**Sexual selection promotes colonial breeding in shell brooding
cichlid fish**

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Colonial species breed in densely aggregated territories containing no other resources than nest sites. This behaviour is usually explained by natural selection, for instance through benefits resulting from reduced predation risk. An alternative hypothesis suggests that like in lek breeding systems, sexual selection may be responsible for the aggregation of competitors, driven by an increased potential for female mate choice among closely assembled males. *Lamprologus callipterus*, a shell-brooding cichlid fish of Lake Tanganyika, provides an ideal test case for the utility of lek evolution models to explain colonial breeding, because breeding territories are established by males before pairing. Large males collect and defend empty snail shells that are then chosen by females for breeding. We checked for a potential influence of sexual selection on colonial breeding in *L. callipterus* by testing predictions of the hot-shot hypothesis and female preference hypothesis of lek mating models. In the field, we found that territories of larger males were more centrally located, and that females preferred to breed with males surrounded by many neighbours, two findings that are consistent with lek mating models. Female preference suggests that sexual selection affects colonial breeding in *L. callipterus*, which implies an influence of sexual selection on the evolution of colonial breeding at large.

Keywords

Coloniality, Female preference, Hot-shot, Hidden lek, Lek breeding

There is ongoing debate about why individuals breed in colonies (Höglund & Alatalo 1995; Danchin & Wagner 1997; Sachs, Hughes, Nuechterlein & Buitron 2007; Neff & Pitcher 2008). In breeding colonies, individuals aggregate and defend nest sites or small territories that contain no other resources (Danchin & Wagner 1997; Perrins & Birkhead 1983; Siegel-Causey & Kharitonov 1990; Wittenberger & Hunt 1985). This breeding pattern is widespread in vertebrates ranging from fish (Gross & Mac Millian 1981; Tyler 1995) to reptiles (Trillmich & Trillmich 1984) and marine mammals (Terhune & Brilliant 1996). Most prominently, this breeding pattern is prevalent in seabirds, where 95% of all species breed in colonies (Brown, Stutchbury & Walsh 1990; Wittenberger & Hunt 1985). Several evolutionary mechanisms have been proposed to explain colonial breeding. ‘Conspecific models’ assume that the fitness benefits of colonial breeding are gained from nesting near conspecifics (Sachs et al. 2007): nesting sites should be aggregated if colonial breeding, for instance, increases foraging efficiency (Beauchamp 1999), facilitates information transfer (Buckley 1997; Barta & Giraldeau 2001), reduces the predation risk for offspring (Gross & Mac Millian 1981; Tyler 1995; Post 1998; Picman, Pribil & Andre 2002; Schaedelin, Fischer & Wagner 2012), or enables parasitic reproduction (Picciulin, Verginella, Spoto & Ferrero 2004). ‘Habitat models’, on the other hand, assume that the fitness benefits of colonial breeding are gained through the characteristics or location of the colony site (Sachs et al. 2007), mainly when suitable breeding habitats are limited (Johnson & Walters 2011; Kiester & Slatkin 1974; Wittenberger & Hunt 1985). Either way, these benefits must outweigh the potential costs of colonial breeding, such as increased parasite transmission, potential infanticide, and competition for mates (Kiester & Slatkin 1974; Danchin & Wagner 1997).

In lek-mating systems, in contrast, males aggregate at display arenas which females visit solely for the purpose of mating (Kiester & Slatkin 1974; Höglund & Alatalo 1995; Loiselle

et al. 2007). Sexual selection is usually held responsible for the evolution of leks. First, the “hot-shot model” assumes that certain males are preferred by females over other males, and that subordinate or less attractive males aggregate around such “hot-shots” in order to increase their mating success (Beehler & Foster 1988; Westcott & Smith 1997; Bednekoff 2002). Second, the “female preference model” assumes that females benefit from clumped male territories either because they can better estimate relative mate quality (Wagner 1998), or because males of high quality tend to be found in larger aggregations (Höglund & Alatalo 1995). According to this model, females preferably settle in large aggregations (Fletcher & Miller 2006). Third, the “hot-spot model” (Bradbury, Gibson & Tsai 1986) hypothesizes that breeding aggregations are a result of males settling in regions with high female abundance (Höglund & Alatalo 1995; Westcott 1997). Females may have large overlapping home ranges relative to male territory size, or well-defined travel routes (Bradbury et al. 1986; Westcott 1997). Males aggregate to increase pairing success, and extra-pair copulations (EPCs) may occur as a by-product of the aggregation (Fletcher & Miller 2006). Finally, the “kin selection model” assumes that subordinate males gain inclusive fitness benefits by aggregating among related males (Bradbury & Gibson 1983; Kokko & Lindström 1996). Only males that are related to the dominant male aggregate (Fletcher & Miller 2006).

The hidden lek hypothesis of colonial breeding provides an alternative explanation, assuming that intersexual selection (female choice) may be responsible for the aggregation of competitors also in non-lekking species that engage in paternal care (Wagner 1998). This hypothesis is an extension of the “EPC hypothesis” (Wagner 1993), which originated from the discovery that socially monogamous birds of both sexes may pursue extra-pair copulations (EPCs; Birkhead & Møller 1992). The hidden lek hypothesis assumes similar mechanisms to be responsible for an aggregation of nests in a colony as in lekking species (Bradbury 1981),

such as the “hot-shot”, “female preference” “hot-spot”, and “kin selection” models (Fletcher & Miller 2006). Five criteria are required for breeding colonies to classify as hidden leks (Fletcher & Miller 2006), four of which focus on the role of extra-pair matings for the accumulation of territories: (1) The species must be socially monogamous or polygynous, where females develop pair bonds with males who provide some form of paternal care; (2) territorial males aggregate, forming arenas where EPCs occur; (3) females seek EPCs and are able to select extra-pair mates; extra-pair males (4) provide no parental care and (5) do not monopolize resources required by females. A study of colony formation in the socially monogamous least flycatcher revealed that some predictions of the hidden lek hypothesis indeed apply in that species (Tarof, Ratcliffe, Kasumovic & Boag 2005).

The current consensus is that colonial and lek mating systems distinctively differ in the parental care system: lekking males provide no parental care while colonial males typically provide substantial and usually essential care. The role of sexual selection is assumed to be restricted to the evolution of leks and its importance is neglected as a driver of colonial breeding, because in many colonial species, breeding pairs choose a nest site together, i.e. after mate choice and pairing. However, if aggregated territories are founded by males only, there is high potential for sexual selection to operate. For example, in bluegill sunfish (Gross & Mac Millian 1981), the cichlid *Lamprologus callipterus* (Sato 1994), boat-tailed crackles (Post 1998), Mediterranean damselfish (Picciulin et al. 2004), lesser kestrel (Calabuig, Ortego & Aparicio 2011), and western sandpipers (Johnson & Walters 2011), males establish a territory first and females settle later. In all these cases, sexual selection mechanisms that are responsible for lek evolution might operate as well, but they have not been explicitly addressed. In boat-tailed crackles, for instance, the difference in reproductive success between colonial and solitary females is explained by the males' defence of colonies from predatory

97 birds (Post 1998). Breeding colonies of lesser kestrel apparently result from neutral colony
98 selection by first-year birds based on nest-site availability (Calabuig et al. 2011). Bluegill
99 sunfish colonies were assumed to result from reduced brood predation through nest
100 aggregations (Gross & Mac Millian 1981), whereas in the Mediterranean damselfish *Chromis*
101 *chromis*, colonial nesting was shown to facilitate parasitic reproduction (Picciulin et al. 2004).
102 *L. callipterus*, a cichlid fish endemic to Lake Tanganyika, provides an ideal opportunity to
103 explore predictions derived from lek evolution models to explain colonial breeding. Here
104 unlike in most colony breeders, males establish their aggregated territories first, i.e. before
105 pairing. Therefore, a direct influence of females on colony formation at the time of territory
106 establishment is very unlikely. Instead, males may be selected to aggregate prior to female
107 arrivals in order to subsequently increase their mating success. Females choose males,
108 established nests or shells contained therein for mating, which yields a high potential for
109 female choice to operate *after* initial colony formation. The breeding system of *L. callipterus*
110 seems to conform to a number of predictions from models of lek mating systems. Males
111 defend territories in clusters (hereafter referred to as ‘nest’), which contain no other resources
112 than empty snail shells attracting females for mating (Sato 1994; Maan & Taborsky 2008;
113 Mitchell, Witz Ocana & Taborsky 2014). However, in contrast to a pure lek, females stay in
114 the male territory after mating, and males indirectly care for the brood by anti-predator
115 defence (Sato 1994; Maan & Taborsky 2008). Hence, the mating system of *L. callipterus*
116 conforms also to resource defence polygyny (Emlen & Oring 1977). Territories of bourgeois
117 males consist of accumulated gastropod shells of the species *Neothauma tanganicense* or
118 *Paramelania damoni*, which serve as exclusive breeding substrate for females (Sato 1994;
119 Gashagaza, Nakaya & Sato 1995; Sato & Gashagaza 1997; Schütz & Taborsky 2000). Shells
120 are collected nearby, or stolen from neighbouring nests (Sato 1994; Maan & Taborsky 2008;
121 Mitchell et al. 2014). Females perform brood care for 10 to 14 days within these shells (Sato

1994).

Throughout the breeding area, sneaker males of all sizes try to steal fertilizations from territory owners (Sato 1994; Taborsky 1998; Schütz & Taborsky 2000; Taborsky 2001). Additionally, small dwarf males attempt to gain fertilizations by entering shells with a spawning female inside (Taborsky 1998; Taborsky 2001; Sato et al. 2004) and releasing sperm from within the shells (Wirtz Ocana, Meidl, Bonfils & Taborsky 2014). Sneakers and dwarf males do not show any parental care (Taborsky 2001), and most sneaker males are too small to claim breeding territories, because they cannot manage to carry shells (Schütz & Taborsky 2005). In contrast to females breeding on hidden leks, which are expected to prefer aggregated males to improve the potential for EPCs (Wagner 1993; Tarof et al. 2005), in *L. callipterus* females are not able to actively seek extra pair matings, since egg deposition and fertilization takes place inside the snail shell (Schütz et al. 2012; Taborsky 2001). The only scope for female choice regarding parasitic males would occur after a female has already chosen a nest and entered a shell, i.e. during her spawning. A dwarf male attempting to enter a shell for spawning needs to pass the female, which can resist this attempt as this passing usually requires a slight backward movement of the female towards the shell entrance (Sato, Hirose, Taborsky & Kimura 2004; own observations).

In this study, we examined the influence of sexual selection on colonial breeding in *L. callipterus* by testing some predictions of lek mating models. In this species nesting males aggregate after a dispersal period extending well over a year (Schütz, Parker, Taborsky & Sato 2006), during which individuals mix and roam about in schools (Sato et al. 2004; own unpubl. data), just like females do between successive broods (Schütz et al. 2006). Therefore relatedness is highly unlikely to affect settlement patterns, and the kin selection model can be

excluded. Since feeding schools of non-breeding fish have no well-defined home ranges or travel routes (own observations), the hot-spot model is also unlikely to apply. Therefore, we focus on predictions of the hot-shot and female preference models. Earlier field studies showed that females did not choose particular males when nest density was high (Schütz & Taborsky 2005), but seemed to preferably mate with larger males when nest density was low (Maan & Taborsky 2008). It is not clear, however, whether females directly prefer large males or males with many neighbours. Hence, we tested in the field whether male size relates to their nest location in a colony, i.e. whether large males have more neighbours (hot-shot model). Furthermore, we tested whether females preferentially settle in large aggregations of nests, i.e. whether they prefer males with many neighbours (female preference model).

Materials and Methods

Study population

We studied a population of *L. callipterus* that was located at Kasakalawe Point near Mpulungu, Zambia (S 08° 45' E 31° 04'). Breeding colonies of *L. callipterus* are temporary, with population density, colony size and nest density within colonies differing extremely between years. To take this variance into account, we analysed two data sets, one from 1997, where population density was low, and one from 2005, where population density was high. In both years, all territories of *L. callipterus* at Kasakalawe Point were mapped. All observations were done by SCUBA diving. For catching of fish a fence net or hand nets were used.

In 1997, we studied *L. callipterus* from 15 January to 4 March and found only one breeding colony with 28 nests. The nests were located between 8 m and 12 m depth. This colony

contained all *L. callipterus* nests found along a stretch of 500 m of coastline between 4 and 15 m water depth, i.e. within an area of approximately 125 000 m². From 16 September to 27 November 2005, we studied *L. callipterus* at the same place and found two colonies (named "West" and "East") within the same area. They contained 80 and 53 nests, respectively, and were approximately 200 meters apart. The nests were located between 9 m and 15 m depth and were the only breeding sites of *L. callipterus* in the above mentioned area during that period.

Data collection

In *L. callipterus*, nest density is highest in the centre of a colony, thus males with more neighbours occupy more central territories. To quantify the density of the area around a nest, all neighbouring nests within a radius of three meters around each nest were counted. The distance to the closest nest was measured to the nearest 10 centimetres for all nests that persisted during the whole observation period ($N = 28$ in 1997 and $N = 118$ in 2005). The distance to the closest nest correlated highly with the number of nests within a radius of 3m in both years (Spearman correlations, 1997: $r_s = -0.543$, $N = 28$, $P = 0.003$; 2005: $r_s = -0.611$, $N = 118$, $P < 0.001$), therefore we report only one of these measures (nests within 3m) as an estimate of nest density.

Catching and measuring of nest males can involve considerable disturbance, which might influence results if males subsequently leave or females are deterred. Therefore, we haphazardly chose a subset of males to determine their sizes to the nearest mm underwater with a measuring plate (standard and total length, SL and TL, $N = 17$ in 1997 and $N = 15$ in 2005).

In 1997, we counted the number of breeding females for each nest every day. Due to the large nest number in 2005, in that year we randomly chose a subset of all nests every day to estimate the number of breeding, spawning and visiting females (on average 14.6 ± 8.3 [mean $\pm$ SD] nests checked per day, with an average time interval of 9.8 ± 7.1 days between subsequent observations of individual nests). We aimed to check every nest once during each moon phase (see below). After counting the number of breeding and spawning females, each focal nest was observed for 4 minutes, and all females within a nest, which were not spawning or breeding but inspecting shells, moving in the nest, sitting on shells or on the ground within nests, were categorized as visiting females. To avoid counting individual females more than once during the observation period, each visiting female was individually tracked after her first visit and not re-counted when she re-visited the nest.

For *L. callipterus*, a lunar synchronization of breeding activity has been reported (Nakai, Yanagisawa, Sato, Nimura & Gashagaza 1990; Maan & Taborsky 2008). To account for this periodicity, we divided the lunar month into four periods: “full moon”, “waning moon”, “new moon” and “waxing moon” (seven days per period). Most spawning, breeding and visiting females in nests were found around full moon (table 1).

Statistics

The distributions of all data were tested for normality with Kolmogorov-Smirnov normality tests. If data differed from a normal distribution ($p < 0.1$), non-parametric statistics were used. As a measure of male reproductive success, we counted the numbers of breeding females per nest at full moon. In 1997 each nest was checked every day, thus we used the numbers of

breeding females on the day of full moon (23.1.97, $N = 20$). In 2005 each nest was only checked once during each moon phase, thus we used the numbers of breeding females during the 7 day period around full moon (13.11.-19.11.2005, full moon at 16.11.2005, $N = 50$). As a measure of male reproductive skew, we determined the frequency distribution of the numbers of breeding females per male at full moon in both years. For both years, we used Spearman rank correlations to test whether (i) male size and (ii) the number of breeding females at full moon correlate with the number of nests within a radius of 3m. In 2005, for each of the 4 moon periods, Spearman rank correlations were used to relate the number of neighbours within a radius of 3 meters to the number of spawning, breeding and visiting females per nest. In addition, we differentiated between ‘high density nests’ (nearest neighbour $\leq 2\text{m}$) and ‘low density nests’ (nearest neighbour $> 2\text{m}$; for details see the Appendix).

Combining data from both years, we tested whether female preference for high density nests can be attributed to female preference of larger males assembling at higher densities. We standardized our data ($[x - \text{mean}] / \text{standard deviation}$) to bring the variables into proportion with each other and to improve model interpretation. We used a linear model (LM) with the number of breeding females as dependent variable, and population density (year), nest density (the number of neighbours within a 3m radius), male size (TL), and the interaction between male size and nest density as co-variables. This model gives estimates for the effects of male size and nest density when the other variable is at average and allows us to compare the influences of both variables on the number of breeding females. Collinearity may affect the outcome of the presented model, therefore we checked for the influence of male size and nest density on the number of breeding females additionally in two separate linear models using the original data. For these and the other LMs used in this study the residuals were checked

for potential deviations from normality, and the assumptions underlying this type of analysis were not violated.

To test for female mate copying, in the year with high population density we tested whether the numbers of (i) visiting and (ii) spawning females are influenced by nest density and the numbers of females already breeding in a nest. We used univariate linear models (LM) with the number of (i) visiting and (ii) spawning females as dependent variable, and the numbers of neighbours within a 3m radius and the number of breeding females as co-variables. Additionally, we used Spearman rank correlations to check whether the presence of breeding females correlated with the number of females visiting a nest separately for all four moon phases. For all analyses, two-tailed tests were applied.

Ethical note

The field work at Lake Tanganyika was conducted with the permission of the Fisheries Department of the Ministry of Agriculture and Co-operatives of Zambia. When fish were caught and measured under water, we immediately released them at the same place where we had caught them. No fish was harmed by our handling.

Results

How big is male mating skew?

In both years, male mating success was moderately skewed, with few males receiving no breeding females in their nests (15% in 1997 and 6% in 2005; fig. 1).

Do larger males have more neighbours?

In the year with low population density (1997), the distance to the nearest neighbouring nest was at least 2m (range: 2-15m), and males had a maximum of 2 neighbours within a radius of 3m (median=1; range 0-2; fig. 2). In the year with high population density (2005), the distance to the nearest neighbouring nest varied between 0.5-22m ($N = 108$ nests) within colonies, and males had up to 13 neighbours within a radius of 3m (median=4; range 0-13; fig. 2). In both years, larger males had more neighbours within a radius of three meters than smaller males (Spearman correlations: 1997: $r_s = 0.62$, $N = 17$, $P = 0.008$; fig. 3a; 2005: $r_s = 0.832$, $N = 15$, $P < 0.001$; fig. 3b).

Do females choose nests with more neighbours?

In the year with low population density, there was no significant correlation between the number of breeding females and the number of nests within a radius of 3m at full moon (Spearman correlation, $r_s = -0.15$, $N = 19$, $P = 0.539$). In contrast, in the year with high population density, there was a positive correlation between the number of neighbours within a radius of 3 meters around the nest and the number of breeding females (Spearman correlation, $r_s = 0.304$, $N = 80$, $P = 0.006$) and visiting females (Spearman correlation, $r_s = 0.447$, $N = 34$, $P = 0.008$) in a nest during the full moon period. At new moon, also more breeding females were found in nests with more neighbours (Spearman correlation, $r_s = 0.266$, $N = 68$, $P = 0.029$), whereas at other moon phases these correlations were non-significant ($0.114 < P < 0.98$). For a comparison between 'high density nests' and 'low density nests' in that year see Appendix tables A1 and A2.

297

298 Are the higher female numbers at high local nest densities attributable to male size effects?

299

300 In both years combined, the number of breeding females per nest was significantly influenced
301 by population density (year), nests density (the number of nests within a 3m radius), and male
302 size (TL; LM, $r^2 = 0.59$, $F_4 = 9.547$, $P < 0.001$). Population density, nest density, and the
303 interaction between male size and nest density significantly determined the number of
304 breeding females, but male size did not (see table 2a). This means that for an average sized
305 male, nest density has a strong effect, whereas for a male with an average nest density, body
306 size has only a very weak effect on the number of breeding females. The estimate of the
307 interaction between nest density and male size was negative, which shows that with
308 increasing numbers of neighbours, body size gets less important, and with increasing male
309 size, the number of neighbours gets less important. When checking for the influence of male
310 size and nest density on the number of breeding females separately, only nest density showed
311 a significant effect (table 2b).

312

313 Do females copy the mate choice of others?

314

315 In the year with high population density, our analysis (LM; $r^2 = 0.189$, $F_2 = 16.178$, $P <$
316 0.001) suggested that the number of females visiting a nest was influenced significantly by
317 the number of females already breeding there ($F_1 = 30.596$, $P < 0.001$), but not by nest
318 density ($F_1 = 0.428$, $P = 0.514$). In contrast, the number of spawning females (LM, $r^2 = 0.031$,
319 $F_2 = 4.278$, $P = 0.015$) was significantly influenced by nest density ($F_1 = 8.28$, $P = 0.004$), but
320 not by the number of females already breeding in the nest ($F_1 = 0.987$, $P = 0.321$). When
321 separated for moon phases, the presence of breeding females was positively correlated with

females visiting a nest around full moon (Spearman correlation: $r_s = 0.429$, $N = 34$, $P = 0.011$) and in the waning moon period (Spearman correlation, $r_s = 0.333$, $N = 43$, $P = 0.029$), but not in the waxing moon period (Spearman correlation, $r_s = 0.342$, $N = 28$, $P = 0.075$) and around new moon (Spearman correlation, $r_s = -0.0199$, $N = 37$, $P = 0.911$).

Discussion

Two models of lek breeding were supported by our results, the hot-shot and the female preference models. Large males occupy central positions in the aggregation of nests, and females prefer to breed in nests at high densities. This suggests a significant influence of sexual selection on the evolution of colonial breeding in *L. callipterus*. We agree with Jiguet & Bretagnolle (2006) that these two models are somewhat complementary, promoting male clustering and the maintenance of leks. We argue that these mechanisms may also promote colonial breeding, especially in species where males settle before pairing (c.f. examples given in the Introduction). Some evidence that sexual selection mechanisms operating in lek breeding may also apply to colony breeders exists also in the banded damselfish (*Abudefduf abdominalis*, Tyler 1995) and in the blue-black grass quit (*Volatinia jacarina*, Almeida & Macedo 2001).

In leks, male-mating success is usually strongly skewed, with one or only a few males obtaining all fertilisations (Ryder, Parker, Blake & Loiselle 2009). For example, in the lekking lance-tailed manakin *Chiroxiphia lanceolate*, non-alpha males can hardly reproduce (DuVal & Kempenaers 2008) and in black grouse *Tetrao tetrix* most males fail to successfully copulate during their lifetime (Kervinen et al. 2012). In contrast, in *L. callipterus* most nest

males are able to attract at least one female, hence male reproductive skew appears to be smaller than in typical lek mating species.

In accordance with predictions of the hot-shot model, in *L. callipterus* large males are located in the centre of male aggregations. Large nest owners, which were shown to be superior competitors (Schütz & Taborsky 2005; Maan & Taborsky 2008), had more nests in their vicinity than smaller males in both years of study and at greatly different population densities. This may suggest that the largest, most competitive males settle first and smaller, less competitive males follow. Large males might also better defend their nest against predators, which could be another reason why smaller males settle near them. Also in least flycatchers (*Empidonax minimus*), which form dense territorial clusters, central males arrived earlier, were heavier and in better body condition than peripheral males (Tarof et al. 2005).

In accordance with the female preference model of lek evolution, females preferentially settled in denser aggregations. In the year with high population density, there was a positive correlation between the number of neighbours and the number of breeding and visiting females per nest. However, in the year with low population density, nest density did not correlate with the number of breeding females, which is probably due to the relatively large distances between neighbouring nests ($\geq 2\text{m}$) and the low numbers of neighbours in this year. When searching for suitable breeding substrates and comparing nests among each other, females cover large distances. If nests are further apart, this entails high travel costs including energy expenditure and predation risk, and comparing males becomes more difficult. Thus, the advantages of reduced travel costs from searching in densely aggregated nests are probably lacking when population density is low. We therefore assume that in *L. callipterus*, female choice is strongly influenced by both population density and local nest density.

371
372 In the year with high population density, more females were breeding at high local nest
373 densities, even though male body size and the number of suitable shells per nest did not differ
374 between the two local densities (see Appendix). This pattern confirms the preference of
375 females for areas with densely aggregated males. The number of spawning and visiting
376 females did not differ between the two local nest densities, which could be due to the lower
377 chance to detect these females. ‘Visiting’ and ‘spawning’ are more transient activities than
378 ‘breeding’, which involves brood care lasting for 10-14 days (Schütz & Taborsky 2005).
379 Visiting females enter the nest for only short periods of time, thus can sometimes be missed
380 during the 4-min observation periods. Spawning is often aborted, especially during the first
381 hour of male spawning, when the female is not yet laying eggs (Schütz et al. 2012). Thus, the
382 count of spawning females is not a very good estimate of nest male success. Instead, the count
383 of breeding females is the most reliable measure of female preference.

384
385 A combined analysis of female settlement patterns including data from both years suggested
386 that mainly nest density, and not male size influenced female preference. A linear model with
387 the standardized values showed a strong and highly significant effect of the number of
388 neighbours: averaged sized males would gain 1.09 breeding females when having one more
389 neighbour. In contrast, the effect of male size on the number of breeding females was
390 insignificant and in fact almost zero: with an increase of 1 cm in total length, males with an
391 average number of neighbours would only receive 0.007 more females. The effect of the
392 interaction between nest density and male size was negative and highly significant, which
393 shows that an increase in one of the two variables leads to a decreasing effect of the other
394 variable on the number of breeding females. The results of the models dealing with each
395 variable separately support the reported effects. Thus, the advantage for females choosing

396 nests with many neighbours is not triggered by the fact that large males are occupying nests in
397 dense areas. Previous experiments showed that females did not prefer large males to small
398 ones when given the choice, but rather use features of their shell collections to decide where
399 to breed (Schütz & Taborsky 2005; Mitchell et al. 2014). We therefore conclude that females
400 mainly choose a spawning territory with males that have many neighbours, and that male size
401 is of secondary importance. However, the comparison between both study years suggests that
402 population density needs to be high enough in order for nest density effects to emerge.
403 Ultimately, one could argue that female preference influences nest site choice of *L. callipterus*
404 males during colony formation, even if females select males only after the latter have settled,
405 i.e. this effect of female choice on male settlement patterns occurs a posteriori.

406
407 The behaviour of visiting females might indicate a potential effect of mate choice copying. In
408 the year with high population density, the presence of already breeding females positively
409 correlated with the number of females visiting a nest during the most active spawning period,
410 i.e. around full moon. However, the number of spawning females was significantly influenced
411 by nest density and not by the number of breeding females. Thus females seem to preferably
412 visit nests containing many breeding females, but then decide to spawn and breed in nests
413 with many neighbours. Since more females are breeding in nests with many suitable shells
414 than in nests with few suitable shells (Schütz & Taborsky 2005), the correlation between
415 visiting and breeding females might be due to a higher number of suitable shells in nests
416 containing more breeding females. Previous experiments refuted the hypothesis that females
417 copy the nest choice of other females (Schütz & Taborsky 2000). In ruffs (Lank & Smith
418 1992) and black grouse (Alatalo, Höglund, Lundberg & Sutherland 1992), larger leks had
419 more female visits and copulations, leading to a higher average male mating success. In *L.*
420 *callipterus*, we cannot test for an influence of colony size on female preference and male

mating success due to limited data, but we assume that colony size might positively influence these parameters as well.

Female choice of nests with many neighbours may yield several benefits, for instance it might lower their travel costs when searching for mates, and it may facilitate their evaluation of male, nest and shell quality. Before spawning, females move from nest to nest to inspect potential shells, nests and males (Mitchell et al. 2014; own observations). More females were visiting high density than low density nests in the period of highest spawning activity (around full moon; see Appendix), which suggests that comparing suitable shells and males may be easier in high density than in low density nests. Another potential advantage of breeding in dense colonies is the reduction of predation risk for females and their offspring (nest defence hypothesis, Gross & Mac Millian 1981; Picman et al. 2002; Schaedelin et al. 2012). Since in *L. callipterus* the female and her brood are located deep inside the shell, offspring predation is not very frequent (own observations). However, reduced travel distances between territories in a dense colony may reduce the predation risk for females during mate search (cf. Alem & Greenfield 2010).

Breeding in dense colonies may also entail potential costs to females. In *L. callipterus*, settling in areas with many close-by nests can increase the risk that the shell in which a female raises her offspring is stolen by a neighbouring male, which is typically followed by expulsion from the shell and offspring loss (Maan & Taborsky 2008). Most naturally occurring shell stealing by bourgeois males occurs from nearest neighbours (Maan & Taborsky 2008). By choosing a nest with a large owner, this risk might be reduced as large males are in general more aggressive and able to hold a territory longer (Schütz & Taborsky 2005; Maan & Taborsky 2008). Costs of breeding in dense colonies have also been suggested

in other species such as the common guillemot (*Uria aalge*), where aggression towards
unattended chicks often results in infanticide, particularly at high nest densities (Ashbrook,
Wanless, Harris & Hamer 2010).

The mating and breeding pattern of *L. callipterus* suggests that colonial breeding can be
subject to sexual selection. This has also been proposed by the hidden lek hypothesis, which
assumes that colony formation in birds may result from female preference for EPCs (Wagner
1993; Wagner 1998). For breeding colonies to be classified as hidden leks it is required that
females are able to actively seek EPCs (Fletcher & Miller 2006). The mating pattern of *L.*
callipterus excludes the possibility that females search for extra-pair fertilizations. A female
ready to spawn enters the shell completely, and the bourgeois and sneaker males release
sperm into the shell opening, without direct contact with the female (Schütz et al. 2012).
Females spawning in regions of high nest density might increase their chances to receive
extra-pair fertilizations from sneaker males, if sneakers preferentially search for spawning
opportunities in regions with high nest density. However, a field study revealed that the
number of spawning females per nest and day does not correlate with the number of sneakers
and dwarfs entering a nest per day (submitted manuscript, Schütz, Tschirren, Pachler,
Grubbauer & Taborsky). Sneaker males are found mainly in feeding schools (Schütz *et al.*
2006) and the mean number of sneakers entering a nest per day is low (0.66; Schütz,
Tschirren, Pachler, Grubbauer & Taborsky, submitted manuscript,). Sneakers seem to take
opportunities to release sperm whenever possible, without comparing different nests.
Additionally, sneaker fertilizations are generally very rare in nature (< 1%, Wirtz Ocana et al.
2014), thus selecting extra mates is an unlikely explanation for female preference of dense
colonies in *L. callipterus*. On the contrary, females seem to avoid the participation of parasitic
males if they can (Sato et al. 2004; own observation), which, if anything, should rather reduce

preference for dense nest areas. Thus, in *L. callipterus* female preference to spawn in dense colonies cannot be explained by a preference for extra-pair fertilizations.

We therefore argue that mechanisms of sexual selection responsible for the formation of leks beyond the potential enhancement of extra pair mating can influence colonial breeding in *L. callipterus*. Such mechanisms may operate in colonial species more generally, i.e. also beyond the enhancement of EPCs. This is especially apparent if territories and nests are founded by the sexually selected sex only, but it may be important, yet more hidden, also in other cases. We propose therefore to expand the hidden lek hypothesis beyond the function of facilitating extra pair matings.

Conclusions

Our results are consistent with both the “hot-shot” and “female preference” hypotheses of lek evolution. In the colonial breeder *L. callipterus*, the formation of aggregated nesting can be at least partly explained by sexual selection. Larger males have more neighbours than smaller ones, which means that they are in the centre of colonies. This is predicted by the hot-shot model. Particularly in the year with high population density, females preferred to mate and breed with males in denser aggregation areas, which is predicted by the female preference model. Regarding the importance of sexual selection for the generation of breeding aggregations, the two allegedly distinct reproductive systems characterised by the absence (lek mating) or presence (colonial breeding) of paternal care may in fact reflect different end points on a continuum. We suggest that models explaining lek evolution via sexual selection can also be used to test predictions of mating patterns in colony breeders, especially (but not only) when males settle first and females follow.

496

497 **References**

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Appendix

Comparison between high and low density nests in 2005

Materials and methods

In the year with high population density, we tested whether female choice is influenced by local nest density. Each nest was either defined as ‘high density nest’ (nearest neighbour distance < 2 meters; $N = 72$, with 36 each in the West and East Colonies) or ‘low density nest’ (nearest neighbour distance ≥ 2 meters; $N = 61$, with 44 in the West and 17 in the East Colony, respectively). To obtain a measure of nest quality, we determined the number of shells suitable for breeding (intact shells > 4.5 cm; Schütz & Taborsky 2000) by measuring them along the axis from the apex to the outer part of the lip (Stresemann 1992). Additionally, the total number of shells (i.e. including broken shells) was evaluated for a sub-sample of nests. Ten to 12 days after spawning, i.e. shortly before young left the shell, a subset of breeding females ($N = 30$) was transported to the surface and the female and her fry were carefully shaken out of the shells. The female was measured to the nearest mm and the number of young was counted. After measuring and counting, the fish were brought back to where we caught them. No fish were harmed by our handling.

We compared the body size of nest owners, the sizes of breeding females, and the number of fry produced between low density nests and high density nests using Student’s t-tests, and the numbers of suitable shells, spawning, breeding and visiting females per nest using Mann-Whitney *U*-tests. To test for a potential influence of the lunar period on the numbers of breeding and visiting females per nest found at high and low local nest densities, we used

669 Mann-Whitney *U*-tests.

670

671 *Results*

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673 In the year with high population density, body sizes of nest owners, sizes of breeding females,
674 number of fry produced, and the numbers of suitable and total shells did not differ between
675 high and low density nests (table A1). Overall, significantly more breeding females were
676 found in high density nests than in low density nests (table A1). Separated for moon phases,
677 the numbers of breeding and visiting females differed between ‘high density nests’ and ‘low
678 density nests’ (table A2, fig. A1).

679

Tables

Table 1: Female abundance during different moon phases

	Spawning females			Breeding females			Visiting females		
	Med.	Quartiles	N	Med.	Quartiles	N	Med.	Quartiles	N
Full moon	1.0	0.0-2.0	88	6.0	3.25-9.75	80	0.0	0.0-1.25	34
Waning moon	0.0	0.0-1.0	102	3.0	1.0-7.0	101	0.0	0.0-0.0	43
New moon	0.0	0.0-1.0	62	2.5	1.0-5.0	68	0.0	0.0-1.0	38
Waxing moon	1.0	0.0-2.0	50	1.0	0.0-3.0	45	0.0	0.0-0.0	28
KW statistic	$\text{Chi}^2 = 37.694, P < 0.001$			$\text{Chi}^2 = 52.206, P < 0.001$			$\text{Chi}^2 = 7.609, P = 0.055$		

Numbers (medians and quartiles) of spawning, breeding, and visiting females per nest during the four moon phases in all nests (2005, ‘high density nests’ and ‘low density nests’ combined). Results of Kruskal-Wallis tests (KW) comparing the female numbers between moon phases are given at the bottom line; all $df = 3$.

Table 2a: Effects on the number of breeding females: combined model

Coefficient	Estimate	Std. error	<i>t</i> value	<i>P</i>
Year	1.05	0.28	3.73	< 0.001
Nest density	1.09	0.27	4.09	< 0.001
Male size	0.007	0.17	0.04	0.97
Nest density $\times$ male size	-0.60	0.16	- 3.854	< 0.001

Results of linear model estimating effects of population density, nest density, male size, and the interaction of male size and nest density on the number of breeding females.

Table 2b: Effects on the number of breeding females: separate models

Coefficient	r^2	df	F	p
(i) Total model	0.296	2	5.876	0.007
Year		1	9.43	0.005
Male size		1	2.669	0.114
(ii) Total model	0.364	2	7.997	0.002
Year		1	5.640	0.025
Nest density		1	5.941	0.021

Results of two linear models estimating the effects of (i) population density (year) and male size, and (ii) population density (year) and nest density on the number of breeding females.

Table A1: Comparison of territories between high and low density nests

	High density nests	Low density nests	Test	p
Size of nest owners	10.17 cm $\pm$ 1.26	10.0 cm $\pm$ 1.18	t = 0.451; df = 40	0.655
Size of breeding females	4.96 $\pm$ 0.34cm	5.0 $\pm$ 0.27 cm	t = 0.339; df=28	0.736
Number of fry per brood	122.92 $\pm$ 38.2	126.8 $\pm$ 33.8	t = 0.290; df=29	0.774
Suitable shells per nest	23.0 (16.5-30.0)	22 (16.25-28.75)	U _{37,24} = 423.0	0.756
Spawning females per nest	0.0 (0.0-1.0)	1.0 (0.0-1.75)	U _{170,132} = 12313.5	0.113
Breeding females per nest	4.0 (2.0-7.0)	2.0 (1.0-5.0)	U _{169,125} = 8105.0	0.001
Visiting females per nest	0.0 (0.0-1.0)	0.0 (0.0-1.0)	U _{69,74} = 2448.5	0.60

For normally distributed data (sizes of fish, SL, and number of fry produced) means $\pm$ standard deviations are shown. For non-normally distributed data, medians and interquartile ranges are shown. The only significant difference is highlighted in bold.

Table A2: Comparison of female abundance between high and low density nests during the 4 moon phases

	Breeding females	Visiting females
Full moon	$U = 566, N_1 = 44, N_2 = 35, \mathbf{P} = \mathbf{0.028}$	$U = 77, N_1 = 14, N_2 = 20, \mathbf{P} = \mathbf{0.027}$
Waning moon	$U = 896, N_1 = 61, N_2 = 40, \mathbf{P} = \mathbf{0.024}$	$U = 227.5, N_1 = 20, N_2 = 23, P = 0.934$
New moon	$U = 372, N_1 = 44, N_2 = 24, \mathbf{P} = \mathbf{0.043}$	$U = 214.5, N_1 = 21, N_2 = 17, P = 0.294$
Waxing moon	$U = 249.5, N_1 = 20, N_2 = 25, P = 0.99$	$U = 98, N_1 = 14, N_2 = 14, P = 1.0$

Results of Mann Whitney U-tests to compare the numbers of breeding and visiting females between high and low density nests at different moon phases. Significant correlations are highlighted in bold (see figure).

Figure legends

Fig. 1. Frequency distribution of the numbers of breeding females per male at full moon in years with low population density (grey bars) and high population density (black bars).

Fig 2. Frequency distribution of the numbers of neighbours within a radius of 3m in years with low population density (grey bars) and high population density (black bars).

Fig 3. Relationship between the total length of nest males and the number of neighbours, and regression lines; (a) low population density (1997), $N = 17$ and (b) high population density (2005), $N = 15$. Note the different scales on the y-axis.

Fig. A1: Numbers of breeding females in high density nests (grey bars) and low density nests (black bars) at different moon phases. Medians and interquartile ranges are shown. The numbers of checked nests are indicated below bars. Asterisks indicate a statistically significant ($p < 0.05$) difference between high and low density nests (see table A2 for statistical details).

740 **Figures**

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742 Figure 1

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Figure 3a

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Figure A1