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Reproducing lizards modify sex allocation in response to operational sex ratios

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Sex-allocation theory suggests that selection may favour maternal skewing of offspring sex ratios if the fitness return from producing a son differs from that for producing a daughter. The operational sex ratio (OSR) may provide information about this potential fitness differential. Previous studies have reached conflicting conclusions about whether or not OSR influences sex allocation in viviparous lizards. Our experimental trials with oviparous lizards (*Amphibolurus muricatus*) showed that OSR influenced offspring sex ratios, but in a direction opposite to that predicted by theory: females kept in male-biased enclosures overproduced sons rather than daughters (i.e. overproduced the more abundant sex). This response may enhance fitness if local OSRs predict survival probabilities of offspring of each sex, rather than the intensity of sexual competition.

Keywords: *Amphibolurus muricatus*;
environmental sex determination; sex ratio;
temperature-dependent sex determination

1. INTRODUCTION

Theory predicts that reproductive females invest equally into producing sons versus daughters when the cost of producing one sex equals that of the other (Fisher 1930). However, fitness returns of daughters can differ from that of sons under a variety of situations, and maternal fitness can be enhanced if mothers adjust their relative investment into each sex accordingly (Charnov 1982). One situation that may favour maternal ability to facultatively adjust offspring sex ratios involves perturbations of the operational sex ratio (OSR; Werren & Charnov 1978; Werren & Taylor 1984). The OSR is defined as the ratio of sexually active males to sexually receptive females within a population (Emlen & Oring 1977), and has important consequences for levels of mating competition and sexual selection (Kvarnemo & Ahnesjö 1996), and potentially for population viability (Le Galliard *et al.* 2005*a*).

The traditional theoretical prediction linking OSR to sex allocation is that frequency-dependent selection will favour overproduction of the less abundant sex when the OSR is perturbed from unity (Fisher 1930). The reasoning is straightforward: an imbalance in OSR reduces mating competition within the less abundant sex (Kvarnemo & Ahnesjö 1996), providing fitness benefits to mothers that adjust their offspring sex ratios so as to overproduce progeny of the less abundant sex. Hence, a sudden perturbation in sex-specific survival (and thus, in adult OSR in the next breeding season) may favour maternal ability for facultative sex-ratio adjustment (Werren & Charnov 1978). Empirical support for this model exists for a variety of organisms (Conover & Van Voorhees 1990; McLain & Marsh 1990; Lummaa *et al.* 1998; Lopez & Dominguez 2003). This model, however, rests upon the critical assumption that the local OSR around the mother at the time of reproductive allocation will predict future levels of mating competition for her offspring when they reach adulthood. Since local OSRs can vary substantially through space and time (Kvarnemo & Ahnesjö 1996; West & Godfray 1997), it seems unlikely that this assumption will be met in many cases—especially for late-maturing species that inhabit heterogeneous habitats.

Habitat patches are likely to vary spatially and temporally in local OSRs (Kvarnemo & Ahnesjö 1996). For example, movement of individuals among patches may result in frequent shifts in local OSRs, thereby reducing the predictability of the OSR that offspring will experience when they mature. However, the current OSR within a given patch may provide a reliable indication of which sex is likely to do well in that patch at that given time. For example, overabundance of males within a given patch suggests the suitability of that patch for male growth and survival. Thus, an alternative sex-allocation strategy in response to OSR perturbations would be to overproduce the sex that is more abundant (rather than less abundant) within a given patch. In this scenario, the OSR predicts sex-specific prospects at the juvenile stage, rather than at the adult stage as suggested by traditional theoretical predictions (Fisher 1930; Werren & Charnov 1978).

In this paper, we experimentally test the above predictions using a multi-clutching lizard, the jacky dragon (*Amphibolurus muricatus*: Agamidae), with environmental sex determination (ESD). This species is well suited to testing the above hypotheses because (i) jacky dragons inhabit extremely heterogenous habitats that vary spatially and temporally (Harlow & Taylor 2000; D. Warner, personal observation), (ii) observations in large experimental field enclosures reveal massive spatial and temporal heterogeneity in OSRs (D. Warner *et al.* unpublished data), (iii) this species produces 2–3 clutches of eggs (3–9 eggs per clutch) throughout an extended reproductive season (October–February; Harlow & Taylor 2000), providing females an opportunity to shift sex-allocation patterns seasonally, and (iv) because jacky dragons have ESD (Harlow & Taylor 2000; Warner & Shine 2005), the potential for sex-ratio adjustment is high (West & Sheldon 2002), and thus sex-ratio adjustment is feasible at appropriate time-scales (as required by Werren & Charnov's 1978 model).

2. MATERIAL AND METHODS

(a) *Experimental protocol*

Adult jacky dragons were collected from natural areas surrounding Sydney during the austral summer of 2003/2004. All lizards were housed in large (2×2 m) field enclosures at Macquarie University, within the natural range of jacky dragons. Lizards were maintained in these enclosures for approximately nine months prior to the

Table 1. Factors contributing to variation in offspring sex ratios in captive jacky dragons. (Interaction terms with p-values greater than 0.25 are not shown. Values in bold face represent statistically significant effects. See text for details on statistical analyses.)

effect	statistic
operational sex ratio	$F_{1,20.06}=0.04, p=0.841$
clutch number	$F_{2,45}=1.6, p=0.218$
operational sex ratio × clutch number	$F_{2,45}=5.0, \boldsymbol{p=0.011}$
egg mass (g)	$F_{1,45}=0.0, p=0.895$
clutch size (number of eggs per clutch)	$F_{1,43}=3.9, p=0.055$

initiation of our experiment (see Warner & Shine 2005 for husbandry details). During this time, each enclosure contained one male and two females.

Immediately after winter emergence (September 2004), lizards were randomly assigned to one of two treatments that differed in OSR. All enclosures contained three lizards. In the male-biased treatment, a single female was housed with two males; this treatment contained nine replicate enclosures (total of nine female lizards). In the female-biased treatment, three females were housed together; this treatment contained four replicate enclosures (total of 12 female lizards). To ensure that clutches in the female-biased enclosures were fertile, we introduced a single male lizard into each enclosure for a 5-day period on a monthly basis. Although jacky dragon densities in the field are generally lower than those in our experimental enclosures, we have observed similar densities and sex ratios under natural conditions (D. Warner, personal observation).

After the females nested, eggs were brought back to the laboratory and weighed. Individual eggs were then half-buried in vermiculite (-200 kPa) within glass jars (125 ml), and sealed with plastic foodwrap. All the eggs were incubated at a constant 28°C, a temperature that generally produces 1 : 1 sex ratios in this species (Harlow & Taylor 2000). All hatchlings were weighed and measured (snout–vent length (SVL) and tail length (TL)) and offspring sex was identified by manual eversion of hemipenes (Harlow 1996).

(b) Statistical analyses
Offspring sex ratios were evaluated with Generalized Linear Mixed Models using the GLIMMIX procedure in SAS (Littell *et al.* 1996). Dependent variables included OSR, clutch number, egg mass, clutch size and higher order interactions in a single model. Clutch sex ratio, expressed as proportion sons, was the dependent variable. Maternal identity was defined as a random effect (because females comprise a random sample of the population from which they were collected) and clutch number was the repeated variable. Only females that produced multiple clutches during the reproductive season ($n=20$) were used in our repeated design; as a result, the data from one female were excluded from our analyses. The model contained a binomial error structure with a logit link function (Wilson & Hardy 2002). Analyses began with the full model including all interactions, and we subsequently eliminated factors backwards, starting with higher order interactions, at p-values of 0.25 (Quinn & Keough 2002). In the final model, significant effects were accepted at $p \leq 0.05$.

We evaluated the effects of the OSR, clutch number and their interaction (independent variables) on hatchling morphology (SVL, mass and TL; dependent variables) using mixed model analyses of covariance. Egg mass (measured at the time of laying) was used as a covariate for analysis of SVL and mass, and SVL was a covariate for analyses of TL. Hatchling body condition was evaluated as hatchling mass using SVL as a covariate. Maternal identity was defined as a random effect. Mean values for each clutch were used as our unit of analysis to avoid pseudo-replication.

3. RESULTS
Oviposition occurred from 14 October 2004 to 11 January 2005. During this period, we obtained 57 clutches yielding 335 eggs. Egg survival was 92.4% and was not influenced by our OSR manipulations or successive clutch number (p-values greater than 0.05).
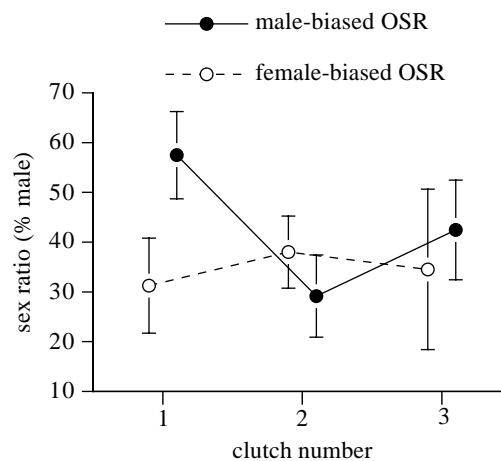
Figure 1. Offspring sex ratios of successive clutches of jacky dragons in response to operational sex ratio (OSR) treatments (adult females were housed in either male- or female-biased groups). Clutches 1–3 represent successive clutches within the same season by reproducing female lizards.

Overall clutch sex ratios tended to be female biased (61.2% female). Sex ratios did not differ between OSR treatments or among successive clutches (table 1). However, the OSR affected offspring sex ratios differently among successive clutches (significant clutch × OSR interaction, $p=0.011$). Individuals exposed to male-biased OSRs overproduced male offspring in their first clutch, but then produced predominately females in their second and third clutches (figure 1). Individuals exposed to female-biased OSRs tended to produce female-biased clutches across the entire reproductive season. Clutch size and egg mass were not associated with offspring sex ratios (table 1), nor was hatchling morphology influenced by OSR, clutch number or their interaction (all p-values greater than 0.05). Analyses performed at the enclosure level (to avoid pseudo-replication) revealed the same patterns described above.

4. DISCUSSION
Previous attempts to evaluate OSR effects on sex allocation in lizards have produced conflicting results. Two of these studies (Olsson & Shine 2001; Robert *et al.* 2003) suggest facultative sex allocation in response to imbalances in the OSR in the predicted direction according to theory (Werren & Charnov 1978), but the other two did not detect any OSR effect (Le Galliard *et al.* 2005b; Allsop *et al.* 2006). In contrast, our OSR manipulations influenced sex allocation in the direction opposite to that predicted by traditional theoretical models (Werren & Charnov 1978), at least for the first clutch in the season.

Our findings provide support for an alternative scenario of sex-ratio adjustment in response to OSR. Since jacky dragon habitat varies both spatially and temporally in OSR, the predictability of future OSRs (at the time when offspring reach adulthood a year later) is likely to be low. And owing to this low predictability, sex-ratio adjustment according to the traditional scenario is unlikely. Thus, females may instead adjust offspring sex ratios to match their offspring sex to sex-specific suitability of habitat for

juveniles. For example, if males are abundant in a given habitat patch, then this information may indicate that this patch is favourable for male growth and survival. Our manipulations indicated that in the first clutches of the season, females overproduced male offspring when exposed to male-biased OSRs. This pattern is consistent with our alternative hypothesis that sex-ratio adjustment is based upon the conditions most suitable at the juvenile stage, which is substantially more predictable than the future mating prospects of offspring 1–2 years later (as assumed in traditional models).

Why did females adjust sex ratio of the first clutch, but not that of later clutches? Previous studies on jacky dragons demonstrate that early hatching (from early clutches) can enhance offspring survival in the field (D. Warner & R. Shine, unpublished data) as well as the probability of reaching sexual maturity at age 1 (Warner & Shine 2005). Thus, the first clutch of the season is likely to be disproportionately important to maternal fitness relative to later clutches, and the sex-allocation strategy seen in our study may benefit both offspring and maternal reproductive success. Interestingly, the second and the third clutches in both OSR treatments were female biased despite all eggs being incubated at a temperature that has previously been shown to produce 1 : 1 sex ratios (Harlow & Taylor 2000). Similar female biases have been shown in another dragon lizard (Uller *et al.* 2006), and probably occur in other reptiles with temperature-dependent sex determination (Freedberg & Wade 2004).

An alternative explanation for the sex-allocation patterns found in our study may involve treatment differences in the opportunity for female mate choice. In our experimental set-up, females exposed to male-biased sex ratios had an opportunity to choose between potential mates, whereas those in the female-biased treatment did not. This situation might affect maternal sex-allocation patterns (Olsson *et al.* 2005). However, virtually nothing is known about mate choice in *A. muricatus*, so this issue needs to be addressed with careful manipulative experiments.

Our study did not identify a mechanism for sex-ratio adjustment. Since eggs from both treatments were incubated at similar temperatures, thermal regimes cannot be invoked. Some intrinsic maternal manipulation of offspring sex ratios may be involved, but differential allocation of steroid hormones is unlikely to explain these patterns because previous work demonstrates no association between maternally derived yolk steroids and offspring sex (Warner *et al.* in press). Further work is required to identify the nature of such proximate links, as well as to evaluate the degree to which spatially variable OSR in a given year predicts sex-specific advantages either during juvenile life (as in our model) or during adult life (as in traditional models).

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