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ORIGINAL PAPER

# Social network predicts loss of fertilizations in nesting males of a fish with alternative reproductive tactics

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**Abstract** Alternative reproductive tactics (ARTs) evolve when there is strong intra-sexual competition between conspecifics for access to mates. Typically, larger "bourgeois" males reproduce by securing the access to reproductive resources while smaller "parasitic" males reproduce by stealing fertilizations from larger males. A number of factors can influence the reproductive success of each tactic, including intrinsic (e.g. size) and extrinsic (e.g. tactic relative frequency) variables. An example where plastic ARTs occur is the peacock blenny *Salaria pavo*, with large males reproducing by defending nests and attracting females (bourgeois tactic) and small males reproducing by achieving sneaked fertilizations (parasitic tactic). In this study, we conducted field observations on individually tagged animals to determine their social network and collected eggs from 11 nests to determine the fertilization success of each male tactic. Paternity estimates for 550 offspring indicated an average fertilization success for nest-holder males of 95%. Nest-holder male morphological traits and social network parameters were tested as predictors of fertilization success, but only the number of sneakers present in the nest-holder's social networks was found to be a predictor of paternity loss. Although male morphological traits had been previously found to be strongly correlated with reproductive success of nest-holder males, as measured by the number of eggs collected in the male's nest, no correlation was found between any of the measured morphological traits and fertilization success for these males. The results suggest a stronger influence of the social environment than of morphological variables in the proportion of lost fertilizations by nest-holder males of this species.

**Keywords** *Salaria pavo* · Social network analysis · Paternity estimates · Blenniidae · Fertilization success

## Introduction

In some species, individuals of the same sex adopt alternative reproductive tactics (ARTs) to get access to mates in response to strong sexual competition (Taborsky et al. 2008). ARTs have been well characterized in teleost fishes (Taborsky 1994), where typically two consistent and discrete male reproductive phenotypes occur within the same population, each one trying to maximize their fitness by allocating resources to one tactic. One male morph makes the investment of getting privileged access to females by defending a nest and presenting attractive traits for females, such as courtship displays and secondary sexual characters, while the alternative male morph exploits the conspecifics' investment by stealing fertilizations in sneaky or coercive ways. The former males are usually called bourgeois or parental, the later parasitic or sneakers (Taborsky 1997). These tactics can be fixed for an entire lifetime, or individuals may switch tactics (plastic ARTs) in a reversible or irreversible manner (Moore 1991; Brockmann 2001; Taborsky et al. 2008). Switching from the parasitic to the bourgeois tactic is characteristic of sequential

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tactics, which are viewed as being conditionally triggered by developmental thresholds (i.e. status-dependent) instead of being genetically determined (Gross 1996). In these cases, fitness is not expected to be equal among alternative morphotypes. Moreover, the fitness of each male type may also be influenced by the frequency of both male tactics in the population (i.e. frequency-dependent), so that the fitness gain of sneakers will be dependent on the proportion of bourgeois males that they can exploit in the population, this balance being closely related to the bourgeois males' loss in fitness (Gross 1996). Fitness can be measured as reproductive success, and in the case of bourgeois males, it consists of the number of eggs spawned in the nest (i.e. mating success) that are fertilized by them (i.e. fertilization success). With the development of molecular techniques to quantify the fertilization success of each male reproductive tactic, it is possible to better understand how these tactics evolve and are maintained in populations.

The peacock blenny, *Salaria pavo*, is an emerging model in the integrative study of proximate and ultimate causes of ARTs and plasticity of mating systems (Oliveira 2006; Saraiva et al. 2013). This blenny is a small intertidal fish inhabiting coastal lagoons and rocky shores along the Mediterranean and adjacent Atlantic coasts (Zander 1986). Bourgeois males are larger than females and have well-developed secondary sexual characters (viz. a head crest and an anal gland), which are used to attract females to their nests for spawning (Gonçalves et al. 2002a; Barata et al. 2008), after which these males provide sole parental care to eggs until hatching (Fishelson 1963; Patzner et al. 1986). Moreover, due to the promiscuous mating system, where males and females may spawn with multiple mates throughout the breeding season, it is possible to find in each nest several batches of eggs in different developmental stages (Patzner et al. 1986).

Peacock blenny males can follow one of two developmental pathways, with some males growing directly into the bourgeois tactic while others first behave as female mimics to sneak fertilizations before switching to the bourgeois tactic at a later stage (Almada et al. 1994; Gonçalves et al. 1996; Fagundes et al. 2015). These alternative life histories are sequential and consistent with body size (i.e. condition) that each male has at sexual maturity in their first breeding season, as a consequence to their hatching time (Fagundes et al. 2015). Early born individuals arrive at their first breeding season with a body size that is too large for them to successfully mimic females, and therefore, they continue growing to become bourgeois males. In contrast, late born individuals arrive at their first breeding season with a small body size that allows them to mimic females' morphology and courtship displays (Gonçalves et al. 2005), switching to the nesting tactic in the following breeding season and going through a phase in which they are reproductively inactive (i.e. transitional males) (Fagundes et al. 2015).

To understand the dynamics of ARTs in *S. pavo*, an estimate of the success of nesting and sneaker tactics is required. Previous work has repeatedly established a strong positive correlation between the nesting male's body size and its mating success, as measured by the number of eggs in the nest, even when controlling for nest size (Oliveira et al. 1999; Gonçalves et al. 2002b). The recent development of genetic markers for *S. pavo* (Cardoso et al. 2013) now allows for estimation of the fertilization success of the male tactics in this species by doing paternity tests in nest-holder male's nests. In this study, we aimed to first confirm that nest-holders are subject to cuckoldry, showing that sneakers fertilize eggs, and then assess how biological and social factors affect the fertilization success of nesting males.

## Materials and methods

### Study site and field observations

This study was conducted during the peak of the breeding season (i.e. beginning of June until the end of the first week of July) in 2009 at Culatra Island (36° 59′ N, 7° 51′ W, Algarve, Southern Portugal). In this population, *S. pavo* nesting sites are very scarce and males mainly nest in artificial reefs made of bricks used by clam culturists to delimit their fields (for a detailed description of the habitat, see Almada et al. 1994). An area of approximately 422 m$^2$ containing four experimental transects, totalling 104 aligned bricks (T1 to T4; Fig. 1), and four sets of tiles distributed in the periphery (sets of tiles A to D; Fig. 1) were monitored throughout the study. A total of 152 fish (58 females, 64 bourgeois males, 24 sneakers and 6 transitional males) were captured during low tide along these transects and adjacent regions. These fish were individually tagged with a combination of coloured beads (diameter = 0.3 cm) attached to a fishing line inserted at the base of the dorsal fin, following the procedure described by Patzner (1984), and previously proved successful in this species (Gonçalves et al. 2003; Gonçalves et al. 2005; Fagundes et al. 2007; Saraiva et al. 2009). To minimize stress, fish were lightly anaesthetized with MS-222 (tricaine methanesulfonate, dilution 1:10,000, Sigma-Aldrich) before manipulation. The male morphotypes were discriminated as follows: bourgeois, males with fully developed secondary sexual characters (SSCs) that are actively defending a nest (i.e. nest-holders) or found wandering the transects (i.e. floaters); sneakers, males lacking male secondary sexual characters and from which sperm could be easily extruded from their vas deferens by gently pressing the abdomen; and transitional, sneakers with poorly developed SSCs undergoing the transition into bourgeois males and so not reproductively active. For each individual, measures of standard length, head height, body height, total length and width of the anal gland (modification

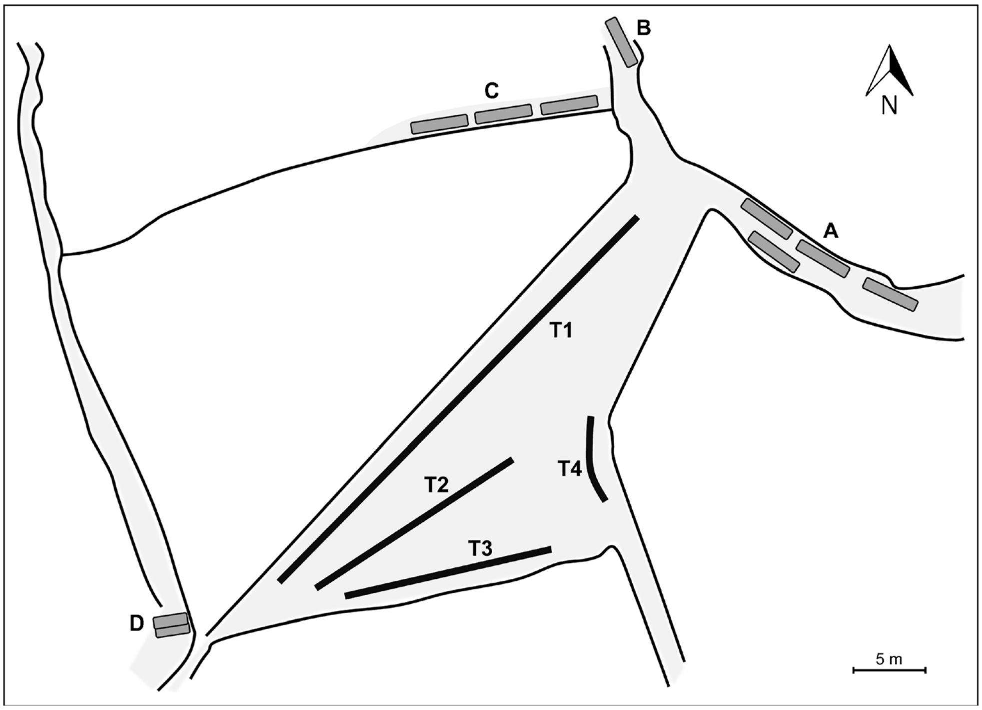

**Fig. 1** Schema of the field site located in the intertidal zone at Culatra Island, representing an area of approximately 422 m$^2$ containing four experimental transects (*T1* to *T4*) and four sets of tiles distributed in the periphery (sets of tiles *A* to *D*). *Shaded areas* represent artificial channels that run through this part of the field and become shallow or submerged during low tide and high tide, respectively

of the first two rays of the anal fin) were taken using a Vernier calliper to the nearest 0.01 cm. A small section of the dorsal fin tissue was also collected and preserved in absolute ethanol at 4 °C for genetic analyses. Fish recovered from anaesthesia in a container with abundant water and aeration and were returned to the brick/shelter from where they were captured. No mortality due to manipulation or anaesthesia occurred. Field observations consisted of inspections ($n$ = 9) during low tide, when all bricks and tiles were exposed, and scans ($n$ = 10) of all bricks during high tide while snorkelling, always recording fish positions (i.e. brick or tile). At the end of the first week of July, a sample of eggs accessible from the nest entrance was collected from 11 nests randomly chosen and preserved in absolute ethanol at 4 °C.

## Genetic analyses

Five microsatellite markers previously isolated from the peacock blenny (*Spavo08, Spavo21, Spavo22, Spavo23*; Cardoso et al. 2013) and shanny *Lipophrys pholis* (*6_6*; Guillemaud et al. 2000) were used to assess paternity at 11 nests. For each of these nests, 50 eggs were randomly selected for genotyping. Larvae were extracted from their eggs using a stereo microscope (VWR SZB 200) to avoid possible contaminations with maternal DNA. In order to determine the allele frequencies of the breeding population, 144 adults (two samples were lost), including the parental male at each nest, were also used for genotyping. Genomic DNA was extracted from larvae and fin samples using Extract-N-Amp™ Tissue PCR Kit (Sigma-Aldrich) following standard procedures.

Polymerase chain reaction (PCR) amplifications were carried out in 25 μl volume reactions on a thermal cycler (Stratagene RoboCycler® Gradient 96) using fluorescent dyed forward primers (Eurofins MWG Operon, Ebersberg, Germany), following the protocol outlined in Cardoso et al. (2013). Locus *6_6* was optimized in this study with an annealing temperature of 59 °C and 1.75 mM of $MgCl_2$. For larvae genotyping, only the $MgCl_2$ concentrations were optimized as follows: *Spavo08* and *Spavo21* at 2.25 mM, *Spavo22* at 2.5 mM and *Spavo23* at 2 mM. Since these microsatellites had different distributions of allele sizes and fluorescent labels, PCR products from each individual were pooled and sent for analysis. DNA fragments were separated on a commercial ABI 3730XL DNA Analyser (Macrogen Inc., Korea) and sized by co-running a GeneScan™ 400HD (Applied Biosystems, Foster City, CA, USA) size standard. DNA fragments were scored manually with the aid of GeneMarker® version 1.95 (SoftGenetics, State College, PA, USA).

Tests for Hardy-Weinberg equilibrium and genotypic linkage disequilibrium were performed using GENEPOP version 4.2.1 (Rousset 2008) with the default setting and the significance levels adjusted for multiple comparisons using the sequential Bonferroni correction with $\alpha = 0.05$ (Rice 1989). Genetic diversity estimates, including expected (He) and observed (Ho) heterozygosities, and exclusion probabilities, for one candidate parent ($P_{E1}$) and for one candidate parent given the genotype of a known parent of the opposite sex ($P_{E2}$), were obtained using CERVUS version 3.0.3 (Kalinowski et al. 2007).

### Paternity analyses

Estimations of paternity for the parental males were obtained using the *two-sex paternity* model developed by Neff et al. (2000a, b) and Neff (2001). This model was selected since it does not require the sampling of all potential parents when the mating system is promiscuous, as in the peacock blenny, where both males and females may spawn with multiple mates and nest-holder males may be the target of sneaked fertilizations. This model requires the genotype of the parental male, the genotypes of the brood in his nest and allele frequencies in the breeding population and gives as an output a paternity measure (*Pat*) expressed as a proportion of $NG_{dad}$ and $ng_{dad}$ (Neff et al. 2000a, b). $NG_{dad}$ is the expected proportion of the offspring that can be genetically compatible with the putative father by chance, calculated independently for each nest using information from all loci; $ng_{dad}$ is the proportion of the offspring that is genetically compatible with the putative father (Neff et al. 2000a). Additionally, paternity estimates were also obtained for tagged sneakers observed near the nests of nest-holder males assessed in this study. In these analyses, we considered all the eggs genotyped in each nest ($n$ = 50 per nest) and also tested if eggs that were not genetically compatible with the parental male could have been fathered by these sneakers.

### Social network analysis

Of the initial 152 individuals marked, only 105 were subsequently observed (35 females, 53 bourgeois males, 15 sneakers and 2 transitional males). A total of 520 observations were registered, 371 from individuals identified at the beginning of this study (mean ± standard error (SE) = 3.53 ± 0.18 observations per individual) and 149 from unknown individuals, which were not used for further analyses. In order to obtain the social network for this population, a two-dimensional matrix was first created, consisting of associations between individuals and the respective brick or tile in which they were seen (two-mode data). As the main focus of this work was to assess the fertilization success of nest-holder males in their nests, we choose to use the location of each individual relative to a brick (where nests are located) or tile (where non-breeding individuals usually seek shelter) and, from there, extract the relationships between individuals. Therefore, a relationship between two individuals is drawn only if they had visited the same brick or were sheltered under the same tile together at the same time or at different times. This type of social network is called an affiliation network and was obtained from the two-mode data matrix by extracting relationships between individuals based on common visits to bricks and tiles (one-mode data) using the function *data > affiliations* tool present in UCInet version 6.488 (Borgatti et al. 2002). This matrix was converted in order to be unweighted, and various measures were obtained using UCInet. As a measure of prominence for each individual in the network, the *Eigenvalue* centrality was selected since it takes into account how well an individual is connected (i.e. degree centrality), weighted by the number of connections his relationships also have (Makagon et al. 2012). To detect different social groups present in the network (i.e. network structure), the Girvan-Newman algorithm (Girvan and Newman 2002) was used. Network graphs of the relational matrix were drawn using the spring embedding based on distance in Netdraw version 2.118 (Borgatti 2002).

### Statistical analyses

In this study, the effects of two types of variables (morphometric and social) on nest-holder males' fertilization success were tested. Morphometric variables comprised measurements of morphological traits related to territoriality and mate attraction (i.e. secondary sexual characters), namely the standard length (SL), head crest size (ratio between head height and body height taken at the insertion of the pectoral fins) and relative anal gland area (determined by assuming an ellipsoid shape for this structure and dividing this value by SL). We did not examine body mass because length and mass are highly correlated in the peacock blenny (Oliveira et al. 1999). Social variables comprised measures obtained from the social network analysis, namely the nest-holder male's *Eigenvector* centrality, egonetwork heterogeneity (EgoNet heterogeneity) and proportion of the egonetwork comprised by sneakers (EgoNet sneaker).

Statistical analyses were performed using R (R Development Core Team 2014). Data were square root transformed to comply with the normality assumption and tested with Jarque-Bera normality test. Correlations between morphometric and social variables were examined with Pearson correlation coefficient ($r_P$) with the significance level adjusted for multiple comparisons using the sequential Bonferroni correction with $\alpha = 0.05$. A multiple regression model was used to predict nest-holder males' fertilization success using as predictors morphometric and social variables previously mentioned. Univariate tests of significance, effect size and observed power ($\alpha = 0.05$) for each predictor variable in the model were also obtained. Descriptive analyses are reported as mean ± SE.

## Results

### Paternity and social network analyses

The number of alleles found in the peacock blenny population of Culatra varied between 6 and 14 alleles per locus (mean of 9.6 alleles; Table 1). All loci were in Hardy-Weinberg equilibrium and were considered statistically independent since no

**Table 1** Summary statistics for five microsatellite loci (Guillemaud et al. 2000; Cardoso et al. 2013) used to characterize the peacock blenny (*Salaria pavo*) breeding population ($n$ = 144) at Culatra Island

| Locus | $N_A$ | Size (bp) | Heterozygosity | | Excl. Prob. | |
|---|---|---|---|---|---|---|
| | | | Obs. | Exp. | $P_{E1}$ | $P_{E2}$ |
| *Spavo08* | 10 | 216–242 | 0.78 | 0.79 | 0.43 | 0.61 |
| *Spavo21* | 11 | 292–336 | 0.81 | 0.77 | 0.38 | 0.56 |
| *Spavo22* | 14 | 129–189 | 0.84 | 0.80 | 0.43 | 0.61 |
| *Spavo23* | 7 | 245–269 | 0.65 | 0.70 | 0.28 | 0.45 |
| *6_6* | 6 | 295–311 | 0.63 | 0.60 | 0.18 | 0.31 |
| Combined $P_E$ | | | | | 0.88 | 0.97 |

$N_A$, number of alleles; Size, range of allele lengths in base pairs (bp); $P_{E1}$, exclusion probability when neither parent is known; $P_{E2}$, exclusion probability when one parent is known

linkage disequilibrium between loci pairs was observed after Bonferroni correction.

Paternity estimates were obtained for 11 different nests, from which 50 eggs were randomly selected for genotyping. Using all paternity estimates calculated, the mean paternity for nest-holder males was 95.34% ± 1.97 (range 83.36–100%; Table 2). Of the 11 nests analysed, we detected cuckoldry in five, with two of these nests having a paternity loss greater than 10%. The mean $\mathrm{NG}_{\mathrm{dad}}$ value obtained for each nest was 0.11 ± 0.01 (range 0.07–0.19), indicating that on average, 89% of all individuals in this population could be excluded as a potential father by chance alone.

**Table 2** Summary statistics of paternity (Pat) analyses obtained from 50 eggs sampled at each nest in peacock blenny population at Culatra Island, calculated according to Neff et al. (2000a, b), with a 95% confidence interval (CI)

| Nest | $\mathrm{NG}_{\mathrm{dad}}$[a] | $\mathrm{ng}_{\mathrm{dad}}$[b] | Pat (%) | 95% CI |
|---|---|---|---|---|
| 1 | 0.08 | 1.00 (50) | 100 | 93–100 |
| 2 | 0.12 | 0.86 (43) | 84.12 | 74–93 |
| 3 | 0.11 | 1.00 (50) | 100 | 93–100 |
| 4 | 0.19 | 0.92 (46) | 90.14 | 81–97 |
| 5 | 0.07 | 0.96 (48) | 95.72 | 87–99 |
| 6 | 0.12 | 0.96 (48) | 95.44 | 86–99 |
| 7 | 0.11 | 1.00 (50) | 100 | 94–100 |
| 8 | 0.08 | 1.00 (50) | 100 | 93–100 |
| 9 | 0.16 | 0.86 (43) | 83.36 | 74–93 |
| 10 | 0.11 | 1.00 (50) | 100 | 93–100 |
| 11 | 0.12 | 1.00 (50) | 100 | 94–100 |
| Mean | 0.11 | 0.96 | 95.34 | – |
| Range | 0.07–0.19 | 0.86–1.00 | 83.36–100 | – |

[a] The expected proportion of offspring compatible with the parental male by chance alone

[b] Proportion of offspring compatible with the parental male and corresponding number of eggs within parentheses

The social network analysis allowed us to examine the social dynamics relative to this subpopulation of *S. pavo* at Culatra Island. The network obtained in this study had 1314 ties between 105 individuals (mean number of ties per individual 12.51 ± 0.93; Fig. 2). The proportion of individuals observed in the consecutive days after being tagged was approximately 69%; transitional males were less re-observed, followed by females and sneakers (see the "Materials and methods" section). The social network presented a structured pattern similar to the spatial structure of experimental transects (Fig. 1), although this was not supported by structural analyses used to identify different social groups. Only nest-holder males defending nests allocated to the largest transect (T1) were distributed in three different "groups" across the network.

From the social network, individual networks (i.e. egonetworks) for each nest-holder male assessed for paternity were obtained. There was great variability in the composition and number of associations across all egonetworks (Fig. 3). For the six nests in which no sneaked fertilizations were detected, only in two cases were sneakers associated (nests 3 and 11), whereas for the five nests with sneaked fertilizations, only in one case (nest 2) was there no association with a sneaker male (Fig. 3). For these sneakers, additional paternity estimates were obtained to confirm whether or not their success in fertilizing eggs in nests with which they were associated (Table 3). When all genotyped eggs in each nest were considered, the paternity estimates obtained for the nine cases of possible sneaking showed that only in four cases sneakers could have fathered some of the eggs, including a nest where all the eggs were compatible with the parental male (nest 11). When analysing only eggs not fathered by the parental male, in two nests, these eggs remained without a known father (nests 2 and 9), while for the other cases, the sneakers associated with the nest could have fathered some of these eggs.

### Fertilization success of nest-holder males

Correlations between the variables used as predictors of fertilization success for the nest-holder males under study were inspected (Supplementary Table 1). For the morphometric variables, a negative correlation between standard length and crest size ($r_P = -0.68$, $n = 11$, $P = 0.021$) was found. For the social variables, only a positive correlation between the *Eigenvalue* centrality and the EgoNet heterogeneity ($r_P = 0.78$, $n = 11$, $P = 0.005$) was obtained, indicating that males who were more central in the network also had more heterogeneous egonetworks. After controlling for multiple comparisons, both correlations became non-significant.

To examine the influence of morphometric and social traits on nest-holder male's fertilization success, we conducted a multiple regression analysis, incorporating standard length, crest size, relative anal gland area, EgoNet sneaker and

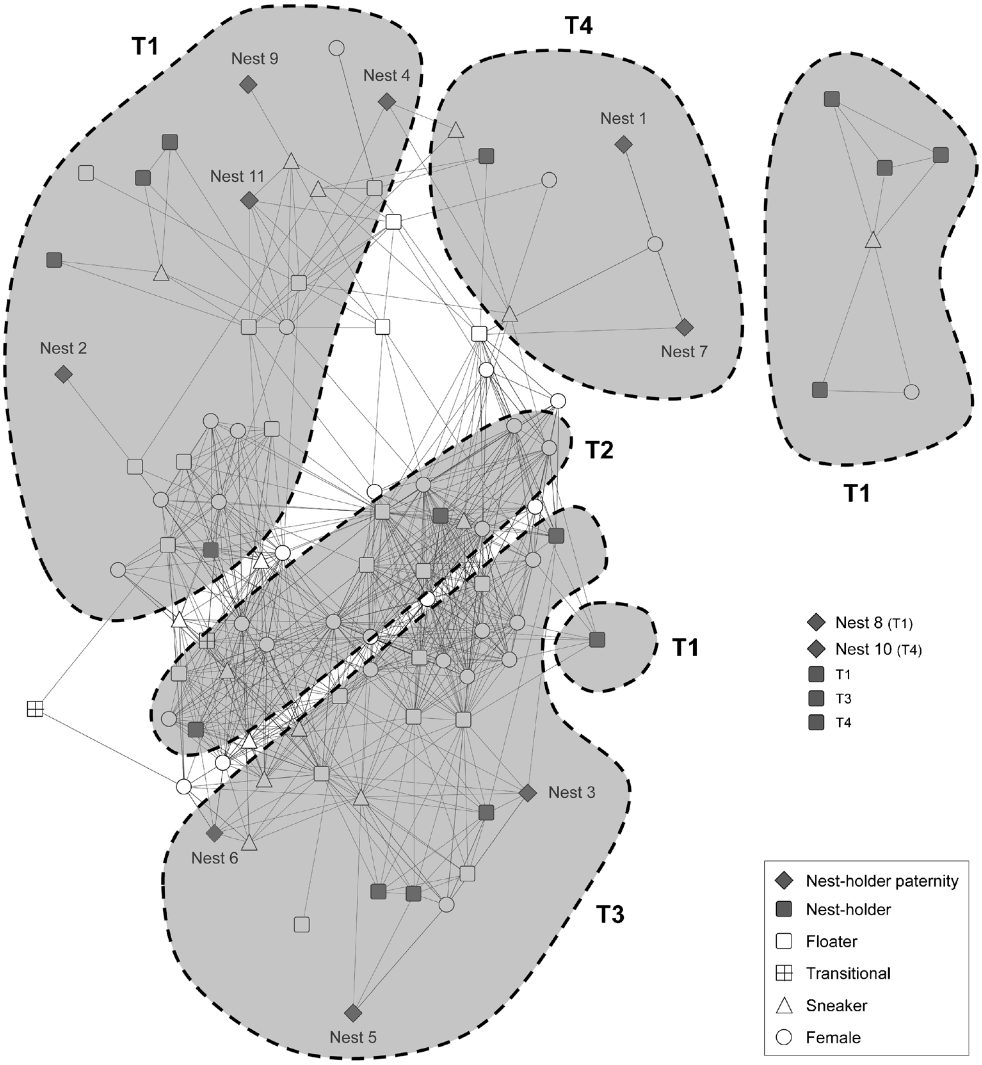

**Fig. 2** Affiliation network depicting social relationships between individuals marked at the beginning of the breeding season. Social ties between individuals are unweighted and present when the individuals visited the same brick or tile. *Symbol colour* designates behaviour: *white symbols* represent wandering and *black symbols* represent site attachment. *Symbol shape* indicates sex and morphotype: *filled diamond*, nest-holder male assessed for paternity; *empty square*, nest-holder not assessed for paternity or floater; ⊞transitional male; *empty triangle* sneaker and *empty circle* female. *Shaded areas* correspond to the location of nests in the experimental transects (*T1* to *T4*)

EgoNet heterogeneity and centrality. The results of this regression indicated the six predictors explained 73.8% of the variance in fertilization success, although not statistically significant ($R^2 = 0.74$, $F_{(6,4)} = 1.88$, $P = 0.282$; Table 4). Only the proportion of sneakers present in the egonetworks was found to be a predictor of the nest-holder male's fertilization success ($\beta = -1.33$, $P = 0.045$; Table 4).

## Discussion

In this study, we report the first estimates of paternity for ARTs in the peacock blenny *S. pavo*; nest-holder males had an average fertilization success of 95%, leaving the remaining offspring assumed to be sired by sneaker males. The incidence of sneaked fertilizations obtained in this work is one of the lowest values published in the literature for species with paternal care of eggs (for a review see Avise et al. 2002; Coleman and Jones 2011), only surpassing the reported estimates in the Centrarchid family (reviewed in Mackiewicz et al. 2005). It is still debated whether the high investment made by males in species with extensive parental care to eggs, which, in blennies, includes nest defence and cleaning, egg fanning (Almada and Santos 1995) and antimicrobial protection (Giacomello et al. 2006), strongly selects against sneaked fertilizations (e.g. Trivers 1972; Westneat and Sherman 1993; Kokko and Jennions 2008; Alonzo 2010). Generally, the proportion of eggs fertilized by sneakers is much smaller than the proportion fertilized by parental males. However, in a recent study, Cogliati et al. (2013) found for the plainfin midshipman fish (*Porichthys notatus*) the lowest levels of fertilization success for the nesting tactic described so far in fish, which was only 63% success when excluding from the analysis potential events of nest takeover.

The fertilization success detected for peacock blenny sneakers in this study could be considered a low "payoff" due to their high frequency and investment in female mimicry. Two main constraints imposed on sneakers may explain their low success. First, in this population, sneakers depend on female mimicry to successfully approach nest-holders, lacking

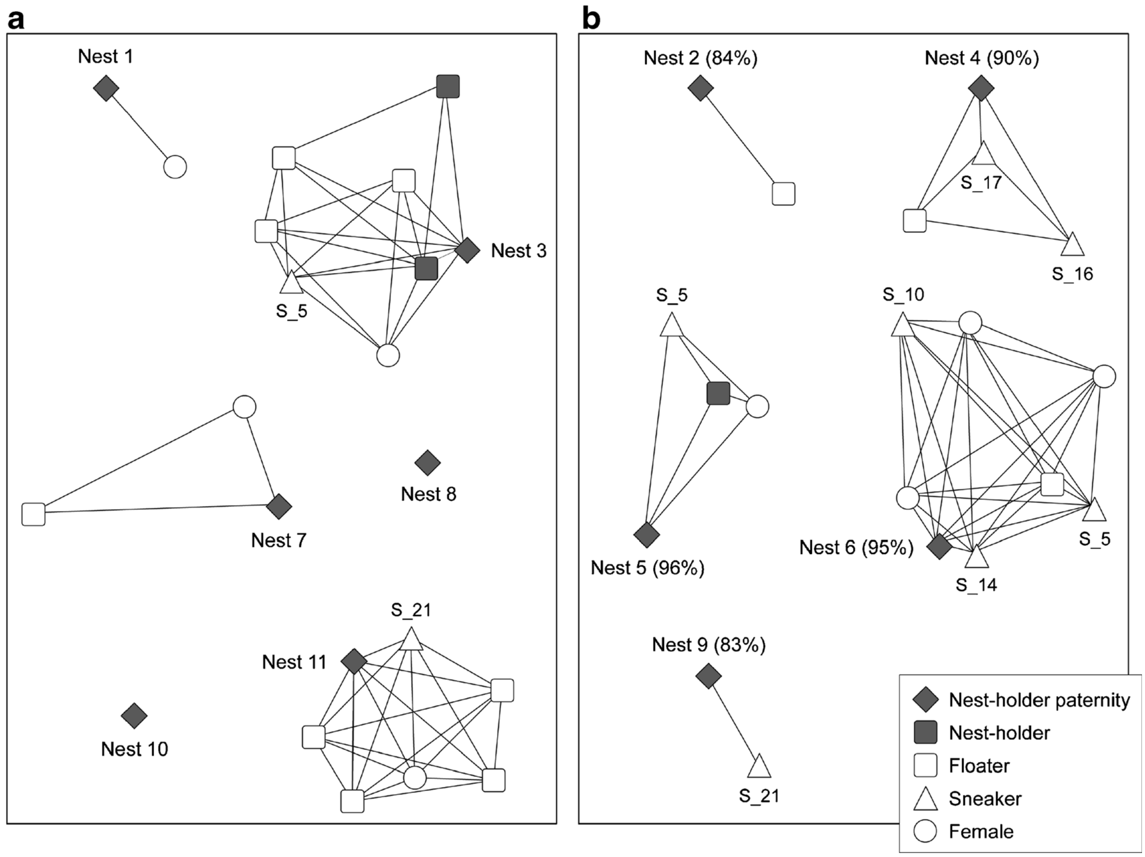

**Fig. 3** Egonetworks depicting social relationships for **a** nest-holder males with no sneaked fertilizations and **b** nest-holder males with sneaked fertilizations, including the respective paternity estimates. Social ties between individuals are unweighted and present when individuals visited the brick where the nest of the focal male was located. *Symbol colour* designates behaviour: *white symbols* represent wandering and *black symbols* represent site attachment. *Symbol shape* indicates sex and morphotype: *filled diamond* nest-holder male assessed for paternity; *empty square* nest-holder not assessed for paternity or floater; *empty triangle* sneaker and *empty circle* female

male secondary sexual characters and adopting female nuptial colouration and courtship behaviours towards nest-holder males (Gonçalves et al. 1996; Saraiva et al. 2013). The efficiency of this tactic is size dependent, as nest-holder males court and attack small sneakers and females with equal frequency, but as sneakers grow, they receive proportionally more attacks and less courts (Gonçalves et al. 2005). Furthermore, due to the existence of a female-biased operational sex ratio in this population (Oliveira et al. 1999), nest-holder males can be 'choosy' regarding potential mates. Consequently, it can be expected that a preference for larger females, as seen in a previous laboratory study (Gonçalves and Oliveira 2003), may play an important role in avoiding sneaking events.

Second, nest characteristics used by nest-holder males as spawning sites, holes in bricks with only one partially obstructed entrance (Almada et al. 1994), may also play a role. The nest characteristics requires sneakers to deceive the nest-holder males in letting them enter inside the nest following a mating event; otherwise, they are left with only the option of releasing sperm from outside the nest during the narrow window of the spawning event. This hypothesis was recently tested in the cichlid *Lamprologus lemairii*, where territorial males use rock holes as nesting sites (Ota et al. 2014). This study showed that when considering both pre- (body size of territorial males and size of nest opening) and post-mating (milt quality traits) factors, larger territorial males spawning in nests with narrower openings had greater siring success (Ota et al. 2014). In contrast, species that reproduce using natural substrates more exposed to conspecifics are expected to be subject to a higher rate of parasitic fertilizations. For example, in the ocellated wrasse (*Symphodus ocellatus*), where territorial males build nests from algae, parasitic fertilizations were detected on 100% of the analysed nests and, on average, 28% of all offspring were not sired by the parental male (Alonzo and Heckman 2010). Therefore, nest characteristics may also be a factor influencing the rate of success of sneakers in the peacock blenny.

However, when these fertilization rates take into account that (1) each nest-holder male has in his nest, on average, approximately 15,000 eggs in different developmental stages (Gonçalves et al. 2002b), and (2) depending on the incubation temperature, eggs can hatch within 8 to 16 days (Westernhagen 1983); sneakers may be able to fertilize thousands of eggs during the whole breeding season.

**Table 3** Summary statistics of paternity (Pat) analyses obtained for sneakers present in the social networks of the nest-holder males assessed in this study

| Nest | Sneaker ID | All eggs | | | | Sneaked eggs | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $NG_{dad}$[a] | $ng_{dad}$[b] | Pat (%) | 95% CI | *n* | $NG_{dad}$[a] | $ng_{dad}$[b] | Pat (%) | 95% CI |
| 1 | – | – | – | – | – | 0 | – | – | – | – |
| 2 | – | – | – | – | – | 7 | – | – | – | – |
| 3 | S–05 | 0.17 | 0.12 (6) | 0 | 0.06–0.24 | 0 | – | – | – | – |
| 4 | S–16 | 0.28 | 0.34 (17) | 8.12 | 0.22–0.48 | 4 | 0.28 | 0.50 (2) | 30.39 | 0.15–0.85 |
| | S–17 | 0.28 | 0.44 (22) | 22.52 | 0.31–0.58 | | 0.28 | 0.50 (2) | 30.83 | 0.15–0.85 |
| 5 | S–05 | 0.17 | 0.08 (4) | 0 | 0.03–0.19 | 2 | 0.17 | 0.50 (1) | 40.13 | 0.09–0.91 |
| 6 | S–05 | 0.17 | 0.28 (14) | 13.79 | 0.18–0.42 | 2 | 0.17 | 0.50 (1) | 40.13 | 0.09–0.91 |
| | S–10 | 0.02 | 0 | 0 | 0.00–0.06 | | 0.02 | 0 | 0 | 0–0.71 |
| | S–14 | 0.08 | 0.06 (3) | 0 | 0.02–0.16 | | 0.08 | 0.50 (1) | 45.41 | 0.10–0.91 |
| 7 | – | – | – | – | – | 0 | – | – | – | – |
| 8 | – | – | – | – | – | 0 | – | – | – | – |
| 9 | S–21 | 0.15 | 0.02 (1) | 0 | 0.01–010 | 7 | 0.15 | 0.14 (1) | 0 | 0.03–0.53 |
| 10 | – | – | – | – | – | 0 | – | – | – | – |
| 11 | S–21 | 0.15 | 0.20 (10) | 6.29 | 0.11–0.33 | 0 | – | – | – | – |
| Range | | 0.02–0.28 | 0–0.44 | 0–22.52 | – | 0–7 | 0.02–0.28 | 0.14–0.50 | 0–45.41 | – |

Estimates were obtained considering either all 50 eggs genotyped in each nest or only the eggs that were genetically incompatible with the parental male (*n*, sneaked eggs)

[a] The expected proportion of offspring compatible with the sneaker male by chance alone

[b] Proportion of offspring compatible with the sneaker male and corresponding number of eggs within parentheses

Reproductive success can be seen as being composed of two components: mating success (i.e. how successful an individual is in obtaining mating events) and fertilization success (i.e. how successful an individual is in fertilizing gametes on each mating event). The distinction between these two components of reproductive success is important when studying factors that affect Darwinian fitness in behavioural ecology, since different sets of factors may be influencing pre- and post-mating selection. In the peacock blenny, mating success, measured as the number of eggs present in the nest defended by nest-holder male, has been shown to correlate with male body size, suggesting that peacock blenny females are using this character as a mate choice criterion (Oliveira et al. 1999; Gonçalves et al. 2002b). However, fertilization success is not related either to male body size or to any other morphometric variable analysed in this study. In contrast, the proportion of sneaker males present in the nest-holder males' networks was the only significant predictor of fertilization success of nest-holder males, with nests in which sneakers were more commonly observed having a higher probability of having sneaked fertilizations. This result is consistent with previous work, where nests that received more eggs during the breeding season had a higher number of different sneakers associated with them (Gonçalves et al. 2003). Together, these results suggest a stronger influence of the social environment than of morphological variables in the proportion of lost fertilizations by nest-holder males. Contrasting this result, Alonzo and Heckman (2010) showed in the ocellated wrasse a positive

**Table 4** Multiple regression model of nest-holder male's fertilization success

| Variable | Coefficient ($\beta$) | SE | *t* | *P* value | eta-squared | Observed power ($\alpha = 0.05$) |
|---|---|---|---|---|---|---|
| Standard length | −0.552 | 0.515 | −1.071 | 0.344 | 0.058 | 0.133 |
| Crest size | −0.789 | 0.533 | −1.478 | 0.214 | 0.111 | 0.209 |
| Relative anal gland area | 0.509 | 0.389 | 1.309 | 0.261 | 0.087 | 0.174 |
| EgoNet sneaker | −1.334 | 0.462 | −2.884 | 0.045 | 0.423 | 0.587 |
| EgoNet heterogeneity | 0.652 | 0.432 | 1.508 | 0.206 | 0.116 | 0.215 |
| Centrality | −0.080 | 0.555 | −0.144 | 0.892 | 0.001 | 0.051 |

The model has the following statistics: $R^2 = 0.74$, $F_{(6,4)} = 1.88$ and $P = 0.282$

relationship between the number of sneakers near nests and the proportion of offspring sired by the parental male. However, in this species, it was previously shown that ocellated females preferred nests with high mating success because nesting males at these sites are more likely to provide parental care (Alonzo 2008; Alonzo and Heckman 2010).

In summary, in the peacock blenny, the two components of reproductive success of nest-holder males depend on two different types of factors. Whereas mating success depends on male characteristics used in female mate choice, fertilization success is influenced by the characteristics of the social network of the nest-holder male. This result highlights the importance that social factors may have on reproductive success and ultimately individual fitness. Finally, it should be stressed that reproductive success should not be viewed as a static trait, but rather as a dynamic characteristic of each of the tactics. The strength of sexual selection may vary between populations (e.g. due to different environment *regimes*; Bessert et al. 2007; Ota et al. 2012) and within populations over time (e.g. seasonal changes in the operational sex ratio; Wacker et al. 2014), thus affecting both spatially and temporally the frequency and relative reproductive success of ARTs. Therefore, future studies in this species should consider both the temporal and spatial dynamics of sexual selection when estimating reproductive success for each male tactic.

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### Compliance with ethical standards

**Ethical approval** All animal protocols were performed in accordance with accepted veterinary practice under a “Group-1” licence issued by the “Direcção-Geral de Veterinária, Ministério da Agricultura, do Desenvolvimento Rural e das Pescas”, Portugal.

**Conflict of interest** The authors declare that they have no conflict of interest.

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