

Home range and social analyses in a mouth brooding frog: testing the coexistence of paternal care and male territoriality

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Abstract

A link between paternal care and territoriality has been described in several anuran species. The southern Darwin's frog (*Rhinoderma darwinii*) has developed a highly specialized form of paternal care known as neomelia, in which males ingest developing embryos and transport them in their vocal sacs until metamorphosis is completed. Based on the main components of territoriality described in amphibians: site fidelity, resource limitation and defence (e.g. of oviposition sites and egg clutches), we hypothesized that *R. darwinii* males exhibit territoriality. To investigate this, we used a multi-method approach that involved estimating home range and movements, performing social network analyses and monitoring potential egg attendance. Forty-five individual frogs and three egg clutches were monitored in a population from southern Chile between December 2010 and February 2011. Site fidelity was found across all groups (juveniles, females, non-brooding males and brooding males) based on small movements between captures (mean \pm 1 SE; 0.96 ± 0.11 m) and small net displacement (2.95 ± 0.55 m). Home ranges were small (1.82 ± 0.54 m; range: 0.1–16 m²) and did not differ significantly among groups. We did not find evidence of male territoriality, instead male frogs exhibited high home range overlapping and intra-group association. No frogs of either sex were ever seen attending eggs. This evidence supports Wells' suggestion; territoriality in anuran species with parental care should be expected only if males defend oviposition sites. Conversely, females did not exhibit home range overlapping and showed evidence of very low intra-group association. This study has thrown up a range of additional questions with regard to temporal segregation between non-brooding males, the roles of male and female calls during reproduction, potential female territoriality and what kind of signals triggers egg ingestion by males.

Introduction

Home range is defined as the area ordinarily traversed by an individual in foraging, mating and caring for its young (Burt, 1943; Stebbins & Cohen, 1995). In amphibians, competition for diverse limited resources such as females or sites for calling, breeding, feeding and sheltering may cause an individual to defend certain (or total) areas within the home range (Duellman & Trueb, 1986; Shepard, 2002). Territoriality has been reported in many amphibian species (Mathis *et al.*, 1995; Shepard, 2004; Wollenberg & Harvey, 2010). In anurans, the primary function of territoriality is associated with reproduction, whereas most urodeles defend non-reproductive resources such as food and refuges (Pröhl, 2005).

Mathis *et al.* (1995) proposed three necessary components of territoriality in anurans: site fidelity, resource limitation (reproductive and non-reproductive) and resource defence, which can range from acoustic advertisement to overt aggression. Occasionally, sites for oviposition may result in a limited resource within an anuran home range (Mathis *et al.*, 1995; Pröhl & Berke, 2001) and a link between territoriality and egg clutch defence by males has been described for several anuran species (Wells, 1977, 2007). Pröhl (2005) suggested that male parental care, in the form of egg attendance and tadpole transport, exhibited by several species of Dendrobatidae, led to site fidelity and subsequently to the evolution of male territoriality.

The southern Darwin's frog (*Rhinoderma darwinii*) is a terrestrial frog that inhabits the temperate forests of south Chile

and Argentina. Although listed as vulnerable by the IUCN Red List (Úbeda *et al.*, 2010), it has recently been proposed that this species be reclassified as Endangered (Soto-Azat *et al.*, 2013). Its sister species, the northern Darwin's frog (*R. rufum*), was last recorded in 1980, and today appears to be extinct (Penna & Veloso, 1990; Soto-Azat *et al.*, 2013). *R. darwinii* has developed a highly specialized form of tadpole transport and nurture, known as neomelia (Bürger, 1905). After larval movement start within eggs (19 to 25 days post-egg fertilization), males incorporate the tadpoles in their vocal sacs for a period of 6–8 weeks, time during which a nourishment fluid is provisioned and metamorphosis takes place; then, the juveniles are regurgitated into the terrestrial environment (Goicoechea, Garrido & Jorquera, 1986). Although tadpole brooding is well described in this species, the existence of egg attendance/defence behaviour remains unclear. Several authors have reported that males stay close to egg clutches providing them with some level of protection (Pflaumer, 1935; Cei, 1962; Rageot, 1978; Crump & Veloso, 2005); while others note that after fertilization, both parents eventually withdraw, without any egg guarding behaviour by either sex (Busse, 2004; Werning, 2009). Since male reproductive success probably depends on the number of eggs that remain viable until the intake, it is rationale to hypothesize that males actively protect egg clutches from environmental or biological damage in order to increase their reproductive success.

R. darwinii is found within native forest, in which they are not homogeneously distributed but clustered in specific sites (Soto-Azat *et al.*, 2013). Ongoing research has identified certain microhabitat variables associated with higher densities of frogs including: lower variation in diurnal temperature and humidity, and substrates with high refuge density (authors's unpubl. data). Site fidelity has been described for *R. darwinii* (Crump, 2002) and field observations have shown that some individuals can be recaptured at the same specific sites for at least three consecutive years (Soto-Azat *et al.*, 2013). Taking this into account, along with potential egg clutch attendance and tadpole brooding by males, and based on the territoriality components proposed by Mathis *et al.* (1995), we hypothesized that male *R. darwinii* exhibit territorial behaviour. To test this hypothesis we used a multi-method approach: (1) determining home ranges and movements of *R. darwinii* in a wild population; (2) comparing home range overlapping by age and sex groups; (3) evaluating the social behaviour and structure of a *R. darwinii* population using social network analyses; and (4) monitoring egg clutches to determine the occurrence of any egg attendance behaviour. If males are territorial, we expect they exhibit a social structure with low intra-sexual home range overlapping, and a low intra-sexual association in comparison with inter-sexual association (unless they fight frequently as a result of resource defence).

Material and methods

Study site

The study was carried out in Tantauco Park, Chiloé Island, Chile. The studied site, which was characterized by an ever-

green coastal temperate rainforest, was located at 43° 21' 47.60" S, 74° 07' 19.65" W, 18 m a.s.l. An area with Darwin's frogs of 15 × 20 m (300 m²; estimated population size: 56.3 frogs; Soto-Azat *et al.*, 2013) was delimited. This area corresponds to a small clearing in the forest, surrounding by a swampy zone and a slow flowing stream. An additional boundary zone of 5 m surrounding the 300 m² study site was searched during each site visit in order to detect any movement of frogs beyond the borders of the delimited area.

Monitoring

Two daily searches of 2 hours each were performed, completing a total of 58 search sessions in 29 days. Frogs were monitored during December 2010 (16 days), January 2011 (10 days) and February 2011 (3 days). This time period was chosen in order to be coincident with the peak of the *R. darwinii* reproductive season (Cei, 1962). As *R. darwinii* is a diurnal frog (Crump, 2002), searches were performed during the day and were done in such a manner that survey effort was equal during each site visit throughout the study plot and the extra boundary zone.

Capture, individual identification and mapping

Frogs were captured by hand using disposable nitrile gloves, temporarily removed for identification and released at the exact site of capture. At first capture, each frog was weighted to the nearest 0.1 g; measured [snout-vent length (SVL)] to the nearest 0.05 mm and photographed (ventral colour patterns are individually distinct and were used for identification purposes – see Soto-Azat *et al.*, 2013). Individuals were classified according to morphologic (body size: ≤15 mm of SVL were considered juveniles, presence of vocal sac in males, enlargement of the coelomic cavity in gravid females) and behavioural characters (calling activity) into four groups: juveniles, adult females, non-brooding adult males and brooding adult males. A plastic, coloured flag with an individual frog ID number was placed at the site of each frog capture to enable subsequent tracking. By the end of the fieldwork, the study site was subdivided into 1 × 1 m squares using strings and the positions of each captured frog were established measuring the distance of the flag to the nearer *x* and *y* square border. A 10-cm grid map of the study site was then created on which the position of each animal capture was plotted.

Home range, site fidelity and spatial overlapping

Home range was estimated using a modified minimum convex polygon method, which included 90% of the individual captures of each frog (MCP 90%; Summers, 1992). To rule out any dependence of the home range size on the number of captures, we preliminary ran non-parametric correlations between these two variables for each group of frogs to determine an excluding cut-off point (or minimum required

captures) when P -value was > 0.1 . As a result, we included only males (brooding and non-brooding) with at least five captures, and females and juveniles with at least four captures for home range and movement analyses.

We used the mean of the distance moved between successive captures (DMC) and net displacement (ND) to assess site fidelity in our study population. Net displacement was calculated by measuring the distance between the initial and final capture point. Animals with high site fidelity should exhibit low values for DMC and ND (Shepard, 2004). To test if body size and/or the time interval between first and last capture (total interval; TI) affects home range size or distance travelled by an individual, we ran non-parametric correlations for body size (a product of body weight and SVL – see below) and TI against home range size and movements (DMC and ND).

In order to explore spatial overlapping, we measured the percentage of the area that each individual home range overlapped with other home ranges. Moreover, we compared intra- and inter-group home range overlapping for the adult classifications.

Social structure

To assess the social structure within the population, we constructed social networks using the software PAJEK (v. 3.1; University of Ljubljana, Ljubljana, Slovenia) where nodes represent an individual frog and ties correspond to associations between two frogs. In this case, all captured individuals were used in the analyses. To increase the probability of detecting an association, we constructed two types of networks based on the closest distance reached by two frogs in the same day. A conservative network was constructed with association defined as an encounter of two frogs at a distance less than or equal to 20 cm, while 90 cm was used in the construction of a relaxed network. Relaxed definition of association used in the relaxed network was based on the mean DMC (95.7 cm) calculated for all tracked frogs across the entire study. We determined the strength of association between each pair of frogs using a simple ratio index (SRI; Cairns & Schwager, 1987), calculated as

$$\frac{X}{X + Y_{ab} + Y_a + Y_b},$$

where X is the number of days during which a pair of animals (a and b) were observed in proximity; Y_{ab} is the number of days during which a and b were observed in separate associations; Y_a is the number of days during which only animal a was observed; and Y_b is the number of days in which only animal b was observed. The SRI provides values that vary from 0 to 1, where 0 indicates that a pair never associated and 1 indicates that a pair was always observed in proximity (Croft *et al.*, 2009). We calculated a group association index (GAI) as the sum of all SRIs within each age/sex group. Therefore, this index incorporates both the number of ties per group association and the strength of these associations. For both networks, we determined degree centrality (defined as the number of

direct ties an animal has; Wey *et al.*, 2008), number of components (groups), size of the main component and the main five centres of activity (hubs). Finally, in order to assess potential egg clutch attendance, we also constructed networks based on egg clutches, where only individuals with direct ties with an egg clutch were incorporated.

Parental care observations

We searched for egg clutches, which are commonly hidden under thick layers of moist vegetation (e.g. moss or ferns; Pflaumer, 1935; Rageot, 1978; Busse, 2004). Due to this, finding an egg clutch proved extremely difficult, even in areas with high frog population densities. When a clutch was located, the number of eggs was counted and the position was plotted on a map of the study site. Each known clutch was carefully monitored (twice daily by 1 h) during every search session to ascertain any interactions with adult frogs.

Data analysis

As weight and SVL are highly correlated, body size was defined as a product of these variables using a principal component analysis. Mann–Whitney U -tests were performed to compare body size between groups. In addition, Kruskal–Wallis tests were carried out to detect differences in home range size, DMC and ND across groups. We also performed Mann–Whitney U -tests to detect any difference in home range overlapping and degree centrality between groups. In order to test if less conservative definition of association was correlated with conservative definition, we ran a Pearson's correlation test between $GAI_{20\text{cm}}$ and $GAI_{90\text{cm}}$. The results are reported as mean ± 1 SE. Significance was considered with P -value < 0.05 . All statistical analyses were carried out using the statistical package SPSS (v. 17.0, IBM Corporation, New York, USA).

Results

Frog captures

A summary of mean SVL and weight per group is shown in Table 1. Juveniles were smaller than any other group (Mann–Whitney U -test for body size factor, all $P < 0.05$). Non-brooding males were smaller than brooding males (Mann–Whitney U -test $Z = -2.706$, $P = 0.005$). Although females were generally larger than both non-brooding males and brooding males, this difference was not significant (Mann–Whitney U -test, both $P > 0.05$). We tracked 13 juveniles, eight females, nine non-brooding males and 15 brooding males. We never found one of these 45 frogs out of the study plot within the 5 m extra boundary area. Of these, 32 frogs (seven juvenile, six females, seven non-brooding males and 12 brooding males) fulfilled the minimum required capture cut-off point for home range and movement analyses.

Home range size and movements

The TI varied from 9 to 71 days (36.3 ± 4.2). Spearman's correlations showed that TI was not correlated with home

Table 1 Body size and spatial ecology data for Darwin's frogs (*Rhinoderma darwinii*) from Chiloé Island, south Chile. Snout-vent length (SVL), home range size (MCP 90%), distance moved between captures (DMC), net displacement (ND) and percentage of home range overlapping with any other individual home range

Group	<i>n</i>	SVL (mm)	Weight (g)	MCP 90% (m ²)	DMC (m)	ND (m)	Overlapping (%)
		mean (±SE)	mean (±SE)	mean (±SE) ^a	mean (±SE) ^a	mean (±SE) ^a	mean (±SE) ^a
Juveniles	13	13.79 (0.29)	0.16 (0.03)	0.52 (0.15)	0.93 (0.37)	3.22 (1.55)	31.09 (5.47)
Females	8	23.56 (0.74)	0.83 (0.07)	1.01 (0.41)	0.74 (0.16)	1.25 (0.31)	12.27 (7.96)
Non-brooding males	9	21.72 (0.49)	0.78 (0.07)	1.98 (0.90)	0.92 (0.10)	3.30 (1.15)	70.96 (12.28)
Brooding males	15	22.92 (0.34)	1.12 (0.07)	2.89 (1.33)	1.09 (0.19)	3.36 (0.94)	44.41 (9.89)

^aCalculated with the minimum required captures for home range estimation (see methods section); juveniles (*n* = 7), females (*n* = 6), non-brooding males (*n* = 7), brooding males (*n* = 12).

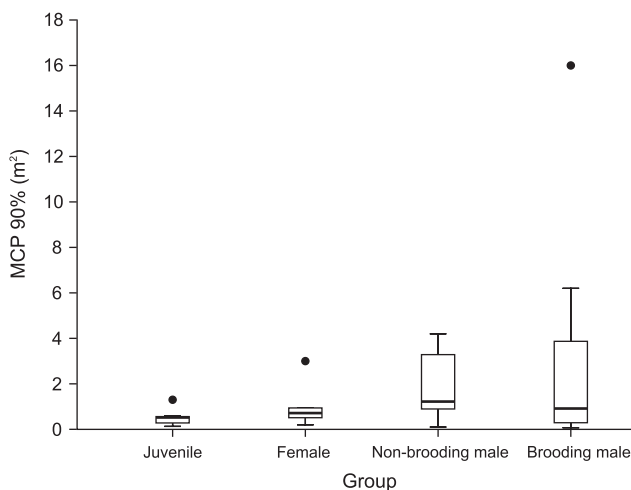


Figure 1 Box and whisker plots of home range size (MCP 90%) per group in a Darwin's frog (*Rhinoderma darwinii*) population from Chiloé Island, south Chile. The centre line in each box indicates the median, the upper and lower box sides represent the interquartile range, the whiskers extend to the 5th and 95th percentiles and dots represent outliers.

range size ($r_s = -0.033$, $n = 32$, $P = 0.858$), DMC ($r_s = 0.039$, $n = 32$, $P = 0.833$) or ND ($r_s = 0.008$, $n = 32$, $P = 0.966$). Home range sizes per group are shown in Fig. 1. A positive correlation was detected between body size and home range size (Spearman's $r_s = 0.480$, $n = 32$, $P = 0.005$); however, differences in home range sizes between groups were not significant (Kruskal–Wallis test $\chi^2 = 3.259$, d.f. = 3, $P = 0.353$). A summary of mean home range sizes per group and a two-dimension map of home ranges are presented in Table 1 and Fig. 2, respectively. Body size was not correlated with DMC ($r_s = 0.226$, $n = 32$, $P = 0.214$) or ND ($r_s = 0.028$, $n = 32$, $P = 0.881$). Although juveniles, non-brooding males and brooding males showed higher DMC and ND compared with females (Table 1), statistical differences were not found between groups (DMC: Kruskal–Wallis test $\chi^2 = 2.442$, d.f. = 3, $P = 0.486$; and ND: $\chi^2 = 3.259$, d.f. = 3, $P = 0.353$).

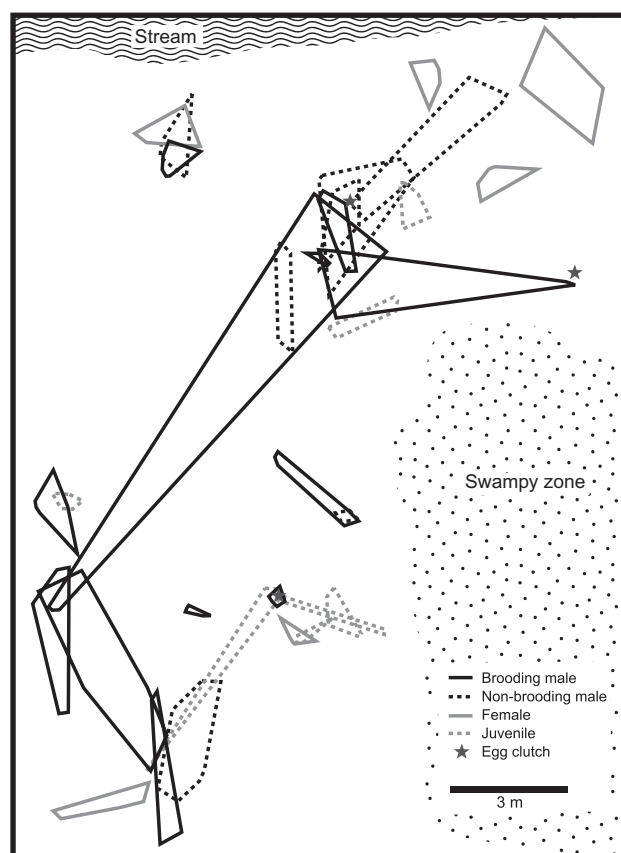


Figure 2 Home range map of 32 southern Darwin's frogs (*Rhinoderma darwinii*) monitored in a temperate forest of Chiloé Island, South Chile.

Home range overlapping

Males (brooding and non-brooding) had in mean a higher percentage of its home range overlapped with any other individual home range (Table 1; Fig. 2), but this difference was statistically significant only when brooding males or non-brooding males were compared with females (Mann–Whitney *U*-test $Z = -2.466$, $P = 0.014$; and $Z = -2.083$, $P = 0.041$; respectively), but not with juveniles (Mann–Whitney *U*-test $Z = -1.855$, $P = 0.073$; and $Z = -0.592$,

Table 2 Summary of social network analyses parameters of a Darwin's frog (*Rhinoderma darwinii*) population from Chiloé Island, south Chile

Parameter	Conservative network ^a	Relaxed network ^b
Pairs of frogs (ties)	17	50
Number of components ^c	30	10
Size of the main component ^d	11.1%	44.4%
Degree centrality (mean ± SE)		
Overall	0.76 (±0.14)	2.20 (±0.26)
Juveniles	1.07 (±0.29)	1.76 (±0.36)
Females	0.37 (±0.18)	1.25 (±0.37)
Non-brooding males	0.40 (±0.22)	2.40 (±0.69)
Brooding males	0.93 (±0.29)	3.14 (±0.48)
Hubs ^e	Two brooding males and three juveniles	Four brooding males and one juvenile

^aConstructed with association defined as an encounter of two frogs at a distance less than or equal to 20 cm.

^bConstructed with association defined as an encounter of two frogs at a distance equal or higher than 90 cm.

^cNumber of units composed by frogs, which have connections (direct or indirect) between them (ties). Frogs with no associations are also considered as individual components.

^dExpressed as a percentage of the total individuals (nodes).

^eOnly the first five hubs were calculated in both networks.

$P = 0.592$; respectively). The home ranges of only two of the six females studied overlapped with other frogs; home range overlapping between females did not occur (Fig. 2). In contrast, males exhibited a higher intra-group overlapping of home ranges (84.21% of the males) in comparison with male/female overlapping (5.21% of the males), a characteristic that was also present when only non-brooding males (more potential 'sexually active' males) were considered (non-brooding males/non-brooding males, 42.85%; non-brooding males/female, 14.28%).

Social structure

A summary of parameters calculated for both conservative and relaxed networks are presented in Table 2, while a graphic representation of all networks is shown in Fig. 3. For both networks, the strongest associations were found between juvenile/brooding male and non-brooding male/brooding male, while juvenile/juvenile and brooding male/brooding male associations were also high. No association between non-brooding male/non-brooding male or female/female were found at ≤ 20 cm, but weak associations between these groups were present at ≤ 90 cm, with only three non-brooding male and one female intra-group pairing found (Fig. 3; see Supporting Information Table S1). Consistent with these findings, there was a strong and statistically significant correlation between $GAI_{20\text{cm}}$ and $GAI_{90\text{cm}}$ (Pearson's $r = 0.879$, $n = 10$, $P = 0.001$; Fig. 4).

Significant differences in the average degree centrality were found only when brooding males were compared with

juveniles or females in the relaxed network (Mann–Whitney U -test $Z = -2.095$, $P = 0.038$; and $Z = -2.470$, $P = 0.013$, respectively), indicating that brooding males have, on average, more direct interactions than these two other groups (Table 2).

Egg clutch observations

Three egg clutches were found during the study (only other two egg clutches were previously detected by the research team during 6 years working with the species), the first detected on 18 December 2010 (egg clutch no. 1, four eggs), the second on 20 December 2010 (no. 2, five eggs) and the third on 24 February 2011 (no. 3, four eggs). All were found under a dense layer (~ 10 cm thick) of moss and ferns. On 27 December, we observed one male incorporating non-hatched eggs from clutch no. 1 into its vocal sac. The process to pick-up all four eggs took 24 h. The new brooding male remained at the oviposition site for 1 week before disappearing; the individual was not observed again. After being monitored, egg clutch no. 2 disappeared 6 days after it was first observed. It is not clear whether the five eggs of this clutch were ingested by a male Darwin's frog or were removed by other means; however, obvious signs of predation were not noticeable. For logistical reasons, egg clutch no. 3 was only tracked for 2 days; all four eggs remained viable at the time of last sighting. During the period of egg clutch monitoring, three different brooding males and two juveniles were found at a distance within 20 cm from egg clutch no.1. Additionally, three and one non-brooding male were observed (only 1 day each) at a distance between 20 and 90 cm from egg clutch no. 2 and egg clutch no. 3, respectively (Fig. 3). Throughout the study, we did not observe these or any other frogs directly attending eggs or providing them with protection.

Discussion

In this study, we investigated the home range, interactions and parental care behaviour of Darwin's frogs (*R. darwinii*). Our results demonstrated a positive correlation between body size and home range size, but no differences in home range size were found between sex or developmental stage (juvenile vs. adults). This may have been due to the high variance in home range sizes found mainly in both brooding and non-brooding males (see Fig. 1). Overall, home ranges were small (< 16 m²) in comparison with anurans from Bufonidae, Leptodactylidae and Hylidae, but similar home ranges have been described in some species from Aromobatidae and Dendrobatidae (for a detailed review of anuran home range sizes, see Wells, 2007). Site fidelity, characterized by low DMC and small ND (Shepard, 2004), was found for all groups. *R. darwinii* has a characteristic sit-and-wait foraging behaviour (Crump, 2002). Such predators are usually sedentary and have smaller home ranges than active foragers (Wells, 2007) and our results indicate that this also follows for *R. darwinii*.

We found no evidence of territorial behaviour in Darwin's frog males based on home range, movement, and social network analyses. In fact both, within and between

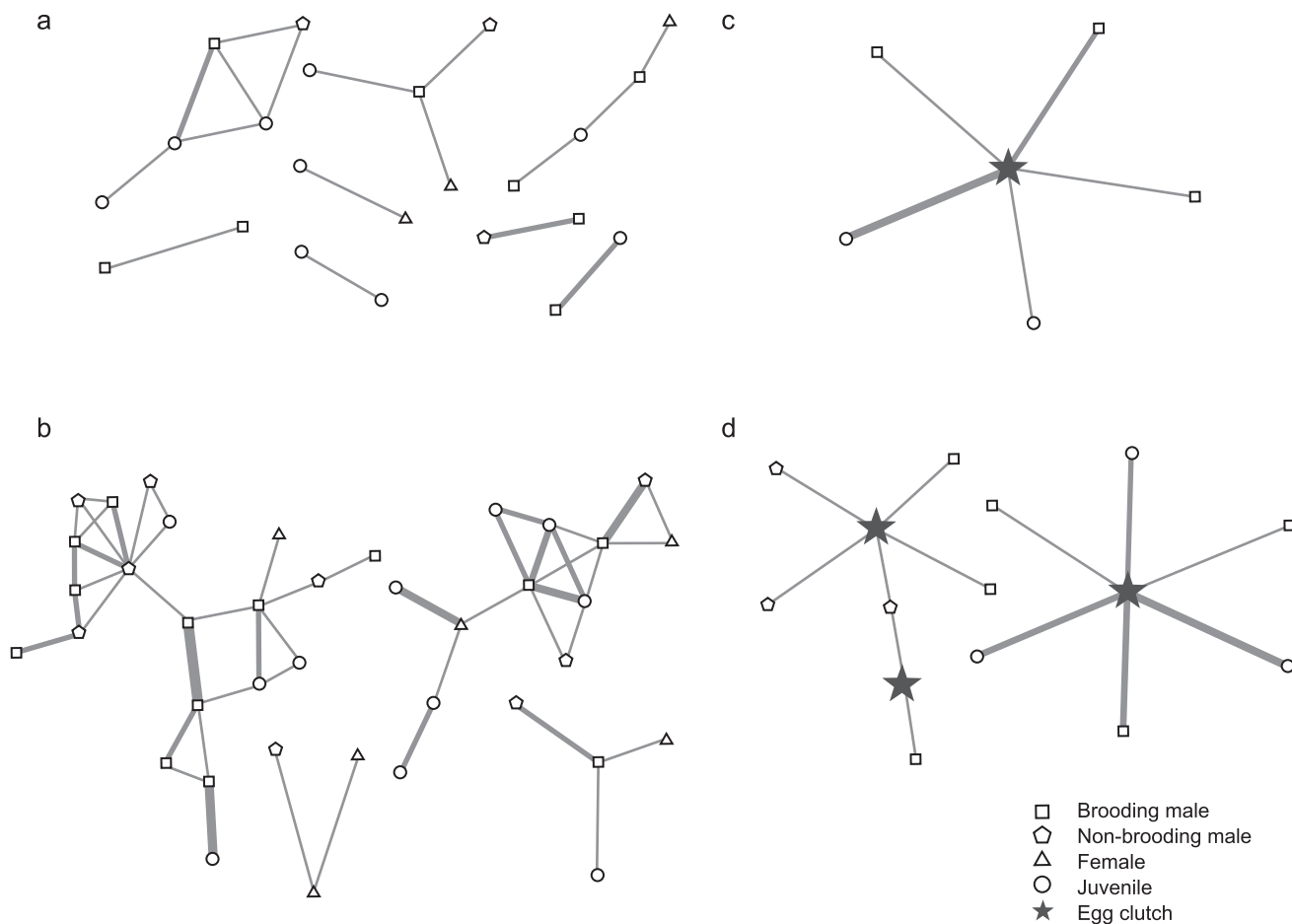


Figure 3 Social networks of a Darwin's frog (*Rhinoderma darwinii*) population from Chiloé Island, south Chile, based on associations at (a) 20 and (b) 90 cm. Networks based on associations between frogs and egg clutches at (c) 20 and (d) 90 cm. All networks were graphed using the Kamada–Kawai algorithm with separate components in software PAJEK (v. 3.1; University of Ljubljana, Slovenia). Thickness of ties represents frequency of interactions.

non-brooding male and brooding male groups, there was a high degree of spatial overlapping. Agonistic visual displays or physical confrontations (Mathis *et al.*, 1995; Gardner & Graves, 2005; Pröhl, 2005) or territorial calls (Penna & Veloso, 1990), which are performed by many territorial anuran species, were not observed during this study. To the best of our knowledge, territorial behaviours have not been described in other field or captivity observations of Darwin's frogs.

The social network analyses showed a lack of non-brooding male/non-brooding male association at a distance of ≤ 20 cm (and lower degree centrality in non-brooding males at this distance; Table 2) and only a very weak association at a distance of ≤ 90 cm. These results indicate that, even when home range overlapping between non-brooding male/non-brooding male was high (42.85%), non-brooding males were almost always observed separated at a finer temporal scale. We did not find evidence of this spacing pattern between either non-brooding male/brooding male or brooding male/brooding

male, where both $GAI_{20\text{cm}}$ and $GAI_{90\text{cm}}$ were high; supporting the hypothesis that call activity, or other behaviour related to courtship, might be implicated in the temporal segregation found between non-brooding males. Previous observations have revealed that brooding males progressively decrease their calling activity as the 'pregnancy' progresses (authors's unpubl. data). When *R. darwinii* females are ready to spawn, they navigate using the male's call to move towards a retreat site, where a calling male is present (Werning, 2009). Spatio-temporal segregation in calling sites, which could result in the temporal non-brooding male/non-brooding male segregation found here, might be used by this species to avoid direct competition between non-brooding males. As observed in other species, the mating success of male Darwin's frogs probably depends on their ability to attract females to their calling sites and to prevent interference from other males (Wells, 1977; Felton *et al.*, 2006).

Home range overlapping between females was not observed and social network analyses showed a low degree centrality

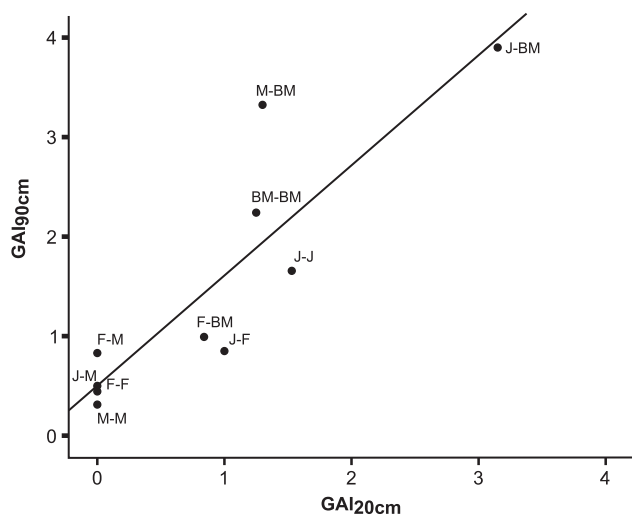


Figure 4 Correlation between group association index at 20 and 90 cm in a Darwin's frog (*Rhinoderma darwinii*) population from Chiloé Island, south Chile. Each dot represents a pair of group associations. BM, brooding male; F, female; J, juvenile; M, non-brooding male.

for this group (Table 2), female/female GAI_{20cm} was zero and only one female pair association was found in the relaxed network. Could this spacing pattern suggest the presence of territoriality in *R. darwinii* females? The small sample size for this group and the lack of territorial behaviour observations impede us to demonstrate female territoriality in Darwin's frogs. In addition, territoriality in females has been described in some amphibian species, with females defending limited resources such as males, refugia and feeding sites (Wells, 1977; Verrell & Brown, 1993; Mathis *et al.*, 1995; Meuche, Linsenmair & Pröhl, 2011). Sex role reversal (i.e. females competing more intensively than males for access to mate) associated with high levels of male parental care investment has been described for some species of fish and birds (Eens & Pinxten, 2000). However, sex role reverse only should occur if females compete for limited receptive males (Summers, 1989). There is no evidence of male limitation for female *R. darwinii* as male/female ratio observed within a population is normally equal or higher than one (Crump, 2002; Bourke, Busse & Bakker, 2011; Soto-Azat *et al.*, 2013; this study), unless brooding males become non-reproductive, and then non-brooding males become rarer than females and therefore a limiting resource. However, we have observed the concurrent presence of froglets (with tail buds) and young tadpoles in the vocal pouches of some males in the wild (also noted in Crump, 2002). This gives the possibility that *R. darwinii* males are polygynous. As proposed by Summers (1989), from a female's point of view, the quality of a male (i.e. quality or quantity of parental care provided to its offspring) would decrease as the number of other females mating with that male increases. In this case, females should aim for mate(s) that reproduce with as few other females as possible (parental quality hypothesis). In *Dendrobates auratus*, a species proposed as an example of

sex role reversal (Trivers, 1972), field and experimental evidence showed that aggression between females is most likely attributable to parental quality rather than sex role reversal (Summers, 1989; Wells, 2007). Then, if female territoriality exists in *R. darwinii*, there is the possibility that it could be associated with sex role reversal or with parental quality hypotheses.

Our hypothesis was that male Darwin's frogs exhibited territoriality because of the high site fidelity and paternal care exhibited by this species. Our field observations suggest that males do not defend oviposition sites or attend egg clutches. This evidence is supported by social network analyses, which showed that associations between non-brooding males and egg clutches were only present in the relaxed network and these associations were in all cases weak. Observations made from a captive-breeding centre further support this; even in relative small terraria, males remain away from their egg clutches and do not provide them with any level of care (Carlos Barrientos-Donoso, pers. comm.). Therefore, parental care in *R. darwinii* could be characterized only by tadpole brooding, rather than egg attendance or defence. Oviposition sites, which are often under a dense layer of moist vegetation (Busse, 2004; this study), should be sufficiently effective protecting eggs from desiccation and other sources of damage. Furthermore, we have observed that these sites are abundant in the forests where Darwin's frogs inhabit, and do not represent a likely limiting resource within suitable habitats. Consistent with Wells' suggestion, territoriality in species with parental care should be expected only if males defend oviposition sites (Wells, 2007). Since males appear to withdraw after egg fertilization, further research is needed to elucidate what kind of signals triggers the ingestion of eggs by males, including those cases in which egg clutches are deposited in areas with high home range overlapping or high density of frogs. Could it be possible, for example, that cross-fostering occurs in *R. darwinii* (i.e. males brooding another male's eggs)?

To our knowledge, this study is the first to integrate home range estimation, social network analyses and egg attendance monitoring to assess territoriality in an anuran species. Our results on home range overlapping and social structure allowed us to reject our hypothesis that male *R. darwinii* exhibit territorial behaviour. This study has thrown up a range of additional questions with regard to temporal segregation between non-brooding males, the roles of male and female calls during reproduction, potential female territoriality and what kind of signals triggers egg ingestion by males. We hope that further research to answer these and other questions will be conducted on this amazing species and that the results will contribute to have a better understanding of the association between parental care and the evolution of territoriality in amphibians.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Summary of associations between different sex and age groups in a Darwin's frogs (*Rhinoderma darwinii*) population from Chiloé Island, South Chile. Group association index (GAI) is shown for both associations at 20 and 90 cm.