

GRAPH THEORY IN ANIMAL BEHAVIOR AND THE SOCIAL ROLE OF GATEKEEPERS ON GROUP
LEARNING IN ZEBRAFISH SHOALS, *DANIO RERIO*

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JHS

I dedicate this document to Ricardo who drove all around Mexico to collect lizards- that after all were not part of this dissertation-, and who has been the best field assistant, tank cleaner, fish catcher, reviewer, fan, doula, nurse, cook, gardener, lover, friend but most of all for the being the best husband. To dad and mom who believed and trusted in my dreams. To my sisters, Citlalli and Metztli, who have always been my muses. To Izel and Naori for always being there to make my day.

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GRAPH THEORY IN ANIMAL BEHAVIOR AND THE SOCIAL ROLE OF GATEKEEPERS ON GROUP
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Social behavior research has moved from a focus on dyads to considering larger group dynamics. Individual animals can adopt particular social roles in a group, such as “gatekeepers”, who control information flow by interacting freely with other animals in the group. In my dissertation, I start with a computer simulation analysis to explore the performance of graph theory metrics to infer information flow in smaller social groups common of animal behavior. By varying different group attributes, we found that some metrics are better than others at describing certain aspects. I found that at least ten interactions should be recorded for each individual in a group. Additionally we make recommendations for specific combinations of metrics and I describe the performance of different metrics under different scenarios. In the experimental part of my dissertation, I looked at how gatekeepers influence the process of group dynamics, group performance and social learning in zebrafish groups. I worked with two different zebrafish strains; a lab reared and a recently derived wild strain. First I identified and removed gatekeepers or non-gatekeepers from different groups. Then I looked at the effect of removal on group dynamics and group learning performance. I found that social dynamics does have an impact on group learning: groups that retained their gatekeeper (removed non-gatekeeper) learned more quickly than did groups from which I had removed the gatekeeper. I found that different strains respond differently to the removal of individuals, suggesting a possible underlying genetic effect. In another set of experiments, I identified, removed and trained individual gatekeepers and non-gatekeepers, returned them to their initial social group, and then trained the entire group on a simple associative task. I found that gatekeepers do play a more important role on information transfer; groups with pre-trained gatekeepers learned more quickly than did groups with pre-trained non-gatekeeper. Finally I found that male tutors were better at transferring information regardless of their gatekeeper status. These results show that individual zebrafish take on specific roles within a social group, and confirm that those roles can impact group dynamics and learning ability.

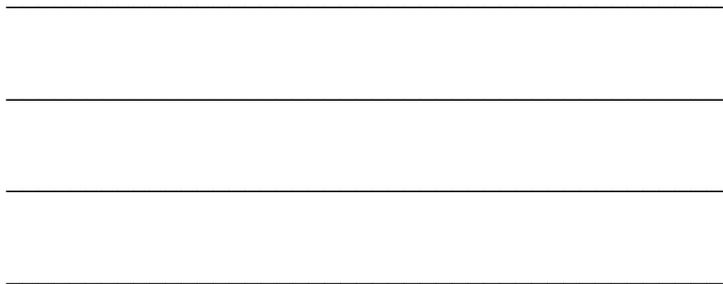


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CHAPTER 1: Introduction

Vital, Cuauhcihuatl

Background

An important challenge in animal behavior is to reconcile the study of a single individual or a dyad with the social environment where gregarious animals live. In recent years there has been a shift from the study of dyads to the study of larger social groups (Krause et al. 2007, Wey et al. 2008). Representation and study of systems with the use of graphs or networks is widely spread in different fields including graph theory (Newman 2002), physics (Hong et al. 2004), sociology (Goh et al. 2002, Lee 2004), more recently it also has been used in biology under the fields of protein studies (Del Sol et al 2004) amino acids (Vendruscolo et al. 2002) neurology (Watts and Strogatz 1998) and metabolic networks (Ravasz et al. 2002). Techniques exploited in these areas have a potential use in the study of animal behavior and only recently more than a handful of studies have starting to explore these metrics and techniques (Croft et al. 2007, Krause et al. 2007, Whitehead 2008, Krause et al. 2009, Sih et al. 2009). Wilson pioneered the idea that social networks were an important technique in studying animals that live in social groups (Wilson 1975). The study of social networks provides insight to the full array of behaviors and interactions in a group rather than focusing in a single individual or in a dyad. In recent years several studies make use of graph networks. Recently applied network theory was used to study the social system of lek-mating wire-tailed manikins. Using four network metrics, degree, eigenvector centrality, information centrality and reach they were able to predict male social rise (Ryder et al. 2008). Clustering coefficient, a metric that describes the cliquishness of a group was used to describe the patterns of social interactions in a population of sticklebacks (Pike et al. 2008). Group

dynamics in animal groups are an important feature that promises to shed light on to many process of animal behavior.

Group dynamics or network structure in gregarious groups is crucial in determining overall group structure and performance. Different aspects such as disease vulnerability, information transfer, resources access, mating, social stability and fusion-fission are influenced by group dynamics. Average number of interactions (degree), social distance between two individuals (average path length) and cliquishness in a whale society influence disease outbreak and vulnerability of killer whales (Guimaraes et al. 2007). In dolphin groups, the removal of highly interactive adult females increases group dispersion (diameter) thus increasing the time and number of individuals information should travel to go from one individual in the group to another (Lusseau 2003) impairing information transfer. Finally society stability in macaques is also strongly influenced by group dynamics. Flack et al. (2006) showed that a decrease in clustering coefficient, after the removal of policing individuals, leads to a higher incidence of aggressive encounters thus risking society stability (Flack et al. 2006). Some individuals however have a higher influence in group dynamics, by controlling the flow of resources in a group playing an important role in group decisions and fitness.

“Gatekeepers” or keystone individuals, play a particular role in shaping interactions in a social group. Individuals differ in their importance on group dynamics, gatekeepers control information flow by interacting freely with many other animals in the group, they influence the distribution of resources such as knowledge, food, disease and mates. Gatekeepers are a common and widespread phenomenon in animal social groups. A female that leads the migration route of brown surgeonfish (Kiflawi and Mazeroll

2006), a dolphin that facilitates interactions among different alliances (Lusseau 2003), matriarchs as the repository of knowledge in African elephants (McComb et al. 2001), hyperaggressive individuals that influence mating success in water striders (Sih & Watters 2005) are all examples of gatekeepers in different animal groups. Although their role may vary significantly from group to group, gatekeepers influence the overall group dynamics and fitness. Understanding the role gatekeepers play in a group will yield insight into group dynamics.

Gregarious animals learn about their social environment through interactions with their group mates. Foraging (Marchetti and Drent 2000, Galef and Giraldeau 2001, Fernandez-Juricic et al. 2004, Leadbeater and Chitka 2007), predator recognition (Chivers et al. 2002, Brosnan et al. 2003, Kelley and Magurran 2003, Vilhunen et al. 2005, Ferrari and Chivers 2008), mate choice (Doutrelant and McGregor 2000, Amy et al. 2008), feeding preference (Benskin et al. 2002) and color preference (Spence and Smith 2008) are all transmitted through both direct and indirect social interactions in various taxa. Earlier Coussi-Korber and Frigaszy (1995) suggested that learning in gregarious groups is influenced by group dynamics (Coussi-Korbel and Frigaszy 1995) and that some individuals may play a more important role in the access to resources. We explore the role of gatekeepers on different aspects of group living in zebrafish shoals. We identify naturally occurring gatekeepers and look at the effect of removing them in group dynamics, group performance and group learning.

Study System

Zebrafish are found in freshwater throughout India, Bangladesh and Pakistan. They have been studied primarily in the Ganges River Basin of Eastern India and Bangladesh, where they are found in still, shallow water with aquatic vegetation along the edges of oxbow lakes and in rice paddies (McClure et al. 2006, Spence et al. 2006, Engeszer et al. 2007b). Throughout India, zebrafish are exposed to similar fish and avian predators (cichlids, catfish, kingfishers, herons). Zebrafish are a popular model organism for geneticists and developmental biologists, and have a huge potential for behavioral research (Guo 2003). There has been recent activity in the study of zebrafish behavior (Wright et al. 2006, Spence et al. 2008). Zebrafish prefer to congregate in small shoals of 2 to 10 fish (Pritchard et al. 2001) and are highly social. Furthermore, zebrafish exhibit social preferences making them a good model for social behavior studies. An association bias is exhibited in particular for stripe patterns (Turnell et al. 2003), color (Snekser et al. 2006) and shape (Saverino and Gerlai 2008). Specific preferences depend on the context in which they are presented (Engeszer et al. 2008), and early social experience (Engeszer et al. 2004, Engeszer et al. 2007a). Furthermore, behavioral differences between strains and wild populations are often large, and appear to have a genetic basis (Wright et al. 2003, Robison & Rowland 2005, Wright et al. 2006, Moretz et al. 2007a). Together, these studies suggest that zebrafish are complex social animals, capable of advanced learning, and suitable for social network studies.

Dissertation overview

We begin this dissertation with a theoretical approach testing the abilities of several social networks with study designs typical of animal behavior; then using the results and suggestions from the theoretical study we conduct three empirical studies to

test different aspects of group dynamics and gatekeepers. In chapter one using computer simulation we test the abilities of several social network metrics to estimate the proportion of gatekeepers in a social group with sample sizes and study designs typical of animal behavior studies. We found that most network metrics are sensitive to the amount of sampling and to variation in group size. We suggest a combination of metrics to provide the most comprehensive description of information flow and we introduce a software package, SocANet, that can be used to conduct similar simulations to determine the best metrics for a particular group of animals and set of conditions.

Following the recommendations from chapter one we move to the empirical part of the dissertation testing the effect of gatekeepers on several aspects of group living. In chapter two we compare social dynamics between two genetically-distinct strains of zebrafish and test the effect of removing individuals with specific social roles from zebrafish shoals. Although we found no difference between strains in the absolute level of group cohesion, SH (Scientific Hatchery) shoals reacted within minutes to recover group structure, whereas PN (a recently wild derived strain) shoals showed no change. Moreover, we found that measures of social dynamics were moderately consistent when taken up to three weeks apart. After finding a strain difference in group response to the removal of a single fish, we then ask in chapter three whether the presence of a single fish facilitates zebrafish group performance in attaining food. We found strain differences with lab reared fish learning by the second day, whereas wild derived fish took three days to learn. Additionally, we found that the presence of a single fish, namely gatekeeper, facilitates the process of group performance, with groups having a gatekeeper performing better than groups where gatekeepers were removed. Finally in chapter four we further

explore the role of gatekeepers. We found that the presence of an experienced gatekeeper facilitates the process of social learning. Both strain and sex influence the effect of gatekeepers on social learning. These results suggest that gatekeeper do directly facilitate the process of group learning, however strain differences might influence the process of information transfer in different strain groups.

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CHAPTER 2: Using graph theory metrics to infer information flow through animal social groups: a computer simulation analysis

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ABSTRACT

Single individuals, termed “gatekeepers”, can have a profound impact on information flow in a group, whether that gatekeeper be a large male despot which strictly controls access to resources and mates, or an infant that is freely passed around among members of a social group. Graph theory offers powerful tools for considering larger aspects of social dynamics such as information flow, and their impact on phenomena such as social learning, social roles, foraging skills transfer and eavesdropping. Here, we use computer simulation to test the abilities of several social network metrics to estimate the proportion of gatekeepers in a social group with sample sizes and study designs typical of animal behavior studies. We find that most network metrics are sensitive to the amount of sampling (number of recorded interactions), and did not give good estimates when fewer than ten interactions were recorded for each animal in the group. Metrics were also quite sensitive to variation in group size, yielding the full range of possible values for groups varying from 20 to 50 animals. We thus recommend against their use with animals that move in and out of groups seasonally. Individual values estimated by each of the metrics were often quite different from each other such that a combination of metrics chosen from each of the following groups provides the most comprehensive description of information flow: a) Closeness or Degree Centrality, b) Betweenness Centrality, c) Density or Clustering Coefficient, and/or d) Diameter, Average Degree or Information Centrality. Finally, we introduce a software package, SocANet, that can be used to conduct similar simulations to determine the best metrics for a particular group of animals and set of conditions.

“Gatekeepers” are animals who control the access to certain resources, such as social interactions, mates, food or space, and who thus have a stronger influence on group behavior and the dynamics of information transfer through the group. These may be dominant animals which mediate the social interactions of other group members (e.g., Flack et al. 2006), animals that live at the fringes of sub-groups, often making contact with neighboring sub-groups (e.g., Lusseau & Newman 2004), or animals in a wide variety of other social roles that influence group behavior. Although these roles are profoundly different in behavioral terms, recent studies (e.g., Newman 2003, Albert et al. 2002) suggest that groups with similar flow of information may share other more general properties of the group as a whole. For example, the number of gatekeepers in a group of song-sharing birds (such as those reviewed by Beecher & Brenowitz 2005) may offer a good estimate of the number of unique songs in a population and the rate at which new songs will become popular. Similarly, in groups with few gatekeepers, mating preferences exhibited by the gatekeepers may be more quickly copied by others. Thus the number of gatekeepers may also be a useful surrogate for mating system and the opportunity for selection (metrics emphasized by Shuster & Wade 2003). When gatekeepers control conflicts (e.g., Flack et al. 2006), the number of gatekeepers may be a good estimate of group stability and vulnerability to disruptions. In endangered iguanas, the number of gatekeepers can serve as an estimate of the degree of anthropogenic disturbance (Lacey & Martins 2003). Thus, although the number of gatekeepers is only one of several group properties, it is an important one that promises insight into group dynamics in a wide variety of behavioral contexts and that is estimated directly by several graph theory metrics.

Social behavior research has recently moved from a focus on dyads to considering larger group dynamics such as alliances, social learning, and eavesdropping. With this shift in emphasis, analytical tools and metrics from graph theory have become popular in the animal behavior literature (see reviews by Krause et al. 2007, Wey et al. 2007, McGregor 2005). For example,

Lusseau & Newman (2004) used Betweenness Centrality and Assortativity to identify alliances within a dolphin social group, and Sundaresen et al. (2006) used Clustering Coefficients to show that Grevy's zebras form tighter social cliques than do onagers. As described by Krause et al. (2007), these metrics offer insights into the roles played by specific individuals in social groups, and the importance of overall social structure to the transmission of information and disease. However, as cautioned by Wey et al. (2007), animal behavior data differ from the data traditionally envisioned by the modern developers of graph theory metrics in ways that may influence estimates and interpretations. Here, we use computer simulation to test the ability of graph theory metrics with behavioral data.

Animal behavior data and the sociological data for which graph theory metrics were developed differ in many aspects of sampling design and community features. For example, animal behavior studies typically include hundreds or thousands of records of social interactions and tens to hundreds of individuals (e.g., 84 individuals, 45 grooming and 29 play data points in Flack et al. 2006, 65 dolphins and 159 data points in Lusseau 2003) whereas most modern graph theory studies typically have several hundred individuals and tens of thousands of data points (e.g., 614 papers and 1036 unique authors in Börner et al. 2005; 16,881 email addresses and 57,029 email messages in Newman et al. 2002). This order-of-magnitude difference can influence both the performance of these metrics and our understanding of their usefulness. Community features such as group size can also change unpredictably from day to day as animals die or migrate. Although changes in group size of 10-50 individuals are relatively small and insignificant in the context of most sociological studies that use graph theory to study communities of tens of thousands of people, they can make a big difference in behavioral research.

Several graph theory metrics have been popular in the recent behavioral literature (see Wasserman & Faust 1994 and Wey et al. 2007 for reviews). We focus on the representative

metrics in Table 1 because of their straightforward interpretation, their likely utility to studies of animal social behavior, and because of the ready availability of calculating software (e.g., Borgatti et al. 2002, Batagelj & Mrvar 1998). We begin by constructing a graph from a list of dyadic interactions, with dots representing individual animals, lines connecting individuals between which interactions have been observed and weights representing interactions frequency (e.g., Fig. 1). Individuals that dominate the network by having numerous and crucial interactions are defined as gatekeepers, and can be identified statistically by Centrality metrics such as Degree, Closeness, Betweenness and Information Centralities (Table 1). To obtain group centrality, we first estimate individual centralities and then calculate a form of variance across Centralities to describe the relative proportion of gatekeepers in that social group as a whole and the relative degree to which information flow is constrained by a small number of individuals. We consider also Diameter, Average Degree, Density, and Clustering Coefficient (Table 1). Although these metrics were designed to describe different aspects of social dynamics, they may also be influenced by the number of gatekeepers in a group and by the unique characteristics of animal behavior data.

Previous studies of network theory metrics have emphasized the effects of missing data on Centrality estimates, showing repeatedly that these give roughly similar values even when a subset of the individuals in the group have been sampled (e.g., Wey et al. 2007, Galaskiewicz 1991, Costenbader & Valente 2003, Frank 2002). Our simulation approach is rather different than that of these earlier studies because we begin with a scenario more typical of animal behavior research in which all individuals in a smaller group have been observed repeatedly. We then consider the ability of each metric to infer social dynamics when critical interactions may have been missed because of limited observation time, with small and sometimes variable group size (e.g., because animals move in and out of the group seasonally), and with groups that vary in amount of information flow (variable number of gatekeepers). By varying these three attributes

(number of gatekeepers, number of interactions recorded and group size) in our simulations, we sample a broad array of situations confronted by behavioral researchers, explore similarities and differences among metrics across these conditions, and make practical recommendations about which metrics are best combined.

METHODS

To begin each simulation, we started with a list of individual animals interacting in a social group (a = number of animals in the group), with a subset of individuals serving as gatekeepers (g = number of gatekeepers in the group). To create a broad range of social settings, we allowed gatekeepers to interact with both gatekeepers and non-gatekeepers, whereas non-gatekeepers interacted only with gatekeepers. Thus gatekeepers were also more likely to be sampled than were non-gatekeepers. More specifically, for each dyadic interaction, we chose two individuals from among the available actors at random with replacement obtaining a weighted or frequency network. If the interaction was between two gatekeepers, we included it in our set of observed interactions. If the interaction was between two non-gatekeepers, we discarded it and chose two new individuals at random. If the interaction was between a gatekeeper and a non-gatekeeper, we included it as an observation 50% of the time (in sensitivity analyses, we confirmed that the general patterns highlighted in the figures below did not change when we used 25%, 75% or 100% instead). We continued this process until we had generated a specified number of interactions, representing the number of dyadic interactions recorded by a human observer (n = number of observed interactions).

We varied also the three main variables, a , g and n , as follows:

1. Social dynamics, as summarized by variation in the number of gatekeepers (g). - We varied the number of gatekeepers from 1 to the total number of animals (e.g., 1, 2, 3, 4, 5, 10, 15, 20, 30, 40 and 50) for two typical group sizes ($a = 20$ and $a = 50$) and two typical number of total interactions recorded ($n = 100$ and $n = 500$). With the above simulation procedure, when all animals are gatekeepers, all animals are equally likely to interact with all other animals in the group with no constraints on information flow.

2. Sampling effort, measured as total number of dyadic interactions observed (n). We created artificial data sets with different numbers of observed dyadic interactions ($n = 50, 100, 150, 200, 250, 350, 450, 800$), holding group size and the number of gatekeepers constant. Specifically, we tested two typical group sizes ($a = 20$ and $a = 50$), and two representative levels of gatekeepers: the situation in which all animals interacted with all other animals in the group ($g = a$) and when half of the animals in the social group were designated as gatekeepers ($g = a/2$).

3. Group size or total number of actors (a). – We varied the group size or total numbers of actors (4, 10, 20, 30, 40, 50), considering two typical values for number of interactions ($n = 100$ and $n = 200$) and social dynamics ($g = a$ and $g = a/2$).

For each of the 94 above scenarios, we repeated the simulation process 100 times, calculating the complete set of statistics for each run and then examining distributions of each statistic (Table 1) using an R script (R developmental team, 2006) developed using *sna* and *igraph* libraries. Dyadic interactions were formed with the aid of a Perl script and Java routines created for this purpose and following the above mentioned guidelines for each simulation. We packaged the necessary software into SocANet which is publicly available on-line (Vital & Martins 2007).

For comparison across metrics, we focused on the 35 runs varying g and n for groups of 50 animals ($a = 50$). For these 35 runs, we calculated Pearson product-moment correlations between the average values of different metrics as a measure of the effect size of the pair-wise

relationships between these metrics. With a sample size of 35 runs, differences of 0.3 in the magnitude of correlation coefficients are statistically significant at the $\alpha = 0.05$ level.

RESULTS

Social Dynamics (number of gatekeepers, g)

As expected, most of the tested metrics varied with the number of gatekeepers (g) and provided rough estimates of the proportion of individual animals that enhance information flow by interacting freely with others in the social group (e.g., Fig. 2). In general, Centrality metrics (lines with symbols) tended to decrease, whereas Density and Clustering Coefficient (lines without symbols) increased with increasing gatekeepers. Diameter and Average Degree varied only slightly, if at all, with the number of gatekeepers. Because these metrics were not designed to estimate the number of gatekeepers and do not appear to provide useful estimators for this aspect of social dynamics, we do not consider them further, except when comparing metrics (below).

Betweenness Centrality dropped steeply with increasing but small number of gatekeepers (Fig. 2, open circles). Other measures of centrality (Degree and Closeness) declined more steadily with number of gatekeepers. Note that Information Centrality (open triangles) is measured on a different scale from other measures of Centrality and so its rate of decline cannot be compared directly. Although designed to estimate potentially different properties of the social group, Density and Clustering Coefficient increased steadily with number of gatekeepers, providing estimates that were comparable, albeit inversely related, to Degree and Closeness Centrality (Fig. 2).

Sampling Effort (number of interactions recorded, n)

The number of interactions recorded (n) had an impact on most network theory metrics (e.g., Fig. 3), especially when sampling was poor. Most Centrality estimates initially increased with increasing sampling effort (left side of Fig. 3), but then dropped off to a more stable value with more than 10 interactions / animal ($n \geq 10 a$). Density and Clustering Coefficient showed a reversed, but similar pattern, increasing to more stable values with more than 10 interactions / animal. Information Centrality (which again is not constrained to fall below 1.0) did not reach a comparable stable point, increasing steadily with sampling effort (Fig. 3).

Group Size (number of actors, a)

Most metrics also varied with increasing number of animals in the social group under at least some conditions (e.g., Fig. 4). Centrality metrics were somewhat less sensitive to an increasing number of group members if all individuals interacted freely with each other ($g = a$, results not shown). For example, Closeness and Degree Centralities remained near or below 0.2 for groups with 20-50 freely interacting individuals. However, when not all individuals interacted freely (e.g., Fig. 4), network metrics were more variable. For example, when only half the animals in the group interacted freely ($g = a/2$), Closeness and Degree Centralities ranged between 0.3 and 0.8 for groups with 20-50 animals (Fig. 4). Density and Clustering Coefficient varied between 1.0 to 0.1 across the same range of group sizes. Betweenness and Information Centrality were somewhat less variable than other metrics in an intermediate range (between 10-40 animals, e.g., Fig. 4). Interestingly, Density and Clustering Coefficient estimates were identical to each other when all of the animals were gatekeepers ($g = a$, results not shown), but different otherwise (e.g., Fig. 4).

Comparing Metrics

Values of Degree and Closeness Centralities were tightly linked to each other ($r = 0.99$), as were values for Density and Clustering Coefficient ($r = 0.96$), and hence equally associated

also with other network metrics (Table 2). Betweenness Centrality was often similar to Degree and Closeness Centralities ($r = 0.8$), whereas Information Centrality was substantially different, showing no association at all with Betweenness Centrality ($r < -0.1$), and only moderate association with Degree and Closeness Centralities ($r = 0.4$). Density and Clustering Coefficient were negatively associated with Betweenness Centrality ($r = -0.6$), but only loosely related to Degree and Closeness Centralities ($r = -0.3$). Average Degree and Diameter, which did not vary with the number of gatekeepers (above), were negatively associated with each other ($r = -0.7$). Average Degree was most closely associated with Information Centrality ($r = 0.9$), but was also moderately associated ($r > 0.7$) with Density and Clustering Coefficient. Diameter was negatively associated with all of the other measures, and showed a tighter relationship with Degree and Closeness Centralities ($r = -0.6$). Note that with a sample size of 35 runs, all of the above correlation coefficients > 0.3 are significantly different from zero at the $\alpha = 0.05$ level.

DISCUSSION

Our results lead to several practical recommendations about the application of social network metrics to the study of information flow through small animal social groups. First, we confirm that Centrality, Density and Clustering Coefficients vary with the number of gatekeepers and are potentially useful to behavioral studies considering this aspect of social dynamics. All of these statistics worked well even when applied to relatively small and poorly-sampled, social groups typical of animal behavior studies. Only Average Degree and Diameter did not vary with the number of gatekeepers (again, not surprising given that they were not designed to measure this aspect of social dynamics). Second, we found that social network metrics depend critically on the extent of sampling, varying with the number of recorded observations. Fortunately, most reached a relatively stable value once the group had been adequately sampled (at least 10 recorded

interactions/animal). Metrics were also sensitive to variation in group size (total number of animals, a), showing the full range of possible values for groups between 20 and 50 animals. Although we expect centrality statistics (which are calculated as variances of individual values) to scale to some extent with sample size, our simulations suggest that the variation may be so large that the metrics are not particularly useful for studying animal groups with variable or shifting group composition. Finally, our results find considerable similarities between metrics that lead to practical recommendations about which combination of metrics may be most useful. Further studies are needed to confirm whether similar combinations are useful in studying other aspects of social dynamics.

Our results confirm that social network metrics can be used to shed light on complex social behavior, even with the small social groups typical of behavioral studies. When Flack et al. (2006) removed “policing” macaques (those that intervened in the conflicts of others), they found decreased willingness to interact with socially-distant animals by estimating Clustering Coefficient from 235 observations of 84 macaques. Similarly, Lusseau & Newman (2004) used Centrality to infer that bottlenose dolphins may be assorting by sex and age, they estimated network statistics based on 1292 observations of 64 dolphins. Our study confirms our initial concerns, but finds that Centrality metrics, Clustering Coefficient and Density can be effectively applied also to dozens of observations from small social groups (5-50 animals). Specifically, our results suggest that a sample of about 10 recorded interactions per animal are enough to obtain consistent values of most network statistics. We have a similar caveat about applying those statistics to groups of variable size. When animals move in and out of the group seasonally, changes in network statistics may reflect differences in the overall size of the group rather than shifts in group dynamics. Using SocANet (Vital & Martins 2007), researchers can explore the effects of sampling and group size on metrics for a particular research design, and generate simulated data for randomization tests of specific hypotheses.

Betweenness Centrality may be the best Centrality metric to distinguish between groups with relatively few gatekeepers. Others have shown that Betweenness Centrality performs better than both Closeness and Degree Centralities in identifying important individuals in a social group (Freeman 1979), and that it conveys the most unique information (Bolland 1988). However, Closeness Centrality is more robust to random errors than is Betweenness Centrality (Bolland 1988). In our study, Betweenness Centrality was effective primarily at distinguishing fine-scale differences between groups with very few to small numbers of gatekeepers (up to 20% of the animals in the group), whereas other metrics were better able to distinguish between social groups with larger proportions of gatekeepers.

Similarities between metric values estimated for different behavioral situations further suggest that a well-chosen combination of measures may provide the best description of any single social group. Specifically, we recommend that in addition to Betweenness Centrality, metrics be chosen from more than one of the following groups: a) Closeness or Degree Centrality, b) Density or Clustering Coefficient, and/or c) Diameter, Average Degree or Information Centrality. Only a combination of these metrics offered a complete picture of the variation in social dynamics. Most recent papers make use of several metrics of graph theory as suggested. For example, Croft et al. (2005) studied the social network of fish with Clustering Coefficient, Average Degree. Corner et al. (2003) studied the disease transmission in brushtail possums using Closeness Centrality and Flow-Betweenness Centrality which is similar to Information Centrality studied here.

Finally, we note that social network metrics and our own emphasis on gatekeeper roles ignore and potentially obscure many important behavioral details. Individual animals change their roles in a social group with age (e.g., Seid & Traniello 2006), learning (e.g., Katsnelson et al. 2007), and even vulnerability to predation (Mathot & Giraldeau 2008). We need additional studies to determine the conditions under which the overall structure of a social group (e.g., relative number

of gatekeepers) is maintained, and to identify the levels at which comparisons of overall group dynamics are most useful. Average Degree and Diameter were not sensitive to variation in the number of gatekeepers in our study, and may provide better estimates of other aspects of social dynamics. Additional simulation studies considering the ability of network statistics to infer other aspects of social dynamics are needed.

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Table 1. Summary of metrics used to describe network dynamics. A social group is defined in terms of individuals ($i = 1, 2, \dots, a$) and a set of connections representing observed interactions between pairs of individuals (Fig. 1). See Wasserman & Faust (1994) for further details (e.g., estimators for standard errors) on all metrics except clustering coefficient (Watts & Strogatz 1998).

CENTRALITY			
	Actor	Group	Description
Degree	$CD_i = d_i$	$CD = \frac{\sum_{i=1}^a (Max[CD_i] - CD_i)}{(a-1)(a-2)}$	Emphasizes the activity level of an individual (total number of interactions per animal). Where d_i and a are as above.
Closeness	$CC_i = \left[\sum_{j=1}^a x_{ij} \right]^{-1}$	$CC = \frac{\sum_{i=1}^a Max[CC_i] - CC_i}{[(a-1)(a-2)]/(2a-3)}$	Emphasizes the minimum distance between individuals in the social group. Where x_{ij} is the number of connections required to travel between animals i and j , and CC_i is the Closeness Centrality for individual i .
Betweenness	$CB_i = \sum_{j < k} P_{jk}(i) / P_{jk}$	$CB = \frac{2 \sum_{i=1}^a Max[CB_i] - CB_i}{(a-1)^2(a-2)}$	Measures the fraction of all shortest paths connecting any two individuals on which individual i resides. Where p_{jk} is the total number of paths linking actors j and k , and $p_{jk}(i)$ is the number of those paths that also include actor i .
Information	$CI_i = \frac{1}{c_{ii} + (T - 2R) / a}$	$CI = \sum_i CI_i$	Focuses on the information contained in all paths originating with a specific actor. We begin with a matrix, C , describing the frequency of dyadic interactions. Element c_{ij} describes the interaction between individual i and itself, T is the trace of the C matrix ($\sum c_{ii}$ for all animals in the social group), and R is the row sum of the C matrix ($\sum c_{ij}$ for all animals; R is equal for all i).
COHESION			
	Formula		Description

Diameter	$DI = \text{Max} [\varepsilon_i]$	Measures the shortest distance between the two furthest individuals in a social group. Eccentricity ε_i is the largest of the shortest distances between individual i and any other individual.
Average Degree	$DG = \frac{\sum_{i=1}^a d_i}{a}$	Average number of dyadic partners per individual, where d_i is the total number of dyadic partners for actor i and a is the total number of individuals in the social group
Clustering Coefficient	$T = \frac{1}{a} \sum_{i=1}^a \frac{2k_i}{d_i(d_i - 1)}$	Average probability that two neighbors (directly connected) of an individual will also be each other's neighbors. Where d_i is the total number of dyadic partners for actor i (as above), k_i is the number of interactions among those partners, and $d_i(d_i-1)$ is the maximum number of interactions that could exist between those partners.
Density	$DN = \frac{\sum_{i=1}^a d_i}{a(a-1)}$	Total number of dyadic partners given the total number of possible dyadic partners. Where d_i and a are as above and $a(a-1)$ denotes the total number of possible dyadic partners.

Table 2. Pearson product-moment correlation coefficients summarizing the magnitude of the relationship between graph theory metrics calculated for data simulated under 35 different conditions of varying number of gatekeepers, group size, and sampling effort. Relationships between Closeness and Degree Centralities and between Clustering Coefficient and Density were very strong ($r > 0.95$). All $|r| > 0.3$ are significantly different from zero at the $\alpha = 0.05$ level.

	Betweenness	Information	Clustering/Density	Degree	Diameter
Closeness/Degree	0.8*	0.4*	-0.3*	0.1	-0.6*
Betweenness	-	0.0	-0.6*	-0.3*	-0.3*
Information		-	0.5*	0.9*	-0.7*
Clustering			-	0.7*	-0.4*
Degree				-	-0.7*

FIGURE LEGENDS

Figure 1. Fictitious example of a social network consisting of 10 individuals to illustrate the calculation of eight network metrics. As explained in Table 1, Diameter is the longest minimum distance between two individuals (e.g., J to D = 5). Average Degree is the average number of lines emanating from each individual. Clustering Coefficient describes the average probability that the social partners of an animal (e.g., D, F, and A are social partners of E) are also each other's social partners. Density is the proportion of the total possible connecting lines that are actually observed in this data set. Low values of the four centrality measures indicate that a few gatekeepers (e.g., H) monopolize information flow, whereas other actors (e.g., I and J) are peripheral and not highly interactive with the rest of the network. Values in parentheses are one standard error.

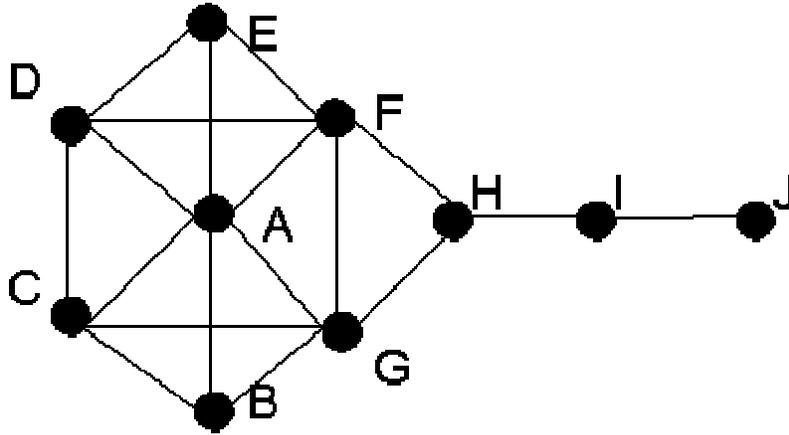
Figure 2. Social network metrics estimated for a well-sampled group of 20 individuals ($a = 20$, $n = 500$) with different numbers of gatekeepers controlling information flow through the group. For illustration, we divided Information Centrality values by the maximum for this set of simulations (6.7). Closed circles = Degree Centrality; closed triangles = Closeness Centrality, open circles = Betweenness Centrality, open triangles = Information Centrality, solid line = Density, dotted line = Clustering Coefficient.

Figure 3. Social network metrics estimated for a social group (20 animals, half of which are gatekeepers; $a = 20$, $g = 10$) with different numbers of recorded interactions (n). Symbols are as in Fig. 2. Information Centrality was divided by the maximum for this set of simulations (5.4).

Figure 4. Social network metrics estimated for social groups with different numbers of animals (a), when half of the animals are gatekeepers ($g = a/2$) and the group was relatively-well sampled ($n = 500$). Symbols are as in Fig. 2. Information Centrality was divided by the maximum for this set of simulations (7.4).

FIGURES

Figure 1



Network metrics	
Diameter	5.0
Average degree	1.6
Density	0.35
Clustering coefficient	0.48
Centrality	
Degree	0.38 (0.170)
Closeness	0.26 (0.010)
Betweenness	0.27 (0.001)
Information	0.16 (0.060)

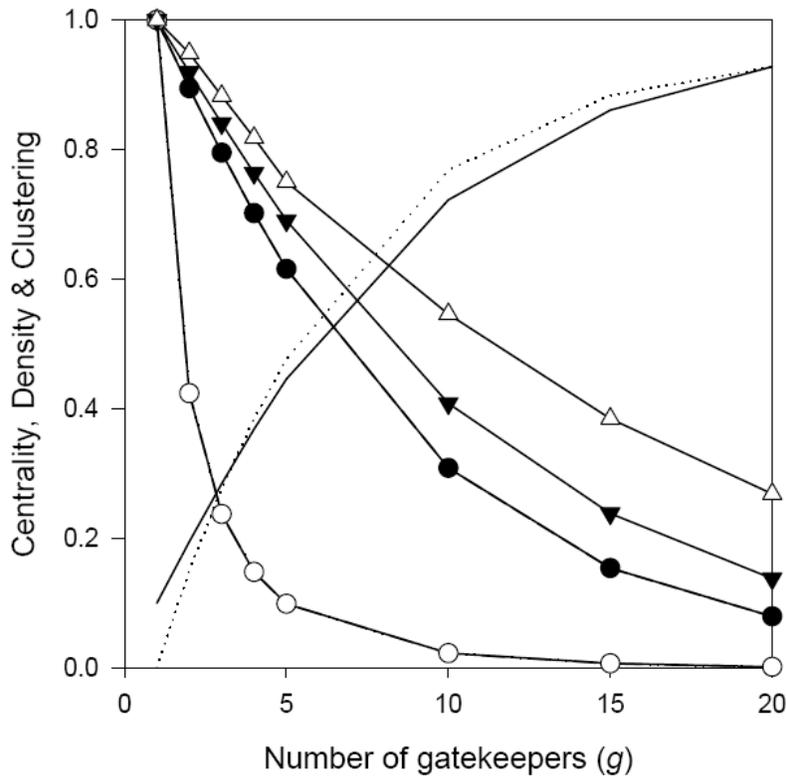


Figure 2. Social network metrics estimated for a well-sampled group of 20 individuals ($a=20$, $n=500$) with different numbers of gatekeepers controlling information flow through the group. For illustration, we divided Information Centrality values by the maximum for this set of simulations (6.7). Closed circles = Degree Centrality; closed triangles = Closeness Centrality, open circles = Betweenness Centrality, open triangles = Information Centrality, solid line = Density, dotted line = Clustering Coefficient.

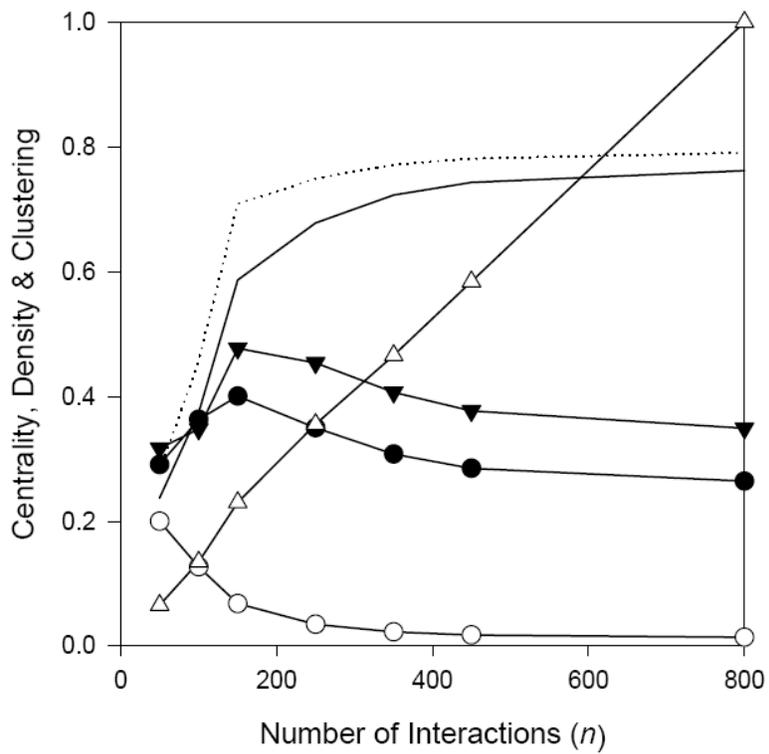


Figure 3. Social network metrics estimated for a social group (20 animals, half of which are gatekeepers; $a = 20$, $g = 10$) with different numbers of recorded interactions (n). Symbols are as in Fig. 2. Information Centrality was divided by the maximum for this set of simulations (5.4).

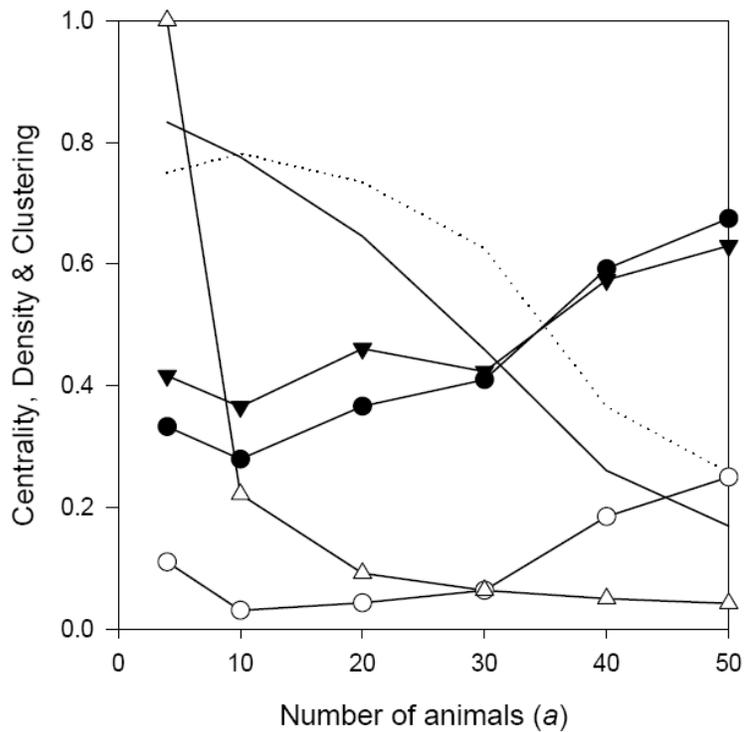


Figure 4. Social network metrics estimated for social groups with different numbers of animals (a), when half of the animals are gatekeepers ($g = a/2$) and the group was relatively-well sampled ($n = 500$). Symbols are as in Fig. 2. Information Centrality was divided by the maximum for this set of simulations (7.4).

CHAPTER 3: Strain differences on the effects of removal of individuals with different social roles on zebrafish group dynamics

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Abstract

Individual animals in a social group often interact in a non-random fashion creating complex social dynamics and structure. Some individuals, such as “Gatekeepers”-individuals that control access to resources through social interactions- may play especially influential roles in shaping group dynamics. Here we compare the social dynamics of two genetically-distinct strains of zebrafish and test the effect of removing individuals with specific social roles from zebrafish shoals. Although we found no difference between strains in the absolute level of group cohesion, we found a significant interaction effect showing that strains differed in group response to removal of a single fish. SH (scientific hatcheries) shoals reacted within minutes to recover group structure, exaggerating the difference in social dynamics between shoals from which gatekeepers vs non-gatekeepers had been removed. In contrast, PN (a recently wild-derived strain) shoals showed no change with experimental treatment and no difference between groups from which gatekeepers and non-gatekeepers had been removed. This result may be due, in part, to a sex difference. Nearly all PN identified as playing distinct social roles (gatekeepers and non-gatekeepers) were male, whereas we found no sex difference in SH fish in terms of the individuals adopting particular social roles. In addition, we found that measures of social dynamics were moderately consistent when taken up to three weeks apart. Our results confirm the importance of social roles in zebrafish shoals, and set the stage for future research into the genetic basis of group dynamics.

Keywords: group dynamics, Information Centrality, Average Degree, cohesion, zebrafish, gatekeepers

Animal group dynamics can be strongly influenced by the presence of certain individuals such as gatekeepers (Flack et al. 2006). Gatekeepers or keystones are individuals that control the flow of information and resources through social interactions (see Vital & Martins 2009 for further explanation and examples). Gatekeepers can play a key role in the arrangement of association patterns, and their removal from a social group can have dramatic impact. For example, removal of policing individuals from pigtail macaque groups decreased the average number of interactions experienced by individuals in the groups while emphasizing cliquishness – exclusiveness- and social elitism –selectivity- (Flack et al. 2006). In cowbirds females play an important role in association patterns; juvenile females are less discriminating than are adult females, such that groups including juvenile females exhibit higher closeness centrality – a pattern of direct and indirect connections allowing them to access all individuals in a group faster - than do groups of adult birds alone (Miller et al. 2008). In this study, we begin to explore the mechanisms influencing whether social roles are important by comparing the impact of gatekeeper removal on two genetically-distinct strains of zebrafish.

Group dynamics play an important role in the ecology and overall well-being of many social groups by influencing disease transmission, information transfer, social stability and alliance formation. For example, Guimaraes et al. (2007) recently showed that the vulnerability of killer whales to infectious diseases is influenced by group dynamics (e.g., average number of interactions, social distance between two individuals, and cliquishness). Social information transfer, which is key in foraging success and social learning, is influenced by group dynamics as well. In dolphin groups, the removal of highly interactive adult females (i.e., gatekeepers) increases group dispersion, and hence hinders information transfer by increasing the time and number of individuals through which information must travel (Lusseau 2003). In macaques,

removal of policing individuals, leads to more aggression thus risking society stability (Flack et al. 2006). Mechanisms that influence group dynamics thus can have a strong influence on many other aspects of group well-being.

Zebrafish are social fish that swim in small shoals (Wright et al. 2006) of 2-10 individuals (Pritchard et al. 2001), and have been used extensively as a model organism for genetic and developmental research. Zebrafish shoaling preferences are influenced by both genetic factors (e.g., Wright et al. 2003; Mann et al. 2003) and early social experience (Engeszer et al. 2004). Here we focus on zebrafish group dynamics and test the impact of removing individuals with special social roles (i.e., gatekeepers) on fish from two genetically-distinct strains. Gatekeepers increase group centrality by controlling information of resources. If gatekeepers play a unique role in a social group, we expect their removal to lead to decreased centralization and poor information flow through the new group. On the other hand, if gatekeepers are not particularly important to group health, their removal should not impact overall group dynamics.

METHODS

We used zebrafish subjects from two strains in this experiment. The Scientific Hatchery (SH) strain was established in the 1990s, whereas we collected zebrafish of the PN strain from their native habitat in West Bengal, India in 2007. In preliminary mtDNA studies, we have found no haplotypes that occur in both strains. We used mass breeding of lab-reared or wild-caught fish to produce lab-reared subjects for our experiment. Once eggs hatched, we housed juveniles in groups of 10-20 individuals, separating them into experimental groups of four as

they reached adulthood (about 4 months). We housed subject fish at all times in 18.9l aquaria under standard conditions (24 – 27°C, 13L:11D cycle, filtered and aerated water, with abundant food) and in single-strain groups. Because of a second experiment that we conducted on these fish immediately after this one (Vital, 2009), each tank was divided by a central plastic plate that left enough room at the bottom to allow fish to swim back and forth between the two sides.

We formed 20 experimental SH groups and 17 experimental PN groups of 4 fish each (two males and two females), size-matching to ensure that all fish in the same group were within 5 mm of each other in standard length. We visually isolated the groups from each other by placing opaque barriers between the aquaria. After two weeks of acclimation, we began behavioral trials. First, we marked each fish for temporary individual identification by painting with the antiseptic Dr. Blu-Kote (a temporary blue dye) on a different body part (e.g., caudal fin, dorsal fin). After 20 min to allow fish to recover from the painting procedure, we conducted one 1-min focal animal sample on each fish in the group, choosing the order of fish at random. To minimize stress, we conducted all behavioral observations from behind a black curtain blind. During each focal animal sample, we continuously recorded the identity of every fish that came within two body lengths of the focal individual (near-neighbor points), gathering at least 10 near-neighbor points for each subject fish in that one-minute sample, as recommended for social network statistics (Vital and Martins 2009).

We used these near-neighbor data to estimate Information Centrality (IC) using software written for this purpose (Vital and Martins 2007). Information Centrality measures the amount of information flow that is channeled through each individual in the social group, or as a combined group measure. In the context of our study, the individual with the highest individual information centrality was the fish that remained in close proximity to other fish in the group.

We follow standard practice by referring to this individual as a “gatekeeper” because they may control the flow of information across the group. We also identified the individual with the lowest individual information centrality -- the fish that was least likely to be within two body lengths of other fish in the group, or which remained near only a single other fish. We refer to this second individual as the “non-gatekeeper”.

Once identifying a single “gatekeeper” and “non-gatekeeper” for each group, we used a net to remove the individual with the highest IC value (i.e., gatekeepers) from half (10 SH, 7 PN) of the experimental groups. We removed the individual with the lowest IC value (non-gatekeepers) from the remaining experimental groups. We then conducted a second series of 1-min focal animal samples, using the resulting near-neighbor data to estimate group levels of Information Centrality (IC) and other social network statistics of each group immediately after experimental treatment. To get an estimate of consistency and repeatability, we repeated the assay one week and then again two weeks later.

We used two-way MANOVAs to test the effects of strain and experimental treatment (whether the removed fish was a gatekeeper or non-gatekeeper) on social dynamics immediately after group manipulation. Second, we used repeated measures ANOVA to test for repeatability of metrics over time. With each model, we used residual analyses to confirm that the usual normality and homoscedasticity assumptions were not violated. As explained in Vital and Martins (2009), not all network statistics are useful for behavioral data given the frequently small group sizes and numbers of observations. We focus below on Information Centrality (IC) and Average Degree (AD), the only two parameters that were variable in our experiment (see Vital & Martins 2009 for a list of metrics used). These two metrics are often negatively related

to each other, with IC describing how tightly a social group is linked, and AD estimating the distance between group members.

RESULTS

No strain differences in baseline social dynamics

Considering only the baseline data collected from the full group of four fish before experimental treatment, we found no significant differences between the two strains in group dynamics (Information Centrality: $t = 0.25$; $df = 35$; $P > 0.8$; Average Degree: $t = 1.1$; $df = 35$; $P > 0.3$). Group Information Centrality (IC) averaged 0.2 for both strains ($SE_{PN} = 0.02$, $SE_{SH} = 0.03$). Average Degree (AD) was also very similar for the two strains with a mean value of 1.7 ($SE_{PN} = 0.09$, $SE_{SH} = 0.08$). As expected for the small group sizes in this study, estimates for most social network parameters were nearly identical for all groups and experimental treatments in this study. Specifically, average Betweenness Centrality = 0.12 ($SE = 0.02$), Clustering Coefficient = 0.78 ($SE = 0.01$), Diameter = 1.89 ($SE = 0.05$) and Density = 0.83 ($SE = 0.01$), regardless of strain.

Estimates were slightly lower after experimental removal than they were in the baseline trials (compare above to Fig. 1a), as expected simply from measuring centrality from three rather than four fish and the consequent decrease in opportunities to interact.

Strains react differently to removal of individuals with different social roles

We found that the two strains of zebrafish reacted differently to removal of group members playing particular social roles (Fig. 1). The social role of the removed fish had a profound impact on social dynamics of groups composed of SH, but not PN fish, resulting in a significant strain x treatment interaction effect in a MANOVA that combined IC and AD (Table 1). SH groups from which the most socially-interactive individual (gatekeeper) had been removed exhibited the highest centrality (Fig. 1a) and lowest average degree. These groups appear to have restructured quickly, with a new fish taking on the role of gatekeeper. Similarly, SH groups from which the least socially-interactive individual (non-gatekeeper) was removed exhibited the lowest centrality and highest average degree (Fig. 1), having become less centrally-focused than before. This difference also led to a significant effect of experimental treatment (i.e., the social role played by the fish that was removed, Table 1). In contrast, the social dynamics of PN groups did not shift with the experimental removal of a group member, regardless of the social role played by that group member. Because the social measures of PN groups were intermediate to those for the two SH treatment conditions, we did not find a significant difference between strains tested as a main effect (Table 1).

The strain difference was confounded, in part, by a sex difference. The removed individual (gatekeeper or non-gatekeeper) was identified by the higher or lower IC value and it was a male in 15 of 18 PN groups, but only 6 of 19 SH groups. Focusing on possible sex rather than strain differences, the social role of the removed individual (gatekeeper or non-gatekeeper) had a somewhat stronger impact if that individual was a female, as opposed to a male (e.g., Fig. 2b), leading to a significant sex x treatment interaction effect on AD (Table 2; results for CI were qualitatively similar, but not statistically significant). Groups were more tightly linked after a female non-gatekeeper had been removed than when a female gatekeeper was removed. In

males, the treatment effect was less dramatic, but in the same direction, contributing to a significant treatment effect on both AD and CI (Table 2). We did not detect a significant sex difference (Table 2).

Repeatability

We found that measures of group dynamics were moderately consistent within two weeks of the experiment. The correlation between measures taken 1-2 weeks apart was moderately high for both IC ($r=0.43-0.6$) and AD ($r=0.51-0.63$) for the three time periods (Table 3). We found no time effect (Wilk's λ , $F=1.91$, d.f. =2,13, $p=0.19$) or strain X time interaction effect ($F=1.06$, d.f. =4,26, $p=0.40$) for IC values measured across three weeks suggesting that Information Centrality is repeatable across time. Even though AD values had higher correlation coefficients than did IC, we did find a significant effect of time (Wilk's λ , $F=6.51$, d.f. =2,13, $p=0.011$) and a time X strain interaction ($F=2.64$, d.f. = 4, 26, $p=0.057$) for this metric, suggesting that absolute values of Average Degree may shift over time.

DISCUSSION

Although we found no significant differences between the two strains in baseline group dynamics, we found a dramatic difference in their response to experimental manipulation. Zebrafish from the domesticated strain (SH) reacted more profoundly to the treatment than did fish from the more-recently-derived strain (PN). Artificial selection appears to have created SH fish that shift social roles quickly, thereby maintaining a stable social hierarchy as indicated by a

higher IC value. The SH strain has been maintained through many generations in high densities comparable to those found in fish hatcheries, and stable social hierarchies may be particularly important for fish in high social densities. SH groups were also more likely to have females with distinct social roles (gatekeepers or non-gatekeepers), whereas PN groups were more likely to have males playing distinct social roles. Thus, domestication may also have acted through a sex difference, making it more likely that females, rather than males, manage information flow.

Major strain differences in zebrafish behavior suggest that there may be genetic differences underlying recent behavioral shifts, and support the use of comparative studies to understand the genetic mechanisms underlying behavioral production and evolution. For example, Robison and Rowland (2005) found strain differences in startle response and surface orientation that appear to be the result of artificial selection on domesticated laboratory zebrafish. Similarly, Wright et al. (2006a) found several genetic regions associated with variation in anti-predator behavior in a QTL cross between a domesticated and recently-derived wild strain. Additionally, age and sex structure found in domesticated strains is more uniform than wild populations (Wright et al. 2006b). Strain differences in simple measures of zebrafish aggression, activity level, and shoaling tendency are also profound (Moretz et al. 2007a). Although Moretz et al. (2007b) describe changes in zebrafish aggression with recent social context, relative strain differences in aggression persisted even after manipulation, and other behavior was completely unaffected by social context. The current study suggests that although there may not be measurable strain differences in zebrafish group structure, different strains respond differently to social manipulation and that zebrafish may provide a useful tool for further studies of the genetic basis of shifting social roles.

Differences in response to females removed but not males suggest that the sex of individuals is key in the social role they play in zebrafish shoals. Social preferences based on sex and phenotype have been demonstrated before (Croft et al. 2004); our results however, suggest that phenotype (especially sex) plays a role in the influence of social roles and group dynamics as well. For example stable female dyads are key to the stability of group dynamics in guppies (Croft et al. 2006) but not so much male dyads. Other characteristics such as harassment level of males influence female centralization in guppies groups. Female guppies in groups with high levels of male harassment are less centralized than groups with lower levels of harassment (Darden et al. 2009). The effect of male dolphins' actions on the group is influenced by the level of knowledge it has about travel times (Lusseau and Conradt 2009). Our results support the idea that the influence of different social roles is dependent on the individual phenotype and calls for more studies about the influence of social roles and phenotypes.

Together these results suggest that genetics differences underlie a recent behavioral shift. There might not be strain differences at the group dynamics level however, different strains respond differently to social manipulations. Additionally our results suggest that the importance of different social roles is dependent upon the individual's phenotype.

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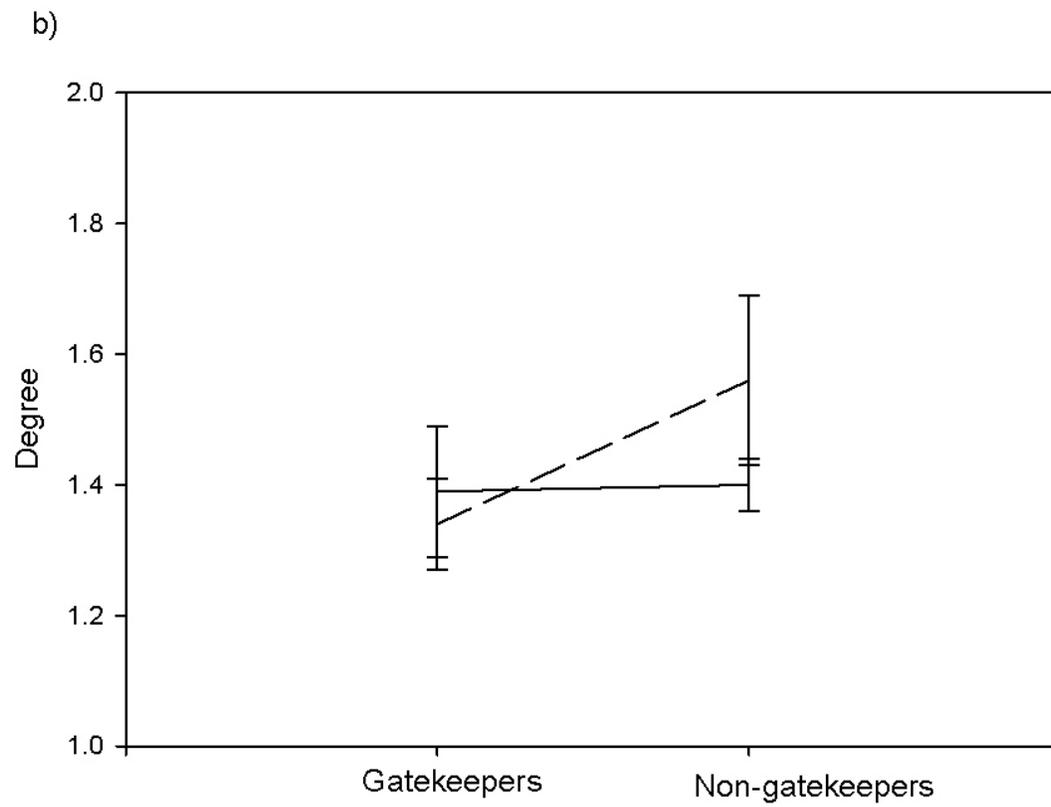
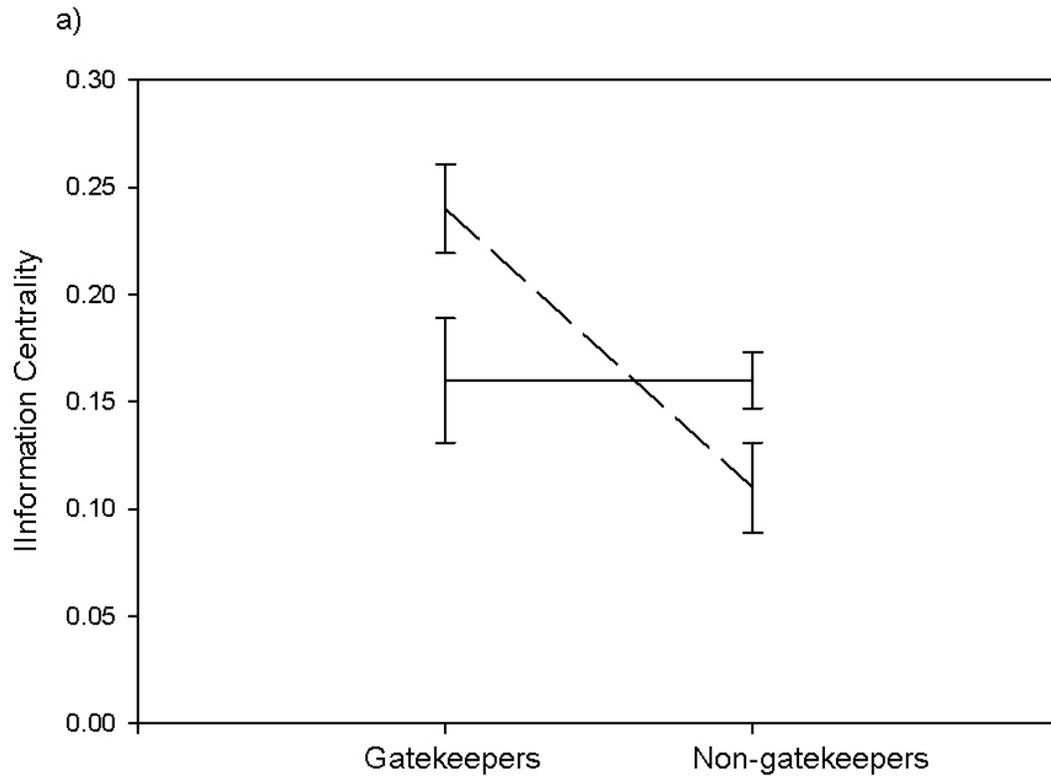
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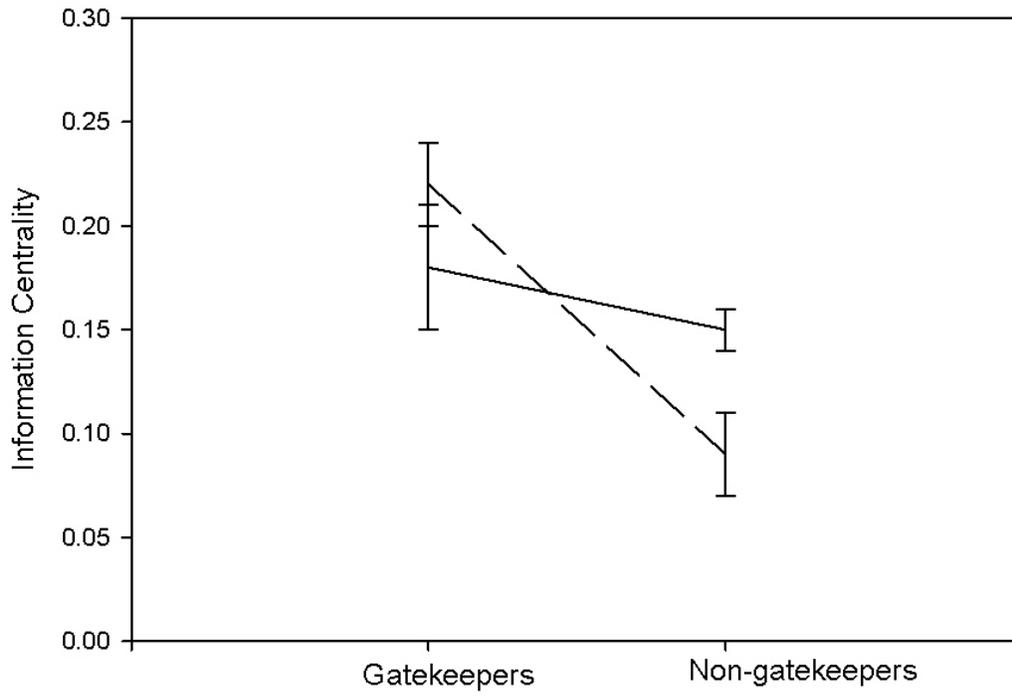
FIGURE LEGENDS

Figure 1. Strain effect on the removal of individuals with different social roles. Mean values of Information Centrality (a) and Average Degree (b) for zebrafish groups a few minutes after experimental removal of one fish with an identified social role. G are groups from which the individual with the highest centrality (gatekeepers) had been removed, whereas NG are groups from which the individual with the lowest centrality (non-gatekeepers) had been removed. Dashed line indicate groups from the SH strain, whereas the solid line marks groups of fish from the PN strain. SH groups had the highest centrality when the gatekeeper had been removed, and the lowest centrality when a non-gatekeeper had been removed, indicating that within minutes another fish had taken on and emphasized the social role of the removed individual. PN groups showed no difference between treatment groups, leading to a significant strain x treatment interaction effect as well as a significant treatment effect (Table 1).

Figure 2. Sex effect on the removal of individuals with different social roles . Mean values of Information Centrality (a) and Average Degree (b) for zebrafish groups a few minutes after experimental removal of one fish with an identified social role. G and NG are “gatekeepers” and “non-gatekeepers”, as in Figure 1. Dashed line indicate groups from which a female fish (gatekeeper or non-gatekeeper) was removed, whereas the solid line marks groups from which a male fish was removed. Groups from which a female was removed had the highest centrality (lowest average degree) when the female was a gatekeeper and the lowest centrality (highest average degree) when the female was a non-gatekeeper. This led to a nearly significant sex x treatment interaction effect (Table 2).



a)



b)

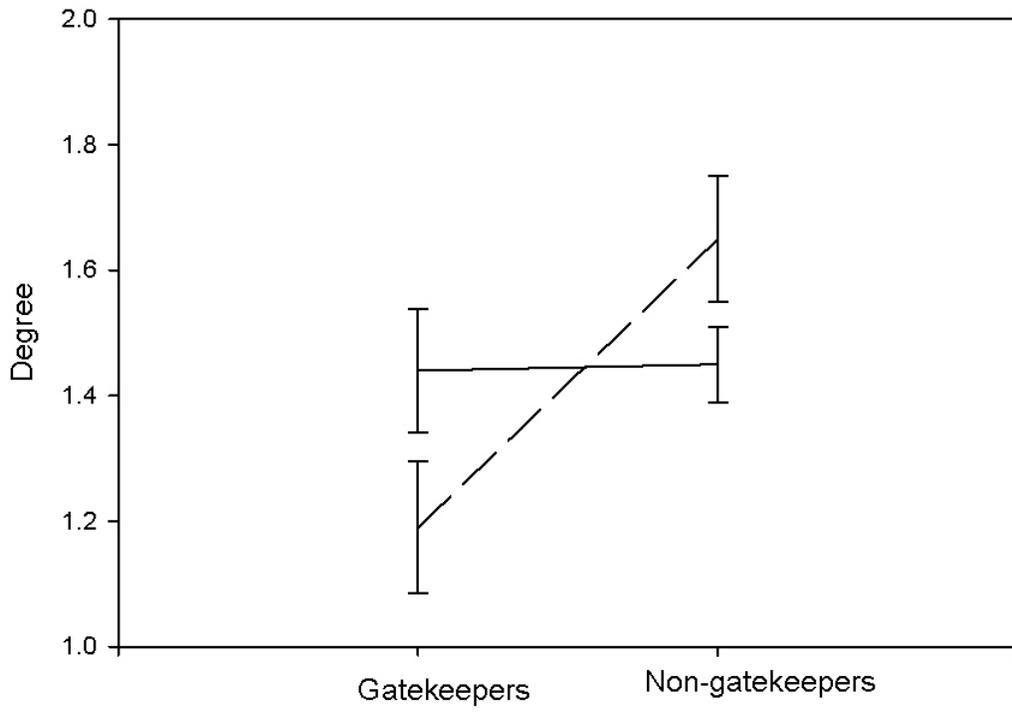


Table 1. Sum of squares tables considering effects of strain (SH and PN) and social role (experimental removal of gatekeeper or non-gatekeeper) on social dynamics. Degrees of freedom = 1, 33 for ANOVA and 2, 32 for MANOVA. * indicates p values for factors that explain a significant proportion of the variance.

	MS	F	p
MANOVA (df = 2, 32):			
Strain		0.68	0.51
Social Role		3.70	0.04*
Strain x Social Role		3.38	0.05*
Information Centrality:			
Strain	0.003	0.64	0.43
Social Role	0.036	7.53	0.01*
Strain x Social Role	0.033	6.93	0.01*
Average Degree:			
Strain	0.025	0.30	0.59
Social Role	0.126	1.54	0.22
Strain x Social Role	0.101	1.23	0.28

Table 2. Sum of squares tables considering effects of sex (male or female) and social role (gatekeeper and non-gatekeeper) of the single fish removed from the group on subsequent social dynamics, combining data from both strains. Degrees of freedom = 1, 33 for ANOVA and 2, 32 for MANOVA. * indicates p values for factors that explain a significant proportion of the variance.

	MS	F	p
MANOVA:			
Sex		0.12	0.88
Social role		4.63	0.02*
Sex x social role		2.72	0.08
Information Centrality:			
Sex	0.001	0.11	0.74
Social role	0.038	7.82	0.01*
Sex x social role	0.015	3.04	0.09
Average Degree:			
Sex	0.004	0.05	0.82
Social role	0.367	5.08	0.03*
Sex x social role	0.328	4.54	0.04*

Table 3. Pair-wise partial correlation coefficients (r) between measures of social dynamics taken one week apart, as estimated from a repeated-measures ANOVA that includes also strain as a factor. P-value for test of whether each correlation coefficient differs from zero is given in parentheses.

	week 1	week 2	week 3
Information Centrality			
week 1	--	0.48 (0.06)	0.62 (0.01)
week 2		--	0.49 (0.06)
Average Degree			
week 1	--	0.40 (0.13)	0.73 (<0.01)
week 2		--	0.42 (0.12)

CHAPTER 4: Social Roles and Foraging Task Performance of Zebrafish Groups

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Author note

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Abstract

Individual animals in a social group often work together to find food and evade predators. In some groups, animals play different social roles, with some individuals appearing to be more important in determining overall group structure and performance. Here, we asked whether the presence of “gatekeepers” facilitates the ability of zebrafish groups to find and attain food. We identified gatekeepers as individuals that readily engaged in social interactions with all other group members and distinguished them from “non gatekeepers” or individuals that interacted only with a small subset of other animals. We then experimentally removed gatekeepers or non-gatekeepers from established groups and trained the reduced shoals in a simple foraging task. We found that the presence of gatekeepers facilitated group performance – groups that retained their gatekeepers performed better than did groups from which gatekeepers had been removed. Additionally, we found significant learning differences between fish from two genetically-distinct strains, with groups from one strain (SH) learning the task significantly more quickly than did groups from a second (PN). These results confirm the importance of social roles to zebrafish groups and set the stage for future research into the genetic basis of social roles and group learning.

Keywords: Group performance, gatekeeper, information centrality, zebrafish

In some animal groups, individuals adopt particular social roles that can impact overall group dynamics (Sih et al. 2009), and the ability of the group to remain together, find food and avoid predators effectively. For example, in pigtailed macaques, policing individuals influence grooming sessions, playing and proximity of their group, and their social role stabilizes the group by lowering the number of aggressive incidents (Flack et al. 2006). Highly connected dolphins play an important role in group learning, altering the number of other individuals through which information must travel in order to reach all the individuals in a group (Lusseau 2003). We recently showed that zebrafish, an important model organism for biomedical research, also form groups in which individuals take on distinct social roles (Vital 2009). Moreover, we found that the impact of experimentally removing a particular individual from the group depended on the social role played by the removed individual and on the genetic strain of zebrafish being manipulated (Vital 2009). Here we test the effects of social role and genetic strain on the group's ability to learn and to perform a simple associative task.

A group's ability to work together effectively depends on a number of factors, including social composition. For example, cowbird flocks with adult females and juvenile males exhibit different group dynamics than do groups with juvenile females, and these differences can influence male song development (Miller et al. 2008). As mentioned above, policing individuals in pigtailed macaques play a key role in maintaining group stability by promoting cooperation and grooming among individuals that would otherwise fight (Flack et al. 2006). Similarly in dolphins, adult females play an especially important role in keeping alliances connected and in maintaining connections with other groups (Lusseau & Newman 2004). Groups of Tonkean macaques readily found food by following previously-trained individuals to a food source

(Ducoing & Thierry 2004). Shy guppies improve foraging by forming shoals with more bold guppies, and vice versa (Dyer et al. 2009).

Some groups maintain stable group dynamics when individuals move in or out of the group by shifting the specific role played by the remaining individuals. For example, social rank of *Anolis* lizards depends on serotonergic activity, and can be easily reversed with behavioral experiences (Summers et al. 2005). In social insects, even though physiology also plays an important role in caste structure, age- and morphologically-determined castes ensure that overall group structure is stable (Robinson 2009). In our preliminary studies of zebrafish social structure, we found that group dynamics were remarkably stable from week to week (Vital 2009). In one strain, this stability was accomplished by individuals shifting social role within minutes after a disruption. In a second strain, stability was the result of less distinct social roles, with all fish in the group sharing the responsibility of information flow.

The stability of the social group and distinctness of social roles can also impact information transfer and the group's ability to learn or to perform a task that involves transferring information (Sih et al. 2009). Gregarious animals rely on social companions to acquire information about their environment (Galef & Giraldeau 2001). Some groups may be better able to find food or avoid predators because of the ways in which they pay attention to each other and transfer information across the group (e.g., Mirabet et al. 2008). Individuals can also learn from one another through observation, imitation, or modeling (Whiten & Mesoudi 2008). The individual identity and social role of both the demonstrator and student may be critical. For instance, whether a zebra finch learned the food preferences of a tutor depended on the sex of both individuals (Katz & Lachlan 2003). In coral reef fishes, groups are more likely to follow adult females to spawning aggregations (Kiflawi & Mazeroll 2006). In bees, group

performance is also influenced by experience (Hofstede & Sommeijer 2006) and population identity (Ings et al. 2005).

Zebrafish are social fish native to southeast Asia usually found in small shoals (Wright et al. 2006) in rivers, small streams, rice paddies, and lakes. Both pigment patterns and early social experience can play an important role in zebrafish shoaling preferences (Engeszer et al. 2004). Zebrafish are a popular model organism for genetics and developmental biology with huge potential for behavioral research (e.g., Guo 2003). Recent studies of zebrafish shoaling have focused on shoaling propensity (Wright et al. 2003), shoalmate choice (Mann et al. 2003), and techniques for studying zebrafish social preferences in a biomedical research context (e.g., Saverino and Gerlai 2008). Furthermore, learning studies suggest that zebrafish are capable of learning active avoidance (Xu et al. 2007), alternation tasks (Williams et al. 2002), color (Spence & Smith 2008) and social preferences (Engeszer et al. 2004). Zebrafish are also motivated by the sight of conspecifics to learn (Al-Imari & Gerlai 2008), and nicotine has been found to affect learning in zebrafish (Levin et al. 2006). Here, we examined the ability of zebrafish groups to perform a simple foraging task and their learning of that task over several days. Using fish measured also as part of an earlier study (Vital 2009), we tested the effects of genetic strain and social role by comparing groups that had been experimentally manipulated by removing either the “gatekeeper” (socially important individual that interacts often with all other members of the group) or a “non-gatekeeper” (socially unimportant individual that interacts only with a small subset of the group).

Method

We began with 37 groups of four zebrafish – two males and two females of roughly the same size) that had been formed and measured as part of an earlier experiment (Vital 2009). Seventeen of the groups were from a well-established lab strain (Scientific Hatcheries, or SH), and 20 other groups were of the more recently-established PN strain. Subjects were born and raised in the lab under standard conditions, being separated into groups of four as they reached adulthood (about 4 months). In the earlier study, we identified “gatekeepers” and “non-gatekeepers” using software specifically developed for this purpose (Vital & Martins 2007). We then removed one fish from each group (10 gatekeepers and 8 non-gatekeepers from SH groups and 7 gatekeepers and 11 non-gatekeepers from PN groups), and measured any resulting shift in social dynamics (Vital 2009).

In the current experiment, we extended this study by training the 37 experimentally-reduced groups of three zebrafish on a simple association task, modified from Williams et al. (2002). We attached a red card cue to one side of the aquarium (zebrafish see and react to colors such as red, (Brockerhoff et al. 1997). Over three days, we trained the reduced groups to associate the red card with food. Eight times each day, we tapped the glass on the upper-middle of the aquarium to attract the attention of the fish. Five seconds later, we dispensed 15 brine shrimp on the side of the aquarium marked by the red card and recorded the number of fish (0 to 3) present on that side of the aquarium (the “correct” side). We then chose whether to move the red card to the other side of the aquarium (at random to avoid a patterned sequence), and waited 30 min before conducting the next trial.

We used repeated-measures ANOVA to test the effects of strain and experimental treatment (whether the fish that was removed prior to the conditioning trials was a “gatekeeper” or a “non-gatekeeper”) on the proportion of the group that was on the “correct” side of the

testing arena at each of 24 trials. Because both males and females were experimentally removed, we tested also for sex differences in performance in similar repeated measures ANOVAs (replacing strain with sex as a factor). We also calculated Pearson product-moment correlations to estimate the magnitude of the relationship between performance (the average proportion of fish that were on the correct side across 24 trials) and measures of group cohesion (Average Degree and Information Centrality, as explained in detail in Vital 2009) across all 37 groups. All analyses were conducted in SAS (9.1.3).

Results

The overall performance of zebrafish groups was influenced by the social role of the fish that had been removed from the group immediately before training began (Fig. 1). Groups from which gatekeepers had been removed did not move much towards the marked card, being only slightly more likely to be on marked side of the testing arena as on the unmarked side (mean proportion of fish on correct side = $58\% \pm 0.04$). In contrast, groups that retained their gatekeepers (a non-gatekeeper was removed) were more likely to be on the marked side and ready to receive food (mean = $67\% \pm 0.03$). This led to a significant between-subjects effect of social role in the repeated measures ANOVA ($F = 4.4$; $d.f. = 1, 25$; $p = 0.045$; Table 1). We found no significant strain or strain x social role interaction effects on overall measures of group performance (Table 1, between subjects effects).

Zebrafish groups learned to move into the marked side of the testing arena over the three days of our training procedure (Fig. 2), as shown by a significant within-subjects effect of time in our repeated measures ANOVA ($F = 1.93$; $d.f. = 23, 575$; $p < 0.01$ with Huynh-Feldt correction; Table 1, within subjects effects). However, this effect was influenced by strain (Table 1, repeated

measures effects). Groups of the domesticated SH strain learned the task more quickly than did groups of fish bred from the more recently established PN strain (Fig. 3). In general, two of the three SH fish in the group had learned the task by day 2, whereas PN fish groups took three days to attain a similar performance level. This led to significant within-subjects effect of strain*time ($F=1.59$; $d.f.=23, 575$; $p=0.05$ with Huynh-Feldt correction) in the repeated-measures ANOVA (Table 1, repeated measures effects).

We did not find significant within-subjects effects of social role or role by time interaction effects (Table 1; $P>0.05$), suggesting that the social role of the removed fish influenced group dynamics and movement towards a food resource rather than learning. Similarly, we found no evidence that the sex of the removed fish impacted group performance or learning ($P>0.05$ in all cases, results not shown). Estimates of Information Centrality and Average Degree were not closely associated with group performance ($r = -0.28$ for IC, $r = -0.34$ for AD; $d.f. = 25$; $p > 0.05$, Table 2).

Discussion

Our results show that the removal of individuals with particular social roles (“gatekeepers”) can influence a zebrafish group’s ability to attain food resources and suggest a genetic basis to differences in group learning.

These results add to the growing body of evidence that metrics from social network theory can be used effectively to identify individuals (e.g., females, dominant individuals) that play critical roles in establishing and maintaining overall group dynamics (Krause et al. 2009; Wey et al. 2008). In theory, the removal of highly connected individuals should have a major impact on group learning because it alters the distance that information must travel in order to

reach all the individuals in a group (Lusseau 2003). Here we confirm empirically that zebrafish groups from which the gatekeepers have been removed were less able to move towards food than were groups from which non-gatekeepers had been removed. Gatekeepers appear to facilitate group performance, perhaps by encouraging the group to move together or by guiding the group more efficiently towards food. Future studies are needed to determine the actual mechanism by which they influence group performance.

Our study also found strain differences in zebrafish group learning. Specifically, we found that SH fish learned more quickly than did PN fish. SH is a highly domesticated strain that has been evolving via artificial selection in the laboratory environment for dozens of generations. In contrast, the PN strain was very recently established from wild fish. Thus, our results may be one more example of behavioral shifts that can be attributed to zebrafish adaptation to a captive environment. Domesticated zebrafish strains differ from recently-established strains in food intake, growth rate, aggression, recovery from disturbance, response to a startling stimulus, and shoaling tendency (Moretz et al. 2007a; Oswald & Robison 2008; Robinson & Rowland 2005). Other studies have found differences in how genetically-distinct zebrafish strains respond to a variety of stimuli including social experience (Moretz et al. 2007b) and alcohol exposure (Gerlai et al. 2008). Because zebrafish are a model organism for which the genome has been well described, these results offer an intriguing opportunity for future research into the genetic mechanisms underlying differences in zebrafish social behavior and learning.

Zebrafish have large brains and are capable of complex learning. Gerlach et al. (2008) showed that zebrafish can imprint on the smell of preferred conspecifics to which they were exposed only 6 days after fertilization. Williams et al. (2002) showed that zebrafish can associate food with a cue in about 14 trials (Williams et al. 2002). Associative learning is reinforced by

visual contact with conspecifics in zebrafish (Al-Imari & Gerlai 2008). Our study confirms that zebrafish also learn effectively in groups. By the third day of our training sessions, two of three fish in the group reliably moved to a color-coded side of the testing aquarium as soon as the observer tapped on the tank. This is a lower learning rate than found by (Williams et al. 2002), suggesting that small groups may learn less quickly than do individual fish, or are otherwise hampered from accomplishing the task as quickly. Future studies are needed to determine whether individuals in the group are learning less quickly or whether the stress to a social animal of performing a task as an individual improved learning.

In summary our results support the idea of different social roles and the importance of these in group performance. Specifically, the removal of individuals with a particular social role, gatekeepers, influences zebrafish group performance in attaining food. However, our results do not fully explain how gatekeepers facilitate this process of group learning. Furthermore, our results show that different zebrafish strains can learn at different rates suggesting an underlying genetic difference, this might have strong implications for zebrafish studies that utilize different strains.

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TABLE 1. Social Role effect of the removed fish. Mean Squares and Wilk's λ values from repeated measures ANOVA testing effects of strain and social role of the removed fish on the proportion of the fish group found on the "correct" side of the testing arena. Degrees of freedom are 23, 575 for within-subjects effects, and 1, 25 for between-subjects effects and 23, 3 for x trial analysis. P-values are in parentheses (with H-F correction for within-subject effects). Asterisk * marks values that are statistically significant at $p < 0.05$ level.

	Between Subjects	Within Subjects	Repeated (Wilk's λ)
Time		0.241* (<0.01)	0.032 (0.11)
Strain	0.9 (0.12)	0.198 (0.05)	0.014* (0.04)
Social Role	1.5* (0.04)	0.111 (0.59)	0.321 (0.97)
Strain x Role	0.3 (0.34)	0.101 (0.70)	0.015 (0.05)
Error	0.3	0.125	

TABLE 2. Relationship between group performance and cohesion. Pearson product-moment correlation coefficients summarizing the relationship magnitude between group performance (average number of fish on the correct side across all 24 trials) and measures of cohesion: Information Centrality (IC) and Degree (Dg). Only $|r| > 0.5$ are significantly greater than zero at $\alpha = 0.05$ level.

Gatekeepers removed (n = 17):

	IC	Dg	Learning rate
IC		0.51*	0.11
Dg			0.13

Non-gatekeepers removed (n=19):

	IC	Dg	Learning rate
IC		-0.16	-0.42
Dg			0.27

Figure 1. Group performance of gatekeepers and non-gatekeepers groups. Group performance depended, in part, on the social role of the removed fish. On average, 67% of fish in groups from which non-gatekeepers had been removed made a correct choice. However, only 58% of fish in groups from which gatekeepers had been removed made a correct choice. This difference was statistically significant when tested as a between subjects effect for social role in a repeated measures ANOVA (Table 1).

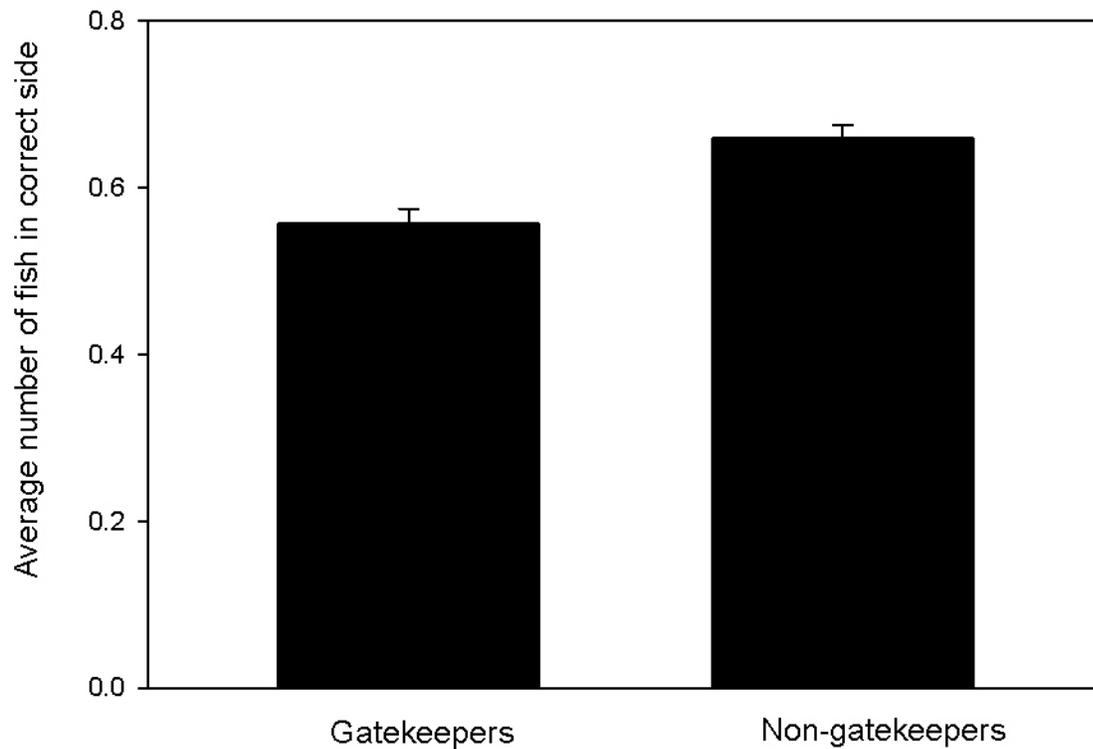


Figure 2. Change in group performance. This figure depicts group performance across 24 trials on 3 days (separated by dotted lines) as measured by the average number of individuals in a group of three fish making a “correct” choice by being on the side of the testing arena at which food was delivered. Fish were equally likely to be on either side of the testing arena on the first day of our learning procedure (average day 1 = 48%). By the third day, 68% of the group was on the color-card-marked side ready to receive food. This difference was statistically significant when tested as an effect of time in a repeated measures ANOVA (Table 1).

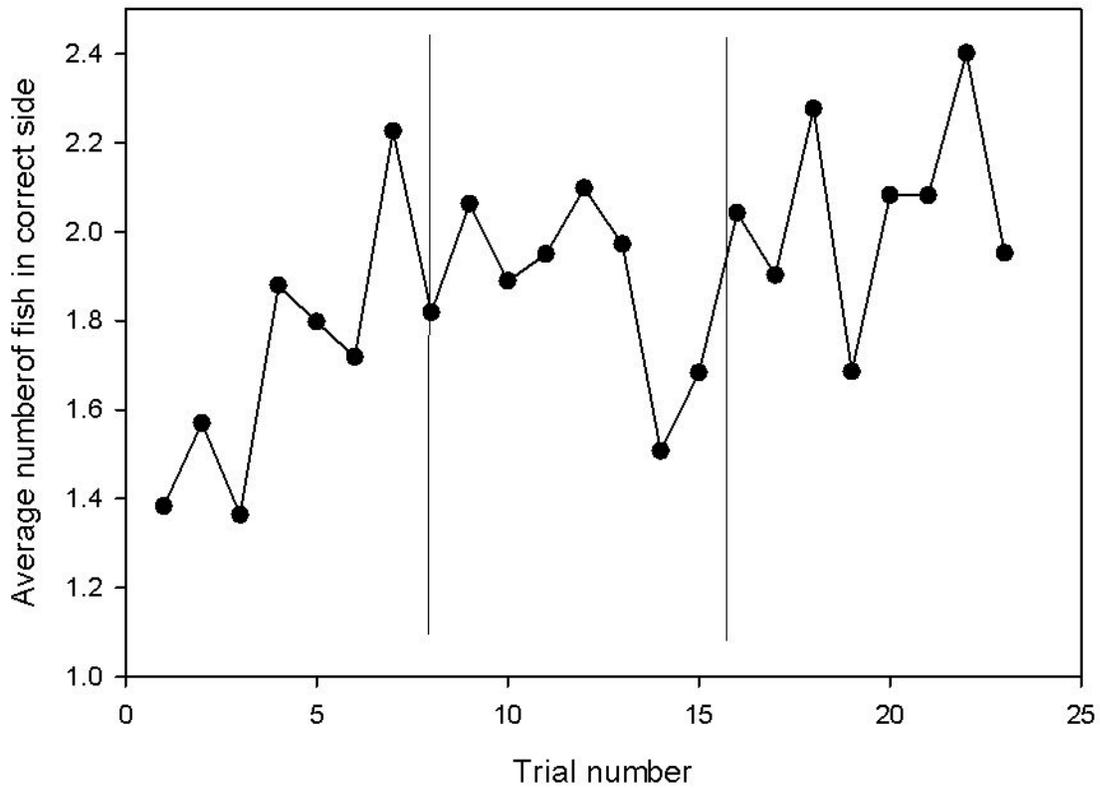
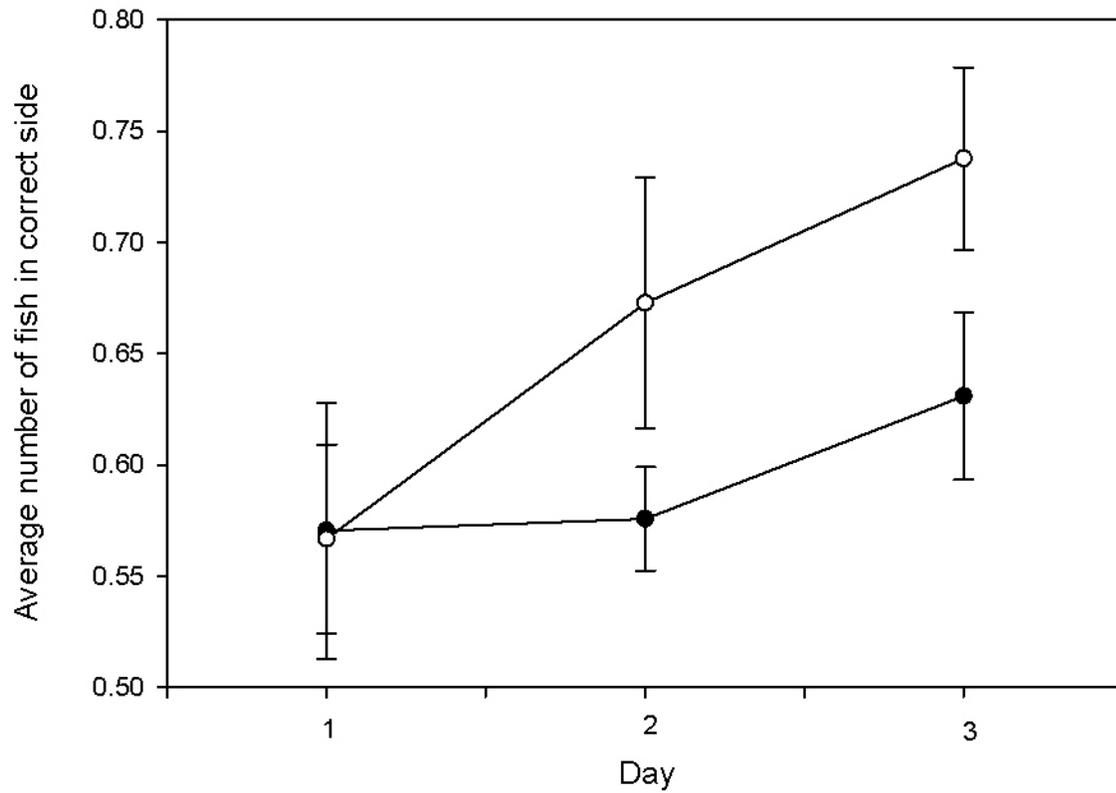


Figure 3. Strain differences in learning. On average, two of the three SH fish (open circles) in the group had learned the task by day 2, whereas PN fish groups (closed circles) took three days to attain a similar performance level. This difference was statistically significant when tested as a repeated measures effect of strain (Table 1).



CHAPTER 5: Gatekeepers influence group learning in zebrafish groups.

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Abstract

In theory, gatekeepers or keystone individuals that interact more regularly with all individuals in a social group can impact group learning by improving the flow of information across the group or by serving as particularly effective learning models. Here, we test this hypothesis empirically in zebrafish groups. We identified naturally occurring gatekeepers (and non-gatekeepers) in zebrafish shoals, removed and trained them individually in a simple associative task. We then placed the now experienced gatekeepers (and non-gatekeepers) back in their experimental group and trained the group on the same association task. Although we found no difference in the initial learning ability of gatekeepers and non-gatekeepers as individuals, We found that the presence of an experienced gatekeeper facilitated the process of social learning. Groups with experienced gatekeepers had either better performance (zebrafish from the PN strain) or learned more quickly than did groups with experienced non-gatekeepers (zebrafish from the SH). Sex of the pre-experienced fish was also important. Groups with experienced females moved away from the aversive stimulus more slowly than did groups with experienced males. However, groups with experienced female gatekeepers showed dramatic improvement over 3 training sessions. Groups with experienced males showed the same learning ability regardless of the social role of the experienced individual. These results suggest that gatekeepers do directly facilitate the process of group learning; however strain differences might influence the process of information transfer in different strain groups.

KEYWORDS: social learning, gatekeepers, zebrafish, association, social dynamics

“Gatekeepers” or “keystone individuals” are animals that interact more readily with others in a social group, and hence facilitate information transfer through the group (e.g., Sih et al. 2009; Vital 2009). They influence the group’s ability to move together around their environment, to find food and to avoid predators through their impact on group dynamics (e.g., Coussi-Korbel & Fragaszy 1995; Vital 2009). By connecting otherwise disconnected groups (Lusseau 2003), facilitating social stability (Flack et al. 2006) or selectively responding to certain bird songs (Miller et al. 2008) gatekeepers may also influence group learning. We recently showed that although the presence of gatekeepers facilitated group performance on a simple foraging task, their removal did not have an adverse impact on group learning (Vital 2009). Gatekeepers, however, may also directly facilitate information transfer by serving as particularly effective models or tutors. For example, social learning of feeding preferences in zebra finches is facilitated by female demonstrators (Katz & Lachlan 2003). Song learning in song sparrows is facilitated by direct social interaction with tutors (Beecher & Burt 2004; Beecher et al. 2007). In the current study, we test whether zebrafish gatekeepers have a greater impact than other fish on group learning by being more effective at sharing previous knowledge.

Learning indiscriminately from other members of the group may not be adaptive and will not increase the mean fitness of individuals in the group (Boyd & Richerson 1985; Boyd & Richerson 1995; Giraldeau et al. 2002). Instead, animals may learn from the group majority, or use phenotypic characteristics to identify and to learn preferentially from more successful, older, or more familiar individuals in a social group (Laland 2004). Some individuals may become social models simply because they are better able to attract the attention of other individuals (Kummer 1978). For example, marmosets observe preferentially members of the other sex during feeding or manipulation of objects (Range & Huber 2007). Ravens show more interest in

food related activities than in object-related activities and their attention was significantly influenced by familiarity and affiliation (Scheid et al. 2007). Siblings of the common raven, *Corvus corax*, who spend significantly more time in each other's close proximity, also showed a learning bias towards siblings demonstrators (Schwab et al. 2008b). Thus, individuals who interact frequently with other animals in the social group (e.g., gatekeepers) may also be better able to attract the attention of other group members, and thus serve as more effective models for transferring new information quickly through a social group.

Complex social dynamics may also influence the effectiveness of particular individuals as social models. For example, both the frequency of interactions as well as the distribution of these within a group may influence the decision of from whom to learn (Coussi-Korbel & Fragaszy 1995). Although close physical proximity may increase the number of opportunities for social learning (Fernandez-Juricic et al. 2004; King et al. 2003), the identity of the most proximal individual may not be as relevant as the identity of another more distant individual. For instance, more physically distant jackdaws individuals provide information about different foraging situations which may therefore be more relevant for social learning (Schwab et al. 2008a). In a simulation study, Voelkl and Noe (2008) showed that the speed of information transfer in a group increased not only according to the number of informed individuals but that it depended also on the complex social structure of that group. In the current experiment, we use Information Centrality and the complex concept of “gatekeepers”, to identify individuals that are socially-important both in terms of physical distance and in their pattern of social interactions with the rest of the social group.

Fish in social shoals gain knowledge about their environment through interactions with shoal mates (Brown & Laland 2003; Croft et al. 2005). This gain of knowledge is sometimes

facilitated by the social characteristics of shoalmates such as familiarity (Morrell et al. 2008), sex (Kiflawi & Mazeroll 2006) and shoal size (Lachlan et al. 1998). Dynamics of shoal encounters has important consequences for the transmission of information through social learning within populations (Croft et al. 2003). We previously found that the removal of important individuals (gatekeepers) in zebrafish shoals influenced group performance (Vital 2009). Specifically, we found that the presence and removal of gatekeepers influenced group performance, with gatekeepers facilitating the process of obtaining food. Additionally we found that domesticated zebrafish learned this task more quickly than did the lab-reared offspring of wild-caught fish.

We now test the ability of experienced gatekeepers and non-gatekeepers to transfer information across a zebrafish group. We began by identifying and training individual fish (some gatekeepers and some non-gatekeepers) in a simple associative task. We then returned the experienced fish to their groups and trained the group as a whole to perform the same associative task. It has been suggested that the time spent with other individuals (Schwab et al. 2008b), physical proximity (King et al. 2003) and demonstrator phenotype (Katz & Lachlan 2003) influence information transfer rate. Additionally, we expect gatekeepers, as identified by Information Centrality, to facilitate the process of information flow (Barzilai-Nahon 2008; Lu 2007). We thus predict that groups with experienced zebrafish gatekeepers learn more quickly than groups with experienced non-gatekeepers.

METHODS

We used wild-type zebrafish for this experiment, employing fish from a lab strain, Scientific Hatchery (SH) that was established in the 1990s and the more recently-established PN

strain, collected from India in 2007. All subject fish had been housed in standard conditions for at least two generations (18.9l aquaria, 24 – 27°C, 13L:11D cycle, filtered and aerated water, with abundant food) and in single-strain groups. As in (Vital 2009), each tank was divided by a central plastic plate that left enough room at the bottom to allow fish to swim back and forth between the two sides.

We formed 17 and 14 groups of PN and SH respectively with 4 adult fish (two males and two females) in each group. These were young, naïve fish that had not been used in any previous experiments. We visually isolated the groups from each other by placing opaque barriers between the aquaria. After two weeks of acclimation, we began behavioral trials. To minimize stress, we measured groups in their home aquaria and conducted behavioral observations from behind a black curtain blind. We identified individual fish using differences in their natural striping patterns, and then observed each fish continuously for a 1-min focal animal sample, recording the identity of every fish that came within two body lengths of the focal individual (near-neighbor points). One minute was sufficient time to gather at least 10 near-neighbor points for each subject fish, as recommended for social network statistics (Vital & Martins 2009).

As in Vital (2009), we used these near-neighbor data to estimate Information Centrality (IC), using software written for this purpose (Vital & Martins 2007). Information Centrality measures the amount of information flow that is channeled through each individual in the social group, or can be combined into a group measure. In the context of our study, the individual with the highest individual information centrality was the fish that most often remained in close proximity to other fish in the group. We follow standard practice by referring to this keystone individual as a “gatekeeper” because they may control the flow of information across the group. The individual with the lowest individual information centrality was the fish that was least

likely to be within two body lengths of other fish in the group, or which remained near only a single other fish. We will refer to this individual as the “non-gatekeeper”.

Once identifying a single “gatekeeper” and “non-gatekeeper” for each group, we used a net to remove the individual with the highest Information Centrality value (i.e., gatekeepers) from 9 of the PN tanks and 8 of the SH tanks, and the individual with the lowest Information Centrality value (non-gatekeepers) from the remaining 8 PN and 6 SH groups. We housed the removed fish by themselves for the rest of the day, training them the following morning on a simple association task.

We attached a blue card to one side of the training aquarium, and trained the individual zebrafish to associate the blue card with an adverse stimulus. During each training session, we tapped the glass in the lower-middle of the aquarium to attract the attention of the fish. We then lowered half way into the tank an adverse stimulus, a 10 x 1 cm stick, into the side of the arena marked by the blue card, and agitated it back and forth in a slow stereotyped fashion until all fish left that side. We recorded the total time (s) from the initial tapping on the glass to when the fish moved out of the side in which the adverse stimulus was presented. We then moved the blue card to the opposite side of the aquarium at random (to avoid a patterned sequence), waited 30 min and repeated the entire process once again.

We trained each removed fish with three two-part trials on the same day: once between 9am-10am, once between 12pm and 1pm, and once between 3pm and 4pm. At the end of the day, we returned the now experienced fish to their original groups, and allowed them to rest for two days. After those two days, we repeated the series of three two-part training trials on the complete groups, training the set of four fish to associate the blue card with an adverse stimulus (or equivalently, to alternate sides of the testing arena between trials). We followed the same

procedure used previously for individual fish, but recorded how many seconds it took for all four fish to leave the side with the adverse stimulus.

We averaged the time (s) it took for individual fish and groups of four fish to escape from the adverse stimulus across the two-parts of each training session, and then used repeated-measures ANOVA to consider differences in learning across the three training sessions spread throughout the day. Specifically, we tested the effects of strain (PN, SH), sex (male, female), social role (“gatekeeper”, “non-gatekeeper”) and their interactions on avoidance behavior (between subject effects), the learning profile of avoidance (x time interaction effects), and avoidance at each training session (univariate within-subjects effects). We then repeated the analysis to consider the predictive value of the same factors in explaining variation in group escape time after the fish had been returned to its group. We conducted all analyses in SAS (2009), and used residual analysis to confirm the usual normality and homoscedasticity assumptions.

RESULTS

Sex and strain influenced individual learning of an adverse stimulus

Individual zebrafish readily learned to associate the blue card with an adverse stimulus (Fig. 1) decreasing their avoidance time from a mean of $3.4 \text{ s} \pm 0.37$ on the first training session to a mean of $0.5 \text{ s} \pm 0.21$ on the third training session (time profile effect in Table 1: Wilk’s $\lambda = 0.35$, $F = 20.1$, $df = 2, 22$; $p < 0.0001$). We found no evidence for differences in the behavior of individual fish with different social roles (gatekeepers vs. non-gatekeepers), but did find differences attributable to sex and strain (Table 1). In general (Fig. 1), SH fish learned the task

more quickly (by the second training session, Y2) than did PN fish, which only learned the task by the third training session (Y3; strain effect on time profile: Wilk's $\lambda = 0.58$, $F = 7.9$, d.f. = 2, 22, $p < 0.002$, Table 1). The strain difference was most dramatic during the second training session when individual PN fish took an average of $4.3 \text{ s} \pm 0.62$ to evade the moving stick in comparison to $1.6 \text{ s} \pm 0.53$ for SH fish (strain effect : $F = 12.2$, d.f. = 1, 23, $p < 0.002$, Table 1). The strain difference was also accentuated by a sex difference in the avoidance behavior of PN fish during the second training session (Y2). Female PN (solid lines, Fig. 1a) were slower to avoid the aversive stimulus ($\bar{X} = 5.8 \text{ s} \pm 1.37$), than were males (solid lines, Fig. 1b, $\bar{X} = 3.2 \text{ s} \pm 0.53$), whereas there was less sex difference in SH fish (dashed lines, Fig. 1a: female $\bar{X} = 0.9 \text{ s} \pm 0.23$; Fig. 1b: male $\bar{X} = 2.3 \text{ s} \pm 0.84$). This resulted in a significant sex x strain interaction effect during this middle training session (Y2: $F = 5.2$, d.f. = 1, 23, $p < 0.04$), and also between subjects overall ($F = 5.7$; $df = 1, 23$; $p < 0.03$, Table 1).

Groups with experienced gatekeepers avoided faster than did groups with experienced non-gatekeepers

Zebrafish groups also learned the task (Fig. 2), leading to a significant effect of time period on avoidance response (time profile effect in Table 2: Wilk's $\lambda = 0.68$, $F = 5.2$, d.f. = 2, 22; $p < 0.02$). Social role of the single experienced fish also had an impact on avoidance behavior (between-subjects effect: $F = 7.9$; $df = 1, 23$; $p = 0.01$), but the effect was complicated by an interaction with strain ($F = 11.3$; $df = 1, 23$; $p < 0.003$; Table 2). PN groups (Fig. 2a) with experienced gatekeepers (black line) avoided the aversive stimulus roughly twice as quickly at every training session as did PN groups with experienced non-gatekeepers (gray line), with no

clear difference in their overall learning rates. In contrast, there was no clear difference in the baseline avoidance behavior of SH groups with experienced gatekeepers or non-gatekeepers (Fig. 2b). However, SH groups that had experienced gatekeepers (black line) learned more quickly (by the second training session, Y2, as in the individual fish training sessions) than did SH groups with experienced non-gatekeepers (gray line: by the third training session, Y3, Fig. 2b). Overall, the effect of social role was significant as a main effect for the second training session (Y2: $F = 8.2$; $df = 1,23$; $p < 0.01$), and as an interaction with strain during the first ($F = 6.8$; $df = 1,23$; $p < 0.02$) and third ($F = 5.1$; $df = 1,23$; $p = 0.03$) training sessions (Table 2).

Groups learned more quickly if experienced female was a gatekeeper

The effect of social role was also complicated by sex differences in avoidance behavior, with experienced females playing an especially important role (Table 2, Fig. 3). Overall, groups with experienced females (Fig. 3a) had slower initial avoidance times ($\bar{X} = 3.0 \text{ s} \pm 0.64$) than did groups with experienced males (Fig. 3b, $\bar{X} = 5.9 \text{ s} \pm 0.84$), leading to a significant between-subjects effect of sex ($F = 15.2$; $df = 1,23$; $p < 0.001$, Table 2). More specifically, groups with experienced female gatekeepers learned quickly, having the fastest avoidance time of all groups by the second and third training sessions (Fig. 3a, black line). Groups with experienced female non-gatekeepers (Fig. 3a, gray line) also learned to avoid the moving stick, but even at the third session remained slower on average than were other types of groups. In contrast, the social role of experienced males had little effect on group avoidance behavior (Fig. 3b), leading to a significant between-subjects social role X sex interaction effect ($F = 7.0$; $d.f. = 1,23$; $p < 0.02$, Table 2).

We did not find evidence for a significant interaction between sex, social role, and strain (Table 2).

DISCUSSION

Our results confirm that some individuals (gatekeepers or keystone individuals) are better able to transfer information to others in a social group, improving the ability of a social group to learn an associative task. This supports previous studies which found that a few knowledgeable individuals can lead a shoal to food (e.g., (Dyer et al. 2009; Reeb 2000), and theoretical studies that show that gatekeepers can impact information transfer (Barzilai-Nahon 2008). In our study, groups with experienced gatekeepers escaped the adverse stimulus more quickly than did groups with experienced non-gatekeepers.

The influence of the social role of the experienced individuals depended on the genetic strain of the zebrafish, setting the stage for future research into the underlying genetic mechanisms. Gatekeepers may be more effective at transferring the information they have learned to other group members, or may simply improve group performance by influencing group stability and thereby allowing other group members to learn quickly. In one strain (SH), groups with experienced gatekeepers learned more quickly (by the second training session) than did groups with experienced non-gatekeepers (by the third training session), suggesting that they serve as more effective learning models. In a second strain (PN), groups with experienced gatekeepers were faster at evading the stimulus even from the first training session. Thus, in the PN strain, the impact of gatekeepers is primarily indirect through improving general group function. A similar pattern has been found in pigtailed macaques, in which the presence of

policing individuals facilitates social stability in a group, reducing conflicts, enhancing infant survivorship and facilitating emergence and spread of cooperative behavior and social learning (Flack et al. 2006). Because of the abundance of genetic tools available for studying zebrafish, this model organism may provide an unparalleled opportunity for understanding the genetic basis of this difference in the social role of gatekeepers on group learning.

Male and female zebrafish are so similar in terms of morphology and behavior that sex differences in zebrafish research studies are often negligible or ignored (e.g., Moretz et al. 2007). Nevertheless, here we found a significant effect of sex and social role on group performance. Other studies have also found that the sex of the demonstrators strongly influences group performance in different tasks. For instance, juvenile males living in close contact to adult females exhibit a different song development pattern than do juvenile males living in close contact with juvenile females (Miller et al. 2008). In zebra finches, sex of the demonstrator influences social learning (Katz & Lachlan 2003) and sex plays a major role in other aspects of social personality (Schuett and Dall 2009). In zebrafish, we found that groups with experienced males (regardless of social role) were better able to avoid the stick quickly than were groups with experienced females. Experienced females only transferred their knowledge to the group if they were also gatekeepers, suggesting that in zebrafish, males are more effective than females at influencing group behavior. Similar sex differences have been observed in other species. For example, common marmosets pay more attention to tutors of the opposite than of the same sex (Range & Huber 2007). In guppies, novel foraging information spreads at a significantly faster rate through subgroups of females than through subgroups of males (Reader & Laland 2000).

In sum our results support the idea that different social roles have an effect on the process of group learning in zebrafish shoals with gatekeepers facilitating the process of group learning.

Furthermore, the effect of social role is deeply influenced by both strain and sex. Differences in strain performance agree with previous studies suggesting an underlying genetic difference (Moretz et al. 2007). Further studies are needed to explore more in depth the social system of zebrafish and how this influences the process of group learning.

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FIGURE LEGENDS

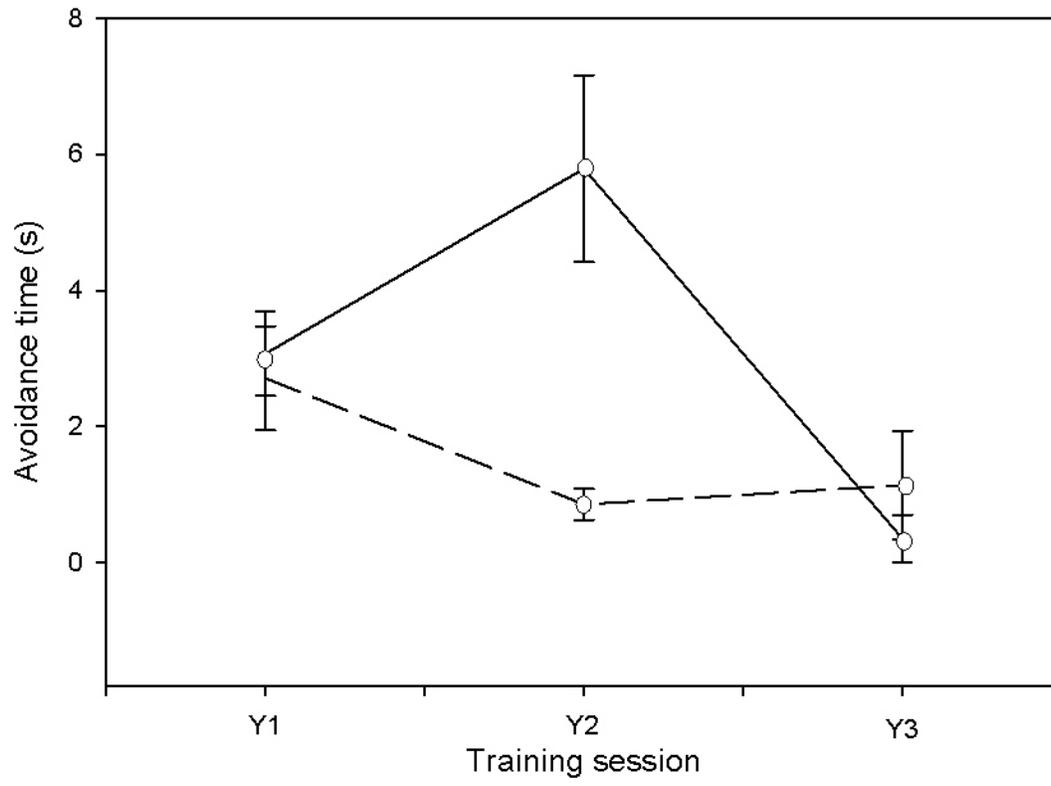
Figure 1. Individual zebrafish learned to avoid an aversive stimulus (decreased avoidance time) across three training sessions (Table 1). 1a) females (open circles); 1b) males (closed circles). The SH strain (dashed lines) learned the task by the second training session, whereas the PN strain (solid lines) generally took until the third training session. There was a greater sex difference in PN fish (solid lines) than in SH fish (dashed lines), leading to a sex x strain interaction effect during the second training session (Table 1). Error bars are one standard error.

Figure 2. Gatekeeper effect on group learning an aversive stimulus. Groups with experienced gatekeepers (black lines) were better able to avoid the stimulus than were groups in which the experienced fish was a non-gatekeeper (gray lines; Table 2). In PN fish (Fig. 2a) the effect of social role was in terms of faster avoidance for groups with experienced gatekeepers (solid black line) than for groups with experienced non-gatekeepers (solid gray line) at all three training sessions. In SH fish (Fig. 2b), the difference was in terms of faster learning: by the second training session (Y2) for groups with experienced non-gatekeepers (gray dashed line), but only by the third training session (Y3) for groups with experienced non-gatekeepers (gray solid line).

Figure 3. Female effect on group learning an aversive stimulus. Groups with experienced females (Fig. 3a, open circles) avoided the aversive stimulus less quickly even at the first training session than did groups with experienced males (Fig. 3b; closed circles; Table 2). However, groups with experienced female gatekeepers (Fig. 3a, black line) learned especially quickly across three training sessions. Groups in which the experienced individual was a male showed little change in performance across the three training sessions (Fig. 3b). Gray lines depict

groups with experienced non-gatekeepers. Symbols are as in Figs. 1 and 2, except that data from PN and SH strains have been combined.

a)



b)

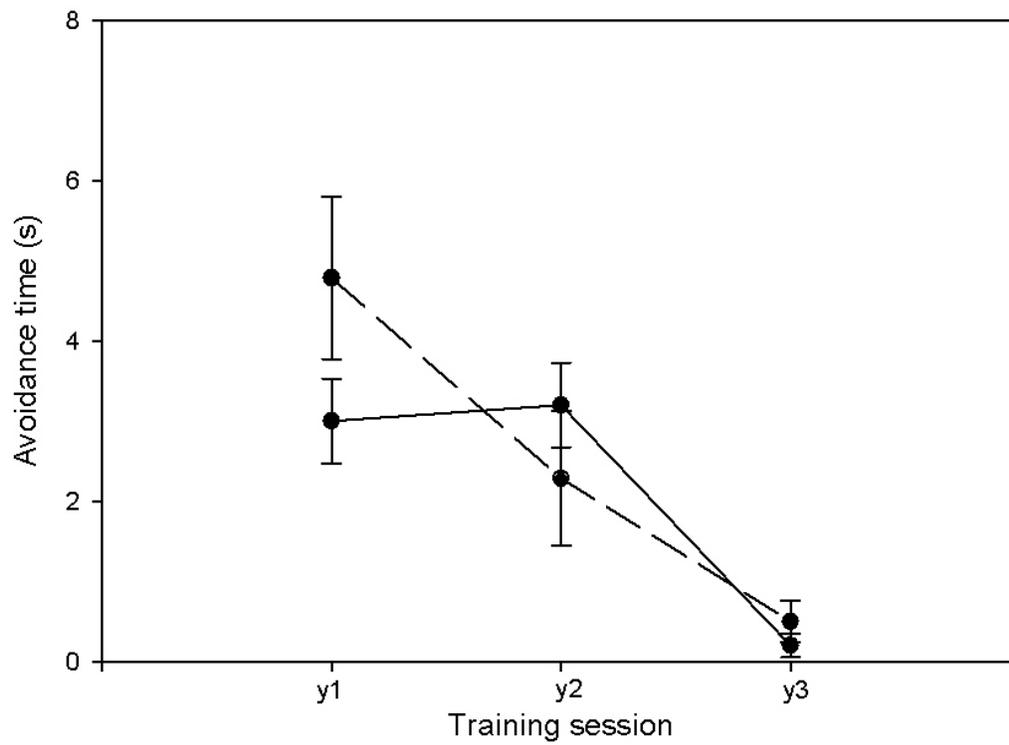
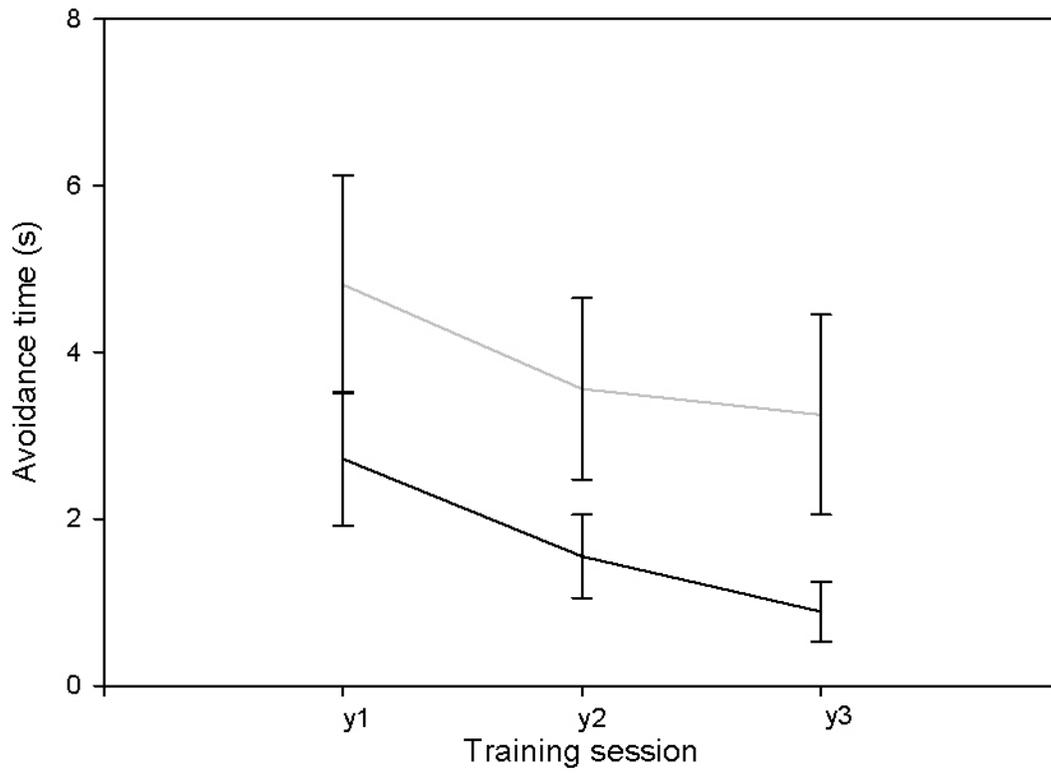


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a)



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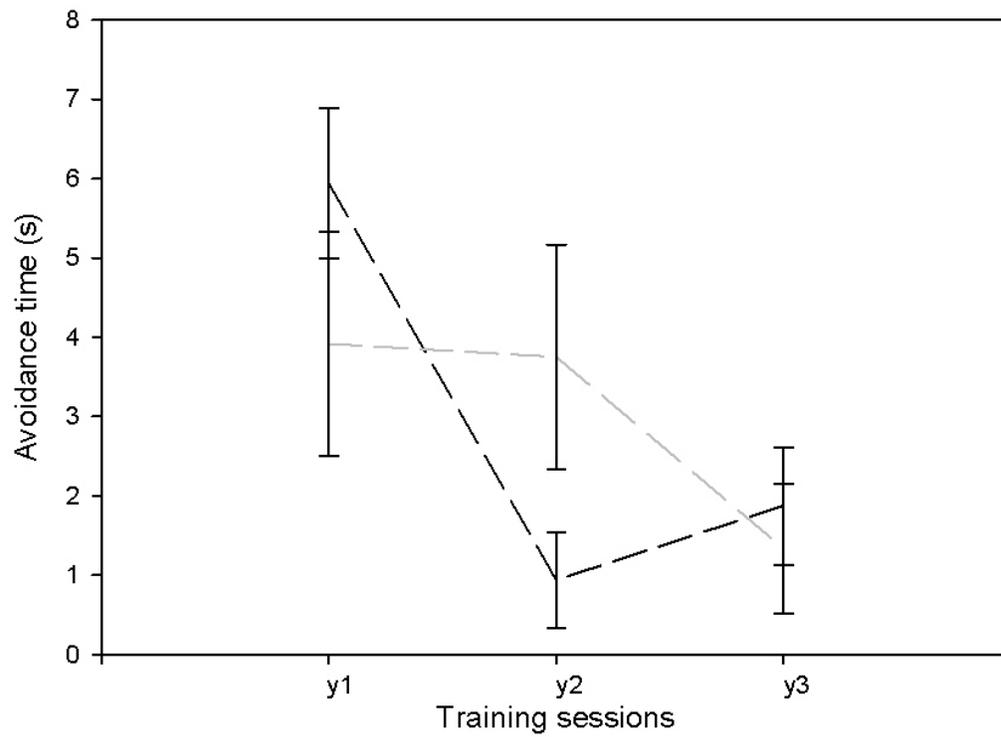
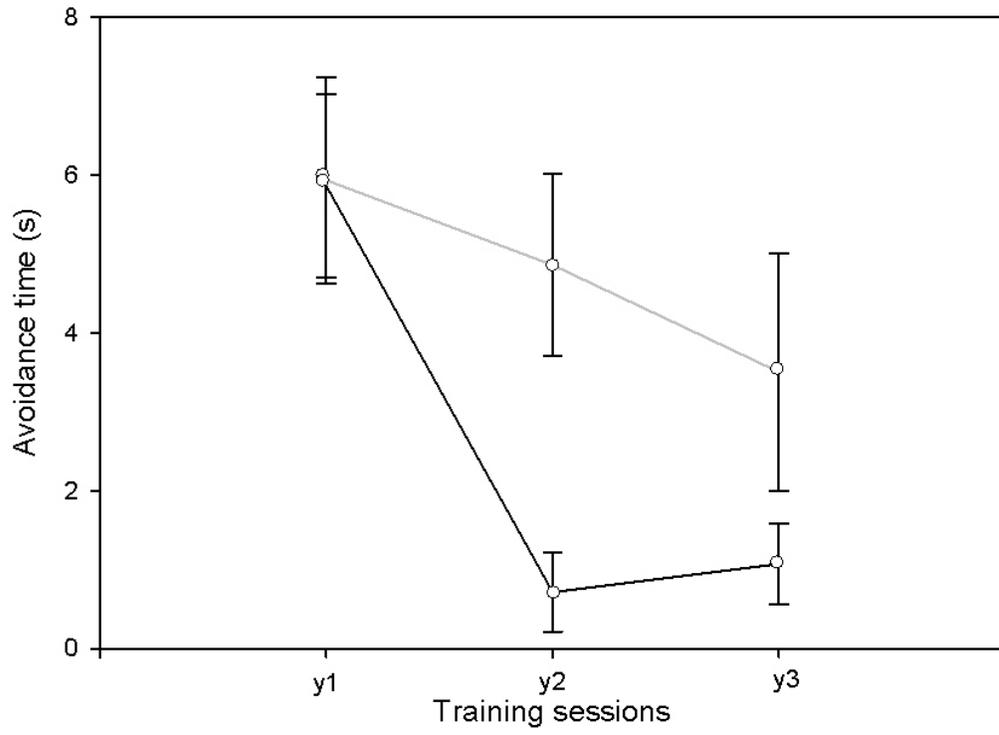


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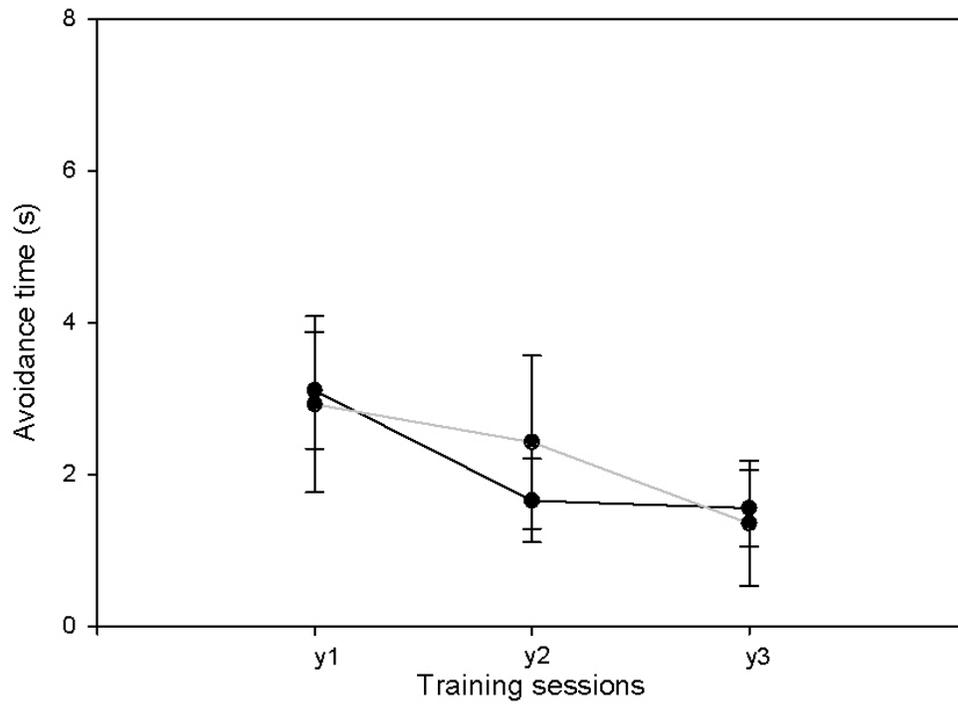


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Table 1. Effects on learning for individual fish. Mean Squares and Wilk's λ values from repeated-measures MANOVA testing effects of sex, strain, and social role of an individual fish on the ability of that fish to avoid an aversive stimulus across three training sessions. Results are presented for each training session (Y1, Y2, Y3), for a time profile across the three sessions (x time), and between subjects (averaging data for each fish across the three training sessions). Degrees of freedom were 1, 23 for each test, except the x time trials in which d.f. = 2, 22. P-values are reported in parentheses. * marks statistically significant effects, when tested at the $\alpha = 0.05$ level.

	Y1	Y2	Y3	x time (Wilk's λ)	Between Subjects
Time				0.35* (<0.01)	0.02
Sex	6.5 (0.22)	3.6 (0.43)	1.3 (0.35)	0.88 (0.25)	0.08 (0.88)
Strain	4.9 (0.28)	67.8* (0.00)	2.5 (0.20)	0.58* (0.00)	6.6 (0.19)
Social Role	0.1 (0.88)	1.8 (0.58)	0.1 (0.82)	0.98 (0.85)	0.6 (0.68)
Sex x Strain	9.6 (0.14)	28.9* (0.03)	0.3 (0.63)	0.82 (0.11)	20.8* (0.03)
Sex x Role	0.7 (0.71)	5.4 (0.33)	0.0 (0.86)	0.95 (0.55)	0.6 (0.68)
Strain x Role	3.9 (0.34)	2.2 (0.53)	0.4 (0.48)	0.99 (0.91)	6.2 (0.20)
Sex x Strain x Role	12.7 (0.09)	0.2 (0.83)	3.4 (0.14)	0.95 (0.58)	11.7 (0.09)

Error

4.0

5.6

1.5

3.6

Table 2. Effects on learning for groups. Mean Squares and Wilk's λ -values from repeated-measures MANOVA testing effects of sex, strain, and social role of an experienced fish on the ability of a group of four fish (including that one experienced member) to avoid an aversive stimulus across three training sessions. Other details are as in Table 1.

	Y1	Y2	Y3	x time (Wilk's λ)	Between Subjects
Time				0.68* (0.01)	0.02
Sex	74.6* (0.00)	3.2 (0.47)	8.4 (0.18)	0.9 (0.28)	59.2* (0.00)
Strain	4.8 (0.44)	2.7 (0.51)	4.6 (0.31)	0.9 (0.59)	0.8 (0.65)
Social Role	0.4 (0.81)	49.6* (0.01)	10.1 (0.14)	0.9 (0.17)	30.5* (0.01)
Sex x Strain	0.0 (0.98)	5.3 (0.36)	7.1 (0.21)	1.0 (0.83)	8.5 (0.15)
Sex x Role	0.3 (0.85)	20.5 (0.08)	15.8 (0.07)	1.0 (0.64)	27.2* (0.01)
Strain x Role	51.5* (0.02)	0.2 (0.86)	22.3* (0.03)	0.8 (0.12)	43.8* (0.00)
Sex x Strain x Role	2.1 (0.60)	1.1 (0.68)	8.2 (0.18)	1.0 (0.83)	3.9 (0.13)
Error	7.6	6.1	4.3		3.9

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Publications

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Vital, C., E.P. Martins. 2009. Using graph theory metrics to infer information flow through animal social groups: a computer simulation analysis. *Ethology* 115:347-355.

Software

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Vital, C., & E.P. Martins. 2004. BEST1.0: Behavioral Stream Analysis. <http://www.indiana.edu/~martinsl/Programs/BeSt/>

Manuscripts in preparation

Vital, C., E.P. Martins. Strain differences on the effects of removal of individuals with different social roles on zebrafish group dynamics. In prep

Vital, C., E.P. Martins. Social Roles and Foraging Task Performance of Zebrafish Groups. In prep

Vital, C., E.P. Martins. Gatekeepers influence group learning in zebrafish groups. In prep

Teaching Appointments

2009 Animal Social Networks (BIO L410, Indiana University)- Instructor

Teaching assistantships

2005 Microbiology laboratory (BIO M255 and BIOM215, Indiana University, Associate Instructor)- Conducted and supervised laboratory. Helped with independent identification of unknowns and proctored exams. Assisted with exam writing.

2003 Introductory biology laboratory (BIO L113, Indiana University, Associate Instructor)- Conducted and supervised laboratory. Led discussion sessions. Managed and assisted students with independent projects.

1999 Pre-calculus online (Math 1508, University of Texas at El Paso, Undergraduate Assistant). Direction and maintenance of online pre-calculus class, writing and grading exams.

2000 Pre-calculus, (Math 1508, University of Texas at El Paso, Undergraduate Assistant).
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Mentoring

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- Joshi Vani (L490 Independent Biology Research 2007)

Presented papers

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Vital, Cuauhcihuatl, Martins, Emilia P. 2008. CISAB

Vital, Cuauhcihuatl, Martins, Emilia P. & Börner, Katy. 2006. Analysis of Cowbird Social Networks with Graph Theory Metrics. International Conference and Workshop on Network Science (NetSci 2006), May 14-25, Bloomington, Indiana.

Vital, Cuauhcihuatl, Martins, Emilia P. 2004. Behavior patterns and transitions matrices of five different species of the lizard sceloporus. Society for the advancement of Chicanos and Native Americans in Science. Santa Fe, NM 2004

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Professional Affiliations

Animal Behavior Society

Association for Women in Science

Center for the Integrative Study of Animal Behavior

Society for the advancement of Chicanos and Native Americans

Society for Integrative and Comparative Biology