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Social Network Analysis of Partner Preference in Lemon Sharks, Negaprion brevirostris, During the Introduction of Unfamiliars to Familiar Groups

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Social Network Analysis of Partner Preference in Lemon Sharks, *Negaprion brevirostris,* During the Introduction of Unfamiliars to Familiar Groups

May 2015

I certify that all material in this thesis which is not my own work has been identified and that no material has been previously submitted and approved for the award of a degree by this or any other University.

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Dedication

My parents, Mary Ann and Dave Keller, have been more supportive of my career than I could ever have dreamed. Without them, I would have never had the opportunities to get to where I am today. Whether it was allowing me to keep a ridiculous amount of fish tanks in our house, even after a faulty light fixture almost burned it down, driving me across the country for graduate school, or motivating me to pursue my career, there are no words to express my deep gratitude for all they have done for me. I certainly do not have enough space in this document to list the specifics of their assistance. As I have gotten older, the sacrifices they have made for me have become so overwhelming apparent. Additionally, I would like to thank Stephen Keller, Gail Westover, and their children for allowing me stay at their home while I interned at the National Aquarium in Baltimore. That position opened up many doors into the field of shark research that I am still benefiting from today.

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I would like to dedicate my thesis to the souls of my Granny, Gertrude Morrison, and my Nana, Mary Motzkin. These women had the kindest spirit that I have ever encountered and truly encompass the term selfless. Their eternal love was so motivating, and I have no doubt they shaped me as the person I have became today.

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Abstract

In order to understand what drives group formations and to predict spatial occurrences, investigations on individual preferences within the groups have proved to be very useful. Groups of sharks have been observed for centuries; however, there is a dearth of quantitative analyses on the mechanisms that drive their formation. In this study, we use controlled, semi-captive behavioural experiments to assess the potential role of familiarity in group formation and on social behaviour in a large marine vertebrate. Juvenile lemon sharks, *Negaprion brevirostris*, (n =23) in Bimini, Bahamas were captured, measured and tagged with external colour codes for individual identification and housed in pens that were exposed to ambient conditions. Sharks were separated into four holding pens according to their size class and given 14 days to familiarize themselves with their cohort. Following familiarization, pairs of sharks were taken from two holding pens and introduced in a social network pen for a behavioural trial (N=27). An overhead video system recorded behaviours for one hour and tracking software transformed the movement patterns into a coordinate system. Multiple algorithms were used to analyze these coordinates and produced a matrix of interactions between familiar and unfamiliar individuals. Social network analysis indicated that juvenile lemon sharks express a significant preference for familiar individuals for the entire trial (P-value = 0.000501) and this preference declines over the one-hour time period. Previous research suggests that the preference for familiars likely occurs to avoid agonistic interactions or to further the advantages of group living. We suggest that preference for familiars is not facilitated by either the 'Dear Enemy' effect or the desire to bolster the benefits of group living. Rather, we believe the two benefits are closely linked as the 'Dear Enemy' effect results in spatial isolation between unfamiliar individuals, which ultimately results in strengthened bonds between familiar individuals that bolster the effects of group living. The decline in avoidance of unfamiliars is most likely caused by forced interactions in our semi-captive trials, and in the wild, this would likely not occur. This research advances our understanding of the mechanisms driving group formation in lemon sharks, a model species for large marine predators.

Key words: partner preference, group formation, social behaviour, familiarity, lemon sharks, nearest neighbour

Introduction

Group Living in the Animal Kingdom

Group living is a widespread phenomenon in the animal kingdom. While animals must ensure benefits outweigh costs via an optimum group size, the observed behaviours increase fitness and provide participants with advantages including enhanced energetic efficiency, social cohesion, decision making, foraging success, and the reduction of predation (Atton et al. 2014; Magurran 1990; Croft et al. 2006; Herksin & Steffensen 1998). The criteria needed to be considered a group vary with life history characteristics, but often include physical proximity, number of animals and orientation of the animal or path of travel (Bejder et al. 1998; Myrberg & Gruber 1974; Pitcher & Parrish 1993**;** Couzin et al. 2002). Participation in groups can maximize reproductive success; although not all individuals benefit from group participation, the risk of an unwanted effect, such as consumption by a predator, is outweighed by the gains and thus, grouping behaviour is palpable (Jacoby et al. 2011). If a group has a net reproductive value that is positive, the behaviours will become more prevalent in future generations. Considering there are many techniques available to maximize success, there is a great amount of variability within animal aggregations.

Aggregations occur when animals are drawn together by a life-sustaining resource such as food, the presence of potential mates, or protection from predation (Krause & Ruxton 2002). If there are social associations occurring within aggregations, they are called social groups; therefore, aggregations are important prerequisites for social groups (Krause & Ruxton 2002). Within social groups, both intra and interspecific variation exist. For example, the lemon shark *(Negaprion brevirostris)* is commonly observed paralleling, following, milling, or circling (Guttridge et al. 2011), whereas the small spotted catshark, *Scyliorhinus canicula,* exhibits far more interactions while resting on the substrate (Jacoby 2012). Among elasmobranch fishes (sharks, skates and rays), aggregations are well known (see Jacoby et al. 2011 for review). These groups are influenced by variables including geographic location (Klimley & Nelson 1984), photoperiod and season (Heupel & Simpfendorfer 2005), temperature (Kessel et a. 2014), and the presence of prey or mates (Hulbert et al. 2005). Elasmobranch aggregations have been reported both scientifically and anecdotally throughout historical literature for centuries; however, there is a dearth of quantitative analysis on the social behaviour within these groups (Bres 1993; Guttridge et al. 2011). Independent of expanding scientific insight, elucidating the mechanisms of group formation is critical for fisheries management. Aggregations and social grouping often display temporal variation. If the mechanism of formation is not properly understood, a fishery opened at an improper time could exploit certain populations.

Variables Affecting Group Membership

To understand what variables can impact social associations, teleost fishes are helpful to consider. Certain teleosts such as the three-spined stickleback (*Gasterosteus aculeatus),* Trinidad guppy (*Poecilia reticulata)* or zebra fish (*Danio rerio)* are model species for reasons including their small size, schooling behaviour, hardiness, and breeding capabilities (Brooks & Endler 2001; Vasta et al. 2004; McKinnon & Rundle 2002)**.** The inherent difficulty of studying sharks both in the wild and captivity has delayed knowledge acquisition of social grouping compared to teleosts. Some factors that have contributed to this are the large size of many sharks, the logistics and expense of maintaining these animals in aquaria, slow reproduction, and the difficulty of observing animals *in situ* (Gruber & Myrberg 1977). To surmount these problems, many social behavioural studies have been conducted on captive juvenile sharks or those in shallow-water habitats (see Myrberg & Gruber 1974; Guttridge et al. 2009b, 2011; Jacoby et al. 2010, 2012). These studies investigated the dynamics and mechanisms influencing this behaviour.

Teleosts are very distinct from sharks and should be used to identify areas of future research, not for drawing ecological conclusions (Guttridge et al. 2009a). Teleosts have been shown to associate based upon species type, size, sex, familiarity, kin, parasite load**,** phenotype, and shoal size **(**Couzin et al. 2002; Krause et al. 2000; Lachlan et al. 1997; Lindström & Ranta 1993; Brown et al. 1993; Gerlach & Lysiak 2006; Ward et al. 2004**)**. While these factors are likely to contribute to partner preferences in sharks, only size, sex, species type, and possibly kinship have indicated an influence on this assortative preference thus far (Guttridge et al. 2009b, 2011; Mourier et al. 2012, Wilson et al. 2013).

Familiarity in Group Behaviour

Familiarity, either measured by time or number of interactions between two animals, is commonly overlooked as a necessary control in social experiments. There are two advantages that likely facilitate the preference for familiars. The first is to reduce agonistic interactions between unfamiliar individuals (‘Dear Enemy’ effect described by Fischer 1954). Seppa et al. (2001) demonstrated that familiar Arctic Charr (*Salvelinus alpinus*) showed increased survival and health due to decreased aggression and subsequently, an increased level of foraging. Secondly, when a preference for familiars exists, the benefits of group living are often further amplified. For example, associating with familiars has been shown to reduce the search time for resources, enhance social learning and social cohesion, reduce aggression, increase growth and health condition, and potentially help develop reciprocal altruism (Ward & Hart 2003; Atton et al. 2014; Hojesojo et al. 1998; Milinksi 1987; Croft et al. 2004; Chivers et al. 1995).

Understanding the impact of familiarity is critical in ensuring the correct interpretation of behavioural trials. Wild studies often conduct trials in a location where animals' refuge (see Guttridge et al. 2011 for example), and researchers are not capable of gauging relationships outside of this zone. For captive trials, if the animals are not given enough time to acclimate, then individuals within the trials may have different levels of familiarity, which could greatly alter the scientific outcomes. Without an adequate understanding of the familiarity between partners, the interactions observed during trials could be misconstrued. Therefore, controlling for familiarity is imperative during wild and captive studies.

Individuals recognize familiars by either learned recognition through repeated exposure, or self-referent matching whereby individuals associate with others who have recently participated in similar activities (Griffiths & Magurran 1997; Ward et al. 2009). Learned recognition of individuals is made difficult due to the "oddity effect." Natural selection eliminates individuals in a group that do not look like their conspecifics, thereby reducing the chance of being captured by a predator if all members look similar (Landeau & Terborgh 1986). While the oddity effect selects for similar phenotypes, variability in odor cues can still allow for recognition (Griffiths 2003). Interestingly, Brown and Smith (1994) showed that preference for familiars existed when individuals were presented with either olfactory cues, or a combination of olfactory and visual cues. There was no preference for familiars when presented with only visual cues.

The ability to distinguish between familiars and unfamiliars is widely documented across vertebrates and has been documented in the taxonomic classes: Mammalia, Reptilia, Aves, Amphibia, and Osteichthyes (Bartal et al. 2014; Osborne 2005; Dalton et al. 2013; Lesbarrèresa & Lodéa 2002; Griffiths & Magurran 1997). The Chondrichthyans (cartilaginous fishes, including sharks, skates, and rays) are the only vertebrate class, sans Agnatha, where this partner preference via familiarity has not been observed. Evidence does suggest that the preference for familiars enhances the benefits of social learning, which has been demonstrated in lemon sharks (Swaney et al. 2001, Guttridge et al. 2013). Additionally, study performed in Bimini, Bahamas showed active partner preference at a refuge site. These associations were unable to be fully accounted for by size-matching and kin preference (Guttridge et al. 2011, 2012). Perhaps the other mechanism influencing these partnerships is the preference for familiars. The observed preference for kin, in one of the two years, could in fact be a preference for familiars, which is probable as kin are often born in close proximity and are thus, more familiar. Preferential association with familiars is dependent on the life history and social dynamics of the species in question (Ward & Hart 2003). When beginning an investigation of a new species, researchers should treat familiarity with as much importance as biases associated with sex, length, and phenotype, as the impacts can be highly significant.

The first experiment testing for familiarity showed that an unfamiliar group of sharks formed fewer aggregations than a group of familiar sharks (Jacoby et al. 2012). These trials conducted on the small-spotted cat shark, *Scyliorhina canicula*, were unable to show significant influence of partner preference via familiarity. Conceivably, an influence of these results was that the sharks were very young and bred in captivity. Studying familiarity is also important as it furthers scientific comprehension of the dynamics behind group formation in large marine vertebrates. Specifically, investigating partner preference is useful as individual partialities influence mechanisms of group formation (Griffiths 2003). In addition to studying familiarity, the use of social network analysis has also proved beneficial for elucidating population dynamics. Social network analysis has been a tool for studying sociology for decades, thereby providing behavioural ecologists with existing, well-developed methods (Croft et al. 2011; Krause et al. 2009).

Social Network Analysis

Social networks (SNs) are comprised of different metrics describing the social aspects of a population such as number of partners, connectivity between all partners, or strength of interactions. SNs can aid researchers in studying population ecology by investigating topics like disease transfer, social hierarchies, or communal isolation (Krause et al. 2009; Mourier et al. 2012). For the purpose of this study, SNs are comprised of individuals, or nodes, connected by edges, representing interactions between individuals. To test for significantly higher dyadic relationships, observed data can be randomized to create a null model, which will serve as a basis for statistical testing (Croft et al. 2011; Mourier et al 2012; Manly 1995; Bejder et al. 1998). The null models will come from binary or weighted matrices, where the edges receive a score of one/zero or a score correlated to the number of interactions, respectively. The use of binary versus weighted networks is dependent on the research question. While investigating partner preference, a weighted network is most useful (Whitehead 2008). The edges of weighted network vary in weight, reflecting the strength of relationship between individuals (Croft et al. 2011; Jacoby et al 2010).

Lemon Sharks and Hypotheses

Lemon sharks occupy coastal zones in the Eastern Pacific and Western and Eastern Atlantic Oceans (Compagno 1984; Guttridge et al. 2013). In Bimini, Bahamas, these sharks are abundant as juveniles and have been used in captive trials for decades (Grubber and Scheniderman 1975; Guttridge et al. 2009b, 2013). Throughout this process, the hardiness of the species has become apparent, in addition to their abundance, making them an ideal species for investigatory trials. The social nature of this species has been well documented in the past decade. These documentations reveal structure to be influenced by size, species type, and possibly kinship (Guttridge et al. 2009a, 2011). Sociality in lemon shark does not ontogenetically dissipate. Aggregations with fully matured adults have been documented in Jupiter, FL (Kessel et al. 2014). In addition to sociality, the intelligence of this species has been demonstrated by operant and classical conditioning and social learning trials (Clark 1959; Gruber and Schneiderman 1975; Guttridge et al. 2013). Northcutt (1977) also demonstrated the family Carcharhinidae, which contains lemon sharks, to have an elevated high brain to body weight ratio. The combination of the aforementioned factors make this species a model species for large-bodied coastal marine vertebrates and an ideal candidate for investigation on the influence of familiarity on partner preference and group dynamics in chondrichthyans.

In this study, we introduced two groups of familiar sharks to each other in a social network pen. The two pairs were unfamiliar with each other, giving each individual the option of associating with one familiar or two unfamiliars. The swimming patterns of the four individuals were recorded for one hour. This captive experiment is unique because individuals were not forced to make an assortative decision such as binary choice experiments, and could interact with any individual present in the arena. In the wild, animals are rarely forced to make a decision that mirror those in binary choice experiments, making our method an improved model for natural behaviour. We hypothesized that familiar sharks would demonstrate a significant preference for each other, and this preference could potentially dissipate with time. This preference could also show intraspecific variability. The lemon sharks of Cape Canaveral, FL have much larger home ranges, i.e. fewer interactions, and might not have had the necessary adaptations to evolve the mechanisms required to show a preference for familiars. As such, investigating life history characteristics before developing hypotheses is of the utmost precedence. Additionally, we expected all interactions, even those between unfamiliar sharks, would increase with time since capture. With the vast knowledge of lemon sharks from previous experiments, we expected sex would not play an important factor in interactions and that length would need to be controlled for to avoid a size-induced bias. The most important facet of this experiment was to determine if familiar sharks, which often have overlapping home ranges in the wild, would show preferred partnerships towards each other compared to unfamiliars. In many other taxa of animals, this is the case, and we expect the lemon shark to be no different.

METHODS

Study Site and Sharks

This study was conducted in Bimini, Bahamas (25°44'N, -79°16'W), located approximately 85km east of Miami, Florida, U.S.A. Bimini was chosen due to the presence of an established research station (Bimini Biological Field Station-BBFS), the ability to conduct behavioural trials on shallow sand flats, the abundance of juvenile lemon sharks (*Negaprion brevirostris*), and the extensive amount of background research on the species (Gruber et al. 2001). A permit (no:MAF/LIA/22) to conduct scientific marine animal research was supplied by the Department of Marine Resources, Bahamas.

Twenty-three juvenile lemon sharks (12F:11M, mean pre-caudal length: 55.73cm, +/- 5.47cm) were captured between January 30th and February 1st 2014 with gillnets off the coast of South Bimini (see Gruber et al. 2001 for capture methods). Upon locating a shark, the animal was removed, placed in a 100-litre plastic box, and transported to holding pens in water ranging from 0.5–1.5 m deep. Each shark was biometrically profiled, which consisted of measuring the pre-caudal length (PCL), determining sex, identifying the individuals via a Passive Integrated Transponder (PIT, Destron Fearing, South St. Paul, MN, USA), and placing the animal in one of four holding pens. Individuals were placed in the different holding pens dependent upon their PCL, creating four replicate pens with similarly sized individuals (See Table 1 for size statistics per pen). These pens, constructed of plastic mesh (5x5cm diamond-shaped holes), were located on the shoreline of the BBFS, exposing the animals to natural environmental conditions (Guttridge et al. 2009b). The random nature of distributing individuals between holding pens allowed for animals that occupied overlapping home ranges to be deemed unfamiliars. Thus, we conducted analysis to determine if initial home range, using capture location as proxy for home range, influenced the number of interactions between animals.

Behavioural Experiment

Sharks were housed in the holding pens for 14 days before trials began, allowing affinity to develop between pen mates, hereafter referred to as ‘familiars’ (Griffiths & Magurran 1997). This time span was derived from research on teleosts and Jacoby et al. 2012, which investigated the effect of familiarity on the small spotted catshark. During the 14-day period, individuals were marked with external coloured tags (Floy Tag Manufacturing, Seattle, WA, USA) to allow for individual recognition by observers (Guttridge et al. 2011). The four holding pens were connected by a series of channels to a larger pen, called the social network (Figure 1). The holding pens and social network were 4m and 6m in diameter, respectively. A trial consisted of ushering two sharks, without physical contact between researcher and subject, from a holding pen to the 1.5 m^2 starting box. Sharks remained in the start box for at least 5 minutes while two other individuals were ushered to the starting box on the other side of the social network. After ushering the individuals into the starting box, and allowing at least 5 minutes to pass since the last shark went into the starting box, the observer climbed onto the observation tower and opened the doors to the social network pen using a series of ropes and pulleys. The trial began as soon as all sharks were in the social network and the doors were closed. A starting map of individual location, identified via colour tags, at specific times was created to aid in data preparation. A GoPro Hero3 Black edition hung above the social network pen and recorded social interactions for one hour. At the hour’s end, sharks were ushered back to their respective holding pens. The four individuals per trial were randomly selected while minimizing the size differential between individuals (mean size difference between all individuals: 5.8 cm, +/- 4.0 cm). The high standard deviation is due to conducting all possible behavioural trials between unfamiliars within a limited size range, then utilizing the remaining groups of sharks that had not been tested against each other despite a relatively large size difference. Length was later investigated to see if there was a significant effect on interactions (see 'Results'). Sex was not used as a factor for creating the groups of four individuals per trial because these animals are 10 years from being sexually mature and previous experiments with juvenile lemon sharks shows sex plays no role in assortative interactions (Guttridge et al. 2009b, 2011). Sharks from different pens were never introduced for more than one behavioural trial.

We conducted a total of 32 trials and used only 27 for analysis. The remaining 5 trials violated key assumptions for this study; they included 1 trial where two individuals from a previous replicate were reintroduced, 1 trial where an individual escaped from the starting box, was recaptured manually, and subsequently placed back in the pen, and 3 trials where injured fish entered the pen and were ultimately consumed by the individuals.

Recently, sharks have been shown to have distinct personalities that vary between individuals (Jacoby et al. 2014; JS Finger personal communication). Two potential factors that could vary with personality include the influence of novel environments and the impact of hunger. The day before a trial, the entire cohort of sharks in a holding pen was allowed four hours of exploration of the social network (if any of the individuals in that pen would be used the following day). To account for hunger, which has also been postulated as a potential factor influencing social behaviours (TL Guttridge personal communication), sharks were fed to near or full satiation the night before a trial on a mixed diet of fresh and frozen local fish (no live prey was used). No sharks died during the experiments and all were released to their natural habitat. Trials were conducted surrounding low tide to ensure a consistent water depth. Environmental conditions have been shown to have no effect on assortative behaviour in lemon sharks (Guttridge et al. 2009b).

Data Preparation

The footage provided by the GoPro was converted to TIFF format, creating a stilled video, and imported into ImageJ (Schneider et al. 2012). MtrackJ (Meijering & Dzyubachyk 2012) was then used to track individual movement. Each shark was manually tracked by the observer by placing four data points (over four seconds) on the shark's snout every 30 seconds. At the end of the hour, each animal had 480 data points summarizing their movement patterns. Upon completion of tracking for one trial, the entire video file was reviewed to ensure no swapping of sharks occurred during the procedure. An algorithm, developed by Jean-Sebastien Finger, analyzed the data provided by MtrackJ to produce behavioural scores for every 30-second period. The possible interactions observed were 'paralleling', 'following', and 'leading'. "Paralleling," a symmetric behaviour, occurred when two sharks swam side by side where one individual's head was in front of the pectoral fin of the other. "Following" occurred when a shark was behind the pectoral fin of another individual. "Leading" is the opposite of "following" and was not considered for this experiment as we were only interested in active participation, which is defined as a behaviour where the focal shark could deviate from interaction if desired. For example, a shark being followed could not dependably dissolve interactions with the following shark, while a shark engaged in a following or paralleling behaviour could easily turn away from it's partner. (See Table 2 for definitions). Myrberg and Gruber (1974) first described these behaviours and required proximity to be deemed an interaction; our study required the sharks to be within 2.5 body lengths of each other. In addition, the four data points ensured that the sharks were traveling in the same direction when the 2.5 body length condition was satisfied. A manual review was conducted and showed this method produced less than a 5% error, a satisfactory value as manually prepared data often would likely carry a higher error rate. Prior to the use of our algorithm, behaviours would be scored during a set time period while observing behavioural interactions in the wild (see Guttridge et al. 2011). Our technique is improved as observer bias is reduced, accuracy is increased with the ability to review tracking footage and more frequent sampling periods, and the definitions of certain behaviours are concrete and based upon Cartesian tracking points.

The tracking algorithm provided the measured interactions at every 30-second interval for the entire study. An assumption of the tracking algorithm was that sharks could be interacting with multiple individuals at the same point in time (i.e. following one shark, paralleling the other two individuals, etc.). For the purposes of this study, the only interactions used for ecologic conclusions were those between nearest neighbors; gambit of the group was not used because the animals did not have freedom to leave the study site, which likely resulted in some instances of forced group membership (White & Smith 2007). However, gambit of the group was investigated (See '*Weighed Edge Permutation')* to demonstrate the importance of using proximity between individuals as a metric for interest of associating. Using gambit of the group, or counting all sharks that meet the criteria to be deemed an interaction, is useful when participation in the group is voluntary, but this semi captive study did not allow that expression. Thus, we used proximity as an indicator of interest. According to nearest neighbor analysis, the only behaviours receiving a score would be the most proximate to the test shark at time X. For example if the test shark was 67 cm behind a leader and paralleled another shark 15 cm away, paralleling would be the only interaction considered for analysis. After eliminating all non-nearest neighbor interactions, a presence/absence score of 1 or 0, respectively, was created for the entire hour at every 30-second period. A score of 1 was awarded if the test shark was interacting with another individual at that time period, and a score of 0 was observed if the animal was solitary. This technique gives equal weight to both following and paralleling. A weighted matrix was then created, combining all presence/absence scores for the 120 sampling periods. This weighted matrix, here after referred to as 'matrix of interactions' detailed the number of interactions between all individuals (Table 3) over the entire hour. At this point, the number of interactions between familiar and unfamiliars could be determined by looking at the matrix of interactions (Table 3).

Statistical Analysis

Weighted edge permutation

Association indices have been used to measure strength of interactions between individuals. Manly (1995) originally used observational data to test against a randomly simulated network that retained features of the original data. This technique has since been widely adopted (Bejder et al. 1998; Croft et al. 2011). Analyzing the strength of interactions can be accomplished by using a weighted network (Croft et al. 2011). 27 matrices of interactions (Table 3) were produced by the aforementioned data preparation and imported into R (R Core Team 2013). The 27 matrices (4x3) were permuted independently, and all cells were combined to create one 4x3 matrix with summed values from the original matrices of interactions. This was replicated 100,000 times. This null model retains original observational data, giving interactions between familiar and unfamiliar individuals an equal weight, which is important for creating a random network (Bedjer et al. 1998). The values of the familiar cells (Table 3) from each of the 100,000 replicates were combined independently to create a distribution of familiar interactions serving as a 'null familiar model' (Figure 2). A Z-score was calculated using the observed number of familiar interactions and the mean and standard deviation of the familiar interactions from the null familiar model. The previous steps were conducted for the 0-20 minute, 20-40 minute, and 40-60 minute time intervals. This analysis was conducted from both nearest neighbor and gambit of the group.

Investigation was conducted to determine if a Markov Chain test could be used for this analysis; however, the 30-second period was not short enough. In some cases, the behavioural state at X + dt was dependent on X, but not enough where we could accurately predict future behavioural states based on current states. Determining reliance of X+dt on X requires creating a null model based on a experimental data to see if observed changes, or lack of changes, in behavioural state differ significantly from a null model with no dependence between states.

Statistical analysis

Mantel test

Croft et al. (2011) suggested that using models, like a General Linearized Mixed Model (GLMM), as an analytical tool for Social Network Analysis is not advised due to the high level of autocorrelation. We used the Mantel test as an additional technique to test significance between two matrices (Guillot & Rousset, 2012). 6 square matrices (20x20) were created containing data on the number of interactions between all individuals per trial, the difference in length between all individuals per trial, the sexual composition of each interaction, if the capture site between individuals was the same, and a presence/absence score of 1 and 0 for familiar or unfamiliar interactions between all individuals per trial. Two matrices were created for sexual composition. First, a presence/absence matrix was created where 1 and 0 represented a same and opposite sex interactions. Another matrix was created with values of 1, 2, and 3, representing a male:male interaction, a heterosexual interaction, or a female:female interaction.

RESULTS

Preference for familiar partners

Familiar sharks showed a significant preference (Z-score =3.48, N=3438 P =0.00504) for each other for the one-hour study. Figure 2 shows a frequency distribution, generated via weighted edge permutation, of familiar interactions if no preference existed, and is our null familiar model. The coloured line represents the observed number of familiar interactions for the entire hour. The Z-score was calculated for 20-minute time intervals and is shown in Table 4. The preference for familiar individuals for 0-20 minutes was still significant (Z-score=3.38, N=1258, P=0.00073). Preference for familiars declined during the 20-40 and 40-60 minute interval (20-40min: Z-score=1.84, N=1162, P=0.0657, 40-60min: Z-score=1.27, N=1064m, P=0.2040). For this analysis, the sample size is also the number of observed familiar interactions. Using a Mantel test to investigate the impact of familiarity on partner preference, we also showed a significant influence on the number of interactions between all individuals (Mantel test correlation =0.1924, N=10 000, P=0.00290).

Using gambit of the group analysis, we were unable to show any significant preference for familiar individuals in our trials. This conflicts with the findings from nearest neighbour analysis for the 0:60minute time scale and 0:20 minute time scale. While the other periods for nearest neighbour analysis did not show significance, they were still trending towards it. Gambit of the group analysis only trended towards significance for the 0:60 minute time period (Z-score=1.51, N=5358, P=0.131043).

Insignificance of Sex and Length on Interaction Rates

Within our controlled range, pre-caudal length had no significant influence on the number of interactions (Mantel test correlation=0.0602, N=10 000, P=0.1799). The presence/absence sexual composition matrix, denoted by 1 and 0s for same and opposite sex interactions had no significant effect on the number of interactions (Mantel test correlation =-0.0460, N=10 000, P=0.2348). The matrix with 1,2, and 3s, representing a male:male interaction, a heterosexual interaction, or a female:female interaction also showed no significant effect on the number of interactions per trial (Mantel test correlation=-0.0628, N=10 000, P=.0164).

Influence of Home Range and Time on Number of Interactions

Using area of capture as a proxy for overlapping home range, we found no significance between pre-existing familiarity and number of interactions (Mantel Test correlation -0.0710, N=10 000, P= 0.1399).

There was a significant correlation between familiar interactions and time (Spearman rank coefficient r_s=0.3869, N=27, P=0.04617), suggesting the sharks became more social throughout the experiment. However, this same trend was not found for interactions between familiar and unfamiliar sharks (Spearman rank coefficient r_s=-0.0497, N=27, P= 0.8055). This lack of significance suggests that the sharks were not becoming more social, but further developing affinities for familiars.

DISCUSSION

Research Findings and Importance

The present study investigated the effect of familiarity on partner preference in juvenile lemon sharks. We found that juvenile *N. brevirostris* showed significant dyadic preferences for familiar individuals during the introduction to unfamiliars. Individuals in trials were matched according to size (mean size difference between all individuals in trial: 5.8 cm, +/- 4.0 cm), but beyond this control, size had no significant impact on partner preference. Guttridge et al. (2009b, 2011) showed lemon sharks preferred sized-matched individuals, and group leaders often led smaller conspecifics; these results were from both semi-captive and wild studies. While we could not exactly size match individuals, the observed preference for familiars provides evidence that familiarity overrides small ranges in size assortment. We would expect size to make a significant difference if it was not controlled for in trials. Interestingly, the preference for familiars was highest during the first 20 minutes of the trial and showed decreases throughout the following 40 minutes. For the entire hour, significant preferences for familiars were still observed. This study provides a framework for future investigation in shark behaviour and demonstrates the importance of controlling for familiarity. Otherwise, the results could be skewed with underlying preferences and initial biases. In the wild, these results can be used to understand social structure within natural aggregation. For example, lemon sharks aggregate off the coast of Jupiter, Fl throughout the late fall and early winter. The findings provided by this study suggest that animals that were familiar before the aggregations would show a significance preference towards each other during the group event.

Explanation of Familiar Preference

While behavioural investigations on partner preference in sharks are relatively limited, there have been numerous studies examining the effect of familiarity on dyadic preferences in other fishes (Centrarchidae: Brown & Colgan 1986,;Poecillidae: Magurran et al. 1994; Griffiths & Magurran 1999; Cyprinidae: Brown & Smith 1994; Osphronemidae: Miklosi et al. 1992; Gasterosteidae: Van Havre & Fitzgerald 1988). Partner preference with familiars can further the benefits of group living compared to the social interaction of two unfamiliar individuals. Therefore, the persistence of familiar interactions could occur in order to build relationships that facilitate high levels of direct fitness (Griffiths 2003; Ward & Hart 2003). In the current study, the familiar interactions did not persist, and in fact, they deteriorated within the hour. If familiars are not being preferred in order to bolster the benefits of group living, what becomes the motivation behind this initial preference?

The 'Dear Enemy' effect, originally introduced by Fisher (1954), is a likely mechanism behind the observed behaviour. Fisher demonstrated the lack of agonistic interactions between neighboring birds. The basis of this relationship is a mutual understanding of complacency between neighbors in order to avoid agonistic interactions, but when an unfamiliar is introduced, the interactions are altered due to the uncertainty of future events. While agonistic interactions are rare between juvenile lemon sharks, it seems as though individuals prefer familiar conspecifics to ensure the lack of potentially harmful interactions with unfamiliars. This hypothesis is bolstered as the unfamiliar individuals interact more as the trials progress, and eventually, the preference for familiars declines because the initial uncertainty of unfamiliars is replaced with the same mutual understanding that originally existed between familiars. However, repeated partnership of lemon sharks has been observed in wild studies where the preference of familiars is the likely mechanism facilitating this (Guttridge et al. 2011). In reality, the reason for preferring familiars does not have to fall within one category completely. We suspect our sharks had a high preference for familiars at first in order to avoid potentially agonistic interactions. After the uncertainty of unfamiliars was erased, the interactions between unfamiliars increased in frequency. Our model system, which provides a framework for natural interactions, is likely different than the wild system (Further explanation in *Experiment Limitations and Management).* In the wild, we suggest that preference for familiars is not facilitated by either the 'Dear Enemy' effect or the desire to bolster the benefits of group living. Rather, we believe the two advantages are closely linked as the 'Dear Enemy' effect results in spatial isolation that results in strengthened bonds that ultimately bolster the effects of group living between familiar individuals.

This is the first instance of dyadic preference for familiar individuals in chondrichthyes. Jacoby et al. (2012) showed familiar individuals formed larger and more frequent groups than would occur randomly; these results did not occur within unfamiliar groups. Furthermore, observed interactions were higher in familiar groups than unfamiliar, but no overall effect of familiarity was observed on partner preference (Jacoby et al. 2012). Why was there a significant effect of familiarity on assortative associations in lemon sharks and not the small-spotted catshark? The purpose behind preferring familiars can be presumed to differ between the species. Also, the young age of the sharks used and the fact that they were bred in captivity could potentially impact experimental results.

Experiment Limitations and Management

An unavoidable flaw of this experiment was taking individuals from a population with overlapping home ranges. The development of familiarity in the wild occurs via overlapping home ranges, and these initial conditions will be criticized as an inherent flaw. Instead, they should be used to further understand the ecological principles because no significant correlation between individuals from the same area of capture and interactions was observed. This suggests original home range, or original familiarity, did not carry over to the experiment. The density of individuals in holding pens was much higher than that experienced in nature. This most likely increased the predilection towards familiar to such an extent that any previous assortative preferences were erased. As this experiment is a model for the natural world, we must realize that the preference for familiars in a normal ecological setting would most likely be a preference for individuals with overlapping home ranges. In the case of our study, overlapping home ranges were represented by co-inhabitation of a holding pen. The lack of significant correlation between capture location and interactions shows the previous levels of familiarity were erased, and unfamiliar individuals were indeed, unfamiliar.

As mentioned, this studied pooled sharks from one nursery. Results could differ if sharks were taken from different nurseries in Bimini, which previous research has shown has no exchange rate (Franks 2007), thereby ensuring no previous interactions or underlying familiarity. However, initial home range showed no significant effect on number of interactions between individuals. Therefore, we expect results would be unaltered even if unfamiliar sharks were taken from different nursery grounds.

The size of the social network pen had two consequences 1) it altered swimming dynamics and 2) it restricted animals from leaving study sight. Initially, the pen was designed to ensure a high number of interactions between animals. The small area required situations for the sharks to turn, thereby avoiding contact with the pen, more often than they would in nature. However, when sharks were forced to make a decision on which direction they turned, they often had to choose between individuals to interact with. In this case, sharks were forced to make a choice between familiar and unfamiliar partners. With this high level of interaction, we were able to observe underlying preferences of these animals. This great benefit is also one of the studies' biggest disadvantages. In the wild, it is likely unfamiliar partners would not have this much time to interact. A pair of familiar sharks could encounter an unfamiliar, and simply swim away as they are in an open system. The familiar partners would then stay together because 1) the uncertainty of unfamiliars would persist and 2) the desire to bolster the benefits of group living. Bolstering the benefits of group living by repeated exposure could be a positive feedback system, where two familiar sharks avoid unfamiliars and strengthen their relationship, thereby increasing the benefits put forth by group living. After this process is completed, the sharks would be more familiar and less likely to interact with unfamiliars than before. This behaviour would allow for the continuance of familiar relationships and the increased social separation between unfamiliars. The significant, positive correlation of familiar interactions with time in captivity (unfamiliar interaction did not show the same significant correlation) supports the argument that the preference for familiars increases with time spent as partners.

Within the first 20 minutes of our trial, the preference of familiars was likely due to the 'Dear Enemy' effect because uncertainty of unfamiliars was high. However, interactions were forced with unfamiliars, and the initial uncertainty was erased; therefore, interactions began to occur with a higher frequency between unfamiliars. We suggest that this would not happen in the wild. The initial reason for avoidance of unfamiliars is due to the 'Dear Enemy' effect, and the isolation between unfamiliars that results from that behaviour would cause prolonged persistent relationships between familiars, which would bolster the benefits of group living. This explanation could likely explain the reason why Guttridge et al. (2011) observed persistent partnerships over different sampling periods. The animals in the wild, whose repeated partnerships were unable to be explained by size, could likely be familiars that rarely interact with unfamiliars, thereby reinforcing their original preference.

Lastly, our tracking model identified behaviours when they met certain qualifications, such as mirrored trajectories and proximity within 2.5 body lengths. Every behaviour that satisfied these requirements was given a score of 1. Due to the size constrains of the pen, it is likely that animals were forced to be in groups and while satisfying the aforementioned requirements, did not actually desire to interact. This theory is validated by looking at the difference in analysis via gambit of the group v. nearest neighbor. The former identified many more interactions and showed no preference for familiar individuals. Therefore, when we look at all animals that meet the requirements of our tracking model, some interactions included were most likely involuntary. Using the nearest neighbour technique was much more accurate in identifying partner preference. This validates the theory that physical proximity is a good metric for behavioural interest in a partner. If the gambit of the group technique were used, we would not have observed a preference for familiars. When working in a closed system where grouping is not voluntary, using nearest neighbor analysis is critical, as physical proximity is a better metric for social interactions than group participation. Gambit of the group is most useful for fission-fusion groups where social participation is voluntary.

Implications for Previous Work

Since influence of familiarity on partner preference has not manifested significance prior to this study, the perception of results in certain behavioural trials could be misinterpreted. In most of the social experiments using captive animals, there is a set of methods in place to control familiarity (see 'Methods' in Guttridge et al. 2009b for example). However, in wild trials the effect of familiarity could be the mechanism influencing social dynamics. Wild investigations are usually unable to determine the level of familiarity between individuals outside of the study site or outside of the time slot for observation. Future work must ensure that all animals are equally familiar with each other so dyadic preference is not inherently biased. As with Guttridge et al. 2011, we do not seek to call previous investigations into question, instead, we hope to provide a framework for elucidating patterns that have no quantifiable explanation.

Future Research

Future research must identify if lemon sharks recognize familiar conspecifics via individual recognition or categorical discrimination. While some researchers postulate the recognition of familiars can occur via individual recognition, we can be certain only that lemon sharks can discriminate between familiar and unfamiliar sharks (Ward et al. 2009). If the sharks are not recognizing individuals, then they are only discriminating amongst a subset of a population. This would be similar to a young child only being able to distinguish between children and adults based on their associated size and not by individual identity. Currently, there have been no investigations looking into individual recognition in sharks. For our study, we hypothesize that visual recognition is used for social behaviours. The sharks in these trials were housed in a similar environmental setting within 20 meters of each other. We argue that recognition via olfactory cues, on an environmental basis and not an individualistic basis, do not contribute to identification or discrimination between familiars and unfamiliars. The mode of recognition is likely to be extremely variable with life history characteristics. Animals that show close associations while resting in close proximity could develop recognition abilities based on olfaction. The evolution of hunting and social behaviours could occur hand in hand, thereby allowing animals that predominantly use visual cues for prey capture to develop the same cues for social recognition. There is a need to determine if the animals can discriminate between individuals without categorical cues like size, sex, or familiarity. These findings will likely vary with the life history of the animal.

Future work must use analysis of microsatellites to determine if these animals show any preference for kin. No experiment has been able to document this behaviour while controlling for familiarity. It is of critical importance to separate kinship and familiarity while analyzing these preferences as the two often directly correlate.

Conclusion

We showed that juvenile lemon sharks display a preference for familiar individuals and that this preference declined over the one-hour trial. The decline in preference suggests the mechanism behind the behaviour is the 'Dear Enemy' effect and the sharks are initially avoiding unfamiliars in order to avoid agonistic interactions. In nature, the 'Dear Enemy' effect would likely result in isolation between unfamiliar sharks, which would further the familiarity between partners and increase the benefits of group living. We suggest that preference for familiars is not facilitated by either the 'Dear Enemy' effect or the desire to bolster the benefits of group living. Rather, we believe the two advantages are closely linked as the 'Dear Enemy' effect results in spatial isolation, which ultimately strengthens bonds that bolster the advantages of group living between familiar individuals. These results further our understanding on what mechanisms are important for the formation of groups in lemon sharks, a model species for large coastal marine vertebrates. The information gathered here, in addition to expanding scientific insight, can also be useful in fisheries management for determining how to best protect aggregate groups, which are of the utmost importance because they represent keystone species from numerous habitats. If these animals were harvested in full, there would be a limited number of predators to return to the original environment and regulate the ecosystem.

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List of figures

Figure 1

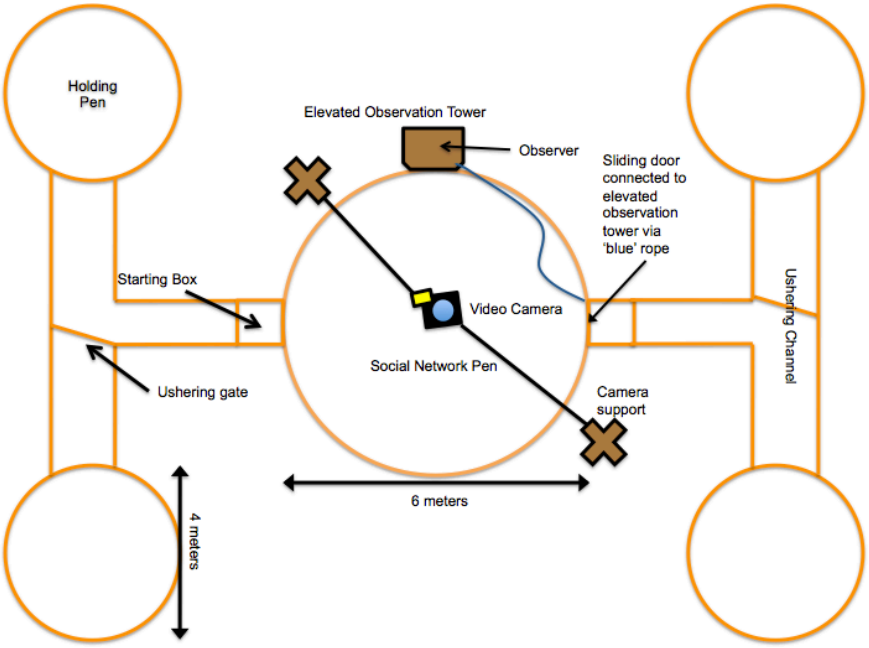

Figure 1. An illustration of the holding pen array used for behavioural trials. Sharks were housed in holding pens and relocated to the social network pen during the 1-hour trials and the exploration periods the day before trials. See 'Behavioural Experiment' for further details.

Figure 2

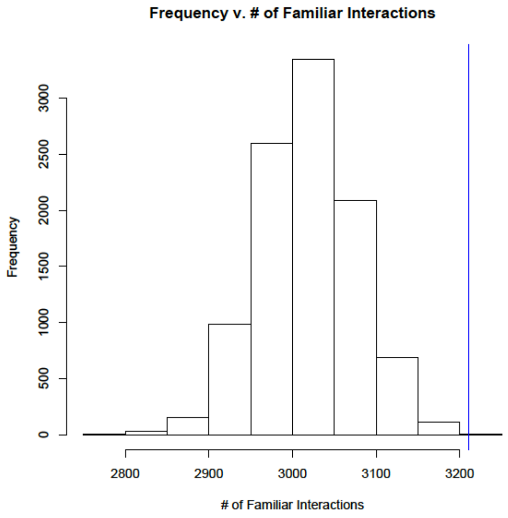

Figure 2. A distribution of familiar interactions that would occur if no preference existed (generated via weighted edge permutation). The vertical line represents number of observed familiar interactions (3438).

List of Tables

Table 1

	Mean Length (cm)	SD (cm)
Pen 1	56.26	5.23
Pen 2	54.98	5.84
Pen 3	55.82	6.08
Pen 4	55.56	6.64

Table 1. The mean length and standard deviation of individuals per holding pen.

Table 2

Behavioural States	Definitions	Considered for this study	Justification of rationale for inclusion in study
Following	An individual mimics trajectory and velocity of leader while within 2.5 body length	Yes	The animals mimic movements and speed while maintaining proximity, thus exhibiting active preference
Paralleling	Two individuals, with their heads aligned in front of their partner's pectoral fin, mimicing trajectories and velocities of their partner while within 2.5 body lengths	Yes	The animals mimic movements and speed while maintaining proximity, thus exhibiting active preference
Milling	Individuals swimming in a non-coordinated manner within 2.5 body lengths	No	The animals do not display maintained speed or trajectory with respect to adjacent shark
Circling	Individuals swimming in circular pattern while following another	Yes	This interaction is deemed 'following' for this study.
Leading	Being in the front of a group of sharks, while within 2.5 body lengths	No	The leader shark does not display an active preference for the sharks' it leads

Table 2. An ethogram of behaviours observed by Myrberg & Gruber 1974, some of which are considered for this study.

Table 3

	2	1	9	11
2	0	37	25	29
1	40	0	32	24
9	27	28	0	28
11	28	28	26	0

Table 3. A weighted matrix denoting the number of nearest neighbour interactions, both paralleling and following, for the 120 sampling periods. The IDs serve as the column and row headers. The IDs of the familiar sharks are identifiable due to their proximity to each other. In addition, the familiar interactions are highlighted.

Table 4

	Gambit of Group		Nearest Neighbor		
	Z-score (sample size)	P-Value	Z-score (sample size)	P-Value	Difference in significance between models?
0-60 min	1.51 (5358)	0.131043	3.48 (3438)	0.000501	Yes
0-20 min	1.16 (1976)	0.246049	3.38 (1258)	0.000725	Yes
20-40 min	.7314 (1752)	0.464535	1.84 (1162)	0.065768	No
40-60 min	1.08 (1630)	0.281042	1.27 (1064)	0.204085	No

Table 4. Time periods throughout experiment with associated Z-score and P-value for gambit of the group and nearest neighbor analysis. Z score was calculated with (number of observed familiar interactions-mean value of null familiar model)/standard deviation of null familiar model. The final column denotes discrepancies in finding significance between the gambit of the group and nearest neighbor technique.