

Table 1 Factors affecting egg size and sex

Response term	Explanatory term	Statistic	d.f.	P value
Embryo sex	Male rank	1.00	1	0.32
Egg volume	Embryo sex	4.27	1	0.04
	Male rank	6.69	1	0.01
	Male rank × offspring sex	0.4	1	0.53
Chick weight	Male rank	6.29	1	0.01
	Embryo sex	0.07	1	0.78
	Male rank × offspring sex	<0.01	1	0.97
Per cent males (2nd clutch)	Per cent males (1st clutch)	2.78	1,15	0.03
Egg volume (2nd clutch)	Egg volume (1st clutch)	3.23	1,15	0.006
Per cent males	Mean egg volume	0.03	1	0.86

Effects of embryo sex and male rank on egg volume and chick weight were investigated using residual maximum likelihood models. There were no differences in chick size at hatching ($\chi^2 = 0.03$, d.f. = 1, $P = 0.47$). Generalized linear mixed models with binomial error structure and logit link function were used to investigate the effects of male rank and a female's average egg volume on the proportion of male eggs laid. Repeated measures within females were controlled for in these analyses ($n = 2$ clutches, 16 females). General linear models were used to investigate the repeatability in sex ratio and egg volume (Genstat 5.4.1, Lawes Agricultural Trust, IACR Rothamsted; 1998) (E.J.A.C., A.F.R., K. Orr, R. Griffiths and D. J. Ross, unpublished results).

in quantity^{2,3} or quality, could this explain these differences in investment? We have shown that female mallards lay larger eggs for preferred males but do not produce more sons¹. This increased investment is not directed at one particular sex, and here we point out the importance of distinguishing between differential investment in the sexes *per se*, as suggested by Petrie *et al.*, and differential investment in the sexes for different males.

Table 1 (top) shows that, within any breeding attempt, female mallards lay larger eggs for male embryos. However, this does not explain the increased investment for preferred males between different breeding attempts. Also, larger eggs for preferred males then produce heavier chicks, irrespective of their sex. Hence, in mallards, both sexes benefit from their mother's increased investment with preferred males.

Maternal characteristics seem to have a strong effect on the sex of their offspring (Table 1, bottom). Females that produce a high proportion of males in their first clutch also produce a high proportion of males in their next clutch, regardless of their partner's rank. Females are also consistent in their egg size after controlling for differential investment for different males, but females that generally produce larger eggs do not produce more males.

Whether or not differences in hormonal levels found in clutches sired by different males translate into 'differential investment' is more complex. Overall increases in hormonal level would suggest differential investment. Alternatively, differential allocation of hormones within a clutch could simply be a consequence of biasing the sex ratio in favour of a particular sex. But if the favoured sex is more costly to produce, then this would still represent a form of differential investment.

The key issue is the effect of this bias in investment. Does biasing the sex ratio of offspring increase the overall viability of the clutch because the preferred sex is more

likely to survive? Or does the less preferred sex suffer in the trade-off to the detriment of their survival? Females may bias investment in the sexes for many different reasons, so understanding how they influence the success of their sons and daughters will also be essential in explaining why birds sired by different males differ in their success.

Finally, could maternal provisioning of egg hormone levels be linked to the sex-determining process, as suggested by Petrie *et al.*? First we need to find out whether male phenotype can influence hormonal levels in the maternal body and ovary, and then whether manipulating hormonal levels within the female body and ovary can bias production of the sexes.

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Ecology

Global amphibian population declines

The decline and disappearance of relatively undisturbed populations of amphibians in several high-altitude regions since the 1970s suggests that they may have suffered a global decline, perhaps with a common cause or causes^{1–3}. Houlahan *et al.*⁴ examined means of trends for 936 amphibian populations and concluded that global declines began in the late 1950s, peaked in the 1960s, and have continued at a reduced rate since. Here we re-analyse their data using a method that accounts for the sampling of different populations over different time periods, and find evidence of a mean global decline in monitored populations only in the 1990s. However it is calculated, the global mean not only masks

substantial spatial and temporal variation in population trends and sampling effort, but also fails to distinguish between a global decline with global causes and the cumulative effects of local declines with local causes.

The first analytical method used by Houlahan *et al.* evaluated patterns in ΔN_t , the annual change in abundance within populations, where

$$\Delta N_t = \log(N + 1)_{t+1} - \log(N + 1)_t$$

They calculated annual mean changes by averaging ΔN_t over populations with recorded abundances in years t and $t + 1$. The temporal pattern of this mean is misleading because each year includes different populations. For example, consider one population studied in years 1, 2 and 3 with $\Delta N_1 = 0.1$, $\Delta N_2 = 0.3$, and another studied in years 2, 3 and 4 with $\Delta N_2 = -0.5$, $\Delta N_3 = -0.3$. Both populations are doing better with time, but the arithmetical averages for the three years, $\bar{\Delta N}_1 = 0.1$, $\bar{\Delta N}_2 = -0.1$, $\bar{\Delta N}_3 = -0.3$, indicate the opposite. The correct approach to estimating $\bar{\Delta N}_t$ uses least-squares means⁵, which estimate the yearly mean averaged over all populations. The least-squares means of ΔN_t , -0.3 , -0.1 and 0.1 , correctly represent the observed trends.

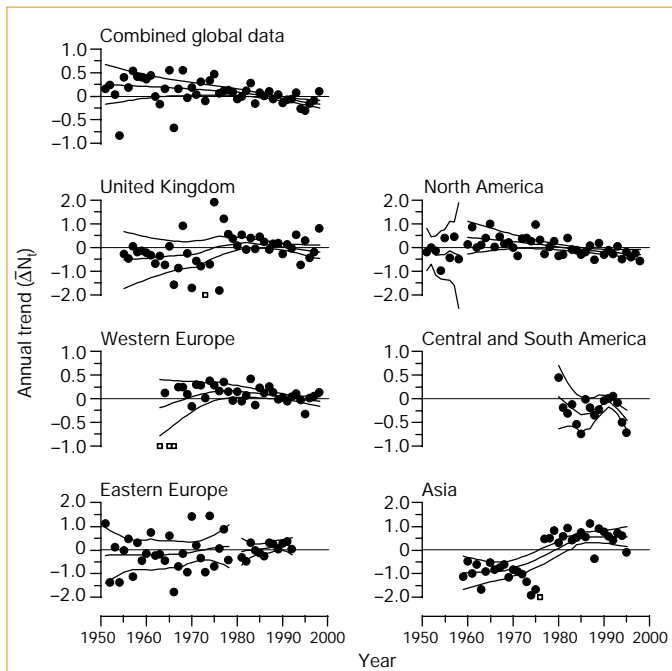
We used least-squares means to estimate annual mean trends for the combined global data and for several geographical regions⁴ (Fig. 1). The global mean trend was significantly positive during 1964–81, indicating that, on average, monitored amphibian populations increased over this period. From 1990 onwards, the trend was significantly and increasingly negative, suggesting a global decline. This decline began more than a decade later than is generally accepted^{1,2}, a quarter of a century later than Houlahan *et al.*⁴ found, and at around the time that concerns about it were first expressed³.

The global mean does not reflect a single worldwide trend. For regions where data allowed separate analysis, trends of the 1990s were significantly negative only in North America and in Central and South America (Fig. 1), where concerns have been raised over amphibian declines^{1–3,6,7}. The trend for Asia was significantly negative during 1959–75 and significantly positive after 1983.

The second analysis carried out by Houlahan *et al.* identified significantly more negative than positive correlations of population size with time, and they interpret this as evidence of a global decline. However, this pattern is expected for many amphibians in which recruitment is more variable than survival, and the exact expectation depends on the population biology of each species¹. It is therefore impossible to establish a correct null hypothesis for the global database.

Extrapolating these results beyond the particular populations studied is tenuous.

Figure 1 Yearly least-squares means for ΔN_t estimated using the statistical-analysis-system procedure mixed⁵ for the combined global data and for different regions. Only those populations with data for three or more consecutive years are included, to allow adjustment for first-order autocorrelation. Data points that fall outside the axes are indicated by hollow squares; the values for these are: UK 1973, -2.12; western Europe: 1963, -2.37; 1965, -1.28; 1966, -2.63; Asia 1976, -3.39. Trend lines are non-parametric regressions using LOESS⁸, weighting points by $1/s.e.^2$; approximate 95% confidence limits for LOESS regressions are indicated by outer lines.



Sampling intensity varied widely within and among regions and over time¹, leading to variation in the power and generality of analyses. Amphibian population declines in less modified habitats are associated with high altitudes^{1,2}, but elevation was not considered in the analyses⁴. Furthermore, the populations included in the data set were studied for many reasons, including concern over possible decline or human impact, and were not a representative random sample of the global amphibian fauna. Determining the true nature and extent of global trends in amphibian populations will require the collection of more data and more detailed analysis.

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Houlahan et al. reply — Alford *et al.* address several questions related to the biological and statistical analysis of declines in global

amphibian populations. They argue that, by emphasizing the global mean, we have masked spatial and temporal variation in amphibian population trends. Admittedly, information is lost when any summary statistic is used, but global amphibian declines should not be inferred by estimating missing values. Furthermore, they contend that we do not distinguish between a global decline with global causes and the cumulative effects of local declines with local causes. But we did not address the issue of causation: we reported widespread declines in extant, mostly lowland populations, whereas the recent focus has been on extinctions at high-altitude sites^{1,2}.

Alford *et al.* disagree with our statistical analysis, and re-analyse our data set using a method that involves estimating about 38,000 unobserved values, some of which are not biologically feasible. The data used in both analyses are annual population growth rates ($dN_{t,p}$, where t is the year and p is the population) from about 900 amphibian-population time series during 1950–97. None of the populations spans the entire period, so the data are a roughly 900×47 matrix of $dN_{t,p}$ with about 5,000 observed values and about 38,000 empty cells.

The least-squares means approach used by Alford *et al.* tacitly estimates the missing values ($dN_{t=1,p=2}$ and $dN_{t=3,p=1}$) by assuming that the difference between $dN_{t=1,p=1}$ and $dN_{t=2,p=1}$ can be used to infer $dN_{t=1,p=2}$ (and, in the same manner, $dN_{t=3,p=1}$); $dN_{t=2,p=1} - dN_{t=1,p=1} : 0.3 - 0.1 = 0.2$. Thus, subtract 0.2 from $dN_{t=2,p=2}$ to get $dN_{t=1,p=2}$: $-0.5 - 0.2 = -0.7$. The mean of $dN_{t=1,p=1}$ (+0.1) and $dN_{t=1,p=2}$ (-0.7) is -0.3, the least-squares means value calculated by Alford *et al.* for year 1.

Estimating 38,000 missing values on the

basis of 5,000 observed values is problematic, and is made worse by ignoring the fact that population sizes are associated with dN values. According to the least-squares means approach, populations can grow without limit and negative dN values can be estimated for extinct populations. Consider again two populations: the sizes of population 1 are $N_1=99$, $N_2=31$ and $N_3=0$ with $dN_{t=1,p=1}=-0.5$ and $dN_{t=2,p=1}=-1.5$. For population 2, $N_2=0$, $N_3=49$ and $N_4=99$, and $dN_{t=2,p=2}=1.7$ and $dN_{t=3,p=2}=0.3$. The estimation procedure used by Alford *et al.* yields $\Delta N_{t=3,p=1}=-2.9$, $\Delta N_{t=1,p=2}=2.7$, implying that population sizes were negative for population 1 in year 4 and for population 2 in year 1. By randomly selecting 20 populations and estimating the missing values for the 1950–97 period, we found that about 20% of the estimated dN values were associated with negative population size.

Alford *et al.* also suggest that the ratio of positive correlations of population size with time to negative correlations is not meaningful. They claim^{3,4} that stationary amphibian population dynamics are characterized by many small, negative dN values and few large, positive dN values. However, in this data set, the average negative change (-0.299 ± 0.007 s.e. ($N=2,464$)) is virtually identical to the average positive change (0.302 ± 0.007 s.e. ($N=2,270$)).

Whether these 936 amphibian populations are representative of the global amphibian fauna of all extant (or recently extinct) populations is unknown and cannot be determined, so the magnitude and direction of any bias cannot be estimated. The choice is therefore whether or not to use the data available to address an important conservation issue. We opted to do so and conclude that amphibians have been and are still declining.

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