Kinship does not predict the structure of a shark social network

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ABSTRACT:

The role of relatedness in animal societies has received important consideration as a process driving social groups. In the marine world, most studies which have investigated this question have focused on marine mammals such as whales and dolphins. For sharks, recent studies have demonstrated preferential associations among individuals from which social communities emerge. Assortment patterns have been found according to phenotypic or behavioural traits but the role of genetic relatedness and family structure in shaping the social structure of adult shark populations has never been investigated. Here, we used a social network analysis crossed with DNA microsatellite genotyping to investigate the role of the genetic relatedness in the social structure of a blacktip reef shark (*Carcharhinus melanopterus*) population. We found an established social network spatially organized but dynamic through time with different communities merging during the mating periods. Such recombination shows the males being more gregarious. Kinship was not a predictor of associations among sharks both at the dyad or community levels as individuals tended to associate independently and randomly regardless of the genetic relatedness between individuals. The lack of parental care in this species may contribute to the breakdown of family links in the population early in life, thereby preventing the formation of kin-based social networks.

Keywords: genetic relatedness, assortment, social organisation, *Carcharhinus melanopterus*, elasmobranch

INTRODUCTION

Socialising is an adaptive strategy which is widespread across the animal kingdom that can take various forms, from temporary unstable associations to long-term stable groups in complex societies (Krause and Ruxton 2002). Understanding the factors that influence the formation and evolution of social groups is important to understand the evolution of animal societies as well as to gain insight into population dynamics and conservation strategy (Snijders et al. 2017). Associations among individuals can provide benefits to improve individual fitness by reducing predation risk or improving foraging efficiency (Krause and Ruxton 2002). While individuals can benefit by simply associating with other conspecifics (Kerth et al. 2011), the benefit of grouping can be enhanced by associating with similar individuals, also called social assortativity. By associating with individuals of the same size or the same sex, individuals are more likely to avoid conflict or harassment (Dadda et al. 2005) and their risk of predation is reduced via the confusion effect (Landeau and Terborgh 1986). Further assorting with kin can also provide indirect fitness benefits (Hamilton 1964). Kin assortment has been shown to provide benefits in reducing aggression (Olsén and JäUrvi), increasing growth rate (Brown and Brown 1993) or allowing cooperative behaviour such as predator inspection (Milinski 1987).

Kin structuring has received extensive attention in cooperative breeding societies in which groups arise from the retention of offspring and delayed dispersal which facilitates the formation of interactions with relative and kin-based groups (Wolf and Trillmich 2008; Hatchwell Ben J. 2010; Wiszniewski et al. 2010). However, the role of relatedness in structuring animal societies that are characterised by a dynamic fission-fusion social system without parental care has not been demonstrated. While the link between social networks and kinship has been extensively studied in terrestrial animals (Holekamp et al. 2012; Carter et al. 2013; Arnberg et al. 2015), kinship structure in social networks of marine and freshwater

organisms has been primarily limited to marine mammals (Wiszniewski et al. 2010; Mann et al. 2012; Reisinger et al. 2017). Several cetacean societies show strong kin-based social network structures. However, in fishes, kin structure is less clear. Work on shoaling fish, for example, did not find kin assortment, even in species that have the abilities for kin discrimination (Croft et al. 2012). While sharks have recently been shown to be able to develop preferred associations and organise into structured social networks (Mourier et al. 2018), kinship has only been explored in one case study that focused on juvenile sharks (Guttridge et al. 2011), highlighting a lack of information on the potential for kin-based associations to arise in shark populations.

Overall, most studies that have explored the relationship between genetic relatedness and social interactions have focused on highly social species and in particular, on species that exhibit parental care (Wolf and Trillmich 2008; Wiszniewski et al. 2010; Kerth et al. 2011). Studying less social vertebrates should significantly improve our understanding of how social and genetic structure interact to shape the evolution of sociality in the animal kingdom.

In this study, we investigate the interaction between socio-spatial patterns and genetic relatedness in a population of blacktip reef sharks (*Carcharhinus melanopterus*) monitored over a 3-year period on the North shore of Moorea island (French Polynesia). Sharks represent an interesting and unique model to explore the extent to which individuals interact with genetically related associates due to ecological traits that differ from most social vertebrates. First, sharks are now increasingly recognised as being capable of complex social interactions, developing preferred social affiliations (Guttridge et al. 2009; Jacoby et al. 2010; Mourier et al. 2012), showing surprising learning abilities (Guttridge et al. 2013; Mourier et al. 2017) and developing patterns of leadership and dominance hierarchy (Guttridge et al. 2011; Jacoby et al. 2016; Brena et al. 2018). Then, as opposed to many social organisms, reef sharks do not show parental care and many shark species drop their progeny in specific

nurseries outside adult habitats (Mourier and Planes 2013). These discrete nurseries are chosen to potentially provide the neonates with a safe environment where they will spend their first months of life. When juvenile sharks reach a certain size or age, they leave their nursery to explore a wider home range (Chapman et al. 2009) and integrate within the adult population and interact with others. Therefore, these shark populations do not show social viscosity (i.e., philopatry and delayed dispersal) as in some kin-based societies (Wolf and Trillmich 2008). In such aggregations, interactions between kin are also diluted by the presence of numerous neighbours and average relatedness quickly drops with increasing group size. In some shark species, the likelihood of associating with a related peer is reduced due to small litter size and a high mortality rate at the juvenile stage, leading to a lack of first order relatives to reach adulthood. However, in a closed system, such as an isolated island, and in the case of blacktip reef sharks which spends its entire life cycle within Moorea (Mourier and Planes 2013), relatives have more chance encounter each other and interact in social groups.

To understand the assortative forces which underpin the structural properties of the system is challenging for elusive underwater animals. As the blacktip reef shark displays a high degree of site attachment (Papastamatiou et al. 2009) and shares some of its areas with a variety of neighbours (Mourier et al. 2012), exploring this network holds the potential to work out the relationship between spatial, social and genetic structure in a reef shark. As reef sharks do not show parental care but display ontogenetic habitat segregation with females leaving their young in discrete nurseries, we will test: (1) whether the social network changes between the non-mating and mating seasons and (2) whether genetic relatedness plays a role in structuring the network at both the individual and community levels. Here, for the first time, we explore the link between social network and relatedness in an adult shark population.

MATERIAL AND METHODS

Field observations and network construction

Between 2008 and 2010, observation surveys were conducted along approximately 10km of coastline of the Northern reef of Moorea Island (French Polynesia) (Figure 1). The surveys consisted of 40 min dives at 6 sites along a 10-km portion of reef in which individual blacktip reef sharks were identified using photo-identification based on the colour-shape of the dorsal fin. Previous surveys of the areas demonstrated that shark social network might be dynamic and change during the mating period (Mourier et al. 2012). Therefore, we split the dataset into non-mating and mating periods to analyse social networks of blacktip reef sharks separately for both periods. This segregation was made based on observed mating scars on the bodies of females as a sign of mating activity (Chin et al. 2015) and pregnancy monitoring from underwater surveys (Porcher 2005; Mourier and Planes 2013). Overall, the non-mating period extends between March and August and the mating period starts in September and lasts until February the following year. Using R package *asnipe* (Farine 2013), we calculated dyadic association strengths for each period (i.e. associations among pairs of individuals) from the spatio-temporal co-occurrences, and the proportion of time two individuals were observed together at the same site, using the half-weight index (HWI) (Cairns and Schwager 1987).

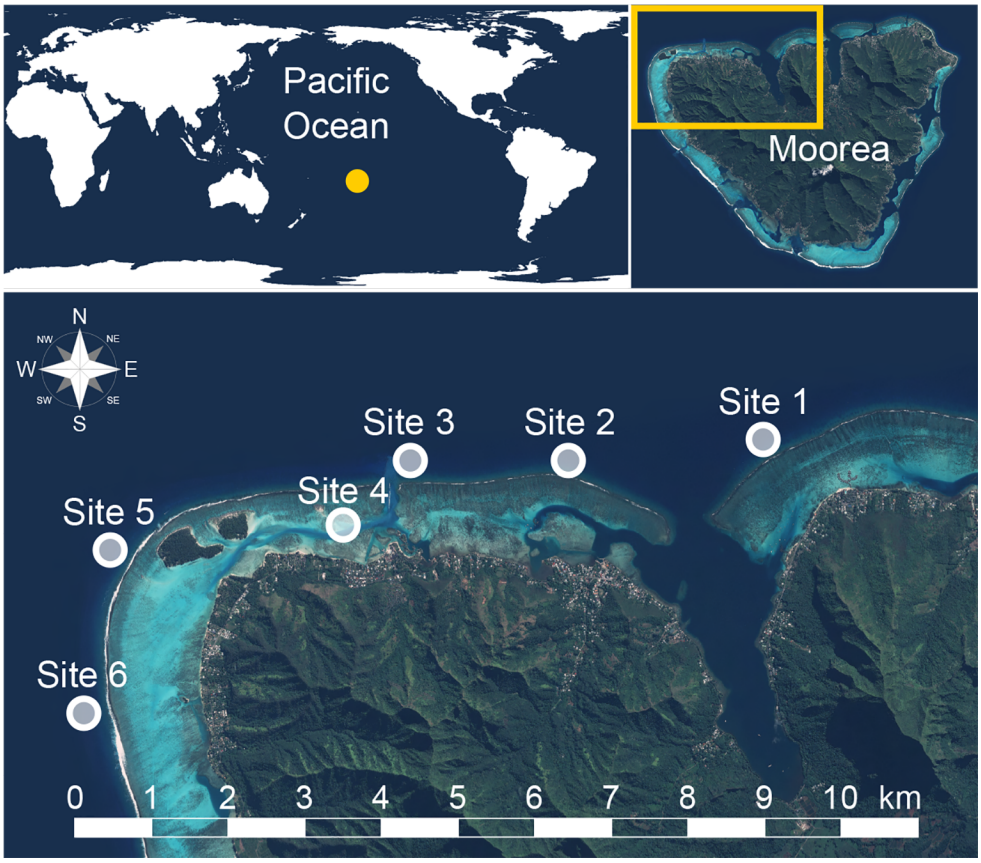

Figure 1. Map of the study location indicating the monitored sites along the 10 km reef edge of the North coast of Moorea.

Network metrics

A randomization procedure was implemented to test if there is a relationship between the social network and factors such as the genetic relatedness, the spatial overlap and the sex category of each dyad. We therefore used a data stream permutation procedure and, based on the stack of individual by individual matrix for each sampling period, swaps individuals between associations within sampling periods, and then recalculated the weighted summary network after each swap. Each of the 1000 permutations implemented in this study created a new weighted network, resulting in a new stack of matrices (Farine 2013). This procedure performed permutations which controlled for the number of observations and group size.

150 We used an eigenvector-based modularity method to determine if the population was divided
151 into different communities (clusters) (Newman 2006), and whether groups of close affiliates
152 (i.e. individuals connected by edges with high HWI values) could be identified. We
153 calculated modularity using the difference between the proportion of total co-occurrences
154 within clusters and their expected proportion given the sum of co-occurrences of inter-cluster
155 individuals.
156 We then compared the gregariousness of individual sharks between the non-mating and
157 mating seasons. For this, we used two measures of gregariousness: node *degree* (or binary
158 degree) which is the number of direct neighbours each individual is connected to in the
159 network and node *strength* (or weighted degree) that is the sum of associations of an
160 individual. We then used these network metrics in order to determine (1) whether individuals
161 differed in their gregariousness between non-mating and mating seasons and (2) whether
162 males and females differed in their gregariousness with respect to season. To test if there is a
163 difference, we used data permutations described above and compared the t-statistic of the
164 observed difference between the two seasonal networks (or between males and females for
165 each network depending on the analysis) to the distribution of t-values from the randomised
166 data (Farine and Whitehead 2015; Farine 2017). If the observed t-statistic sits outside the
167 random distribution, then the difference is significant. The significance is therefore calculated
168 by counting the number of randomised test statistics that had a greater value than the
169 observed statistic if the statistic is positive (or a lower value than the observed statistic if the
170 statistic is negative), and then dividing by the number of randomisations.
171
172 **DNA sampling and laboratory procedures**
173 Shark fishing sessions using rod and reel and barbless hooks were conducted to obtain tissue
174 samples for genetic analysis. Once hooked, sharks were brought alongside the boat where

175 they were inverted and placed in tonic immobility while biological data and tissue samples
176 were collected. Each shark was identified by photo-identification of the dorsal fin, sexed and
177 measured (Mourier et al. 2012; Mourier, Mills, et al. 2013). Fishing sessions were conducted
178 directly after underwater surveys to avoid perturbations of the experimental setup (Mourier et
179 al. 2017) and to increase the chance of getting DNA samples from sharks that were part of
180 the social network. Fishing effort was maintained until sharks failed to respond to the bait
181 (generally <30 min and after catching 2-3 individuals). A fin clip was collected from the
182 second dorsal fin or anal fin and samples were individually preserved in 95% ethanol and
183 returned to the laboratory for genotyping (Mourier and Planes 2013). DNA was extracted
184 using the QIAGEN DX Universal Tissue Sample DNA Extraction protocol. PCR
185 amplification and the microsatellite loci used are described in detail in previous studies
186 (Mourier and Planes 2013; Vignaud et al. 2013; Vignaud et al. 2014). The software
187 MICROCHECKER (Van Oosterhout Cock et al. 2004) was used to test for null alleles and
188 other genotyping errors.
189 We compared the suitability of four pairwise relatedness estimators, Queller and
190 Goodnight (QG) (Queller and Goodnight 1989), Lynch and Ritland (LR) (Lynch and Ritland
191 1999), Li et al. (LL) (Li et al. 1993) and Wang (W) (Wang 2002) in the R package *related*
192 (Pew et al. 2015) and determined that the Wang estimator was best suited to our
193 microsatellite panel (Fig. S1). This function generates simulated individuals of known
194 relatedness based on the observed allele frequencies and calculates the genetic relatedness
195 using four different estimators. The correlation between observed and expected genetic
196 relatedness was obtained for each estimator, and the one with the highest correlation
197 coefficient was selected for further analysis.
198
199

200 **Spatial profiles**

201 We describe spatial patterns for all individuals at each survey site and for each season using a
202 residency index R that takes into account the unequal sampling effort between sites using the
203 following equation:

204 $$R_{ij} = \frac{S_{ij}}{\sum_j S_i} * \frac{\sum_i S_j}{\sum_{ij} S}$$

205 with R_{ij} representing the Residency index of individual i at site j and S_{ij} represents the number
206 of sighting of individual i at site j. The left part of the equation is the siting rate of individual i
207 at site j while the right part of the equation represents a weight, where the sampling bias is
208 considered. We then generated a Bray-Curtis similarity matrix to construct a matrix of spatial
209 overlap between individuals for each season.

210

211 **Crossing network analysis with genetic relatedness**

212 Several different approaches were used to investigate the role of genetic relatedness in
213 driving social structure in this population. First, across both non-mating and mating social
214 networks, we asked what most influenced social association strength among sharks: genetic
215 relationships, sex or spatial relationships. We used the multiple regression quadratic
216 assignment procedure (MRQAP) [49] modified by Farine (Farine 2013) that enables null
217 models built from pre-network data permutations to be used in conjunction with a MRQAP
218 regression. This approach was shown to be more accurate than classic MRQAP procedures
219 (Farine 2017). The dependent matrix in this procedure contained the observed association
220 strengths (HWI values) in the social network. The three predictor matrices came from the
221 spatial overlap among dyads, the sex homophily and the genetic relatedness values among
222 dyads, allowing us to explore how spatial preferences, sex and genetic relationships,
223 respectively, influence network structure during both seasons. In the sex homophily matrices,
224 similar dyads received a value of 1, while dissimilar dyads received a value of 0. We

225 performed all the network analyses, including permutations and MRQAP tests, using the
226 package asnipe (Farine 2013) in R v. 3.3.0 (R Core Team 2019).
227 Next, to determine whether individuals within communities were more or less closely related
228 than expected, we compared the observed values for each community against a distribution of
229 expected relatedness values generated by randomly shuffling individuals between community
230 groups for 1000 permutations, where size was kept constant using the R
231 package *related* (Pew et al. 2015). If the observed mean relatedness was greater than that of
232 the permuted data ($P > 0.95$), then the null hypothesis which predicted that the mean within-
233 community relatedness is random, was rejected. If only a few closely related individuals were
234 present, then it is possible that their within-community overabundance compared to between
235 social communities might not be detected using mean coefficient of relatedness (Buston et al.
236 2009).
237 Similarly, we verified whether the proportion of closely related pairs was higher within than
238 between social communities using a chi-squared test following the same approach as the
239 precedent analysis with mean relatedness. We compared the χ^2 statistics of the observed
240 difference in proportions of relatedness values above a certain threshold between within- and
241 between-communities to that of expected relatedness values generated by randomly shuffled
242 individuals between community groups for 1000 permutations and keeping size constant. We
243 tested with the two threshold relatedness values 0.125 and 0.25 corresponding to the
244 theoretical relatedness of cousins and half-sibs, respectively.
245

246 # RESULTS

247 **Data summary**

248 The network was built with 105 individual sharks observed at least 3 times (range 3-20)
249 during 136 sampling weeks. The threshold of three observations per shark was chosen to

remove transient sharks from the network. The non-mating season network consisted of 103 individuals (67 males and 36 females) and the mating season network of 101 individuals (67 males and 34 females), with both networks having 99 individual sharks in common (65 males and 34 females). Both networks are presented in Figure 2. The non-mating season network was divided into five distinct communities while the mating season network was only divided into three large communities (Figure 2). Gregariousness was generally higher during the mating season, with a significantly higher degree during the mating season (t = -2.803, P_{rand} = 0.001) and a higher strength, although the latter was not significant (t = -2.883, P_{rand} = 0.052) (Figure 3). Males were generally more gregarious than females, as they interacted with more individuals (higher degree) and had stronger relationships (higher strength) for both seasons, although only degree was significantly higher for males during the non-mating period (Figure 3; Table 1).

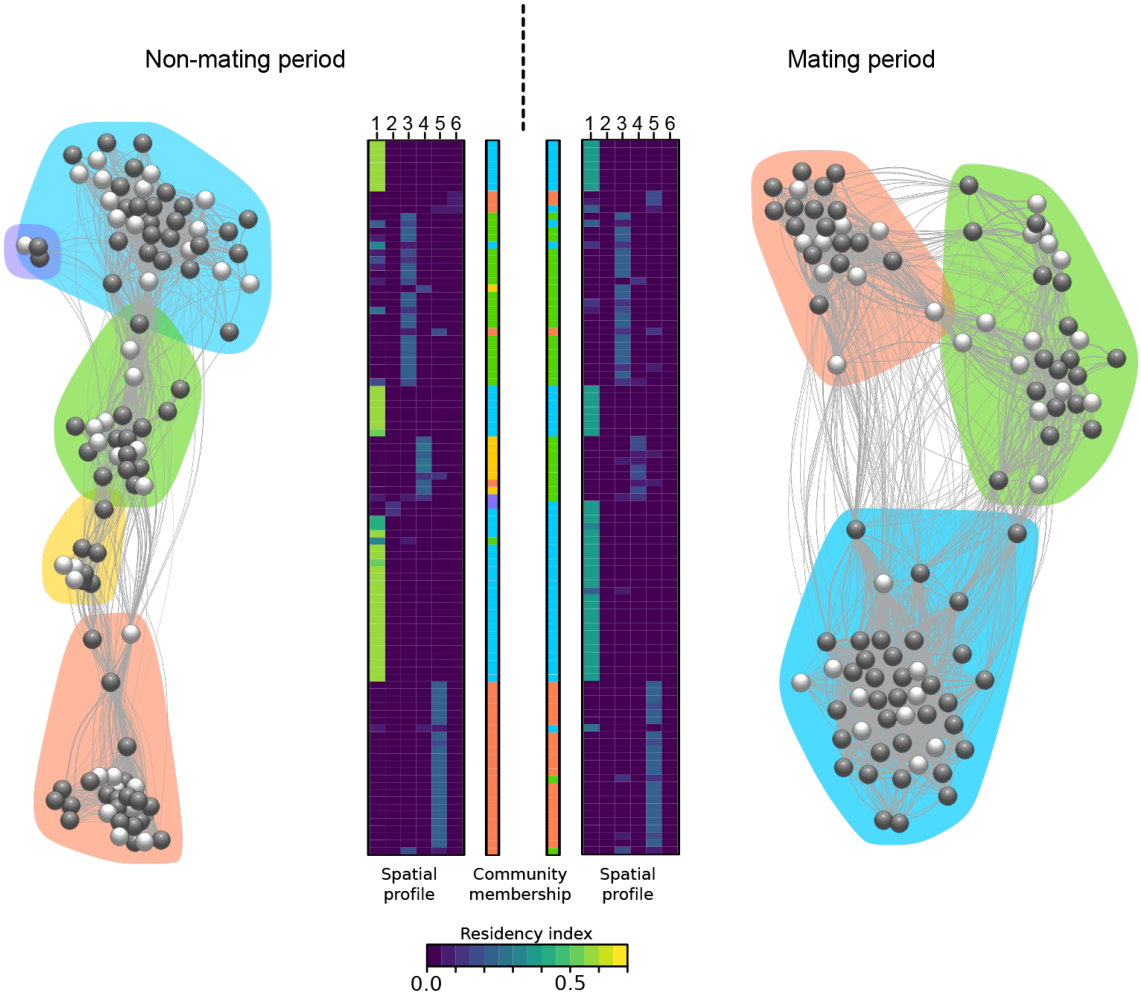

Figure 2. Social networks of the studied blacktip reef shark population in Moorea during non-mating and mating seasons. Nodes represent individual sharks with black nodes representing males and white nodes representing females. Communities are represented by the background coloured areas. Each network is associated with a heatmap representing the spatial profile of each individual common to both networks (n = 99) on each row with a colour proportional to its residency index at each site on each column. Community membership is also indicated for each individual to highlight potential shift in membership between seasons.

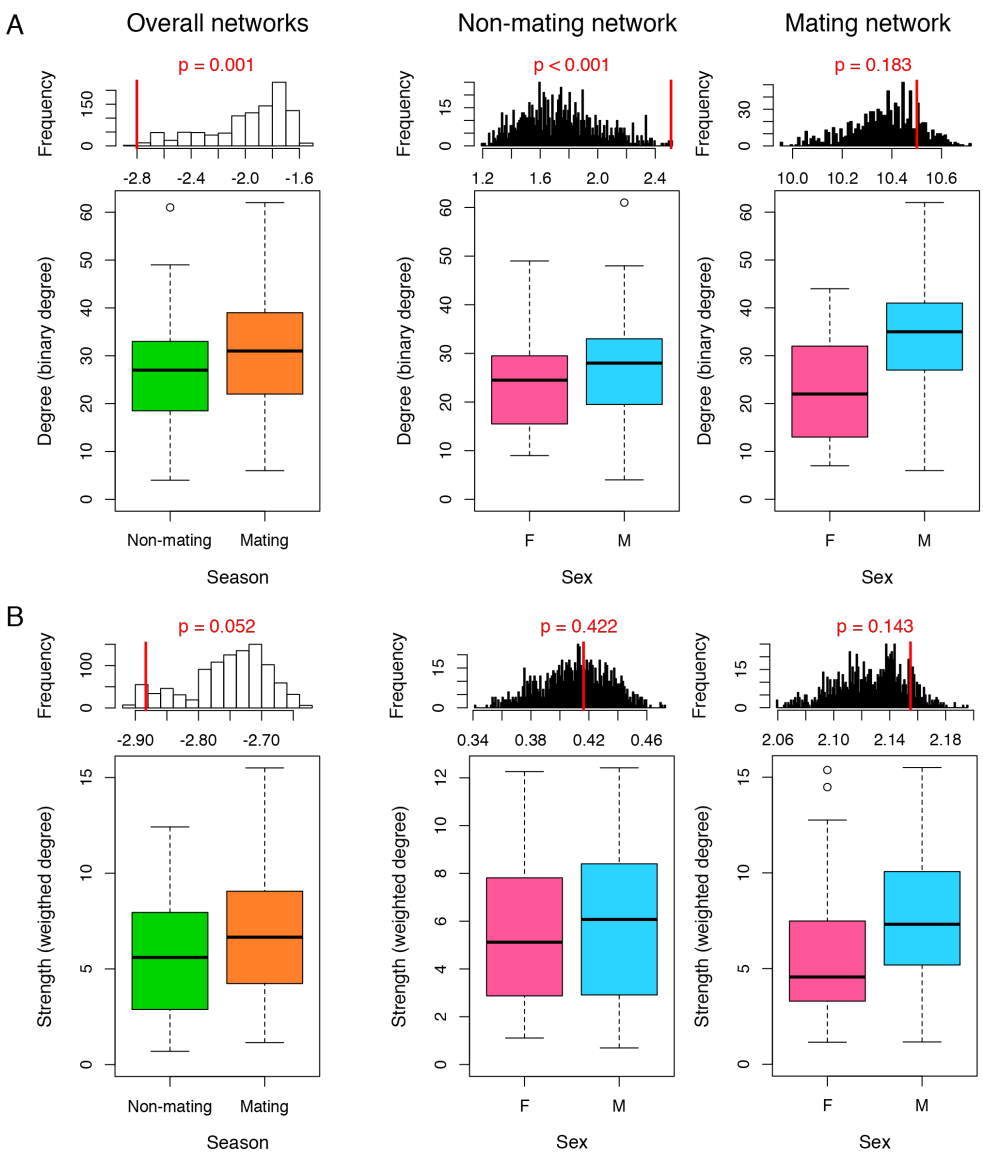

Figure 3 Differences in gregariousness (degree and strength) of individual sharks between both seasons and between males and females for each season (colours: green indicates the overall non-mating season's network; orange indicates the overall mating season's network; pink indicates females and blue indicates males for each season). Comparison of the coefficient from the GLM based on the observed data (red vertical line) and the frequency distribution of coefficients from the same model based on the randomized data are indicated over each box plot to report significance of the difference.

282 **Table 1** Effects of sex on shark gregariousness (degree and strength) in social networks for
283 both seasons.

		Non-mating season				Mating season			
		Coefficient	SE	*t* statictic	P_{rand}	Coefficient	SE	*t* statictic	P_{rand}
Degree	*Intercept*	24.250	1.802	13.457		23.471	1.938	12.109	
	Sex (male)	2.511	2.234	1.124	**< 0.001**	10.500	2.380	4.412	0.183
Strength	*Intercept*	5.466	0.538	10.145		5.687	0.594	9.570	
	Sex (male)	0.416	0.668	0.623	0.422	2.154	0.7298	2.952	0.143

284

285 A total of 225 adult sharks were genotyped from the studied area. About 80% of the
286 individuals included in our social network analyses were genotyped: 79.6% for the non-
287 mating period (i.e. 82/103 individuals) and 78.2% for the mating period (79/101 individuals).
288 From the 17 microsatellite markers taken from our previous study (Mourier and Planes
289 2013), the presence of null alleles was detected at Cli12 which was then removed from our
290 dataset for further genetic analyses. We conducted the genetic analyses with 16 loci (Table
291 S1).

292

293 **Crossing of genetic relatedness and association patterns**

294 MRQAP regressions revealed that only spatial overlap was a significant predictor of
295 associations among sharks for both mating and non-mating seasons ($p < 0.05$; Table 2; Table
296 3). Indeed, sex homophily was not a significant factor and this was also confirmed by the
297 lack of significance of male and female homophily in explaining association patterns among
298 sharks.

299 With a negative regression coefficient, genetic relatedness was not a significant predictor of
300 association patterns in both seasons ($\beta = -0.014$ for the non-mating season and $\beta = -0.002$ for
301 the mating season; Table 2 and Table 3).

302

303 **Table 2** Results of MRQAP analysis of the effects of genetic relatedness, sex and spatial
304 relationships (spatial overlap) on observed association strength in the social networks during
305 the non-mating and mating seasons.

	Non-mating period		Mating period	
	Regression coefficient	*P*	Regression coefficient	*P*
Relatedness	-0.014	0.598	-0.002	0.725
Sex	0.004	0.123	0.008	0.785
Spatial overlap	0.418	**0.018**	0.407	**0.012**

306 Significant *P* values are shown in bold.

307

308 **Table 3** Results of MRQAP analysis of the effects of genetic relatedness, sex homophily and
309 spatial relationships (spatial overlap) on observed association strength in the social networks
310 during the non-mating and mating seasons.

	Non-mating period		Mating period	
	Regression coefficient	*P*	Regression coefficient	*P*
Relatedness	-0.014	0.602	-0.002	0.765
Male homophily	0.006	0.146	0.010	0.863
Female homophily	-0.003	0.891	-0.001	0.566
Spatial overlap	0.418	**0.018**	0.407	**0.012**

311 Significant *P* values are shown in bold.

312

313 Average pairwise relatedness among individuals was 0.0006 ± 3.98 10^{-6} (mean ± SE) ranging
314 from -0.462 to 0.909. Within-community relatedness estimate was inferred for each
315 community with five or more samples for both non-mating and mating seasons; therefore,
316 during the non-mating period, the purple community (Figure 2) which had only three
317 individuals, was excluded from this analysis. In general, relatedness within all communities
318 was not higher than expected if communities were randomly organized (non-mating period:

mean ± SE = 0.017 ± 0.005, p < 0.322; mating period: mean ± SE = 0.016 ± 0.005, p < 0.297) (Figure 4). Relatedness within each community was also not higher than expected: for communities green (mean ± SE = 0.058 ± 0.017, p < 0.069), blue (mean ± SE = 0.011 ± 0.007, p < 0.378), red (mean ± SE = -0.004 ± 0.008, p < 0.535) and yellow (mean ± SE = -0.014 ± 0.041, p < 0.563) during the non-mating season, and green (mean ± SE = 0.038 ± 0.012, p < 0.245), blue (mean ± SE = 0.012 ± 0.006, p < 0.339) and red (mean ± SE = -0.001 ± 0.009, p < 0.534) during the mating season (Figure 4).

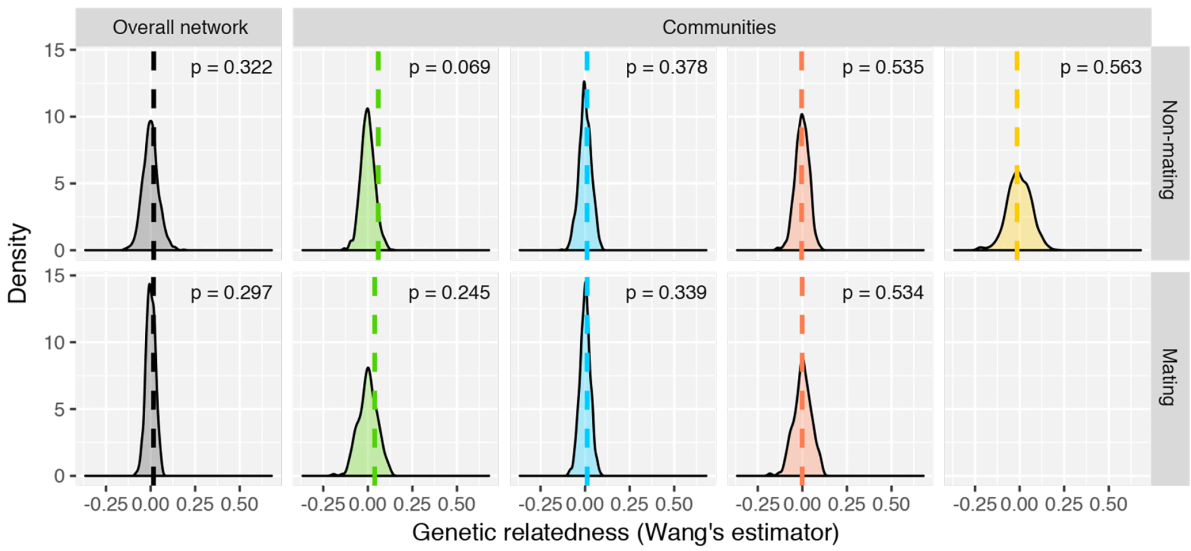

Figure 4 Relatedness analysis for communities of the non-mating and mating shark social networks. Dotted-lines indicate average observed within-community relatedness; density plots show the expected distribution of within-community relatedness values if communities were randomly organized at their original sample size and based on 1000 iterations. P-values indicate the percentage of randomized iterations where the expected values were greater than or equal to the observed value. Similarly, for the overall network, the observed average within-community relatedness across all communities is compared with expected values. Communities are based on community detection procedure from the networks presented in Figure 1.

Among the 25 200 potential pairs, 5 614 (22.3%) had relatedness values higher than 0.125 and 1910 (7.5%) had values higher than 0.25. In addition, there was no difference in the proportion of close relatives within and between communities for either threshold relatedness value during the non-mating season (chi-squared test for threshold 0.125: d.f. = 1, $\chi^2 = 0.543$, p = 0.684; chi-squared test for threshold 0.25: d.f. = 1, $\chi^2 = 1.539$, p = 0.344) and during the mating season (chi-squared test for threshold 0.125: d.f. = 1, $\chi^2 = 1.524$, p = 0.491; chi-squared test for threshold 0.25: d.f. = 1, $\chi^2 = 2.040$, p = 0.314) (Table 4).

Together, these results suggest that no differences exist for within- and between-community membership with respect to the genetic relatedness of their members.

Table 4 Community composition of highly related pairs. For each community, the number and percentage of within community pairs of relatedness values >0.25 and >0.125 are indicated.

	Non-mating period			Mating period		
Community	Pairs with R > 0.25	Pairs with R > 0.125	Total	Pairs with R > 0.25	Pairs with R > 0.125	Total
Green	41 (8.2%)	119 (23.9%)	496	31 (14.7%)	64 (30.4%)	210
Blue	22 (18.3%)	42 (35%)	120	53 (7.9%)	157 (23.5%)	666
Red	18 (5.5%)	60 (18.4%)	325	11 (5%)	38 (18.1%)	210
Yellow	2 (13.3%)	2 (13.3%)	15			
Purple	0	0	1			

356 # DISCUSSION

357 We found that the social network of blacktip reef sharks in Moorea was dynamic with
358 five mixed-sex communities during the non-mating season merging into three larger
359 communities during the mating season (Figure 2). Social proximity was not predicted by the
360 genetic relatedness between sharks both at the association and community levels and was
361 only explained by spatial overlap between individuals. A genetic relatedness analysis also
362 revealed that individuals had low probabilities of interacting with a close-kin which could
363 explain the lack of influence of kinship in structuring the social network in this population.
364 These results therefore suggest that genetic relatedness does not drive the structure of the
365 social network in this shark population.

366 Our previous work on this population suggested that association patterns between
367 sharks could be dynamic and may be seasonally dependent (Mourier et al. 2012). By
368 analysing the non-mating and mating seasons separately, we revealed that the overall
369 population network changed between seasons. During the non-mating season, the network
370 was composed of five communities in which members were more connected to each other
371 that they were with members of other communities. During the mating season, the network
372 communities merged to form three larger communities. This dynamic appears to be driven by
373 the movements and increased activity of some individual sharks during the mating season,
374 switching from one community to another (Figure 2). Males that move among communities
375 during the mating season may gain a selective advantage in courting novel females as shown
376 in other fishes (Kelley et al. 1999). Individuals tend to be more connected during the mating
377 period and males are more gregarious than females during both seasons (Figure 3). Having
378 strong and stable social affinities, especially in females, has been shown to increase resilience
379 of associations to male harassment or novel intruders (Jacoby et al. 2010). This could partly
380 suggest that, even if genetic relatedness does not influence the structure of the network,

381 developing strong associations in shark populations can provide fitness benefits, at least
382 within a reproductive context.
383 Among the adult sharks in our population, there was a generally low level of
384 relatedness, and only a small number of dyads had close familial relatives. Interactions
385 frequently occurred between distant kin and non-kin. This implies that the social structure
386 among adult blacktip reef sharks was not based on associations between close kin as
387 demonstrated by our analyses which compared association patterns with genetic relatedness
388 among dyads at the pairwise or community levels. This is confirmed by the low number of
389 close kin available for each shark in the population (<8 % pairs with R>0.25), thereby
390 limiting the probability of an individual to encounter a family member and to develop strong
391 associations with them. These results can be explained in part by the life history and life
392 cycle of blacktip reef sharks. In fact, in contrast to most social animals that show some forms
393 of family structure and parental care, female reef-associated sharks such as blacktip reef
394 shark, leave their pups in their nursery after birth (Mourier and Planes 2013). Moreover, litter
395 size in this species does not exceed five pups (Mourier, Mills, et al. 2013) while litter size in
396 Moorea was limited to a maximum of two pups (Mourier and Planes 2013). In addition,
397 blacktip reef sharks follow a yearly breeding cycle with females giving birth every year and
398 potentially being fertilized by multiple males within or across years, which increases the
399 probability of having maternal and paternal half-siblings. Our ongoing long-term nursery
400 monitoring shows that capture probabilities rapidly decline after March (unpublished data), a
401 couple of months after parturition, which suggests a dramatic mortality rate within the
402 nursery areas during the first months of life (i.e. survival rate expected to be inferior to 50%
403 during the first year of life). Together with a small litter size and absence of parental care,
404 this high mortality rate, which is common in many shark species, is likely to limit the
405 opportunity to find family members and develop strong affiliations with close relatives at

406 adulthood. Even in nurseries, juvenile lemon sharks did not clearly assort by relatedness
407 (Guttridge et al. 2011), even if the probability of finding a relative is higher for this species
408 with a larger litter size. When juvenile sharks grow, they progressively explore their
409 environment and increase their home range (Chin A et al. 2013), creating an opportunity to
410 find related individuals such as parents or maternal half-siblings from previous reproductive
411 seasons. At adulthood, our results confirm that preferred associations (Mourier et al. 2012)
412 are not driven by genetic relatedness as sharks are associating with conspecifics of variable
413 genetic distances. This suggests that sharks might not have the ability for kin recognition
414 simply based on visual or olfactory cues and that kin-based preferred associations may only
415 develop within nursery areas from increased familiarity with littermates.
416 The emerging literature suggests that genetic structure of animal social networks can vary
417 dramatically, from highly cohesive kin-based groups like African elephants (*Loxodonta*
418 *africana*) (Archie et al. 2006) or spotted hyenas (*Crocuta crocuta*) (Holekamp et al. 2012), to
419 groups with moderate levels of genetic relatedness due to limited dispersal like the Galápagos
420 sea lion (*Zalophus wollebaeki*) (Wolf and Trillmich 2008) or the eastern grey kangaroo
421 (*Macropus giganteus*) (Best et al. 2014), or to groups with little to no genetic relatedness like
422 guppies (*Poecilia reticulata*) (Croft et al. 2012), the common raccoon (*Procyon lotor*)
423 (Hirsch et al. 2013) or migratory golden-crowned sparrows (*Zonotrichia atricapilla*)
424 (Arnberg et al. 2015). These patterns of variation provide opportunities to explore how
425 ecological factors interact with kinship to produce variations in the structures of animal
426 societies. Kinship is expected to promote the evolution of cooperation and sociality in
427 animals (Hamilton 1964). However, our understanding of the evolution of sociality results to
428 a great extent from the study of closed societies, in which interactions mainly involve
429 relatives and can hence be explained by kin selection (Hamilton 1964). However, the kin
430 selection theory has recently been challenged by results from studies showing that fitness

benefit can emerge in social groups composed mainly of non-relatives (e.g., Cameron et al. 2009; Riehl 2011; Wilkinson et al. 2016). In many natural populations, dispersal tends to be limited, favouring local competition between neighbours and the emergence of a social component, whether it be selfish, aggressive, cooperative or altruistic (Lehmann and Rousset 2010). But how social behaviours translate into fitness costs and benefits depends considerably on life-history features, as well as on local demographic and ecological conditions. The fission – fusion social dynamics lead to unstable group membership, and dispersal and occasional recruitment of unrelated individuals lead to low average relatedness in groups. Then under such conditions, selection is not expected to favour kin recognition mechanisms based on familiarity alone.

Therefore, contrary to the kin selection hypothesis which predicts stronger associations among kin, sharks tended to assort randomly according to relatedness. As kinship does not explain the strength of social affiliations in blacktip reef sharks, the question remains as to how and why sharks form preferred associations organised in social communities (Mourier et al. 2012). Although cooperation has been mainly explained in the context of kin selection, there might be potential benefits of non-kin sociality in blacktip reef sharks. While evidence of shark cooperation has not been confirmed, grouping can have several benefits in sharks (Jacoby et al. 2012), including increased foraging success by hunting in groups (Weideli et al. 2015; Mourier et al. 2016), protection from predators (Mourier, Planes, et al. 2013), or increased tolerance relationships and reduced aggression rate (Brena et al. 2018). These benefits do not necessarily imply kin selection and can simply build on the development of familiarity from repeated interactions. Social structure in reef sharks can arise from multiple simple ecological factors such as the distribution of resources in space and time leading to aggregations of individuals even in the absence of benefits of direct social affiliation (Ramos-Fernández et al. 2006) or mitigation of the cost of unnecessary aggression when competing

for resources mediated by individual recognition (Brena et al. 2018). Regardless of the exact cause of social preferences in reef sharks, the absence of kinship as an important factor in structuring association patterns suggests that there are important benefits of sociality in sharks that we still need to uncover. With an increasing use of social network analyses applied to shark populations (Mourier et al. 2018), future work on social networks and genetic relatedness in different populations or species is necessary to confirm our results and to improve our understanding of population dynamics in sharks and the evolution of sociality.

SUPPLEMENTARY MATERIAL

Supplementary information including a table and a figure can be found online.

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REFERENCES

Archie EA, Moss CJ, Alberts SC. 2006. The ties that bind: genetic relatedness predicts the fission and fusion of social groups in wild African elephants. Proc R Soc Lond B Biol Sci. 273(1586):513–522. doi:10.1098/rspb.2005.3361.

Arnberg NN, Shizuka D, Chaine AS, Lyon BE. 2015. Social network structure in wintering golden-crowned sparrows is not correlated with kinship. Mol Ecol. 24(19):5034–5044.

Best EC, Dwyer RG, Seddon JM, Goldizen AW. 2014. Associations are more strongly correlated with space use than kinship in female eastern grey kangaroos. Anim Behav. 89:1–10. doi:10.1016/j.anbehav.2013.12.011.

Brena PF, Mourier J, Planes S, Clua EE. 2018. Concede or clash? Solitary sharks competing for food assess rivals to decide. Proc R Soc B. 285(1875):20180006. doi:10.1098/rspb.2018.0006.

Brown GE, Brown JA. 1993. Social dynamics in salmonid fishes: do kin make better neighbours? Anim Behav. 45(5):863–871. doi:10.1006/anbe.1993.1107.

Buston PM, Fauvelot C, Wong MYL, Planes S. 2009. Genetic relatedness in groups of the humbug damselfish Dascyllus aruanus: small, similar-sized individuals may be close kin. Mol Ecol. 18(22):4707–4715. doi:10.1111/j.1365-294X.2009.04383.x.

Cairns SJ, Schwager SJ. 1987. A comparison of association indices. Anim Behav. 35(5):1454–1469. doi:10.1016/S0003-3472(87)80018-0.

Cameron EZ, Setsaas TH, Linklater WL. 2009. Social bonds between unrelated females increase reproductive success in feral horses. Proc Natl Acad Sci. 106(33):13850–13853. doi:10.1073/pnas.0900639106.

Carter KD, Seddon JM, Frère CH, Carter JK, Goldizen AW. 2013. Fission–fusion dynamics in wild giraffes may be driven by kinship, spatial overlap and individual social preferences. Anim Behav. 85(2):385–394. doi:10.1016/j.anbehav.2012.11.011.

Chapman DD, Babcock EA, Gruber SH, Dibattista JD, Franks BR, Kessel SA, Guttridge T, Pikitch EK, Feldheim KA. 2009. Long-term natal site-fidelity by immature lemon sharks (Negaprion brevirostris) at a subtropical island. Mol Ecol. 18(16):3500–3507. doi:10.1111/j.1365-294X.2009.04289.x.

Chin A, Heupel MR, Simpfendorfer CA, Tobin AJ. 2013. Ontogenetic movements of juvenile blacktip reef sharks: evidence of dispersal and connectivity between coastal habitats and coral reefs. Aquat Conserv Mar Freshw Ecosyst. 23(3):468–474. doi:10.1002/aqc.2349.

Chin A, Mourier J, Rummer JL. 2015. Blacktip reef sharks (Carcharhinus melanopterus) show high capacity for wound healing and recovery following injury. Conserv Physiol. 3(1):cov062. doi:10.1093/conphys/cov062.

Croft DP, Hamilton PB, Darden SK, Jacoby DMP, James R, Bettaney EM, Tyler CR. 2012. The role of relatedness in structuring the social network of a wild guppy population. Oecologia. 170(4):955–963. doi:10.1007/s00442-012-2379-8.

Dadda M, Pilastro A, Bisazza A. 2005. Male sexual harassment and female schooling behaviour in the eastern mosquitofish. Anim Behav. 70(2):463–471. doi:10.1016/j.anbehav.2004.12.010.

Dekker D, Krackhardt D, Snijders TAB. 2007. Sensitivity of MRQAP Tests to Collinearity and Autocorrelation Conditions. Psychometrika. 72(4):563–581. doi:10.1007/s11336-007-9016-1.

Farine DR. 2013. Animal social network inference and permutations for ecologists in R using asnipe. Methods Ecol Evol. 4(12):1187–1194. doi:10.1111/2041-210X.12121.

Farine DR. 2017. A guide to null models for animal social network analysis. Methods Ecol Evol.:n/a-n/a. doi:10.1111/2041-210X.12772.

Farine DR, Whitehead H. 2015. Constructing, conducting and interpreting animal social network analysis. J Anim Ecol. 84(5):1144–1163. doi:10.1111/1365-2656.12418.

Gruber SH, Marignac JRC de, Hoenig JM. 2001. Survival of Juvenile Lemon Sharks at Bimini, Bahamas, Estimated by Mark–Depletion Experiments. Trans Am Fish Soc. 130(3):376–384. doi:10.1577/1548-8659(2001)130<0376:SOJLSA>2.0.CO;2.

Guttridge TL, Dijk S van, Stamhuis EJ, Krause J, Gruber SH, Brown C. 2013. Social learning in juvenile lemon sharks, Negaprion brevirostris. Anim Cogn. 16(1):55–64. doi:10.1007/s10071-012-0550-6.

Guttridge TL, Gruber SH, DiBattista JD, Feldheim KA, Croft DP, Krause S, Krause J. 2011. Assortative interactions and leadership in a free-ranging population of juvenile lemon shark Negaprion brevirostris. Mar Ecol Prog Ser. 423:235–245. doi:10.3354/meps08929.

Guttridge TL, Gruber SH, Gledhill KS, Croft DP, Sims DW, Krause J. 2009. Social preferences of juvenile lemon sharks, Negaprion brevirostris. Anim Behav. 78(2):543–548. doi:10.1016/j.anbehav.2009.06.009.

Hamilton WD. 1964. The genetical evolution of social behaviour. I. J Theor Biol. 7(1):1–16. doi:10.1016/0022-5193(64)90038-4.

Hatchwell Ben J. 2010. Cryptic Kin Selection: Kin Structure in Vertebrate Populations and Opportunities for Kin-Directed Cooperation. Ethology. 116(3):203–216. doi:10.1111/j.1439-0310.2009.01732.x.

Hirsch BT, Prange S, Hauver SA, Gehrt SD. 2013. Genetic relatedness does not predict racoon social network structure. Anim Behav. 85(2):463–470. doi:10.1016/j.anbehav.2012.12.011.

Holekamp KE, Smith JE, Strelioff CC, Van Horn RC, Watts HE. 2012. Society, demography and genetic structure in the spotted hyena. Mol Ecol. 21(3):613–632. doi:10.1111/j.1365-294X.2011.05240.x.

Jacoby DMP, Busawon DS, Sims DW. 2010. Sex and social networking: the influence of male presence on social structure of female shark groups. Behav Ecol. 21(4). doi:10.1093/beheco/arq061. [accessed 2017 Jan 4]. http://beheco.oxfordjournals.org/content/early/2010/05/09/beheco.arq061.

Jacoby DMP, Croft DP, Sims DW. 2012. Social behaviour in sharks and rays: analysis, patterns and implications for conservation. Fish Fish. 13(4):399–417. doi:10.1111/j.1467-2979.2011.00436.x.

Jacoby DMP, Papastamatiou YP, Freeman R. 2016. Inferring animal social networks and leadership: applications for passive monitoring arrays. J R Soc Interface. 13(124):20160676. doi:10.1098/rsif.2016.0676.

Kelley JL, Graves JA, Magurran AE. 1999. Familiarity breeds contempt in guppies. Nature. 401(6754):661–662. doi:10.1038/44314.

Kerth G, Perony N, Schweitzer F. 2011. Bats are able to maintain long-term social relationships despite the high fission–fusion dynamics of their groups. Proc R Soc Lond B Biol Sci. 278(1719):2761–2767. doi:10.1098/rspb.2010.2718.

Krause J, Ruxton GD. 2002. Living in Groups. OUP Oxford.

Landeau L, Terborgh J. 1986. Oddity and the ‘confusion effect’ in predation. Anim Behav. 34(5):1372–1380. doi:10.1016/S0003-3472(86)80208-1.

Lehmann L, Rousset F. 2010. How life history and demography promote or inhibit the evolution of helping behaviours. Philos Trans R Soc Lond B Biol Sci. 365(1553):2599–2617. doi:10.1098/rstb.2010.0138.

Li CC, Weeks DE, Chakravarti A. 1993. Similarity of DNA fingerprints due to chance and relatedness. Hum Hered. 43(1):45–52.

Lynch M, Ritland K. 1999. Estimation of Pairwise Relatedness With Molecular Markers. Genetics. 152:1753–1766.

Mann J, Stanton MA, Patterson EM, Bienenstock EJ, Singh LO. 2012. Social networks reveal cultural behaviour in tool-using dolphins. Nat Commun. 3:980. doi:10.1038/ncomms1983.

Milinski M. 1987. TIT FOR TAT in sticklebacks and the evolution of cooperation. Nature. 325(6103):433–435. doi:10.1038/325433a0.

Mourier J, Brown C, Planes S. 2017. Learning and robustness to catch-and-release fishing in a shark social network. Biol Lett. 13(3):20160824. doi:10.1098/rsbl.2016.0824.

Mourier J, Lédée EJI, Guttridge TL, Jacoby DMP. 2018. Network analysis and theory in shark ecology - methods and applications. In: Shark Research: Emerging Technologies and Application for the Study of Shark Biology. Carrier J, Heithaus M and Simpfendorfer C. CRC Press. p. 337–356.

Mourier J, Maynard J, Parravicini V, Ballesta L, Clua E, Domeier ML, Planes S. 2016. Extreme Inverted Trophic Pyramid of Reef Sharks Supported by Spawning Groupers. Curr Biol. 26(15):2011–2016. doi:10.1016/j.cub.2016.05.058.

Mourier J, Mills SC, Planes S. 2013. Population structure, spatial distribution and life-history traits of blacktip reef sharks Carcharhinus melanopterus. J Fish Biol. 82(3):979–993. doi:10.1111/jfb.12039.

Mourier J, Planes S. 2013. Direct genetic evidence for reproductive philopatry and associated fine-scale migrations in female blacktip reef sharks (Carcharhinus melanopterus) in French Polynesia. Mol Ecol. 22(1):201–214. doi:10.1111/mec.12103.

Mourier J, Planes S, Buray N. 2013. Trophic interactions at the top of the coral reef food chain. Coral Reefs. 32(1):285–285. doi:10.1007/s00338-012-0976-y.

Mourier J, Vercelloni J, Planes S. 2012. Evidence of social communities in a spatially structured network of a free-ranging shark species. Anim Behav. 83(2):389–401. doi:10.1016/j.anbehav.2011.11.008.

Newman MEJ. 2006. Finding community structure in networks using the eigenvectors of matrices. Phys Rev E. 74(3):036104. doi:10.1103/PhysRevE.74.036104.

Olsén KH, JäUrvi T. Effects of kinship on aggression and RNA content in juvenile Arctic charr. J Fish Biol. 51(2):422–435. doi:10.1111/j.1095-8649.1997.tb01676.x.

Papastamatiou YP, Lowe CG, Caselle JE, Friedlander AM. 2009. Scale-dependent effects of habitat on movements and path structure of reef sharks at a predator-dominated atoll. Ecology. 90(4):996–1008. doi:10.1890/08-0491.1.

Pew J, Muir PH, Wang J, Frasier TR. 2015. related: an R package for analysing pairwise relatedness from codominant molecular markers. Mol Ecol Resour. 15(3):557–561. doi:10.1111/1755-0998.12323.

Porcher IF. 2005. On the gestation period of the blackfin reef shark, Carcharhinus melanopterus, in waters off Moorea, French Polynesia. Mar Biol. 146(6):1207–1211. doi:10.1007/s00227-004-1518-0.

Queller DC, Goodnight KF. 1989. Estimating Relatedness Using Genetic Markers. Evolution. 43(2):258–275. doi:10.1111/j.1558-5646.1989.tb04226.x.

R Core Team. 2015. R: a language and environment for statistical computing. Vienna, Austria: R Computing Foundation for Science.

Ramos-Fernández G, Boyer D, Gómez VP. 2006. A complex social structure with fission–fusion properties can emerge from a simple foraging model. Behav Ecol Sociobiol. 60(4):536–549. doi:10.1007/s00265-006-0197-x.

Reisinger RR, Beukes (née Janse van Rensburg) C, Hoelzel AR, de Bruyn PJN. 2017. Kinship and association in a highly social apex predator population, killer whales at Marion Island. Behav Ecol. 28(3):750–759. doi:10.1093/beheco/arx034.

Riehl C. 2011. Living with strangers: direct benefits favour non-kin cooperation in a communally nesting bird. Proc R Soc Lond B Biol Sci. 278(1712):1728–1735. doi:10.1098/rspb.2010.1752.

Snijders L, Blumstein DT, Stanley CR, Franks DW. 2017. Animal Social Network Theory Can Help Wildlife Conservation. Trends Ecol Evol. 32(8):567–577. doi:10.1016/j.tree.2017.05.005.

Van Oosterhout Cock, Hutchinson William F., Wills Derek P. M., Shipley Peter. 2004. micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes. 4(3):535–538. doi:10.1111/j.1471-8286.2004.00684.x.

Vignaud T, Clua E, Mourier J, Maynard J, Planes S. 2013. Microsatellite Analyses of Blacktip Reef Sharks (Carcharhinus melanopterus) in a Fragmented Environment Show Structured Clusters. PLOS ONE. 8(4):e61067. doi:10.1371/journal.pone.0061067.

Vignaud TM, Mourier J, Maynard JA, Leblois R, Spaet JLY, Clua E, Neglia V, Planes S. 2014. Blacktip reef sharks, Carcharhinus melanopterus, have high genetic structure and varying demographic histories in their Indo-Pacific range. Mol Ecol. 23(21):5193–5207. doi:10.1111/mec.12936.

Wang J. 2002. An Estimator for Pairwise Relatedness Using Molecular Markers. Genetics. 160(3):1203–1215.

Weideli OC, Mourier J, Planes S. 2015. A massive surgeonfish aggregation creates a unique opportunity for reef sharks. Coral Reefs. 34(3):835–835. doi:10.1007/s00338-015-1290-2.

Wilkinson GS, Carter GG, Bohn KM, Adams DM. 2016. Non-kin cooperation in bats. Phil Trans R Soc B. 371(1687):20150095. doi:10.1098/rstb.2015.0095.

Wiszniewski J, Lusseau D, Möller LM. 2010. Female bisexual kinship ties maintain social cohesion in a dolphin network. Anim Behav. 80(5):895–904. doi:10.1016/j.anbehav.2010.08.013.

Wolf JBW, Trillmich F. 2008. Kin in space: social viscosity in a spatially and genetically substructured network. Proc R Soc Lond B Biol Sci. 275(1647):2063–2069. doi:10.1098/rspb.2008.0356.