Herpetological Journal

How to form a group: effects of heterospecifics, kinship and familiarity in the grouping preference of green and golden bell frog tadpoles

Lígia Pizzatto, Michelle Stockwell, Simon Clulow, John Clulow & Michael Mahony

School of Environmental and Life Sciences, University of Newcastle, Callaghan 2308, NSW, Australia

Social aggregations are widespread among animal groups. They are relatively common in amphibian larvae, likely conferring protection against predators, advantages for microhabitat selection, foraging efficiency, and thermoregulatory efficiency. Group formation involves selection of individuals to group with by the other members, and several tadpoles are reported to recognise and prefer to aggregate with siblings or familiar individuals. In Australia, tadpoles of the endangered green and golden bell frog, *Litoria aurea*, are attracted to conspecifics and form schools. We conducted two choice experiments for captive breed tadpoles of this species to test their grouping preferences. Tadpoles preferred to aggregate with conspecifics to heterospecifics of a sympatric species; however, when conspecifics were absent they preferred to aggregate with the heterospecifcs than to remain alone. Tadpoles also preferred unfamiliar kin to unfamiliar non-kin conspecifics, but had no preferences between unfamiliar and familiar siblings. Once widespread in southeast Australia, the green and golden bell frog has suffered considerable declines and local extinctions in recent decades. Susceptibility to chytridiomycosis is likely the major threat for most remaining fragmented populations and the major challenge for reintroduction programs. The strong gregarious behaviour of this species may affect disease dynamics, especially chytridiomicosis that continues to threaten remaining wild populations.

Key words: amphibian, conspecific attraction, familiarity, grouping, kin recognition, *Litoria aurea*

INTRODUCTION

Spatial aggregations are the most common distribution patterns in nature, and understanding how animal groups are formed, along with their advantages and disadvantages, has attracted the attention of ecologists for decades (Rubenstein, 1978). Some animals may group as a result of resource patchiness, but social grouping also has evolutionary advantages. Group living is shown to be particular advantageous in decreasing individual predation risk and increasing foraging efficiency in several species, but may also confer aid in rearing young, facilitate mating, increase protection against extreme weather conditions, and improve swimming or flying performances (Rubenstein, 1978). On the other hand, aggregation can in some cases increase competition, attract predators, and increase disease transmission (Krause & Ruxton, 2002).

Animals often use assortative behaviour based on specific traits to select mating partners or group members, such as species (Graves & Gotelli, 1993), kinship (Waldman & Adler, 1979), and familiarity (Magurran et al., 1994), and the benefits of the differential assortment varies among animal groups. For example, bluegill sunfish are able to recognise and will associate with familiar mates with whom they have foraged more successfully (Dugatkin & Wilson, 1992).

Conspecific aggregations can be particularly advantageous as individuals match in terms of habitat and food requirements, and are physically similar to each other, contributing to predator confusion and consequently decreasing individual predation risk (Krause & Ruxton, 2002). However, heterospecific aggregations (including facultative mutualism) can minimise competition within the group (Mönkkönen et al., 1999) and are common in a wide range of animal taxa including amphibians and their larvae (Glos et al., 2007).

Kinship grouping seems to increase growth rates in tadpoles (Blaustein & Waldman, 1992; Hokit & Blaustein, 1994; Jasieński, 1988); and in fish, it increases shoal cohesiveness and consequent protection from predators (Hain & Neff, 2009). Kin attraction or repulsion can also affect dispersal rates and thus influence population structuring. Grouping based on familiarity and kinship to other individuals is well reported in fishes (Barber & Wright, 2001; Frommen et al., 2013), tadpoles (Waldman & Adler, 1979; Blaustein & O'Hara 1987; Halverson et al., 2006), and some reptiles (Werner et al., 1987; Clark et al., 2012).

Correspondence: Lígia Pizzatto (ligia.oceanica@gmail.com)

In tadpoles, the intensity of mutual attraction seems to be at least partially dependent upon school density, and school formation and dynamics can be variable. For example, in some species individuals are in tight contact with each other, whereas in others, individuals are regularly spaced. Similarly to some fishes, tadpole schools can be polarised with individuals distributed in parallel and moving as an unit, or be unpolarised and exhibit low mobility (Wassersug, 1973). Social aggregations in tadpoles have been known to occur for a long time (Wassersug, 1973), but are recorded in only few species worldwide (Blaustein & Waldman, 1992; Duellman & Trueb, 1994). Wassersug (1973) refers to a personal communication to report that several (non-specified) species of anuran larvae in Australia form active swarming schools. In northern Australia *Cyclorana longipes, C. australis,* and *Litoria dahlia* have been seen to form very tight schools (M. Crossland, pers. comm.). However, the only published work showing conspecific attraction and formation of social groups in an Australian tadpole is for the green and golden bell frog, *Litoria aurea* (Leu et al., 2013).

The green and golden bell frog was once a common species widely distributed in south eastern Australia (Mahony et al., 2013). It has some general traits of a weed or colonising species (e.g., long-lived; highly fecund; habitat and dietary generalist; non-sensitive to disturbed areas), and has been introduced into New Zealand, New Caledonia, Vanuatu Loyalty Islands and Wallis and Futuna (Pyke & White, 2001). However, the extreme sensitivity to the infectious chytrid fungus has been the major cause of the species' decline in its native range, currently restricted to small isolated populations along the coast of New South Wales to eastern Victoria (Mahony et al., 2013).

In tadpoles, protection against predators, increased foraging efficiency and growth are cited as the major advantages for grouping (Steinwascher, 1978; Watt et al., 1997; Wells, 2010). Considering these advantages, social grouping can be expected in *L. aurea* because eggs are laid in large clumps (Antis, 2013); both eggs and tadpoles are highly palatable (Morgan & Buttemer, 1996); and tadpoles live in ponds (including temporary ponds) where predator and food detection can be compromised due to water turbidity (Wells, 2010). The mechanisms driving school formation, such as assortative behaviours, have not been investigated in this taxon, and may contribute to a better understanding of the species' distribution pattern and disease dynamics in natural conditions. For instance, proximity to other individuals, especially heterospecifics that carry chytridiomicosis but show no signs of illness, could increase transmission. In a broader context, identifying the 'rules' of assortment for group formation in a species from a continent not previously studied adds to our ability to generalise patterns that have been studied in only a handful of species with limited geographic range (Roche, 1993).

Using an experimental approach we addressed four questions: (Q1) Do green and golden bell frog tadpoles discriminate and prefer to group with conspecifics rather than non-conspecifics? (Q2) Do these tadpoles prefer to aggregate with heterospecifics if conspecifics are absent? (Q3) Do these tadpoles discriminate and prefer to group with kin rather than non-kin? (Q4) Do these tadpoles discriminate and prefer to group with familiar kin rather than unfamiliar kin?

Table 1. Testing conditions for grouping preferences in *Litoria aurea* tadpoles. Gosner stage and snout-urostyle length (SUL) are for testing individuals. Averages±standard errors, minimum and maximum values. N=sample size (per clutch in the clutch column), NA=non-applicable.

Question	Date	Clutch ID - testing individuals (N)	Clutch ID - non-kin conspecifics stimulus (N)	SUL (mm)	Gosner stage	Air temperature °C	Water temperature °C	N tested
Q1	27–29 July 2013	s2-2013 (10) s4-2013 (10)	s4-2013 (10) s2-2013 (10)	28.6±0.7 (11.6–22.4)	30–37	20.7±0.15 (19.8–21.5)	24.6±0.22 (23.3–26.1)	20
Q2	14–15 April 2014	Unknown, mixed	NA	13.2±0.3 (9.9–14.4)	25–29	22.0±0.53 (22.0–23.3)	23.3±0.13 (22–23.3)	15
Q3	29 November –20 December 2012; 13–16 December 2013	s3-2012 (26), k3-2012 (12), k1-2013 (14)	s1-2012 (12), k1-2012 (14), k2-2012 (12), s1-2013 (5), k5-2013 (5), k6-2013 (4)	9.6±0.6 (7.2–15)	25	26.3±0.14 (24.9–27.5)	25.1±0.15 (22.8–27.0)	52
Q4	3–18 December 2012	s3-2012 (24), k3-2012 (18), k1-2013 (15)	NA	8.9±0.3 (6.7–11.2)	25	25.5±0.10 (24.9–27.2)	24.5±0.30 (22–30.3)	55

Q1: Do green and golden bell frog tadpoles discriminate and prefer to group with conspecifics rather than non-conspecifics? Q2: Do these tadpoles prefer to aggregate with heterospecifics if conspecifics are absent? Q3: Do these tadpoles discriminate and prefer to group with kin rather than non-kin? Q4: Do these tadpoles discriminate and prefer to group with familiar kin rather than unfamiliar kin?
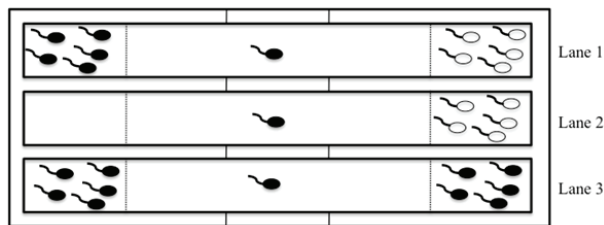
Fig. 1. Illustration of the arenas used in the experiments. The plastic core tray has three independent lanes separated from each other by solid opaque plastic - lanes and tray limits are represented by full thicker lines. The dashed lines represent fly screen mesh that isolated the end compartments where stimuli tadpoles were placed. The full vertical thinner lines on the top sides of the lanes represent lines drawn to visually demarcate the middle arena into three 20 cm sections, two adjacent to each end compartment and one mid-section. Black tadpoles are *Litoria aurea,* and white tadpoles are *Limnodynastes peronii.* Individuals inside the end compartments are stimuli and the ones in the centre are testing tadpoles. Lane 1 is representing Q1, in which tadpoles chose between conspecifics and heterospecifics, lane 2 is representing Q2, in which tadpoles chose between heterospecifics and an empty compartment, and lane 3 is representing Q3 or Q4 in which tadpoles chose between kin and non-kin unfamiliar conspecifics, or between familiar and unfamiliar kin.

MATERIALS AND METHODS

Captive *Litoria aurea* were bred in the Australian spring and summer of 2012 and 2013 at the University of Newcastle amphibian research facilities. Breeders (five pairs) were originally collected from Koorangang Island (32°51'47" S, 151°43'39" E) in 2010, and the eggs used in the experiments were from these animals (one clutch) or their offspring (six clutches; Table 1). Each clutch used in experiments belonged to different parents. For three of those clutches, eggs were collected within 24 hours of being laid and each clutch was split into two opaque plastic tubs (60x40x40 cm) filled with rainwater. This design created three groups of tadpoles (i) kin and familiar to each other; (ii) kin and unfamiliar to each other; and (iii) non-kin and unfamiliar to each other. *Litoria aurea* tadpoles from other clutches laid during the same period and not used as testing individuals were used as stimuli in the experiments (Table 1). Tadpoles of striped marsh frogs *Limnodynastes peronii* were collected from Koorangang Island (KI: 35°50'29" S 151°41'59" W) and the Watagans National Park (WNP: 35°01'25"S 151°25'53"W), NSW for inclusion as heterospecifics. Striped marsh frogs are sympatric with green and golden bell frogs, and tadpoles of both species co-occur in ponds in several areas where bell frog populations remain, including KI, but not WNP. This second location was sourced due to easier availability and catchability at the time of the experiment. All tadpoles were fed on a mixture of thawed lettuce, trout pellets (Ridley Aqua-feed, Ridley AgriProducts Pty Ltd, Narangba, Australia) and spirulina powder (Bioglam®, Australia) daily. On

experimental days tadpoles were fed only after all trials were complete.

Experiments were conducted in plastic trays (1 m length x 0.1m width) with fly screen mesh barriers fixed 20cm from both ends to form two end-compartments and a 60cm long middle arena. Trays were filled with rainwater to a depth of 5cm and their tops were marked with black lines to visually demarcate the middle arena into three 20 cm sections (two adjacent to each end compartment and one mid-section; Fig. 1). Water and air temperatures were recorded at the beginning of each trial using a digital thermometer with external probe.

In three of the experiments the testing individuals had choice between two groups of stimulus tadpoles, placed in each end compartment. Each experiment was differentiated by the group condition that stimulus tadpoles were raised: in experiments of conspecific preference (Q1), stimuli tadpoles were *L. aurea* (non-kin and unfamiliar to the testing individual) vs. *Li. peronii* from KI*;* in experiments for kinship preference (Q3) stimuli groups were *L. aurea* tadpoles that were unfamiliar kin vs. unfamiliar non-kin to the testing tadpole; and in experiments for familiarity preference (Q4) stimuli were familiar kin vs. unfamiliar kin *L. aurea* tadpoles. The fourth experiment to test preference for heterospecific preference over being asocial (Q2) had only five heterospecifics *Li. peronii* from WNP in one of the compartments.

Tadpoles used in all experiments varied in size from 7 to 22mm in snout-urostyle length (SUL), and Gosner stage 25 to 33 (Gosner, 1960; Table 1). However, stimuli and tested individuals were similar in size and Gosner stage within each replica to ensure any preference by testing individual was not based on body size and developmental stage of the stimuli. In all experiments stimuli tadpoles were left to acclimatise for five minutes in the experimental arena before a testing *L. aurea* was released in the centre of the tray and filmed for 45 minutes using a CCTV system. Each tadpole was tested only once, but tested individuals also were used as stimuli. Stimulus tadpoles may have been used multiple times, however not in consecutive trials. The end of the tray that each stimulus group was presented was alternated. After each trial all tadpoles were removed from the tray, water was discharged, tray rinsed, and refilled with clean water.

From the video recordings of the experiments we scored the time tadpoles spent in each section of the tray, and calculated the proportion of time they spent in each end section (i.e. adjacent to each group of stimulus) from the 45 min total time. Individuals that did not move from the centre of the arena, or did not visit both sides of the arena were excluded from the analyses (two in Q2, four in Q3, and nine in Q4). Experiments were run in batches from 0900 to 1700 hours, from November 2012 to May 2014 (Table 1), according to availability and sizes of tadpoles. The proportion of time tadpoles spent near conspecifics when the alternative choice was heterospecific tadpoles in Q1; heterospecifics when the alternative choice was the empty compartment in Q2; kin when the alternative choice was non-kin in Q3; and familiar when the alternative choice was unfamiliar

Table 2. Main effects of generalised linear models on grouping preference parameters in *Litoria aurea* tadpoles. Snout-urostyle length (SUL) Gosner stage and clutch ID are for testing individuals. Clutch IDs of stimulus tadpoles were not included in any analyses due to extreme low variation. NA=non-applicable (low/no variation, or unknown ID).

Question	SUL			Gosner stage			Clutch ID			Water temperature		
	X^2	df	*p*-value	X^2	df	*p*-value	X^2	df	*p*-value	X^2	df	*p*-value
Q1	0.27	1	0.604	3.97	7	0.784	0.02	1	0.894	1.33	1	0.248
Q2	1.59	1	0.207	3.88	3	0.275	NA	NA	NA	0.07	1	0.796
Q3	0.30	1	0.586	NA	NA	NA	0.54	2	0.765	0.22	1	0.636
Q4	0.02	1	0.896	NA	NA	NA	0.55	2	0.758	0.50	1	0.481

conspecifics in Q4, was compared to the expected mean proportion of time of 0.5 (two choices without expected preference) using a non-parametric Wilcoxon test. Because this type of test cannot incorporate multiple factors into the analyses, we also ran GLMs with log-link function to investigate the effects of clutch origin, SUL and Gosner stage of tested tadpoles, and water temperature, on the proportion of time tadpoles spent near conspecifics in Q1; heterospecifics in Q2; kin in Q3; and familiar in Q4. We compared the strength of preference for conspecifics in Q1, heterospecifics in Q2, kin in Q3, and familiar in Q4 using a Wilcoxon test on the proportion of time tadpoles spent near those groups. Analyses were performed in the software JMP 11; statistical significance was set at <0.05 for one-tail mean tests (expected mean < observed mean).

All procedures used in this work meet the requirements of the NSW Animal Research Act and Regulation, the Australian code of practice for the care and use of animals for scientific purposes; were approved by the University of Newcastle Animal Care and Ethics Committee protocol A-2012-237, and licensed by the NSW National Parks & Wildlife Service (permits SL101097 and SL100190).
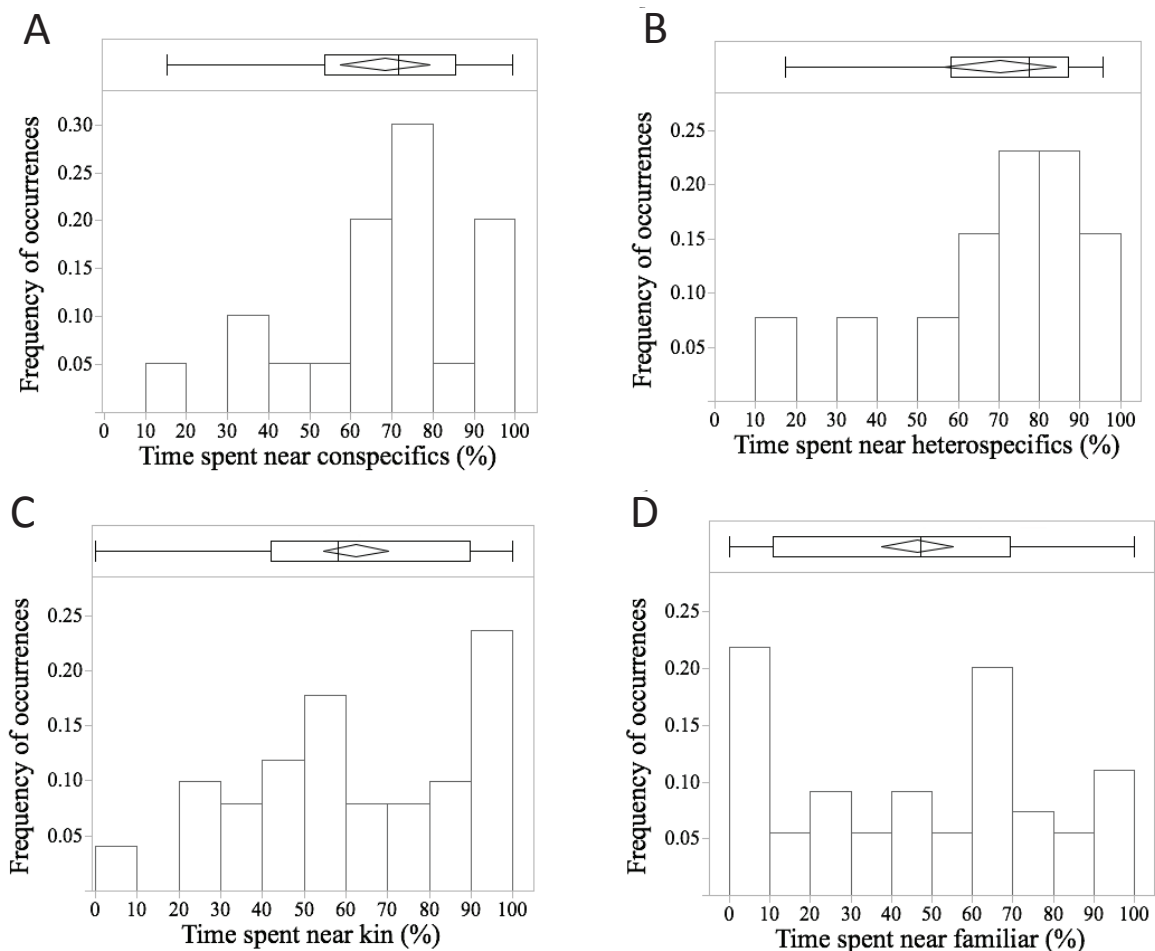

Fig. 2. Histogram of the proportion of time *Litoria aurea* tadpoles spent near (A) conspecifics when alternative was a heterospecific group (*Limnodynastes peronii*), (B) heterospecifics (*Limnodynastes peronii*) when alternative was an empty compartment, (C) unfamiliar kin when alternative was unfamiliar non-kin, and (D) familiar kin when alternative was unfamiliar kin. The vertical bar on the top of the figure represents the median, the right side of the box is the first quartile, the left side of the box is the third quartile, whiskers represent maximum and minimum values, the inner diamond represents the upper and lower 95% confidence intervals of the mean.

RESULTS

Tadpole SUL, Gosner stage, clutch ID and water temperature during the trials had no effect on the proportion of time tadpoles spent near conspecifics in Q1, heterospecifics in Q2, kin in Q3 or familiar tadpoles in Q4 (Table 2).

Litoria aurea tadpoles spent a median of 71% of the trial time (upper quartile=85%, lower quartile=54%) adjacent to their conspecifics, when they had the choice between conspecifics and *Li. peroni*. This proportion is significantly higher than the 50% expected by chance (W=78.00, *p*<0.001, Fig. 2A). In this trial, 75% of all tested tadpoles spent over 60% of the time adjacent to the conspecifics (Fig. 2A). In the absence of conspecifics, tadpoles still exhibited gregariousness preference, spending significantly more time than expected adjacent to heterospecifics when the other choice was an empty compartment (W=34.50, *p*=0.007, Fig. 2B). Those tadpoles spent a median of 77% of the time adjacent to the heterospecifics (lower quartile=58%, upper quartile=87%), and 67% of all individuals spent over 60% of the time near the heterospecifics in this trial (Fig. 2B).

When tadpoles had the choice between groups of unfamiliar kin and non-kin conspecifics, 48% of the individuals spent over 60% of the time adjacent to kin (Fig. 2C). Tadpoles spent significant more time next to their kin than non-kin (W=299.00, *p*=0.0005), with a median time of 58% (lower quartile=42%, upper quartile=90%, Fig. 2C). Only 42% of tested tadpoles spent more than 60% of the time near the familiar group of kin (Fig 2D). In general, tadpoles did not spend more time than expected (median=47%, lower quartile=11%, upper quartile=69%) near a familiar group of kin when the other option was unfamiliar kin (W=-96.00, *p*=0.835, Fig. 2D).

There was an apparent stronger preference for conspecifics over heterospecifics, and heterospecifics over isolation, when compared with the preference for kin over non-kin (Fig. 2). Although, those differences were not significant; the only significant difference was the lower time tadpoles spent near the familiar group, when compared to the time spent near conspecifics, heterospecifics or kin (X^2=12.1, df=3, *p*=0.0069, Fig. 3).

DISCUSSION

Our experiments show the strong preference of *Litoria aurea* tadpoles for forming social aggregations, as habitat types and temperature were homogenous in the arena as a whole. Several of the advantages of gregarious behaviour that are applicable to tadpoles, such as increased defence against predators (Spieler & Linsenmair, 1999; Spieler, 2002), habitat selection (Pfennig, 1990), foraging efficiency (Steinwascher, 1978b; Bazazi et al., 2012), and thermoregulatory efficiency (Espinoza & Quinteros, 2008) can occur in both single and multi-species groups. However, *L. aurea* tadpoles discern and prefer to aggregate with conspecifics than heterospecifics. Conspecifics are phenotypically more similar to each other than heterospecifics, probably
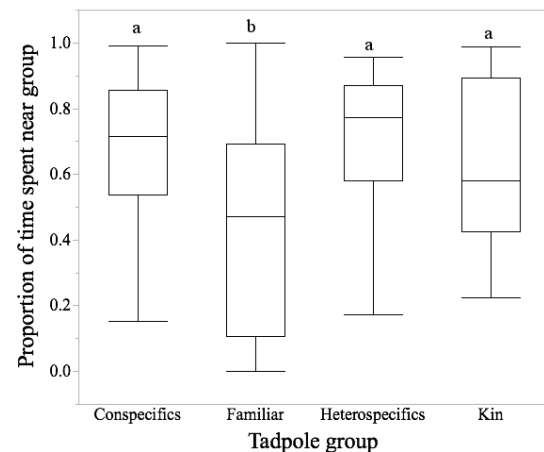

Fig. 3. Box-plot of the proportion of time *Litoria aurea* tadpoles spent adjacent to conspecifics, heterospecifics (*Limnodynastes peronii)*, unfamiliar kin, and familiar kin tadpoles, when alternative choices were, respectively, heterospefics (*Limnodynastes peronii*), isolation (empty compartment), unfamiliar non-kin, and unfamiliar kin. Vertical lines inside the rectangles represent medians, bottom and top of the rectangles represent first and third quartiles, and whiskers are the minimum and maximum values. Letters above the whiskers represent statistical differences among the groups.

minimising the oddity effect and decreasing individual predation risk within a group (Krause & Ruxton, 2002). Conspecific aggregation can also be beneficial in terms of habitat selection and foraging performance, as individuals of the same species are often a better match in terms of habitat and food requirements than heterospecifics (Krause & Ruxton, 2002). Two life-history traits of *Litoria aurea* tadpoles may favour formation of conspecific aggregations over heterospecific groupings: first, tadpoles are diet generalists (Bower et al., 2014) and thus food limitation leading to strong intraspecific competition may be low in most cases (except in situations where ponds are drying out). Second, tadpoles are apparently palatable and predated upon by a wide range of animals, including visually oriented wading birds (Pyke & White, 2001), in which case oddity effect on predation may be strong (Mathis & Chivers, 2003; Croft et al., 2009). Low intraspecific competition, especially in terms of interference when food availability is not too low, has been shown in other tadpoles that form conspecific aggregations (Laufer & Maneyro, 2008).

Litoria aurea tadpoles also prefer kin to non-kin, but had no preference between familiar and unfamiliar kin. Anurans were among the first vertebrates shown to recognise kin (Waldman & Adler, 1979). While kinship recognition has been challenged in some cases and kin aggregations were shown to be a result of familiarity (Waldman, 1985; Griffiths & Magurran, 1999), other studies show some fish and tadpoles are indeed able to recognise kin (Blaustein & Waldman, 1992; Hain & Neff, 2007, present work), and groupings based on kinship discrimination has been reported under natural

conditions (Halverson et al., 2006). In many cases, both innate recognition and learning are suggested to be involved in kin discrimination, and kinship and familiarity recognition are now well known in several species of schooling fishes and some amphibians (Waldman 1986; Blaustein & Waldman, 1992; Olsén, 1999). As in other vertebrates, the major histocompatibility complex (MHC) has been shown to significantly influence on the odours used for kin discrimination in tadpoles (Villinger & Waldman, 2008; Villinger & Waldman, 2012).

Kin preference may be advantageous in several ways. For example, tadpoles are shown to have higher growth rates in groups of sibling than in mixed-clutch groups (Blaustein & Waldman, 1992; Hokit & Blaustein, 1994; Jasieński, 1988). In *Leptodactylus ocellatus,* a schooling species, interference competition within tadpoles was not detected in experimental tests (Laufer & Maneyro, 2008). Despite the effect of kinship not being directly tested in that experiment, all tadpoles belonged to the same clutch. Given that intra-specific competition, by both exploitation and interference, can lead to reduced growth in tadpoles (Wilbur, 1976; Alford, 1999), the absence of interference competition among kin could lead to the increased growth often recorded in kin aggregations (Laufer & Maneyro, 2008).

In tadpoles and salamanders, individuals that discriminate kin may also be able to direct intra-specific competition (e.g., via cannibalism) towards non-kin (Pfennig & Collins, 1993; Pfennig et al., 1994). Indeed, kinship discrimination is more likely to occur in cannibalistic tadpole species than non-cannibalistic ones (Pfennig, 1999), and cannibalism is quite common among *L. aurea* tadpoles (L.P. pers. obs.). *Litoria aurea* lays a large clutch of aggregated eggs and tadpoles apparently remain together after hatching, at least in captivity (L.P. pers. obs.); thus, individuals in schools should be kin. If food is relatively abundant, cannibalism towards school members would be disadvantageous, despite the nutritional value of the prey (Pfennig, 1997). In this scenario, testing cannibalism rates in kin groups vs. non-kin groups raised at the same per capita food ratios may enlighten on the benefits of kin discrimination in *L. aurea* tadpoles.

Kinship grouping can also be advantageous in terms of resistance to diseases and pathogens, which may also affect growth. *Xenopus laevis* tadpoles exposed to water conditioned by immunologically (MHC genes) dissimilar conspecifics present higher mortality and fast development without proportional increase in growth, when compared to tadpoles exposed to MHC-similar conspecifics (Barribeau et al., 2012). Because MHC determines immunological repertoires and social preferences, the authors suggest that by associating with genetically similar individuals tadpoles would be more adapted to the microbial assemblage they share, and better pathogen resistance may be an important factor mediating kinship preference (Barribeau et al., 2012). Such an elegant hypothesis deserves attention and further testing.

In general terms, the strong aggregative nature of *L. aurea* tadpoles can also have costs that influence the species' conservation. Grouping often affects disease dynamics, increasing transmission, incidence, and/or prevalence (Patterson & Ruckstuhl, 2013). As for many other amphibian species, chytridiomycosis is a current major threat for the already disjointed populations of *L. aurea* and its control is a major conservation challenge (Stockwell et al., 2008; Mahony et al., 2013). Chytrid fungus attacks the mouthparts of tadpoles (Berger et al., 1998; Marantelli et al., 2004) and while usually not fatal, sub-lethal effects such as slower development, smaller size at metamorphose (which may result in death post-metamorphose), decrease in activity and changes in anti-predator behaviour are reported as a result of the infections (reviewed by Kilpatrick et al., 2010). In spite of physical contact not being required for pathogen acquisition, as zoospores disperse in the water (Carey et al., 2006), grouping is likely to increase zoospore density and consequently infection transmission. Larva of other species, such as *Rana catesbeiana,* avoid conspecifics infected with *Candida humicola,* likely reducing disease transmission (Kiesecker et al., 1999); however, it is currently unknown if *L. aurea* tadpoles (and frogs) can recognise and avoid diseased individuals. In our experiments, despite the preference for conspecifics, *L. aurea* tadpoles did not avoid proximity to heterospecifics, and indeed preferred to be close to *Li. peronii* than asocial. This result reinforces the strong social behaviour of this species and suggests that benefits of gregariousness overrides the costs. However, it does not necessarily mean that tadpoles will form tight heterospecific aggregations in the field. Heterospecific schools have not been recorded to date and may be prevented if sympatric heterospecifics are not strongly gregarious, which may be the case of *Li. peronii.* For disease dynamics, however, even if tadpoles avoid diseased conspecifics and do not school with heterospecifics, co-occurrence and proximity with other carrier species such as *Li. peronii* and *Litoria fallax,* which to not show signs of chytridiomicosis, could still contribute to increasing transmission in natural situations.

More specifically, for breed and release programs of *L. aurea*, raising kin individuals together may improve success, at least in captivity. Further studies can clarify if kin aggregations are advantageous in most field conditions, in which case releasing individuals as clutches would be also advised.

ACKNOWLEDGEMENTS

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