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 organised 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.

KEY WORDS: Assortment; Group; Shoaling; Social behaviour; Social information; Social organisation

INTRODUCTION

Group living in one form or another is extensive among animals, shaping and shaped by a range of ecological and evolutionary processes (Krause and Ruxton 2002; Ward and 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 (Croft et al 2009; Wey et al. 2008; Whitehead 2008; Pinter-Wollman et al. 2013; Krause et al. 2015).

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; 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; 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 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 utilising 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 (e.g. Lusseau 2003; Croft et al. 2004; 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 and Ruxton 2002; Ward and 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.). 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; 2013; 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 (Hoare et al. 2000a; 2000b; Krause et al. 2000). 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

Threespine sticklebacks 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 around 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 to 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. In total 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. 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 to 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 and Laland 2011). Fish were unsexed and groups were presumed to contain both non-reproductive males and females. To enable us to recognise 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 and 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 (approximately 18100 cm^2 in area) and the smaller one 91.5 cm diameter (6600 cm^2 in area). 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 cm x 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.

148 *Experimental procedure*

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150 For each observation the group of fish to be tested was taken from its holding aquarium and
151 introduced to the arena, close to the edge. They were allowed to settle and move throughout the
152 arena for 12 min before the observations began. These lasted for a further 138 min, during which
153 time the entire arena was filmed. Each group was filmed in the arena on two occasions, separated
154 by either 5 or 48 h. Following the first observation the fish were removed and returned to their
155 holding aquarium. At the end of the second observation the tags were removed from the fish and
156 they were transferred to a separate housing aquarium, playing no further role in the study.
157 Groups in the 5 h inter-trial period treatment were observed on the morning and afternoon on the
158 same day, with the first trial taking place at 09:00 hours. Groups in the 48 h inter-trial period
159 treatment were tested at either 09:00 or 15.00 hours for both of their observations.

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161 From the videos, the location of each fish was recorded every 6 min using the tracking program
162 LoggerPro (Vernier Software and Technology, www.vernier.com Fish were considered to be
163 associating if the distance between them was less than 7.5 cm (corresponding to approximately 2
164 standard body lengths). A ‘gambit of the group’ approach was used (Croft et al. 2008), with all
165 members of a group connected to at least one other member by less than 7.5 cm considered to be
166 associating. Pairwise association data were used to construct association matrices describing the
167 frequency of associations of all members of each group. From this, the network metrics
168 described below were determined, and compared between groups. Blinded methods were not
169 used.

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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 09.00 hours) or afternoon (16.00 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

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195 Using the association data we calculated separate network metrics. These were: *Network density*,
196 the number of pairwise interactions observed divided by the total number of possible pairwise
197 interactions. *Network differentiation*, a measure of the evenness of the distribution of the total
198 pair-wise interactions between individuals within an association matrix (Edenbrow 2011), was
199 derived from the co-efficient of variation (standard deviation of observed interactions for a given
200 pair within the group / mean number of interactions per pair for the group). A greater network
201 differentiation score suggests more variation in the extent to which individuals associate with
202 one another. *Number of elements* described the number of subunits (lone individuals or groups of
203 individuals) that were separated from other subunits by more than 2 body lengths. *Size of largest*
204 *element* referred to the number of fish seen in the largest subunit. Network density and
205 differentiation were calculated from the association matrices compiled for each observation. For
206 the number of elements and the size of the largest element we used mean values (determined
207 from the number of elements and size of the largest element recorded at each 6 min sampling
208 interval) for each group. These were analysed using repeated measures GLMs, comparing data
209 for the first and second observation, with arena size (small or large) and duration between
210 observations (5 or 48 h) included as categorical covariates. A separate GLM was performed for
211 each of the four metrics.

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213 Time-of-day effects

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215 We first used GLMs to compare the four metrics between the groups tested in the morning and
216 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 criticised 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 (see Results).

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-

240 analysis using Stouffer's weighted z method identified positive correlations in each of these
241 (small arena, 5 h: n=5, P<0.001; small arena, 48 h: n=5, P<0.001; large arena, 5 h: n=5,
242 P<0.001; large arena, 48 h: n=5, P<0.005).
243
244 *Network metrics*
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246 For all four network metrics we saw variation between the first and second observations, with
247 these differences largely being driven by changes in behaviour in the shorter inter-observation
248 duration treatments (Fig. 2). In general, fish in these treatments interacted less frequently in the
249 second observation than in the first, engaging in fewer pair-wise interactions and forming more
250 and smaller subunits. For network density an effect of arena size was seen too, with density
251 being lower in the smaller arenas. Weaker effects of arena size were seen upon the number of
252 separate elements and the size of the largest element as well; there were fewer elements in the
253 smaller arenas, with more fish in the largest unit. Test statistics are presented in Table I.
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255 *Time of day effects*
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257 We saw no differences in any of the four network metrics between groups of fish tested in the
258 morning or afternoon (Table II). We then compared these metrics between groups tested in the
259 morning and afternoon and those of the main experiment groups tested twice in the short inter-
260 observation period treatment of the main experiment, comparing them to their first and second
261 test metrics. We saw no differences in any of these metrics obtained from their first test of the
262 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 to those tested only once in the morning or the afternoon. Similarly, the fish were organised 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 sticklebacks. 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 groupmates across both trials. Non-random assortment can occur through a number of different mechanisms

(Hoare et al. 2000a; 2000b; 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 and Krause 2001; Croft et al. 2009a), relatedness (Frommen et al. 2004; 2007; Piyapong et al. 2011), familiarity (Griffiths and Magurran 1997; Croft et al. 2004; Frommen et al. 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; 2008a; 2008b; Kleinhappel et al. 2014; 2016) and competitive ability (Metcalf and 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. 2009b). 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 (e.g. 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 reencountering 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 groupmates 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 impact 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 'cliquier' 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 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 recognised the potential for personality trait expression to both shape and be shaped by social interactions in a number of ecologically relevant ways (reviewed by Webster and Ward 2011; Magnhagen 2012; Wilson et al. 2013; Wolf and 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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609

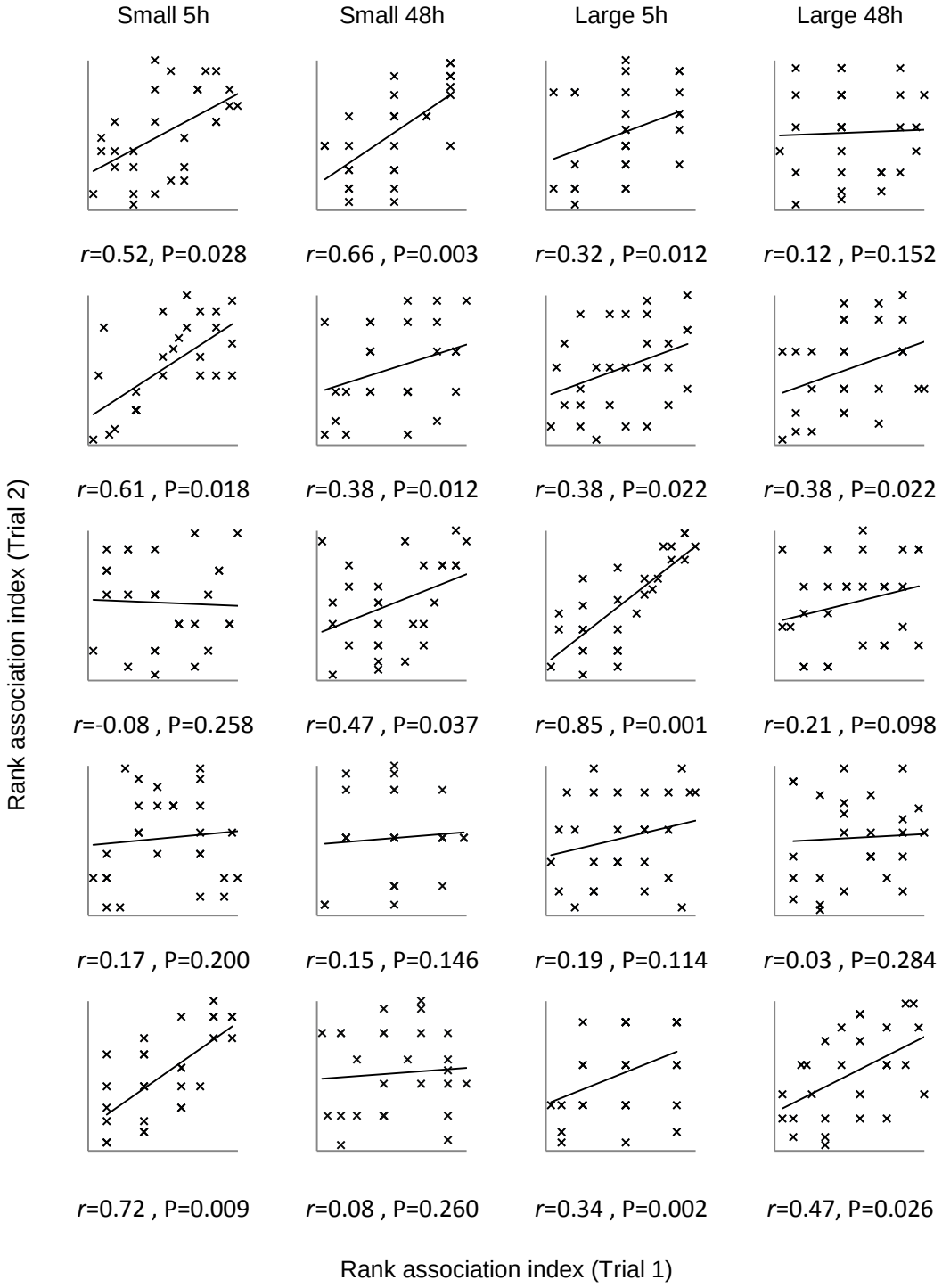
FIGURE CAPTIONS

Figure 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 5h, interval between tests; small pool 48h interval; large pool 5h interval; large pool 48h interval) are arranged by column, with each column displaying a plot for each of the five replicate groups. Within plots each point 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.

Figure 2. The mean (+/- 95% confidence interval) score per group for each of four network metrics. Groups were either tested twice in small or large arenas at 5 or 48h intervals (main experiment), or only once in the morning or afternoon in the time-of-day effects experiment. See Main Text and Tables I and II for further details.

626 **Figure 1.**

627



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631 **Figure 2.**

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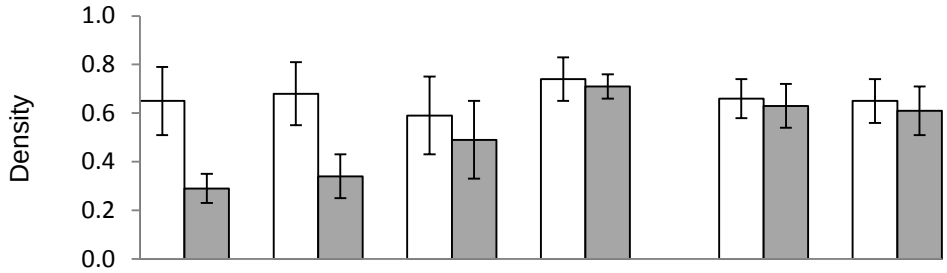
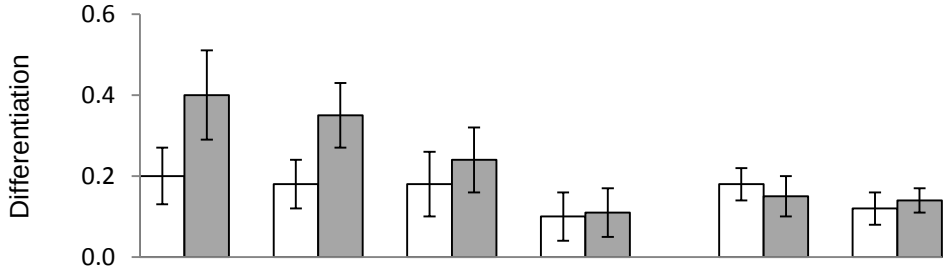
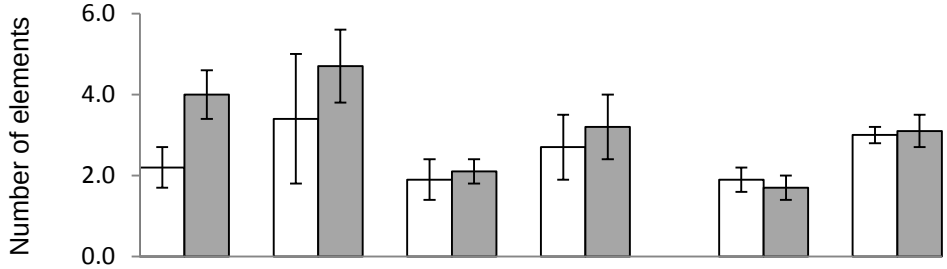
(a) Network density



(b) Network differentiation



(c) Number of separate elements



(d) Size of largest element

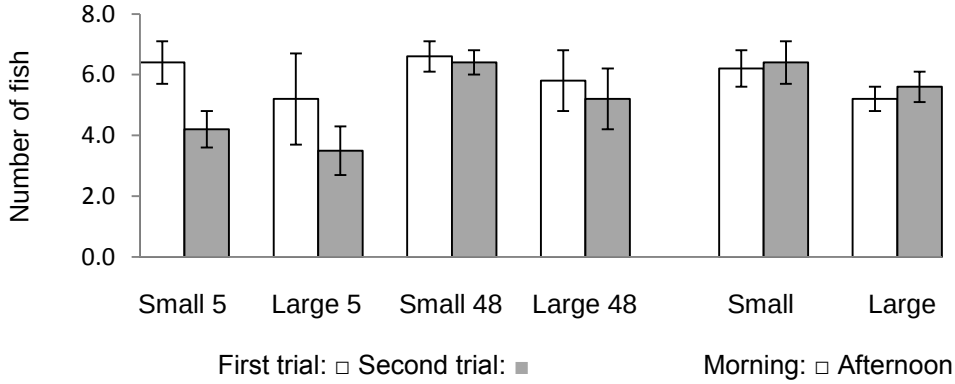


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Table I. Output from repeated measures GLMs investigating the effects of arena size and inter-trial duration on four different network metrics.

		d.f.	*F*	*p*
(a) Density	(i) *Within subjects effects*			
	Trials	1	25.56	<0.001
	Trials*Arena size	1	0.01	0.989
	Trials*Inter-trial duration	1	11.15	0.004
	Trials*Arena size* Inter-trial duration	1	0.99	0.341
	Error	16		
	(ii) *Between subjects effects*			
	Arena size	1	5.12	0.036
	Inter-trial duration	1	7.48	0.015
	Arena size* Inter-trial duration	1	0.09	0.763
	Error	16		
(b) Differentiation	(i) *Within subjects effects*			
	Trials	1	8.87	0.009
	Trials*Arena size	1	0.11	0.745
	Trials*Inter-trial duration	1	3.59	0.076
	Trials*Arena size* Inter-trial duration	1	0.26	0.616
	Error	16		
	(ii) *Between subjects effects*			
	Arena size	1	2.86	0.110
	Inter-trial duration	1	5.85	0.028
	Arena size* Inter-trial duration	1	0.23	0.635
	Error	16		
(c) Separate elements	(i) *Within subjects effects*			

	Trials	1	34.19	<0.001
	Trials*Arena size	1	0.11	0.750
	Trials*Inter-trial duration	1	14.28	0.002
	Trials*Arena size* Inter-trial duration	1	1.57	0.227
	Error	16		
	(ii) *Between subjects effects*			
	Arena size	1	4.32	0.054
	Inter-trial duration	1	5.99	0.026
	Arena size* Inter-trial duration	1	0.01	0.952
	Error	16		
(d) Largest element	(i) *Within subjects effects*			
	Trials	1	83.87	<0.001
	Trials*Arena size	1	0.09	0.768
	Trials*Inter-trial duration	1	35.89	<0.001
	Trials*Arena size* Inter-trial duration	1	2.71	0.119
	Error	16		
	(ii) *Between subjects effects*			
	Arena size	1	4.12	0.059
	Inter-trial duration	1	5.91	0.027
	Arena size* Inter-trial duration	1	0.96	0.969
	Error	16		

641 **Table II.** Output from GLMs (i) investigating the effects of time of testing (morning or afternoon) and
642 arena size for four different network metrics, and comparing these behaviours in fish tested in the
643 morning or afternoon against those tested in the first (ii) and second (iii) trial in the repeated measures
644 experiment.
645

		d.f.	*F*	*P*
(a) Density	(i) *Time of testing: AM versus PM*			
	Arena Size	1	0.04	0.842
	Time	1	0.32	0.577
	Arena size* Time	1	0.01	0.957
	Error	16		
	(ii) *Time of testing and first test of repeated measure*			
	Arena Size	1	0.19	0.663
	Time	2	0.30	0.739
	Arena size* Time	2	0.55	0.579
	Error	34		
	(iii) *Time of testing and second test of repeated measure*			
	Arena Size	1	0.42	0.522
	Time	2	5.73	0.007
	Arena size* Time	2	0.88	0.420
	Error	34		
(b) Differentiation	(i) *Time of testing: AM versus PM*			
	Arena Size	1	1.64	0.217
	Time	1	0.05	0.828
	Arena size* Time	1	1.04	0.324
	Error	16		
	(ii) *Time of testing and first test of repeated measure*			
	Arena Size	1	2.27	0.141
	Time	2	1.25	0.299
	Arena size* Time	2	0.93	0.402
	Error	34		
	(iii) *Time of testing and second test of repeated measure*			
	Arena Size	1	1.94	0.173
	Time	2	5.67	0.007
	Arena size* Time	2	0.88	0.422
	Error	34		

(c) Separate elements	(i) *Time of testing: AM versus PM*			
	Arena Size	1	41.9	<0.001
	Time	1	0.14	0.708
	Arena size* Time	1	0.50	0.489
	Error	16		
	(ii) *Time of testing and first test of repeated measure*			
	Arena Size	1	13.74	0.001
	Time	2	0.14	0.873
	Arena size* Time	2	0.11	0.896
	Error	34		
	(iii) *Time of testing and second test of repeated measure*			
	Arena Size	1	11.84	0.002
	Time	2	6.59	0.004
	Arena size* Time	2	0.17	0.842
	Error	34		
(d) Largest element	(i) *Time of testing: AM versus PM*			
	Arena Size	1	8.14	0.012
	Time	1	1.02	0.326
	Arena size* Time	1	0.08	0.786
	Error	16		
	(ii) *Time of testing and first test of repeated measure*			
	Arena Size	1	2.12	0.154
	Time	2	0.26	0.771
	Arena size* Time	2	0.26	0.773
	Error	34		
	(iii) *Time of testing and second test of repeated measure*			
	Arena Size	1	6.37	0.016
	Time	2	4.77	0.015
	Arena size* Time	2	0.17	0.983
	Error	34		

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Table III. Spearman correlation co-efficients for two measures of each of four different network metrics.

	n	*r*	*P*
(a) Small arena, 5h inter-trial duration			
Network Density	5	0.70	0.188
Network differentiation	5	0.60	0.285
Number of separate elements	5	0.67	0.219
Size of largest element	5	1.00	<0.001
(b) Small arena, 48h inter-trial duration			
Network Density	5	0.56	0.320
Network differentiation	5	0.20	0.747
Number of separate elements	5	0.90	0.037
Size of largest element	5	0.97	0.005
(c) Large arena, 5h inter-trial duration			
Network Density	5	0.60	0.285
Network differentiation	5	1.00	<0.001
Number of separate elements	5	0.70	0.188
Size of largest element	5	1.00	<0.001
(d) Large arena, 48h inter-trial duration			
Network Density	5	1.00	<0.001
Network differentiation	5	0.70	0.118
Number of separate elements	5	0.70	0.118
Size of largest element	5	1.00	<0.001