Do operational sex ratios influence sex allocation in viviparous lizards with temperature-dependent sex determination?

D. J. ALLSOP, D. A. WARNER, T. LANGKILDE, 1 W. DU 2 & R. SHINE
School of Biological Sciences, The University of Sydney, Sydney, New South Wales 2006, Australia

Abstract
Under certain environmental situations, selection may favour the ability of females to adjust the sex ratio of their offspring. Two recent studies have suggested that viviparous scincid lizards can modify the sex ratio of the offspring they produce in response to the operational sex ratio (OSR). Both of the species in question belong to genera that have also recently been shown to exhibit temperature-dependent sex determination (TSD). Here we test whether pregnant montane water skinks (Eulamprus tympanum) utilise TSD to select offspring sex in response to population wide imbalances in the OSR, by means of active thermoregulation. We use a combination of laboratory and field-based experiments, and conduct the first field-based test of this hypothesis by maintaining females in outdoor enclosures of varying OSR treatments throughout pregnancy. Although maternal body temperature during pregnancy was influenced by OSR, the variation in temperature was not great enough to affect litter sex ratios or any other phenotypic traits of the offspring.

Introduction
In most sexually reproducing species, females are expected to allocate equal effort to producing sons and daughters, assuming that the fitness returns from the sexes are equal (Fisher, 1930). However, sex specific fitness returns may differ under a wide variety of circumstances (Bull, 1981; Bull & Charnov, 1988). For example, when relatives interact with each other in competition for mates or resources, and these interactions differ between the sexes, fitness returns may be sex specific (Hamilton, 1967). In circumstances where fitness returns differ between sons and daughters, selection may favour maternal ability to shift sex allocation to produce the ‘best’ sex for the prevailing social and ecological conditions (Hamilton, 1967; Trivers & Willard, 1973; Charnov, 1982; Frank, 1990; West et al., 2002; West & Sheldon, 2002). Here we investigate a case where the environment might have sex specific fitness effects on the offspring, and ask whether mothers facultatively adjust their offspring sex ratios in response to imbalances in the population adult sex ratio (termed the Operational Sex Ratio (OSR)).

The OSR is defined as the ratio of males to females that are ready to mate in a population at any one time (Emlen & Oring, 1977), and this ratio can have a large impact on the level of mating competition, and thus sexual selection (Kvarnemo & Ahnesjo, 1996, 2002). A bias in the OSR will usually result in increased competition for mates or resources amongst individuals of the more abundant sex, and the extent of the OSR bias will determine the level of competition and the intensity of sexual selection (Clutton-Brock & Parker, 1992). For example, males of the common spider mite (Tetranychus urticae) increase their level of mating competition as the OSR shifts towards a male bias, and consequently sexual selection leads to an increase in male body size relative to female size (Enders, 1993). In a sex-role-reversed shorebird (Wilson’s phalarope, Phalatropus tricolor), the intensity of female–female competition diminishes as more males arrive at breeding grounds, levelling out the OSR (Colwell & Oring, 1988).

Sex allocation theory predicts that if the OSR is perturbed from unity (either in time or space), facultative
sex ratio adjustment should cause females to over-produce the rare sex. In this way, mothers can reduce competition within the over-abundant sex, and thus maximise offspring fitness (Werren & Charnov, 1978; Werren & Taylor, 1984; West & Godfray, 1997). For such an effect to occur, there must be considerable overlap in generations, such that offspring have the opportunity to compete with their mothers' adult conspecifics. In addition, mothers must have the ability to accurately perceive the OSR and to make the relevant sex ratio adjustments (Werren & Charnov, 1978). Empirical evidence that OSR can influence sex ratio has come from disparate phylogenetic lineages, including plants (Lopez & Dominguez, 2003), insects (McLain & Marsh, 1990), and humans (Lummaa et al., 1998; Lazarus, 2002).

Two recent studies on viviparous scincid lizards in Australia have suggested the same ability in reptiles. The first of these studies, using free ranging populations of the Tasmanian snow skink, Niveoscincus microlepidotus, used correlational evidence to suggest that females perceive the OSR through the number of potential mates they encounter during the breeding season. They then shift their litter sex ratios to track the OSR both among and within years (Olsson & Shine, 2001). The second example used the southern water skink, Eulamprus tympanum, and conducted laboratory experiments with animals held in small sex-biased populations (Robert et al., 2003). Robert et al. (2003) concluded that pregnant females use facultative sex allocation in response to OSR, by overproducing the sex that is in short supply. However, the mechanism by which female E. tympanum perceives the OSR remains elusive (Robert, 2003). Intriguingly, although the taxa involved belong to two widely separated phylogenetic lineages, both belong to genera that have been reported to shift offspring sex ratios in response to thermal conditions during gestation (temperature-dependant sex determination (TSD): Robert & Thompson, 2001; Wapstra et al., 2004).

The mechanism of sex determination in these viviparous lizards is poorly understood and remains controversial (Valenzuela et al., 2003). In the southern water skink, E. tympanum, offspring sex ratio is sensitive to maternal temperature during gestation, suggesting a possible role for TSD (Robert & Thompson, 2001). Temperature-dependant sex determination is commonly associated with a lack of heteromorphic sex chromosomes (Bull, 1980), and karyotype analysis demonstrates that the Eulamprus genus indeed shows no overt sex chromosome dimorphism (Donnellan, 1985). However the co-occurrence of TSD and genetic sex determination (GSD) in sister taxa and even within a single species (Wibbels et al., 1994; Shine et al., 2002) suggest that the boundary between TSD and GSD is blurred, and that the two mechanisms comprise points on a continuum rather than a strict dichotomy (Sarre et al., 2004).

Here we return to E. tympanum to test for facultative sex allocation in response to OSR in a more natural context. To do so we combine the realism of field conditions with the power of experimentation, by allowing females to gestate in field enclosures in which we have manipulated OSR. We further extend previous work by asking whether the two environmentally labile sex allocation strategies (OSR and TSD) are linked in this species, by measuring the thermal behaviour of pregnant females throughout gestation in the laboratory whilst housed in varying OSR treatments. Our prediction is that pregnant females utilise TSD to alter their sex allocation decisions in response to an unbalanced OSR, via active maternal thermoregulation during gestation.

Materials and methods

The model system

The southern water-skink, E. tympanum is a medium sized (mean adult snout-vent length (SVL) ≈ 82 mm) insectivorous scincid lizard with a geographic range stretching throughout much of montane SE Australia (Cogger, 2000). Eulamprus tympanum is viviparous and is predominantly lecithotrophic, providing nutrients for the developing embryos in a yolk sac (Thompson et al., 2001a). Ovulation and mating occur in October, and the females gestate their young over summer, giving birth to between one and five offspring in January/February (Schwarzkopf & Shine, 1991).

Laboratory experiment

Adult male and female E. tympanum were collected early in the breeding season (early October, 2004) from Kanangra-Boyd National Park (33 °58.270S, 150 °03.346E), 160 km west of Sydney, New South Wales, in eastern Australia. Lizards were measured (mass, SVL, tail length), sexed (by hemipene eversion: Harlow, 1996), and given a unique toe clip for individual identification. Lizards were then kept in experimental groups in animal housing facilities at the University of Sydney.

Animals were kept in plastic nally bins (645 × 413 × 276 mm) and provided with basking heat using under-cage heating tape set to ~40°C (average field basking temperature in field is 30°C (Schwarzkopf & Shine, 1991). Thus, lizards in these enclosures were able to regulate their own temperatures behaviourally whenever the heating tape was switched on, from approximately 16°C (air temperature) to 40°C (the hottest point above the heat tape). Heat strips were controlled with an electronic timer set to one of two contrasting basking length regimes in an attempt to mimic thermal variation in the field. Half of the animals were offered a limited availability of hot basking temperatures (3 h per day) and the other half were given an extended basking opportunity (10 h per day). Room lights rotated on an 11 h on: 13 h off cycle and
Basking heat was only offered when lights were on. Outside of basking time, enclosure temperatures fell to ambient field night-time temperatures (~16°C). Thermochron iButton temperature data loggers (Robert & Thompson, 2003) glued to the dorsal surface of the female lizards recorded body temperature every 20 min (see statistical analysis below).

In order to test the idea that females adjust their offspring sex ratios in response to OSR (Robert et al., 2003), males and females were randomly distributed amongst two adult sex ratio treatments: either a male biased OSR (one pregnant female and two males per enclosure) or an all female OSRs (three pregnant females per enclosure). Lizards were then housed in experimental groups in field enclosures at the site of capture.

The field enclosures were circular (diameter = 1.5 m) and constructed of sheet metal (450 mm high) dug 50 mm into the ground to prevent lizards from escaping. All enclosures contained several hollow logs and dense alpine grass, which provided basking and retreat sites for the lizards. Water was provided in a bowl in the centre of each enclosure. Lizards were fed crickets (dusted in vitamin/mineral mix) twice weekly and checked for offspring twice daily (morning and afternoon).

Field experiments
Adult male and female E. tymanumns were collected part way through the breeding season during the summer of 2003 (15–16 December) from Kanangra-Boyd National Park (see above). After collection, lizards were measured (mass, SVL, tail length), sexed (by hemipene eversion: Harlow, 1996), and given a unique toe clip for individual identification. Lizards were then housed in experimental groups in field enclosures at the site of capture.

The field enclosures were circular (diameter = 1.5 m) and constructed of sheet metal (450 mm high) dug 50 mm into the ground to prevent lizards from escaping. All enclosures contained several hollow logs and dense alpine grass, which provided basking and retreat sites for the lizards. Water was provided in a bowl in the centre of each enclosure. Lizards were fed crickets (dusted in vitamin/mineral mix) twice weekly and checked for offspring twice daily (morning and afternoon).

To determine the effect of OSR on the sex ratios and phenotypic traits of offspring, we divided the pregnant females into two treatments that differed in male and female abundance, as per the laboratory experiments (above). The female biased treatment housed four pregnant females together per enclosure, whereas the male biased treatment housed one pregnant female and three adult males per enclosure.

Because this species has previously been shown to modify sex allocation in response to maternal thermal regimes during pregnancy (Robert & Thompson, 2001), it was critical that basking opportunities did not differ among treatments. To ensure this equivalence, we took a hemispherical (‘fish-eye’) photograph with a Nikon Coolpix digital camera (Nikon, Lidcombe, NSW, Australia) facing directly upwards from ground level at the centre of each enclosure. The photographs were analysed using Gap Light Analyser software to quantify canopy cover and calculate the total amount of incident solar radiation reaching each enclosure (Frazer et al., 1999: Langkilde et al., 2003). We assigned the two OSR treatments among enclosures such that there was no significant difference between treatments in either canopy cover or solar radiation (one factor ANOVA with treatment as the factor and canopy cover and incoming solar radiation as the dependent variables: canopy cover, F1,6 = 0.0, P = 0.99; incoming radiation, F1,6 = 0.0, P = 0.95). We also measured the surface temperatures of basking sites (in four enclosures per treatment) with tidbit temperature loggers (Onset corporation) over one week during the gestation period; temperatures did not differ significantly among enclosures (ANOVA: F1,6 = 0.1, P = 0.94).

Hatchling husbandry and measurement of phenotypes
Females in the field enclosures were transported to the animal housing facility at the University of Sydney towards the end of pregnancy (12–13 January 2004), and kept individually in plastic containers (200 × 200 × 200 mm) with water and shelter. Room temperature was set at a constant 15°C and heat for thermoregulation was provided by a strip of heating tape running along one end of each container on a 9 h on: 15 h off cycle. Room lights were set to a cycle of 11 h on: 13 h off. The lizards were fed crickets (dusted in vitamin/mineral mix) twice weekly and checked for offspring twice daily (morning and afternoon). Females from the laboratory experiment were left in their experimental treatments until parturition.

At birth offspring were measured (mass, SVL and tail length), sexed, and given a unique toe clip for individual identification, as described for the adults above. The running speeds of neonates were measured 3–4 days after birth on an electronically timed racetrack 1 m long and containing four infrared photocells (connected to a stopwatch) spaced at 25 cm intervals along the length of the track. All locomotor trials were conducted in a room at 25°C, and each lizard was given 30 min to acclimate to room temperature before its first trial. Individuals were placed at one end of the racetrack and encouraged to run by gently prodding their tails with an artist’s paintbrush. Each lizard was raced four times with a 30 s rest between successive trials. In our analyses, we use two measures of locomotor speed: the average speed recorded over the entire 1 m distance, and the average speed recorded over any 25 cm interval. To obtain data on growth, all lizards were remeasured prior to being released at the field site where their mothers had been captured.

Statistical analysis
We estimated gestation length as the number of days between ovulation and parturition. As previous work has demonstrated synchronous ovulation in cool-climate viviparous montane skinks (Wapstra et al., 1999), we assumed a single date (October 15) for ovulation at the
start of the breeding season, when the animals emerge from winter inactivity and begin courtship and mating (Schwarzkopf & Shine, 1991). We quantified the thermal profiles of pregnant females from thermochron records by analyzing temperature data from only the active daytime periods when the under-cage heating strips were on. We excluded the first and last hour of data during that time so as to allow for any cage differences in heating and cooling rates. The body temperatures of pregnant females are summarized as the Mean Temperature Selected (Mean T. Sel.), the Maximum Temperature Selected (Max. T. Sel.) and the Variance in Selected Temperature (Var. T. Sel.).

We refer to the sex ratio as the proportion of male offspring. As proportion data such as sex ratios usually have nonnormal error variances and unequal sample sizes, we first analysed the sex ratios using a GLM approach. The null model was specified with a binomial error structure and a logit link function, assigning the number of males in a litter as the response variable and the litter size as the binomial denominator (Crawley, 1993; Wilson & Hardy, 2002; Allsop & West, 2004). Models were fit to laboratory and field data separately, and at the level of the litter and averaged for the enclosure. In all cases, after fitting the maximal model, we checked for over dispersal in the data by inspection of the heterogeneity factor (HF), and re-scaled the GLM analysis by the HF for subsequent model simplification wherever necessary. All sex ratios reported here are back-transformed (original) values. For our analysis of the effects of OSR on female thermoregulatory behaviour and offspring phenotypes, we use standard ANOVA, ANCOVA and MANOVA techniques, the details of which are specified in the relevant sections below. To avoid pseudo-replication, we present all analyses averaged for the enclosure, as the litters from mothers in the female-biased OSR treatment cannot be treated as independent units for analysis. However, our results remain unchanged when analysed at the litter level, or using a nested ANOVA with litter sex ratio nested within enclosure.

**Results**

A total of 65 female *E. tympanum* were captured for the experiments (45 in the laboratory experiment and 20 in the field experiment), ranging in size from 68 mm SVL to 91 mm SVL, with a mean of 82.6 mm (SE = 0.57). Gestation times varied from 80 to 193 days with a mean of 121 days, and there was no significant difference between mean gestation times in the laboratory or the field (Lab mean gestation = 122.5 days, SE = 5.13, n = 45; Field mean gestation = 118.8 days, SE = 1.42, n = 20: F$_{1,63}$ = 0.23, P = 0.63). Females gave birth to a total of 182 offspring in 65 litters ranging in size from one to five neonates, with a mean litter size of 2.18 (SE = 0.092), with no significant difference in mean litter size between the laboratory and the field experiments (lab mean litter Size = 2.71, SE = 0.17; field mean litter size = 3, SE = 0.19: F$_{1,63}$ = 1.02, P = 0.32).

Although litter sex ratios from the laboratory experiment were more male biased (mean lab litter sex ratio = 0.7, SE = 0.05) than sex ratios from the field enclosures (mean field litter sex ratio = 0.5, SE = 0.08), the sex ratios were not significantly different (F$_{1,64}$ = 1.81, P = 0.18).

**The effects of OSR on thermal profiles of pregnant females**

Operational sex ratio significantly affected the selected body temperatures of pregnant females in our laboratory experiments (one factor MANOVA: Pillai’s trace, F$_{3,29}$ = 3.1, P = 0.04, n = 32 enclosures). Pregnant females housed with other females achieved significantly higher maximum body temperatures than did females housed with males (Female OSR Max. T. Sel. = 32.4°C, SE = 0.2: Male OSR Max. T. Sel. = 31.6°C, SE = 0.2; F$_{1,31}$ = 7.1, P = 0.04; Fig. 1). However, OSR had no significant effect on mean selected temperature (F$_{1,31}$ = 0.04, P = 0.84) or on thermal variances (F$_{1,31}$ = 0.02, P = 0.89). The long and short basking treatments had no effect on the thermal profiles of pregnant females (one factor MANOVA: Pillai’s trace, F$_{1,31}$ = 0.9, P = 0.44, n = 32 enclosures).

**The effects of the OSR on offspring sex ratio**

Sex ratios in the laboratory experiments

Although OSR affected maternal thermal profiles (see above and Fig. 1), we found no significant effect of OSR on offspring sex ratios produced by females in the laboratory (F$_{1,32}$ = 0.41, P = 0.53; Fig. 2), and no direct or interacting effect of the long or short basking treatment on sex ratio (F$_{1,30}$ = 2.09, P = 0.16). In support of these findings, the females’ thermal preferences also had no significant effect on offspring sex ratios.
Neither did we detect any significant effects of any higher order interactions between thermal preference and the OSR treatment on offspring sex ratio.

Sex ratios in the field experiments
Similar to our laboratory findings, field manipulation of the OSR had no significant impact on sex allocation by the pregnant females, with OSR unable to explain significant variance in offspring sex ratios in the field ($F_{1,8} = 0.07, P = 0.79$; Fig. 2).

The effects of the OSR on offspring phenotype
Even if reproducing females do not modify sex ratio in response to the OSR, they could potentially adjust offspring phenotypes in ways that enhance offspring fitness (Shine & Downes, 1999; Wapstra, 2000). To evaluate the effects of OSR on neonatal phenotypes, we used one-factor MANOVA with experimental treatment as the factor, and mean trait values for each litter as the dependent variables. Sex differences in offspring traits were also evaluated with MANOVA, but all male and all female litters were omitted from the analysis to avoid an unbalanced design. Our data show strong differences in the phenotypes of neonates born in the laboratory and the field (Pillai’s trace, $F_{8,39} = 11.78, P < 0.0001$). Field born offspring were larger-bodied (SVL: $F_{1,46} = 13.9, P = 0.0005$; Mass: $F_{1,46} = 33.9, P < 0.0001$), and faster runners (1 m sprint speed: $F_{1,46} = 15.8, P < 0.0002$; 25 cm sprint speed: $F_{1,46} = 7.1, P = 0.01$). We report the results for both field and laboratory experiments.

Offspring phenotypes in the laboratory experiments
Offspring phenotypes were not significantly influenced by the OSR in which the mother gestates her young (Pillai’s trace, $F_{8,20} = 0.4, P = 0.86$). The MANOVA for the effect of offspring sex on offspring phenotype was significant (Pillai’s trace, $F_{8,25} = 12.8, P < 0.0001$), however when we inspected the one-way ANOVA’s within the enclosure level analysis of offspring phenotype, none of the offspring traits were significantly different between the sexes (Table 1).

Offspring phenotypes in the field experiments
Unfortunately, there were insufficient data to perform MANOVA on the offspring phenotype information averaged at the enclosure level to assess the affect of OSR on phenotype. However, the individual ANOVAs suggest that OSR had no effect on phenotypic traits in the field data (Table 1). Hatchling sex also did not significantly affect phenotypic attributes in the field experiment (Pillai’s trace, $F_{8,5} = 0.53, P = 0.79$).

Discussion

OSR and sex allocation
Despite the significant effect of OSR on the females’ thermoregulatory behaviour (Fig. 1), we detected no
shift in maternal allocation to male versus female offspring in response to the OSR, either by altering litter sex ratios, or by altering respective body sizes of male and female offspring (Fig. 2; Table 1). There was also no subsequent impact of OSR on any of the phenotypic traits, or by altering respective body sizes of male and female offspring in response to the OSR, either by altering litter size at hatching, or by altering respective body sizes of male and female offspring (Fig. 2; Table 1). These findings are in agreement with previous studies of E. tympanum in the present study did not facultatively shift the sex ratios of their litters in response to our OSR manipulations. These findings are in agreement with a recent study on the viviparous common lizard Lacerta vivipara (Le Galliard et al., 2005). However, our results are contrary to those from previous studies on the same species as we use in the present study (Robert et al., 2003), and to those on another viviparous lizard reported to shift sex allocation in response to OSR (Olsson & Shine, 2001). These conflicting results require an explanation.

**Differences between the present study and previous work on E. tympanum**

What is the source of the different findings between the present study and those of Robert et al. (2003)? The laboratory component of the present study used the same protocol as for Robert et al. (2003), and both our laboratory and field experiments used the same study population. Although the OSR treatments in our field enclosures were similar to those used in the laboratory study of Robert et al. (2003), the field enclosures were larger and more complex, reducing contact between animals and allowing far greater opportunity for females to evade males. Thus, the density of males (as perceived by the pregnant females) may have been substantially lower under field conditions than in the laboratory study of Robert et al. (2003).

The statistical analysis by Robert et al. (2003) treated each neonate as an independent unit for analysis, thus inflating statistical power. However, despite the reduction in degrees of freedom, reanalysis of their data using females held in equivalent OSRs to ours (i.e. excluding those females held individually), and analyzing at the level of the litter, still revealed a significant effect of OSR on offspring sex ratios (F1,11 = 10.7, P < 0.01, n = 49 offspring from 12 litters). However, our current set of experiments showed no such effect with much larger sample sizes (F1,63 = 0.23, P > 0.05, n = 182 offspring from 65 litters). Our lack of support for the previous finding is therefore not due to reduced power – if the effect shown in Robert et al. (2003) was present in our study, our larger sample sizes should have detected it.

**Differences between E. tympanum and N. microlepidotus**

The timing of OSR assessment by pregnant females could potentially explain differences between our study and those for the Tasmanian snow skink, N. microlepidotus (Olsson & Shine, 2001). Although our E. tympanum females were exposed to different OSR treatments for most of gestation, they were brought into the laboratory post-ovulation and post-conception. Similarly, although both the laboratory and field animals in the present study were held in their respective OSR treatments over the middle third of their gestation period (generally believed to be most important for sexual differentiation: e.g. Bull, 1987), it remains possible that female lizards assess OSR at some other phase of the reproductive cycle. Females could assess the OSR prior to ovulation and then either make the relevant allocations immediately, or use the information gathered at that time to make subsequent allocations. For example, Olsson & Shine’s (2001) analysis of snow skinks (N. microlepidotus) inferred OSR from mating scars, implying a pre-conception (indeed, preovulation) assessment of the OSR. The timing of OSR assessment does not however explain the differences between our current study and that of Robert et al. (2003) using the same species, as in both studies

---

**Table 1** Effect of operational sex ratio (OSR) and offspring sex on offspring phenotypes. Analyses of variance were based on mean trait values of each enclosure (for OSR effects), and mean trait value of each sex within enclosures (for sex effects amongst the offspring). Body condition was calculated as the residual score from the general linear regression of ln mass on ln snout-vent length (SVL). Hatchling growth was calculated as the size increment between release and birth divided by the number of days between measurements.

<table>
<thead>
<tr>
<th>Trait</th>
<th>OSR treatment effect</th>
<th>Offspring sex effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snout-vent length (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body mass (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tail length (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body condition (residuals)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Running speed (m s⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over 25 cm</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Over 1 m</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Growth rate (size days⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In SVL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In mass</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For each sex, the expected mean values were calculated from the respective mean trait values of the sex within each enclosure (Table 1).Trait effects were assessed using one-way ANOVAs, with OSR treatment or offspring sex as factors. Mean trait values of offspring from 12 litters. However, our current set of experiments showed no such effect with much larger sample sizes (F1,63 = 0.23, P > 0.05, n = 182 offspring from 65 litters). Our lack of support for the previous finding is therefore not due to reduced power – if the effect shown in Robert et al. (2003) was present in our study, our larger sample sizes should have detected it.

**Differences between E. tympanum and N. microlepidotus**

The timing of OSR assessment by pregnant females could potentially explain differences between our study and those for the Tasmanian snow skink, N. microlepidotus (Olsson & Shine, 2001). Although our E. tympanum females were exposed to different OSR treatments for most of gestation, they were brought into the laboratory post-ovulation and post-conception. Similarly, although both the laboratory and field animals in the present study were held in their respective OSR treatments over the middle third of their gestation period (generally believed to be most important for sexual differentiation: e.g. Bull, 1987), it remains possible that female lizards assess OSR at some other phase of the reproductive cycle. Females could assess the OSR prior to ovulation and then either make the relevant allocations immediately, or use the information gathered at that time to make subsequent allocations. For example, Olsson & Shine’s (2001) analysis of snow skinks (N. microlepidotus) inferred OSR from mating scars, implying a pre-conception (indeed, preovulation) assessment of the OSR. The timing of OSR assessment does not however explain the differences between our current study and that of Robert et al. (2003) using the same species, as in both studies

---


JOURNAL COMPILATION © 2006 EUROPEAN SOCIETY FOR EVOLUTIONARY BIOLOGY
the animals were placed in their respective OSR treatments at approximately the same time during pregnancy.

If the assessment of OSR is made before the start of gestation, any spatial or temporal variation in OSR could cause conflicting results between studies carried out at different times or locations. Field studies of *E. tympanum* have revealed considerable variation in the proportion of females that breed each year (Schwarzkopf & Shine, 1991; Schwarzkopf, 1993; Doughty & Shine, 1998), causing fluctuations in OSR from year to year (Kvarnemo & Ahnesjo, 1996). Thus, we might expect similar temporal or spatial variation in facultative sex allocation response to the OSR. Although such variation will generate predictable and repeatable sex allocation responses in the wild, it is a potential source of difference between our current experimental findings and those of the previous study on the same species (Robert et al., 2003). Such variation might also explain the contrast between our results and those reported for *N. microlepidotus* (Olsson & Shine, 2001; Robert & Thompson, 2001), if the information on OSR is gathered before the animals were placed into experimental treatments.

The level of placental differentiation is another potential source of differences between species in the ability to facultatively adjust sex ratios. The placenta of *N. microlepidotus* is relatively complex, allowing the passage of organic nutrients to the embryo (Thompson et al., 2001b) and thus confers a potential for ongoing facultative shifts in sex allocation throughout pregnancy. In comparison, *E. tympanum* appears not to be capable of passing organic nutrients across the placenta, and so must make any sex-specific nutrient allocations at ovulation (Thompson et al., 2001a). Nonetheless, it may be possible for the mother to adjust hormone allocation after ovulation (K. Robert, personal communication).

**Conclusion**

One logistical difficulty in statistical analyses of sex-ratio data is their relatively low power to convincingly refute the null hypothesis of a 50:50 sex ratio (Wilson & Hardy, 2002). Hence, it would be premature to conclude that no such effect exists; instead, all we can say is that in the present study, there is no clear evidence that female water skinks modify sex ratios or phenotypic traits of their progeny in response to OSR. The assumptions of the original model predicting facultative sex allocation in response to OSR (Werren & Charnov, 1978) are actually quite restrictive, requiring that generations overlap sufficiently to generate competition between adults and offspring, and that the perturbation to the OSR is of sufficient magnitude to cause a facultative response (West & Godfray, 1997). In addition, the imbalance in the adult sex ratio at the time of breeding must translate into a sex specific fitness differential for the offspring being gestated at the time that the OSR is perceived and acted upon (S. A. West, personal communication). Nonetheless, the discovery of TSD in at least three widely separated lineages of scincid lizards (Robert & Thompson, 2001; Shine et al., 2002; Wapstra et al., 2004) indicates that the ability to facultatively adjust sex ratios in response to environmental cues is widespread within this group. Identifying the proximate cues used to adjust sex allocation in these animals remains an exciting challenge for future research.

**Acknowledgments**

We thank A. Lane, M. Elphick, J. Herbert, S. Iglesias. K. Murphy, B. Phillips, T Schwartz and J. Thomas for assistance, and F. Seebacher, M. Thompson, M. Crowther, N. Colegrave, S. West, B. Sheldon and an anonymous reviewer for comments and discussion. K. Robert constructed the field enclosures, encouraged us to think about sex allocation in viviparous skinks, and provided valuable input to our discussions. This project was approved by the University of Sydney Animal Care and Ethics Committee (protocol numbers L04/10-2003/1/3832 and L04/10-2004/1/3991). All lizards were collected with permission from the New South Wales National Parks and Wildlife Service (licences numbers S10658 and S11367). This work was supported financially by the Royal Commission for the Exhibition of 1851 (fellowship to DJA) and the Australian Research Council (grants to RS).

**References**


