CSIRO PUBLISHING

Wildlife Research, 2012, **39**, 350–354
http://dx.doi.org/10.1071/WR11193

Estimates of sex ratio require the incorporation of unequal catchability between sexes

Evan J. Pickett[A,B], *Michelle P. Stockwell*[A], *Carla J. Pollard*[A], *James I. Garnham*[A], *John Clulow*[A] *and Michael J. Mahony*[A]

[A]School of Environmental and Life Sciences, University of Newcastle, University Drive, Callaghan, NSW 2300, Australia.

[B]Corresponding author. Email: evan.pickett@uon.edu.au

Abstract

Context. Estimates of the sex ratio of a population are a common summary statistic used for ecological studies and conservation planning. However, methods to determine the sex ratio often ignore capture probability, which can lead to a perceived bias in the sex ratio when the sexes are detected at different rates.

Aims. To illustrate the bias from conventional count-based analysis methods for determining sex ratio by comparison with analytical methods that include capture probability.

Methods. Closed-population mark–recapture analysis was used to determine the population size of each sex within a population of green and golden bell frogs (*Litoria aurea*). This was then compared with the traditional count-based methods of estimating sex ratio to determine the effect of incorporating capture probability on the sex ratio estimate.

Key results. More males than females were detected during surveys, producing a male-biased sex ratio when there was no incorporation of capture probability. Mark–recapture results indicated a similar population size between the two sexes, suggesting that the sex ratio is closer to even.

Conclusions. Methods to estimate sex ratio that incorporate capture probability can significantly reduce the bias obtained from count data.

Implications. We suggest that population studies must incorporate capture probability to determine the sex ratio of a population.

Additional keywords: Anuran, conservation, frog, *Litoria aurea*, mark–recapture, population ecology.

Received 24 November 2011, accepted 21 March 2012, published online 26 April 2012

Introduction

The sex ratio is a commonly used summary statistic in ecology because it immediately identifies heterogeneity of processes within a system (Bolen and Robinson 1995). In diocecious species, the sex ratio changes over time through the following two major processes: discordant survival rates between the sexes and hermaphroditism. Because hermaphroditism is rare among tetrapod species, adult populations that exhibit a skewed sex ratio often indicate discordant survival between the sexes during at least one life-history stage (Hamilton 1967). Heterogeneity in survival can be caused by either natural ecological processes or human-induced threatening processes, and can therefore provide vital information in ecological studies and early conservation assessment of species or populations (Hailey and Willemsen 2000; Solberg *et al*. 2002).

Despite the interpretative capacity of sex ratios, many studies use count data to determine the sex ratio of a population, which does not account for variation in detection probability between sexes. This is evident in many taxa, including insects (Davis and Rendón-Salinas 2010; Wehi *et al*. 2011), birds (Ewen *et al*. 2001; Jaatinen *et al*. 2010), reptiles (Hailey and Willemsen 2000) and amphibians (Berven 1990; Lemckert 2005), and risks false interpretation of population dynamics.

Differences in behaviour, visibility and catchability can lead to a bias in the perceived sex ratio of a species or population. This is particularly evident in anurans, because males are often conspicuous and aggregated around breeding habitat, whereas females avoid breeding habitat until they mate (Lodé *et al*. 2005). Therefore, different survey techniques have led to different perceived biases, such as active searches resulting in male bias (Barbieri and Bernini 2004; Goldingay and Newell 2005) and trapping has resulted in both male and female bias, depending on the location of traps (Berven 1990; Lemckert 2005; Alho *et al*. 2008).

The application of recently available software allows conservation biologists to validate assumptions of important summary statistics and correct for false bias. Absolute estimates of the population size, which incorporate the variation in catchability between sexes, provide a method to accurately compare population sizes of each sex. Mark–

recapture models estimate the probability of capture and its associated variability, which can be incorporated into sex-ratio estimation. However, few studies have used mark–recapture software to determine the sex ratio of a population (for examples, see Kéry and Juillerat 2004; Alho *et al*. 2008).

The aim of the current study is to illustrate the bias from conventional count-based analysis methods for determining sex ratio by incorporating different capture probabilities between sexes by using closed-population mark–recapture modelling.

Materials and methods

Study species and site

The green and golden bell frog (*Litoria aurea*) is a threatened species that inhabits ponds and wetlands of eastern Australia. It is sexually dimorphic by size and conspicuous during the breeding season, when it is most frequently surveyed. Previous population studies of *L. aurea* have involved visual searches for individuals, which has resulted in a male-biased sex ratio (Pyke and White 2001; Goldingay and Newell 2005; Hamer and Mahony 2007), despite an even sex ratio at birth (Greer and Byrne 1995). It has been hypothesised that the male bias is due to the behaviour of males to form chorus aggregations within ponds, whereas females are more secretive and are more likely to be widely dispersed away from ponds (Goldingay 2008).

The study site was located within known *L. aurea* habitat in the Brickpit, at Sydney Olympic Park, Australia. The Brickpit is a 16-ha disused quarry that contains 46 freshwater ponds, varying in size from large wetlands to small ephemeral ponds. The current study included 12 of these waterbodies, and the surrounding terrestrial habitat in which the density of adult *L. aurea* was highest.

Field techniques

Mark–recapture was conducted over 18 nights in January of 2009 and 2010, with each year consisting of three surveys of three nights.

Individuals were located by nocturnal surveys involving visual searches of vegetation, aquatic and terrestrial habitat, and then captured using a disposable plastic bag to prevent disease transmission among individuals. Individuals greater than 45 mm in length were marked by subcutaneous injection with a passive integrated transponder (PIT) tag according to the methods of Christy (1996). Males develop nuptial pads ~45 mm in length, so the presence of nuptial pads indicated a male, whereas absence of this feature indicated a female (Christy 2000). Frogs were then released at the site of capture.

Analysis

Count data

Two analyses were conducted for determining the sex ratio using count data; these were counts with replacement and counts without replacement. Surveys with replacement involved counting the number of males (m) and females (f) that were captured within each survey. Similarly, surveys without replacement did not count animals that were recaptured, and had previously been marked (Skalski *et al*. 2005).

The sex ratio $\left(R\frac{M}{F}\right)$ was determined for both these techniques using the equation of Skalski *et al*. (2005), as follows:

$$R\frac{M}{F} = \frac{m}{f},$$

where m is the count of males and f the count of females for each survey. Variance and standard error were determined using the methods in Skalski *et al*. (2005), as follows:

$$Var\left(R\frac{M}{F}\right) = \frac{R\frac{M}{F}\left(1 + R\frac{M}{F}\right)^2}{n}$$

Mark–recapture

To determine the population size of the two sexes, each year was analysed independently, using the same set of *a priori* candidate models in a closed-capture analysis with program MARK Version 6.1 (White and Burnham 1999). This method of analysis assumes that the population is closed to the effects of mortality, migration and recruitment during the survey period; therefore, the interval between surveys was kept short (0–4 days).

An encounter history for each individual was created on the basis of the three survey periods for each year, where '1' represents a capture and '0' represents no capture (e.g. '101' represents an individual that was captured on the first and last survey period). The sex of each individual was entered as a group covariate.

Capture probability is subject to three major mechanisms which can have an effect on the eventual outcome of the model. Time variation (t) occurs when the capture probability is different for each survey period. Behavioural response (b) is the response of an individual to being captured influencing the probability of it being recaptured; for example, trap shyness commonly occurs in small mammal trapping surveys. Finally, individual heterogeneity (h) is the event where each individual has a different capture probability. The eight models of Otis *et al.* (1978) which account for these effects and their interactions on capture probability were run to determine the level of impact.

Two models were compared for each year, which represented either equal or unequal sex ratios. This was achieved by maintaining constant population size between sexes, N(.), and varied population size between sexes, N(g). The effect of sex on capture and recapture probability was also tested.

Model selection was based on Akaike's information criterion with correction for small samples (AICc), produced by program MARK. The most parsimonious model was determined as the model with the smallest AICc value. Program MARK produced negative AICc values, because a negative constant is removed from calculation to hasten processing time. The most parsimonious model with negative values was therefore interpreted as the value furthest from zero. The ΔAIC value is the difference between a model and the most parsimonious model, and ΔAIC values of less than two could not be considered different enough to reject (Burnham and Anderson 2002). To remedy this, each model was weighted according to the ΔAIC value, and parameter outputs were averaged according to the methods of Burnham and Anderson (2002). Models that failed to converge were removed from the candidate model set to prevent influence on the model averaging results.

Results

Count data

In both years, there were a greater number of males captured than there were females (Table 1). The male bias decreased when recaptured animals were omitted from the analysis (Fig. 1).

Mark–recapture

The most parsimonious model for 2009 included time variation in capture probability, with equal population sizes for both sexes. However, models that included different population sizes for each sex and variation in capture probability owing to sex, could not be distinguished from the most parsimonious model (Table 2). The most parsimonious model for 2010 included variation in capture probability by sex, with equal population sizes for both the sexes. The heterogeneous mixed-effect model indicating heterogeneity in capture probability and a sex varying capture probability model with different population sizes for each sex could not be distinguished from the most parsimonious model (Table 3).

Model-averaged estimates for male and female population size indicated a slightly male-biased sex ratio. The difference between the population sizes, however, were not significant, thus indicating less male-bias compared with the count-data analysis.

Discussion

Incorporation of unequal catchability using mark–recapture methods had an appreciable effect on the sex-ratio estimates for the Brickpit population of *L. aurea*. The field techniques to collect count data resulted in the over-representation of males, which has been observed in other population surveys for this species.

Development of methods for estimating population parameters should aim at minimising the number of assumptions of biological consequence (Soetaert and Herman 2009). In this case, removal of the assumption of equal catchability had a significant effect on the perceived sex ratio, transforming a male-biased sex ratio to a sex ratio which was not significantly different from 1 : 1.

The implication of such inaccuracies can be seen in the application of sex ratios. Ecological and conservation projects are increasingly utilising sex ratios to detect impacts and as a parameter of population models to project fluctuation and viability (Lacy 1993; Jenouvrier *et al*. 2010), particularly for threatened species (e.g. Haig *et al.* 1993). Sex ratio can be an indication of threatening factors that have a greater impact on a particular sex, such as disease (Davis and Rendón-Salinas 2010)

Table 1. Summary of the number of captured *Litoria aurea* individuals for each survey

The number of newly captured animals during each survey period is shown in parentheses

Parameter	Survey 1	Survey 2	Survey 3	Total (captured)	Total (individuals)
2009					
Males captured	49 (49)	48 (21)	34 (11)	131	81
	25 (25)	30 (15)	22 (13)	77	53
2010					
Males captured	44 (44)	47 (19)	39 (8)	130	71
Females captured	24 (24)	28(15)	27 (8)	79	47

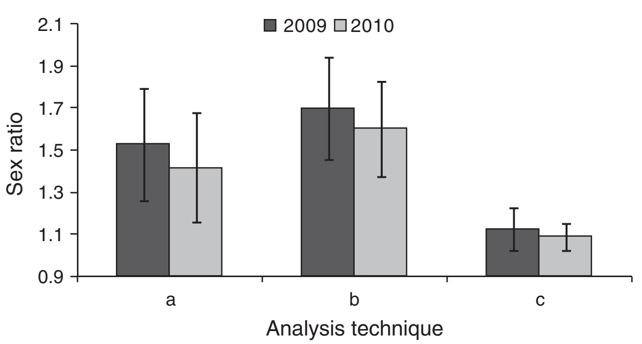

Fig. 1. Estimates of sex ratios (male : female) based on count data (*a*) without replacement and (*b*) with replacement and (*c*) using mark–recapture analysis to determine absolute population size for each sex. Error bars indicate standard error.

Table 2. Summary of model selection results used to predict population size of *Litoria aurea* in the Brickpit, Sydney Olympic Park, for the 2009 breeding season

Capture probability (p), recapture probability (c) and population size (N) included the effects of time (t), sex, their interaction (sex × t) and a mixed-effect parameter (pi) accounting for individual heterogeneity in capture probability

Model	Deviance	K	AIC_c	ΔAIC_c	w_i
p(t) = c(t) N(.)	25.95	4	–351.65	0.00	0.33
p(t) = c(t) N(sex)	24.83	5	–350.72	0.93	0.21
p(sex) = c(sex) N(.)	29.84	3	–349.80	1.85	0.13
p(sex) = c(sex) N(sex)	29.75	4	–347.85	3.80	0.05
p(sex × t) = c(sex × t) N(sex)	21.78	8	–347.56	4.09	0.04
p(pi) pi(sex) N(.)	28.00	5	–347.55	4.10	0.04
p(sex) c(sex) N(.)	28.59	5	–346.96	4.69	0.03
p(.) c(.) N(sex)	30.92	4	–346.68	4.97	0.03
p(sex) c(sex) N(sex)	27.34	6	–346.14	5.50	0.02
p(pi) pi(.) N(.)	31.63	4	–345.97	5.68	0.02
p(.) c(sex) N(sex)	30.47	5	–345.09	6.56	0.01

Table 3. Summary of model selection results used to predict population size of *Litoria aurea* in the Brickpit, Sydney Olympic Park, for the 2009 breeding season

Capture probability (p), recapture probability (c) and population size (N) included the effects of time (t), sex, their interaction (sex × t) and a mixed effect parameter (pi) accounting for individual heterogeneity in capture probability. K is number of parameters and w_i is model weight

Model	Deviance	K	AIC_c	ΔAIC_c	w_i
p(sex) = c(sex) N(.)	19.39	3	–341.56	0.00	0.23
p(pi) pi(sex) N(.)	16.04	5	–340.81	0.74	0.16
p(sex) = c(sex) N(sex)	19.31	4	–339.60	1.96	0.09
p(t) = c(t) N(.)	19.70	4	–339.21	2.35	0.07
p(sex) c(sex) N(.)	18.71	5	–338.15	3.41	0.04
p(.) c(.) N(sex)	20.81	4	–338.10	3.46	0.04
p(t) = c(t) N(sex)	19.21	5	–337.64	3.91	0.03
p(sex) c(sex) N(sex)	18.56	6	–336.23	5.32	0.02
p(g × t) = c(g × t) N(sex)	16.71	8	–333.93	7.62	0.01

and predation (Wehi *et al*. 2011). In addition, population models require an estimate of the breeding population, which is the number of reproductively active individuals that are available to contribute towards recruitment. This is primarily based on the following three parameters: overall population size, mating system of the species and sex ratio (Lacy 1993). A sex ratio away from 1 : 1 reduces the breeding population size of monogamous species, because the excess individuals of the more abundant sex cannot breed. Alternately, polygynous species can have a female-biased sex ratio, because a single male can breed multiple times (Leopold 1933; Bolen and Robinson 1995).

The male-biased sex ratio recorded in previous studies of *L. aurea* would indicate lower survival in females, which are generally more important for long-term sustainability of polygynous species (Bolen and Robinson 1995). The implication of this discrepancy for the conservation of the species is therefore important if a biased estimate were used to determine management actions for the protection of the species.

The problem of unequal catchability is ubiquitous in animal surveys and population ecology. Sex is one of the most important factors for unequal catchability, and should not be ignored. The present study has illustrated this point, and we recommend that estimates of sex ratio avoid the use of count data where capture probabilities of each sex cannot be determined separately.

Acknowledgements

We thank the many volunteers who helped with fieldwork, particularly Daniel O'Brien and Rodney Wattus, who assisted on multiple occasions. We thank Katie Oxenham, Jenny O'Meara and Kerry Darcovich of the Sydney Olympic Park Authority for operational and logistic assistance and Deborah Bower for revision of the manuscript. This study was funded as part of the ARC linkage project (LP0989459). We were supported by the following industry partners: Sydney Olympic Park Authority, NSW Roads and Traffic Authority, Strathfield Council, the Office of Environment and Heritage and the South Australia Museum. The project was conducted under NPWS Scientific Licence (SL100092) and approved by the University of Newcastle Animal Care and Ethics Committee (ACEC number A-2008-165).

References

Alho, J. S., Herczeg, G., and Merilä, J. (2008). Female-biased sex ratios in subarctic common frogs. *Journal of Zoology* **275**, 57–63. doi:10.1111/j.1469-7998.2007.00409.x

Barbieri, F., and Bernini, F. (2004). Distribution and status of *Rana latastei* in Italiy (Amphibia, Ranidae). *The Italian Journal of Zoology* **71** (Suppl 1.), 91–94.

Berven, K. A. (1990). Factors affecting population fluctuations in larval and adult stages of the wood frog (*Rana sylvatica*). *Ecology* **71**, 1599–1608. doi:10.2307/1938295

Bolen, W. L., and Robinson, E. G. (1995). 'Wildlife Ecology and Management.' (Prentice Hall: Englewood Cliffs, NJ.)

Burnham, K. P., and Anderson, D. R. (2002). 'Model Selection and Multimodel Inference: a Practical Information-Theoretic Approach.' (Springer-Verlag: Heidelberg, Germany.)

Christy, M. T. (1996). The efficacy of using passive integrated transponder (PIT) tags without anaesthetic in free-living frogs. *Australian Zoologist* **30**, 139–142.

Christy, M. T. (2000). The ecology and conservation of the green and golden bell frog *Litoria aurea* (Lesson) (Anura: Hylidae). Ph.D. thesis, University of Sydney, Sydney.

Davis, A. K., and Rendón-Salinas, E. (2010). Are female monarch butterflies declining in eastern North America? Evidence of a 30-year change in sex ratios at Mexican overwintering sites. *Biology Letters* **6**(1), 45–47. doi:10.1098/rsbl.2009.0632

Ewen, J. G., Clarke, R. H., Moysey, E., Boulton, R. L., Crozier, R. H., and Clarke, M. F. (2001). Primary sex ratio bias in an endangered cooperatively breeding bird, the black-eared miner, and its implications for conservation. *Biological Conservation* **101**, 137–145. doi:10.1016/S0006-3207(01)00022-2

Goldingay, R. L. (2008). Conservation of the endangered green and golden bell frog: what contribution has ecological research made since 1996? *Australian Zoologist* **34**, 335–349.

Goldingay, R. L., and Newell, D. A. (2005). Aspects of the population ecology of the green and golden bell frog *Litoria aurea* at the northern end of its range. *Australian Zoologist* **33**, 49–59.

Greer, A. E., and Byrne, M. (1995). Sex ratio and frequency of the oestological abnormalities in the Australian hylid frog *Litoria aurea* from two apparently unpolluted localities in Sydney, New South Wales. *Australian Zoologist* **30**, 43–47.

Haig, S. M., Belthoff, J. R., and Allen, D. H. (1993). Population viability analysis for a small population of red-cocked woodpeckers and an evaluation of enhancement strategies. *Conservation Biology* **7**, 289–301. doi:10.1046/j.1523-1739.1993.07020289.x

Hailey, A., and Willemsen, R. E. (2000). Population density and adult sex ratio of the tortoise *Testudo nermanni* in Greece: evidence for intrinsic population regulation. *Journal of Zoology* **251**, 325–338. doi:10.1111/j.1469-7998.2000.tb01083.x

Hamer, A. J., and Mahony, M. J. (2007). Life history of an endangered amphibian challenges the declining species paradigm. *Australian Journal of Zoology* **55**, 79–88. doi:10.1071/ZO06093

Hamilton, W. D. (1967). Extraordinary sex ratios. *Science* **156**, 477–488. doi:10.1126/science.156.3774.477

Jaatinen, K., Lehikoinen, A., and Lank, D. B. (2010). Female-biased sex ratios and the proportion of cryptic male morphs of migrant juvenile ruffs (*Philomachus pugnax*) in Finland. *Ornis Fennica* **87**, 1–10.

Jenouvrier, S., Caswell, H., Barbraud, C., and Weimerskirch, H. (2010). Mating behaviour, population growth, and the operational sex ratio: a periodic two-sex model approach. *American Naturalist* **175**, 739–752. doi:10.1086/652436

Kéry, M., and Juillerat, L. (2004). Sex ratio estimation and survival analysis for *Orthetrum coerulescens* (Odonata, Libellulidae). *Canadian Journal of Zoology* **82**, 399–406. doi:10.1139/z04-004

Lacy, R. C. (1993). VORTEX: a computer simulation model for population viability analysis. *Wildlife Research* **20**, 45–65. doi:10.1071/WR9930045

Lemckert, F. L. (2005). Population structure, individual growth and survival of an Australian frog *Crinia signifera* at a pond. *Acta Zoologica Sinica* **51**, 393–400.

Leopold, A. (1933). 'Game Management.' (Scribner: New York.)

Lodé, T., Holveck, M.-J., and Lesbarréres, D. (2005). Asynchronous arrival pattern, operational sex ratio and occurrence of multiple paternities in a territorial breeding anuran, *Rana dalmatina. Biological Journal of the Linnean Society. Linnean Society of London* **86**, 191–200. doi:10.1111/j.1095-8312.2005.00521.x

Otis, D. L., Burnham, K. P., White, G. C., and Anderson, D. R. (1978). Statistical inference from capture data on closed animal populations. *Wildlife Monographs* **62**, 1–135.

Pyke, G. H., and White, A. W. (2001). A review of the biology of the green and golden bell frog *Litoria aurea. Australian Zoologist* **31**, 563–598.

Skalski, J. R., Ryding, K. E., and Millspaugh, J. J. (2005). Estimating population sex ratios. In 'Wildlife Demography: analysis of Sex, Age, and Count Data'. pp. 49–87. (Elsevier: Sydney.)

Soetaert, K., and Herman, P. M. J. (2009) 'A Practical Guide to Ecological Modelling Using R as a Simulation Platform.' (Springer: Verlag, NY)

Solberg, E. J., Loison, A., Ringsby, T. H., Sæther, B.-E., and Heim, M. (2002). Biased adult sex ratio can affect fecundity in primiparous moose *Alces alces*. *Wildlife Biology* **8**, 117–128.

Wehi, P. M., Nakagawa, S., Trewick, S. A., and Morgan-Richards, M. (2011). Does predation result in adult sex ratio skew in a sexually dimorphic insect genus? *Journal of Evolutionary Biology* **24**, 2321–2328. doi:10.1111/j.1420-9101.2011.02366.x

White, G. C., and Burnham, K. P. (1999). Program MARK: survival estimation from populations of marked animals. *Bird Study* **46**, S120–S139. doi:10.1080/00063659909477239