead to changes in behaviour. The biological significance of the interaction between duration between observation and network metrics in our study may be unclear, but this finding nevertheless has significant implications both for the design of experiments and for the extent to which comparisons can be drawn between the findings of separate studies that sample network structure over different time intervals.

A final and unexpected finding of our study was that some group network metrics, most prominently the size of the largest subunit, were positively correlated across observation periods. Because the sample sizes within each treatment were low and because this observation was not something we set out to investigate, we suggest these findings be treated as provisional and that they need to be followed up with further research that explicitly investigates the consistency of these and other metrics.

Nonetheless, these data do at least suggest the possibility of group-level stability in certain behavioural measures, functionally similar to and potentially arising from individual level personality differences. A growing number of studies have recognized the potential for personality trait expression to both shape and be shaped by social interactions in a number of ecologically relevant ways (Webster & Ward, 2011; Magnhagen, 2012; Wilson *et al.,* 2013; Wolf & Krause, 2014). Further research into how group composition affects group-level behaviour and function and especially the degree to which this is consistent and predictable would be useful. Such work could go beyond quantifying network metrics, as we have done here, to also consider behaviours more functionally related to the kinds of personality traits quantified in individual animals, such as activity, exploration rate and use of risky areas of the environment.

In summary, our study provides evidence of consistency in association network structure, both at the dyad and group level. We have shown that arena size can affect certain group-level metrics in laboratory studies and, more interestingly, that the length of the duration between observations can substantially affect network structure. While the biological basis and implications of this finding are not immediately clear, we suggest that this is an important factor that should be accounted for by researchers designing experiments that call for repeated observations of interactions between social groups of animals.

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References

Allen, J., Weinrich, M., Hoppitt, W. & Rendell, L. (2013). Network-based diffusion analysis reveals cultural transmission of lobtail feeding in humpback whales. *Science* **340,** 485–488.

Aplin, L. M., Farine, D. R., Morand-Ferron, J., Cole, E. F., Cockburn, A. & Sheldon, B. C. (2013). Individual personalities predict social behaviour in wild networks of great tits (*Parus major*). *Ecology Letters* **16,** 1365–1372.

Aplin, L. M., Farine, D. R., Morand-Ferron, J., Cockburn, A., Thornton, A. & Sheldon, B. C. (2015). Experimentally induced innovations lead to persistent culture *via* conformity in wild birds. *Nature* **518,** 538–541.

Atton, N., Hoppitt, W., Webster, M. M., Galef, B. G. & Laland, K. N. (2012). Information flow through threespine stickleback networks without social transmission. *Proceedings of the Royal Society B* **279**, 20121462.

Atton, N., Galef, B. J., Hoppitt, W., Webster, M. M. & Laland, K. N. (2014). Familiarity affects social network structure and discovery of prey patch locations in foraging stickleback shoals. *Proceedings of the Royal Society B* **281**, 20140579.

Boogert, N. J., Nightingale, G. F., Hoppitt, W. & Laland, K. N. (2014). Perching but not foraging networks predict the spread of novel foraging skills in starlings. *Behavioural Processes* **109,** 135–144.

Croft, D. P., Albanese, B., Arrowsmith, B. J., Botham, M., Webster, M. & Krause, J. (2003). Sex-biased movement in the guppy (*Poecilia reticulata*). *Oecologia* **137,** 62–68.

Croft, D. P., Krause, J. & James, R. (2004*a*). Social networks in the guppy (*Poecilia reticulata*). *Proceedings of the Royal Society B* **271,** S516–S519.

Croft, D. P., Arrowsmith, B. J., Webster, M. & Krause, J. (2004*b*). Intra-sexual preferences for familiar fish in male guppies. *Journal of Fish Biology* **64,** 279–283.

Croft, D. P., James, R., Thomas, P. O. R., Hathaway, C., Mawdsley, D., Laland, K. N. & Krause, J. (2006). Social structure and co-operative interactions in a wild population of guppies (*Poecilia reticulata*). *Behavioural Ecology and Sociobiology* **59,** 644–650.

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Croft, D. P., James, R. & Krause, J. (2008). *Exploring Animal Social Networks*. Princeton, NJ: Princeton University Press.
Croft, D. P., Darden, S. K. & Ruxton, G. D. (2009*a*). Predation risk as a driving force for phenotypic assortment: a cross-population comparison. *Proceedings of the Royal Society B* **276,** 1899–1904.
Croft, D. P., Krause, J., Darden, S. K., Ramnarine, I. W., Faria, J. J. & James, R. (2009*b*). Behavioural trait assortment in a social network: patterns and implications. *Behavioural Ecology and Sociobiology* **63,** 1495–1503.
Cross, P. C., Lloyd-Smith, J. O., Bowers, J. A., Hay, C. T., Hofmeyr, M. & Getz, W. M. (2004). Integrating association data and disease dynamics in a social ungulate: bovine tuberculosis in African buffalo in the Kruger National Park. *Annales Zoologici Fennici* **41**, 879–892.
Edenbrow, M., Darden, S. K., Ramnarine, I. W., Evans, J. P., James, R. & Croft, D. P. (2011). Environmental effects on social interaction networks and male reproductive behaviour in guppies, *Poecilia reticulata*. *Animal Behaviour* **81,** 551–558.
Farine, D. R., Garroway, C. J. & Sheldon, B. C. (2012). Social network analysis of mixed-species flocks: exploring the structure and evolution of interspecific social behaviour. *Animal Behaviour* **84,** 1271–1277.
Farine, D. R., Spencer, K. A. & Boogert, N. J. (2015). Early-life stress triggers juvenile zebra finches to switch social learning strategies. *Current Biology* **25,** 2184–2188.
Firth, J. A., Sheldon, B. C. & Farine, D. R. (2016). Pathways of information transmission among wild songbirds follow experimentally imposed changes in social foraging structure. *Biology Letters* **12,** 20160144.
Frommen, J. G. & Bakker, T. C. (2004). Adult three-spined sticklebacks prefer to shoal with familiar kin. *Behaviour* **141,** 1401–1409.
Frommen, J. G., Mehlis, M., Brendler, C. & Bakker, T. C. (2007). Shoaling decisions in three-spined sticklebacks (*Gasterosteus aculeatus*) – familiarity, kinship and inbreeding. *Behavioural Ecology and Sociobiology* **61,** 533–539.
Griffiths, S. W. & Magurran, A. E. (1997). Familiarity in schooling fish: how long does it take to acquire? *Animal Behaviour* **53,** 945–949.
Hamede, R. K., Bashford, J., McCallum, H. & Jones, M. (2009). Contact networks in a wild Tasmanian devil (*Sarcophilus harrisii*) population: using social network analysis to reveal seasonal variability in social behaviour and its implications for transmission of devil facial tumour disease. *Ecology Letters* **12,** 1147–1157.
von Hippel, F. (2010). *Tinbergen's Legacy in Behaviour: Sixty Years of Landmark Stickleback Papers*. Leiden: Brill.
Hoare, D. J., Krause, J., Peuhkuri, N. & Godin, J. G. (2000*a*). Body size and shoaling in fish. *Journal of Fish Biology* **57,** 1351–1366.
Hoare, D. J., Ruxton, G. D., Godin, J.-G. J. & Krause, J. (2000*b*). The social organization of free-ranging fish shoals. *Oikos* **89,** 546–554.
Kleinhappel, T. K., Burman, O. H., John, E. A., Wilkinson, A. & Pike, T. W. (2014). Diet-mediated social networks in shoaling fish. *Behavioural Ecology* **25,** 374–377.
Kleinhappel, T. K., Burman, O. H., John, E. A., Wilkinson, A. & Pike, T. W. (2016). A mechanism mediating inter-individual associations in mixed-species groups. *Behavioural Ecology and Sociobiology* **70,** 755–760.
Krause, J. & Ruxton, G. D. (2002). *Living in Groups*. Oxford: Oxford University Press.
Krause, J., Hoare, D. J., Croft, D., Lawrence, J., Ward, A., Ruxton, G. D., Godin, J.-G. J. & James, R. (2000). Fish shoal composition: mechanisms and constraints. *Proceedings of the Royal Society B* **267,** 2011–2017.
Krause, J., Ward, A. J., Jackson, A. L., Ruxton, G. D., James, R. & Currie, S. (2005). The influence of differential swimming speeds on composition of multi-species fish shoals. *Journal of Fish Biology* **67,** 866–872.
Krause, J., James, R. & Croft, D. P. (2010). Personality in the context of social networks. *Philosophical Transactions of the Royal Society B* **365,** 4099–4106.
Krause, J., James, R., Franks, D. W. & Croft, D. P. (Eds) (2014). *Animal Social Networks*. Oxford: Oxford University Press.
Leblond, C. & Reebs, S. G. (2006). Individual leadership and boldness in shoals of golden shiners (*Notemigonus crysoleucas*). *Behaviour* **143,** 1263–1280.

Lusseau, D. (2003). The emergent properties of a dolphin social network. *Proceedings of the Royal Society B* **270,** S186–S188.
Magnhagen, C. (2012). Personalities in a crowd: what shapes the behaviour of Eurasian perch and other shoaling fishes? *Current Zoology* **58,** 35–44.
McGregor, P. K. (Ed) (2005). *Animal Communication Networks*. Cambridge: Cambridge University Press.
Metcalfe, N. B. & Thomson, B. C. (1995). Fish recognize and prefer to shoal with poor competitors. *Proceedings of the Royal Society B* **259,** 207–210.
Moran, M. D. (2003). Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* **100,** 403–405.
Pike, T. W., Samanta, M., Lindström, J. & Royle, N. J. (2008). Behavioural phenotype affects social interactions in an animal network. *Proceedings of the Royal Society B* **275,** 2515–2520.
Pinter-Wollman, N., Hobson, E. A., Smith, J. E., Edelman, A. J., Shizuka, D., De Silva, S., Waters, J. S., Prager, S. D., Sasaki, T., Wittemyer, G. & Fewell, J. (2013). The dynamics of animal social networks: analytical, conceptual and theoretical advances. *Behavioural Ecology* **25,** 242–255.
Piyapong, C., Butlin, R. K., Faria, J. J., Scruton, K. J., Wang, J. & Krause, J. (2011). Kin assortment in juvenile shoals in wild guppy populations. *Heredity* **106,** 749–756.
Reebs, S. G. (2002). Plasticity of diel and circadian activity rhythms in fishes. *Reviews in Fish Biology and Fisheries* **12,** 349–371.
Ward, A. J. (2012). Social facilitation of exploration in mosquitofish (*Gambusia holbrooki*). *Behavioural Ecology and Sociobiology* **66,** 223–230.
Ward, A. J. & Krause, J. (2001). Body length assortative shoaling in the European minnow, *Phoxinus phoxinus*. *Animal Behaviour* **62,** 617–621.
Ward, A. J. W. & Webster, M. M. (2016). *Sociality: The Behaviour of Group Living Animals*. Zurich: Springer International Publishing.
Ward, A. J., Hart, P. J. & Krause, J. (2004). The effects of habitat-and diet-based cues on association preferences in three-spined sticklebacks. *Behavioural Ecology* **15,** 925–929.
Ward, A. J. W., Holbrook, R. I., Krause, J. & Hart, P. J. (2005). Social recognition in sticklebacks: the role of direct experience and habitat cues. *Behavioural Ecology and Sociobiology* **57,** 575–583.
Ward, A. J. W., Webster, M. M. & Hart, P. J. B. (2007). Social recognition in wild fish populations. *Proceedings of the Royal Society B* **274,** 1071–1077.
Ward, A. J. W., Webster, M. M., Magurran, A. E., Currie, S. & Krause, J. (2009). Species and population differences in social recognition between fishes: a role for ecology? *Behavioural Ecology* **20,** 511–516.
Ward, A. J. W., James, R., Wilson, A. D. M. & Webster, M. M. (2013). Site fidelity and localised homing behaviour in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behaviour* **150,** 1689–1708.
Weber, N., Carter, S. P., Dall, S. R., Delahay, R. J., McDonald, J. L., Bearhop, S. & McDonald, R. A. (2013). Badger social networks correlate with tuberculosis infection. *Current Biology* **23,** R915–R916.
Webster, M. M. & Laland, K. N. (2009). Evaluation of a non-invasive tagging system for laboratory studies using three-spined sticklebacks *Gasterosteus aculeatus*. *Journal of Fish Biology* **75,** 1868–1873.
Webster, M. M. & Laland, K. N. (2011). Reproductive state affects reliance on public information in sticklebacks. *Proceedings of the Royal Society B* **278,** 619–627.
Webster, M. M. & Ward, A. J. W. (2011). Personality and social context. *Biological Reviews* **86,** 759–773.
Webster, M. M., Goldsmith, J., Ward, A. J. W. & Hart, P. J. B. (2007). Habitat-specific chemical cues influence association preferences and shoal cohesion in fish. *Behavioural Ecology and Sociobiology* **62,** 273–280.
Webster, M. M., Adams, E. L. & Laland, K. N. (2008*a*). Diet-specific chemical cues influence association preferences and prey patch use in a shoaling fish. *Animal Behaviour* **76,** 17–23.

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Webster, M. M., Ward, A. J. W. & Hart, P. J. B. (2008*b*). Shoal and prey patch choice by co-occurring fishes and prawns: inter-taxa use of socially transmitted cues. *Proceedings of the Royal Society B* **275,** 203–208.

Webster, M. M., Atton, N., Hart, P. J. B. & Ward, A. J. W. (2011). Habitat-specific morphological variation among threespine sticklebacks (*Gasterosteus aculeatus*) within a drainage basin. *PLoS ONE* **6,** e21060.

Webster, M. M., Atton, N., Hoppitt, W. & Laland, K. N. (2013). Environmental complexity influences association network structure and network-based diffusion of foraging information in fish shoals. *American Naturalist* **181,** 235–244.

Wey, T., Blumstein, D. T., Shen, W. & Jordán, F. (2008). Social network analysis of animal behaviour: a promising tool for the study of sociality. *Animal Behaviour* **75,** 333–344.

Whitehead, H. (2008). *Analyzing Animal Societies: Quantitative Methods for Vertebrate Social Analysis*. Chicago, IL: University of Chicago Press.

Whitlock, M. C. (2005). Combining probability from independent tests: the weighted *Z*-method is superior to Fisher's approach. *Journal of Evolutionary Biology* **18,** 1368–1373.

Wilson, A. D. M., Krause, S., Dingemanse, N. J. & Krause, J. (2013). Network position: a key component in the characterization of social personality types. *Behavioural Ecology and Sociobiology* **67,** 163–173.

Wilson, A. D. M., Krause, S., Ramnarine, I. W., Borner, K. K., Clément, R. J. G., Kurvers, R. H. & Krause, J. (2015). Social networks in changing environments. *Behavioural Ecology and Sociobiology* **69,** 1617–1629.

Wolf, M. & Krause, J. (2014). Why personality differences matter for social functioning and social structure. *Trends in Ecology and Evolution* **29,** 306–308.

Wolf, J. B., Mawdsley, D., Trillmich, F. & James, R. (2007). Social structure in a colonial mammal: unravelling hidden structural layers and their foundations by network analysis. *Animal Behaviour* **74,** 1293–1302.