Sex Ratio and Survivorship in the Australian Freshwater Crocodile *Crocodylus johnstoni*

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SYNOPSIS

Crocodylus johnstoni embryos have their sex determined by incubation temperature. Females result from the highest and lowest temperatures at which embryo survivorship is possible. Consequently, embryos with the highest probability of dying from inadequate temperatures become females. Low temperature incubation prolongs development, increasing the probability of death (especially of females) through drowning in wet season floods. Sex ratio selection may be constrained by survivorship considerations. Both time of hatching and the sex ratio of hatchlings vary between river systems and within the one river system over time. A possible advantage of temperature-dependent sex determination is speculated upon, but not demonstrated.

INTRODUCTION

The endemic Australian freshwater crocodile, *Crocodylus johnstoni*, is widely distributed in the lagoons, rivers and streams which drain the north coast of the Australian continent (Worrell, 1952, 1964; Cogger, 1979). It is one of the more abundant extant crocodilians (Groombridge, 1982), and is neither endangered nor threatened (Jenkins, 1979). The species is particularly amenable to demographic study because individuals congregate during annual dry seasons and nest within a contracted two- to three-week period (Webb, 1982). In addition, it is small in size (maximum sizes are 2–3 m total length and 30–60 kg body weight), and compared to some crocodilians, is easy to handle.

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Of particular relevance to the present study, *C. johnstoni* has tempera-ture (environmental?) dependent sex determination (TSD) (Webb, Buckworth & Manolis, 1983d). This complicates demography, because it means that the sex of offspring in any one year or area is likely to be the interactive result of: nest sites available in a particular area or year; nest sites selected; time of nesting relative to environmental temperature cycles (and perhaps cycles of other parameters); the bias of selected nests (and perhaps eggs; Bull, Vogt & Bulmer, 1982) to one sex or the other; and possible long-term selection influences on the sex ratio (Bull & Vogt, 1979, 1981; Bull, 1980, 1981a, b; Mrosovsky, 1980; Mrosovsky & Yntema, 1980; Miller & Limpus, 1981; Bull, Vogt & Bulmer, 1982; Bull, Vogt & McCoy, 1982; Bulmer & Bull, 1982; Ferguson & Joanen, 1982, 1983; Vogt & Bull, 1982; Vogt, Bull, McCoy & Houseal, 1982). Many of the same factors affect embryo survivorship (Webb, Buckworth & Manolis, 1983d; Webb, Sack, Buckworth & Manolis, 1983), and it would be surprising if survivorship, sex determination and the sex ratio of annual recruits were independent of each other.

In the present study we investigate the extent to which these factors are interrelated in *C. johnstoni*, and specifically examine:

(i) Whether or not TSD, as determined by constant temperature incubation in the laboratory, reflects accurately the way in which sex determination operates in the field.

(ii) Whether or not as a result of TSD there is differential survivor-ship of eggs destined to become males or females.

(iii) The extent to which differential survivorship interacts with TSD to vary sex ratio at hatching (recruit sex ratio) within the one population over time, and between populations.

(iv) The extent to which survivorship constraints could be expected to limit sex ratio selection.

The three lines of evidence pursued are: an examination of field nests from laying to hatching; an examination of geographical variation in the time of hatching and sex ratio of recruits; and an analysis of variation in the population age structure. On the basis of these and other results we hypothesize that: the relationship between tempera-ture and sex may be an indirect one mediated through the profound effect temperature has on embryonic metabolic and development rates; sex ratio selection is constrained by selection for maximizing embryo survivorship; sex-specific survivorship may be a fitness charac-ter consistent with the model of Charnov & Bull (1977), but a selective advantage in directing low probabilities of surviving at females would need to be demonstrated; an advantage of TSD over genotypic sex

determination (GSD) would exist if TSD was itself an unavoidable consequence of a mechanism related primarily to enhancing embryo survivorship.

THE McKINLAY RIVER *C. JOHNSTONI* POPULATION: A SELECTED REVIEW

Study Area and History of Protection

The McKinlay River drains an extensive black soil plain of grassland, savannah and light eucalypt forest (Webb, Manolis & Buckworth, 1983). The substrate of the mainstream bed is typically sand, and sandbanks are often associated with flood plain billabongs (lagoons). During the dry season (April/May to November) water is restricted to isolated pools in the mainstream bed and flood plain creek lines, where *C. johnstoni* congregate, and ultimately nest (August/September). The wet season is usually heralded by isolated storms in September/October, but 92% of the 1400 mm annual average rainfall is between November and April, and this is associated with widespread flooding.

Crocodylus johnstoni was legally hunted throughout the Northern Territory in 1960–1963, when it was protected. Density has been steadily increasing since that time.

Population Size and Age Structure

In 1979 the McKinlay River population was estimated as 1000 animals (Webb, Manolis & Buckworth, 1983), and the approximate age struc-ture (Fig. 1) was derived from a sample of 240 recaught individuals (Webb, Buckworth & Manolis, 1983a). The method of predicting age was based on the relationship between age and size, but with two corrections. First, the difference between the observed growth rate of an individual of given size, and the predicted mean growth rate of that sized individual in the population, was used to compute an approximate age-size relationship for the individual (a major correction). Second, predicted ages were rounded to the nearest November hatching period. Capture methods (almost exclusively fine nets; Webb & Messel, 1977) resulted in random capture of most size groups. In the younger animals (< six years), ages were considered accurate to the month.

The Population Sex Ratio

Of 697 *C. johnstoni* caught and sexed, the sex ratio (expressed as the proportion of males) has been 0.33 (0.31 among recaught individuals).
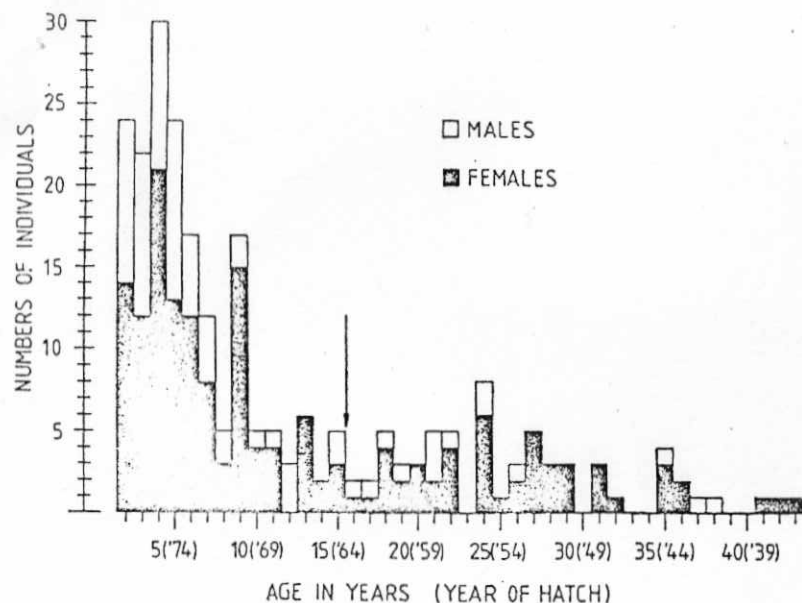
FIG. 1. The estimated 1979 age structure of 240 McKinlay River *C. johnstoni*. The arrow indicates when hunting ceased (protection).

The sex ratio of immatures (0.36) is significantly higher than that of matures (0.17) (Webb, Buckworth & Manolis, 1983a, d).

Post-hatching Mortality and Growth

Mortality between hatching and one year of age occurs mainly during the first wet season. It was estimated as 98% in one experiment (Webb, Buckworth & Manolis, 1983d), although 90–95% may be a more typical range (unpublished data).

Post-hatching male and female survivors grow at the same rate until about five years of age after which males grow faster than females (Webb, Buckworth & Manolis, 1983a). Recapture results demonstrate no significant sex-specific mortality in immature animals (Webb, Buckworth & Manolis, 1983a).

Movement and Dispersal

A high percentage (96.4%) of recaught animals are within 6 km of where they had been marked a year before. Long-distance movements (for example between the upstream and downstream parts of the study area) were rare (< 1%). The proportions of males and females which

move are not significantly different, and both sexes have a well-developed homing ability (Webb, Buckworth & Manolis, 1983b).

Reproduction and Nesting

Females mature at 74–78 cm snout-vent length (SVL) and 11–14 years of age, and males at about 87 cm SVL and 16–17 years of age (Webb, Buckworth & Manolis, 1983a, d). Reproductive senescence is about 40–45 years of age. Each year 84.4% of females > 11 years of age and 8% of females ≤ 11 years of age lay eggs. Mean clutch size is 13.2 ± 3.2 eggs. The egg-laying period is contracted (August 21 ± 6 days (SD)), and appears constant (within two weeks) from year to year. Nest sites are chosen after a series of "test" excavations in sand or other friable substrates close to water. The availability and location of friable substrates is dependent on water levels, which themselves depend on the extent of the previous wet season. Nesting banks used in one year may be inundated and unavailable in another.

Eggs are typically deposited within a distinct moisture band (~ 5% moisture in oven dried samples), and depth to the top egg (19.6 ± 7.5 cm) is partly dependent on substrate friability. There is a significant tendency for large clutches of large eggs (from older or larger females) to be laid early in the egg-laying period.

Clutch temperatures show considerable daily variation (3.5°C range in one nest) and baseline levels depend on the degree of exposure, depth, moisture content and thermal characteristics of the substrate around and beneath the clutch. Some of the hottest nests at the time of egg-laying have been those in which sand overlies a solid clay bank. Incubation takes 9–14 weeks. Females rarely attend nests during this period, but excavate nests from which young are calling at the end of incubation.

Egg mortality is high (60–70% in one year). It results from varanid predation, inundation and/or washing out of nests with subsequent exposure of eggs, females excavating the nests of other females, trampling by buffalo, wild pig predation, overheating and/or possibly desiccation. Predation is significantly greater at colonial nesting sites than at sites where only one or two females nest.

Temperature-dependent Sex Determination

Sex varies with incubation temperature (Fig. 2) (Webb, Buckworth & Manolis, 1983d; additional data), but differs from *Alligator mississippiensis* (Ferguson & Joanen, 1982, 1983) and *C. porosus* (unpublished data) in the following features:
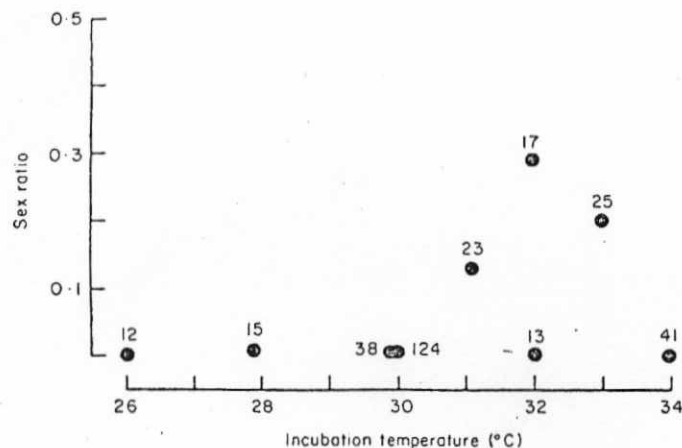
FIG. 2. The general trend in the relationship between sex ratio (proportion of males) and incubation temperature ($< \pm 1°C$) as indicated by laboratory trials. Numbers are sample sizes.

(i) Females have been produced at all temperatures so far tested.

(ii) High (34°C) and low (26–30°C) temperatures produce exclusively females.

(iii) There is a narrow range of temperatures in which males are produced (31–33°C), and thus there are likely to be two temperature thresholds (where 0.5 sex ratios result) for sex determination.

At 31–32°C sex is determined at approximately 20–30 days of age (unpublished data), although the times and embryonic stages at which sex is determined are probably temperature-dependent (Yntema, 1979; Bull, 1980; Bull & Vogt, 1981; Ferguson & Joanen, 1983; unpublished data on *C. porosus*).

At the time of hatching, the gonads of most males [Fig. 3(A), (B)] and females [Fig. 3(C), (D)] are well differentiated. Unlike *A. mississippiensis* (Ferguson & Joanen, 1983), the cliteropenis is also differentiated, and most *C. johnstoni* hatchlings can be externally sexed (Webb, Manolis & Sack, in press).

The gonads and clitoris of females produced at the highest incubation temperatures (34°C) are macroscopically similar to those of 30°C females. Histologically [Fig. 3(E)] these females have a thin cortex and a greatly vacuolated and distorted medulla. This is consistent with gonadal development being severely retarded (relative to embryo stage) and the relatively young (small) gonad being distorted by enhanced development of the mesonephros, metanephros and adrenal to which it is attached.

In two of some 40 females produced at an extremely (~ 34°C) high
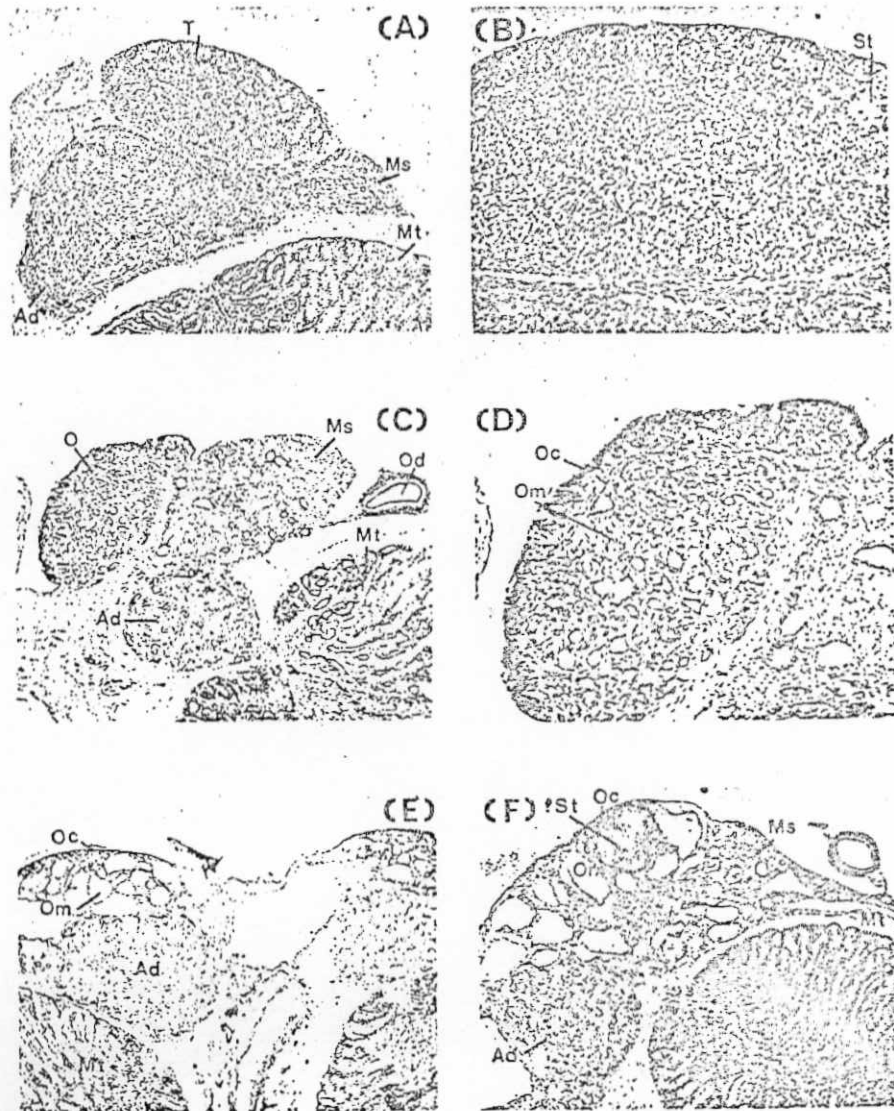

FIG. 3. Histological differences in the gonad region of hatchling *C. johnstoni*. (A), (B) A wild male. (C), (D) A wild female, but with similar gonads to a 30°C incubated female. (E) A 34°C incubated female with a thin cortex and greatly vacuolated medulla. (F) A 34°C female which contains an apparent seminiferous tubule. Ad, adrenal; Ms, mesonephros; Mt, metanephros; O, ovary; Oc, cortex; Om, medulla; Od, Oviduct; St, seminiferous tubule; T, testis.

incubation temperature that were examined histologically, an apparent seminiferous tubule was present [Fig. 3(F)], suggesting some may be hermaphroditic. That others retain their female sex was indicated by five 34°C females incorporated into a raising experiment (Webb, Buckworth & Manolis, 1983c). All retained female characteristics. Two had ovaries, oviducts and a distinct clitoris when examined after four months, and three had a distinct clitoris when released after seven months.

Temperature-dependent Survivorship

At 30°C, 63–100% of fertile eggs develop successfully. Incubation time is 84–85 days, and few hatchlings are abnormal (< 1%). In contrast, at 34°C only 21% hatch, and 74% of hatchlings have obvious abnormalities; incubation takes 68–69 days. At 26°C all embryos die before hatching, and most advanced dead embryos are abnormal. Based on development rates at 26°C (2.1 times slower than at 30°C), total incubation time would be 170–180 days if any animals survived (Webb, Buckworth & Manolis, 1983d).

Annual Variation in the Number and Sex Ratio of Hatchlings

On 10 November 1980, 1981 and 1982, all hatchlings sighted in the same 7 km section of the Mary River (adjacent to the McKinlay River) were collected. The number and sex ratio varied from year to year (Webb, Buckworth & Manolis, 1983d):

	Males	Females	Sex ratio	Total
1980	57	54	0.51	111
1981	22	63	0.26	85
1982	49	40	0.55	89

METHODS

Field Nests

In August–September 1982, 28 nests were located in the McKinlay River area soon after laying. Clutch temperatures (one to two eggs deep) were measured, and the top egg was removed for aging (Webb, Buckworth & Manolis, 1983d) unless a clutch was known to have been laid the previous night (prior visits; abundant mucus on the eggs). Most clutches were 1–6 days old ($N = 15$), six clutches were 7–13 days

old and five were 14–24 days old. Two clutches could not be aged because the eggs removed were infertile. Nests were re-covered and 1m² pieces of wire mesh pegged over them to restrict predators.

At or near hatching, each nest was revisited. Predators had burrowed beneath the mesh at eight nests and removed all eggs. At the others, a second clutch temperature was measured, then each egg was removed, numbered and its position mapped in relation to other eggs in the clutch (Ferguson & Joanen, 1982, 1983). Eggs which were not hatching were returned to the laboratory and incubated at 31–32°C (13 clutches); laboratory incubation took < 6 days for four clutches, 7–13 days for two clutches and 14–21 days for seven clutches. On the basis of the second clutch temperature measured, and information on development rates at different temperatures (Webb, Buckworth & Manolis, 1983d; Webb, Buckworth, Manolis & Sack, 1983), minor corrections were made to the laboratory incubation times so that total field incubation times could be estimated. Two new nests were located at the time the others were revisited.

Hatchlings were consecutively numbered (independent of their clutch of origin), measured, weighed and sexed by examination of the cliteropenis. They were maintained in a heated raising chamber (Webb, Buckworth & Manolis, 1983c) for up to three weeks before being sexed again. If any doubts or inconsistencies existed in the sexing, individuals were killed and their gonads examined macroscopically and histologically (haematoxylin and eosin). Individuals which either hatched prematurely, or were abnormal, or were dead in eggs at advanced embryonic stages, were also examined histologically. All 22 animals thus examined were of the sex allocated by cliteropenis examination.

Geographic Variation in Hatchling Sex Ratios and Time of Hatching

In November–December 1982, 4569 hatchlings were collected in repetitive trips to a number of different Northern Territory rivers (Fig. 4). All were weighed and had snout-vent length (SVL) and the width of the umbilical scar measured, and 4556 were sexed by examination of the cliteropenis (in 13 animals sex was unclear). On the basis of information from hatchlings released and recaught, yolk scar width was used as an indicator of week of hatch: ≥ 5 mm = day of hatch; > 1.8 mm = one week; 0.3–1.7 mm = one to two weeks; 0.0–0.2 mm = two to three weeks plus. In 12.5% of animals the yolk scar was healed at the time of sexing. The majority of such hatchlings were in the same size class as animals with a 0.1 mm yolk scar from the same batch: they were assigned an age of two to three weeks, but in a few cases their size, and
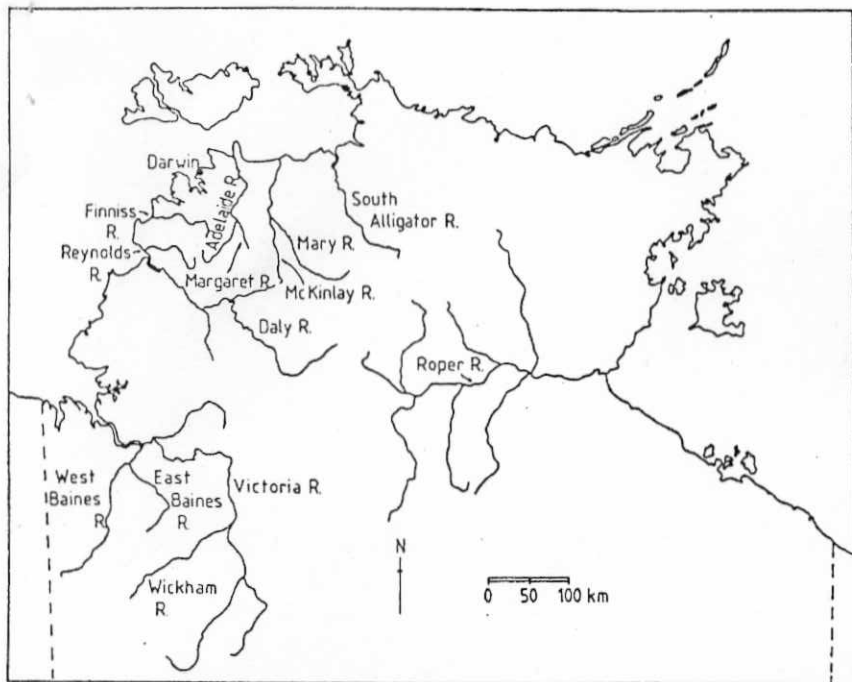
FIG. 4. The location of rivers in which *C. johnstoni* hatchlings were collected.

information from prior visits, were used to age them at three to four or four to five weeks.

Age Structure Analysis

General

Implicit within the population age structure (Fig. 1) is variation in sex ratio and survivorship (in the one area) over a number of years. In order to examine that variation we made the following assumptions:

(a) The aging method was sufficiently accurate for year class sex ratios up to nine years (on Fig. 1) to be accepted as realistic estimates of cohort sex ratio, if cohorts were represented by > 10 individuals. This assumption is supported, because the aging method was accurate to the month up to six years of age and contained few errors up to nine years. There is a high proportion (24%) of each population year class represented in the age structure. Using > 10 individuals effectively rendered one cohort sex ratio unusable (eight years).

It should be recognized that Fig. 1 is the 1979 age structure, whereas

that originally presented (Webb, Buckworth & Manolis, 1983a) was the 1978 age structure.

(b) Recaptured animals were a random sample of the population. This assumption is supported by: the capture method (fine nets) being random for most size groups; the high recapture rates achieved (61.5% of animals marked in 1978 were recaught once in two subsequent annual recapture efforts); the higher proportion (24%) of the population represented in the age structure; and the sex ratio of initial captures (0.33) in comparison to that of recaptures (0.31).

(c) That age-specific survivorship is an important variable affecting the size of cohorts on Fig. 1 (Caughley, 1976).

(d) That for the purposes of estimating age-specific survivorship, and examining environmental variables which may have had a significant influence on survivorship, the size of year classes up to 16 years could be considered correct. This assumption is supported by the age-specific survivorship curve being primarily dependent on the younger year classes, and by the normal distribution of aging errors up to 16 years. Very high or very low survivorship should be reflected, even in the less accurately aged year classes (10–16 years).

(e) That up to 16 years, sex-specific trends in mortality, emigration and immigration would be negligible compared to the extent of annual variation in the number and sex ratio of recruits. This assumption is supported by the recapture data, which indicated no significant sex-specific mortality or dispersal predisposition, and by the extent of annual variation in the number and sex ratio of hatchlings in one area (see p. 326).

Estimating the number and sex ratio of recruits in past years

On Fig. 1, each year class effectively represents the animals that have survived from the number of eggs laid in a particular year to two years of age, three years, and so on. If the population had been at equilibrium in the past, and the number of eggs laid each year could be assumed to have been more or less constant, those survivors could be plotted as the proportion of the number of eggs laid which survived to different years of age. The difference in survival proportion (*SP*) between, for example years 3 and 4, contains an estimate of age-specific mortality from three to four years of age.

To use this approach with the McKinlay River population, which has been recovering since protection, the number of eggs laid in past years (the number of mature females present) cannot be assumed to have remained constant, and needs to be estimated (more females have

been maturing than dying as the population was recovering and expanding). Animals > 11 years were assumed to be mature, and 1.35 females were assumed to have died each year for the previous 15 years (the mean number of animals per year class between 27 and 43 years on Fig. 1; animals mature at the time of protection). By recapitulating the age structure one year at a time and making an addition for dead animals, the number of mature females (> 11 years) in past years was estimated (Table I). It was assumed that 92.4% of these nested annually (the estimate was increased from the recorded 84.4% to account for younger females nesting), with a mean clutch size of 13.2 eggs. Annual egg production for each year was calculated (Table I), and survivorship from eggs to two, three, and up to 16 years of age (as discussed above) was calculated (Table I; Fig. 5). The relationship between SP from eggs to consecutive years (Y) was fitted to an exponential curve:

$$SP = 0.0505\,e^{-0.1632Y}\ (r^2 = 0.75;\ P < 0.001).$$

Seventy-five per cent of the variation in year class size between two and 16 years (on Fig. 1) could be accounted for by age-specific mortality. In the results, the extent to which the remaining variation can be attributed to environmental conditions during the relevant incubation periods is examined.

On the basis of Fig. 5, the SP to one year of age can be approximated for each year (Table II), by assuming the curve could be extrapolated to year 1 (two years was the youngest year class). However, it could not be extrapolated to zero, because mortality from zero (hatching) to one year is known to be much greater than that estimated by the curve, and may be equally variable. Accordingly, SP_1 reflects the combined egg to hatching and hatching to one year survivorship, and SP_0 (survival proportion to hatching) cannot be estimated.

RESULTS

Field Nests

Sex ratios from individual clutches (Fig. 6) paralleled constant temperature incubation results (Fig. 2), with a female bias in the fast and slow developing nests. The peak of the male bias (sex ratios near 1.0) was in nests taking 72–74 days to incubate. At constant temperature, this incubation time would be achieved at 32–33°C (Webb, Buckworth & Manolis, 1983d), which is similar to the male peak in Fig. 2. The

TABLE 1
Estimated egg production and survival proportions to increasing years of age

| Year of hatch	No. mature females still alive in 1979	Addition for dead females (1.35 per year)	Total no. of mature females per year	Estimated number of eggs produced	No. of offspring alive in 1979	Survival proportion to (N) years
1977	54	2.70	56.7	691.6	23	0.0347 (2)
1976	52	4.05	56.05	683.6	22	0.0322 (3)
1975	49	5.40	54.40	664.7	30	0.0451 (4)
1974	48	6.75	54.75	667.8	24	0.0359 (5)
1973	47	8.10	55.10	672.0	17	0.0253 (6)
1972	43	9.45	52.45	639.7	12	0.0188 (7)
1971	41	10.80	51.80	631.8	5	0.0079 (8)
1970	38	12.15	50.15	611.7	17	0.0278 (9)
1969	36	13.50	49.50	603.7	5	0.0083 (10)
1968	32	14.85	46.85	571.4	5	0.0087 (11)
1967	32	16.20	48.20	587.9	3	0.0051 (12)
1966	26	17.55	43.55	531.2	6	0.0113 (13)
1965	25	18.90	43.90	535.4	2	0.0037 (14)
1964	23	20.25	43.25	527.5	5	0.0095 (15)
1963	18	21.60	39.60	483.0	2	0.0011 (16)
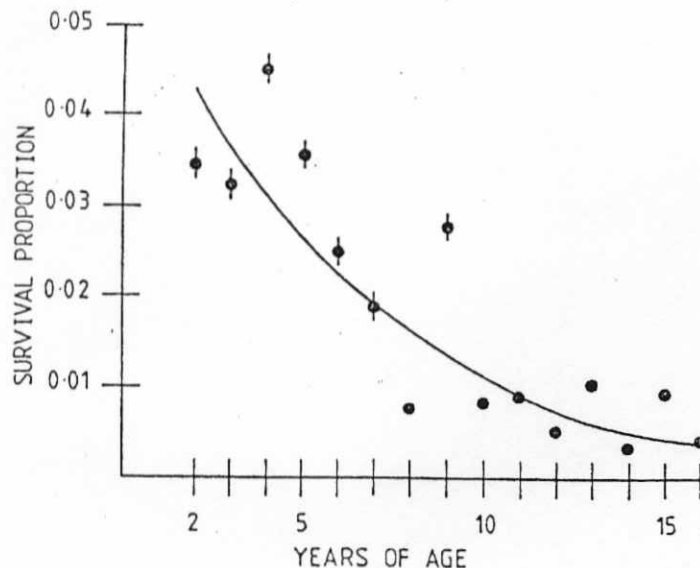
FIG. 5. The estimated proportion of eggs laid in previous years which are represented by survivors in subsequent years. Bars indicate the estimates for which there is also a sex ratio estimate.

distribution of clutches with regard to sex ratio (Fig. 7) was skewed towards females, and was not bimodal.

Females produced from the fastest developing clutches (65 days) were histologically similar to those from 34°C constant temperature incubation, although vacuolation of the medulla was not as pronounced. No animals with hermaphroditic tendencies were detected.

Within nests that produced both males and females, nest maps (Fig. 8) indicated a tendency for eggs producing either sex to be clumped, though not necessarily at the top or bottom of a nest. This is consistent with thermal or other gradients not necessarily being in the vertical plane.

With clutches lumped by mean incubation time (Fig. 9), survivorship of fertile eggs was reduced in the fast and slow developing nests, and within one group (76–79 days) in the middle of the range. The frequency of advanced dead embryos was distributed similarly.

Final clutch temperatures (T_f; 34.3 ± 1.8°C (SD); $N = 17$) were significantly higher than initial clutch temperatures (T_i; 30.2 ± 1.6°C; $N = 17$), and this difference was unchanged when both T_i and T_f were corrected (± 1°C) according to time of day at which they had been measured. T_i and T_f were not significantly correlated with each other ($r^2 = 0.003$) but the change in temperature ($\Delta T = T_f - T_i$) was predictable from T_i: $\Delta T = 34.2 - 1.0 T_i \pm 1.5$°C ($r^2 = 0.54$; $0.01 > P > 0.001$).

TABLE II

Water height (WH) and rainfall characteristics of previous years, the estimated survival proportion from eggs to one year of age and sex ratio. Numbers in brackets are based on samples of less than 10 individuals.

| Year of hatch	Estimated survivorship to one year of age	Sex ratio	Maximum Aug. WH (m)	Maximum Sept. WH (m)	Maximum Oct. WH (m)	WH Oct.–Aug. (m)	Total October rainfall	Total October raindays
1977	0.041	0.42	0.74	0.69	0.67	−0.07	12	3
1976	0.045	0.45	0.80	0.76	0.74	−0.06	18	2
1975	0.074	0.30	0.71	0.68	1.43	0.72	137	7
1974	0.069	0.46	0.93	1.00	1.63	0.70	57	4
1973	0.053	0.29	0.57	0.27	0.60	0.03	27	6
1972	0.050	0.33	0.47	0.49	0.29	−0.18	31	4
1971	0.025	(0.40)	0.03	0.01	1.69	1.66	145	12
1970	0.103	0.12	0.20	0.02	1.42	1.22	102	7
1969	0.036	(0.20)	0.72	0.61	2.66	1.94	74	10
1968	0.045	(0.20)	—	0.88	0.69	—	54	7
1967	0.031	(1.00)	—	—	—	—	14	3
1966	0.080	(0.00)	0.70	—	—	—	65	6
1965	0.031	(0.00)	0.95	0.91	0.91	−0.04	27	2
1964	0.093	(0.40)	0.93	0.91	1.70	0.77	143	7
1963	0.047	(0.50)	0.95	0.91	0.91	−0.04	64	6
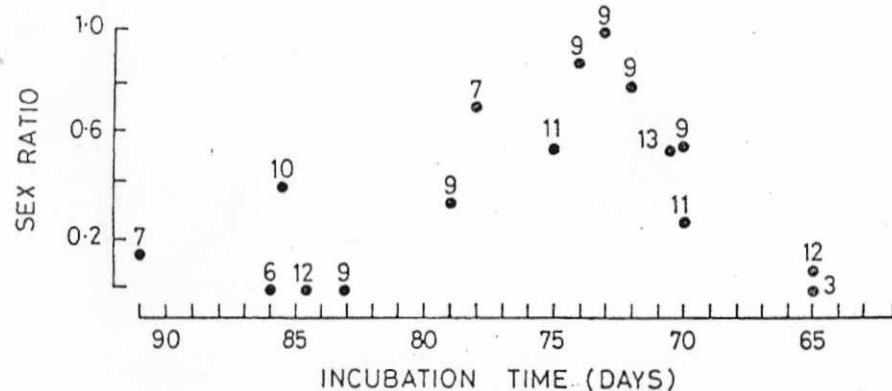
FIG. 6. Sex ratios of wild *C. johnstoni* clutches as a function of their mean incubation time. Numbers are the number of individuals in each clutch which could be sexed.
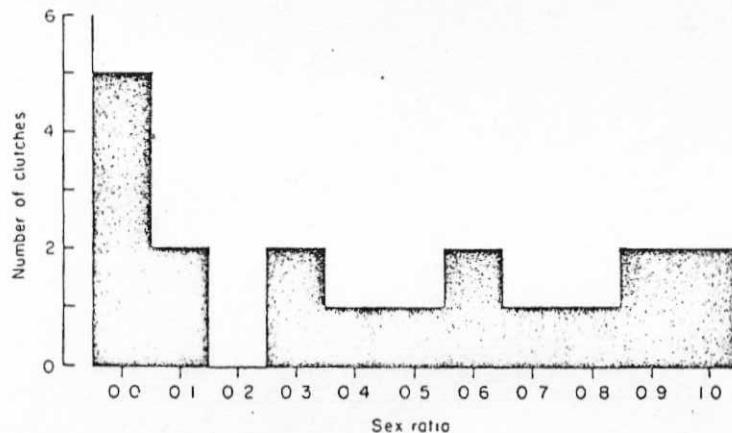

FIG. 7. Frequency distribution of clutch sex ratios for 19 *C. johnstoni* nests.

This indicates that independent of the range of T_i's, temperatures would tend to standardize at the same T_f's.

When nests were lumped according to T_i and T_j (Fig. 10), some trends in sex ratio and survivorship were apparent:

(i) Sex ratio was normally distributed with T_i, which is consistent with T_i being measured closer to the time that sex was actually determined.

(ii) The most commonly selected T_i range (87 viable eggs; 47%) was 30.0–30.9°C, which corresponded with a sex ratio of 0.49.

(iii) High and low T_i's gave a pronounced female bias, and only one T_i range (31.0–31.9°C) gave a male bias (0.60; 17% of viable eggs).
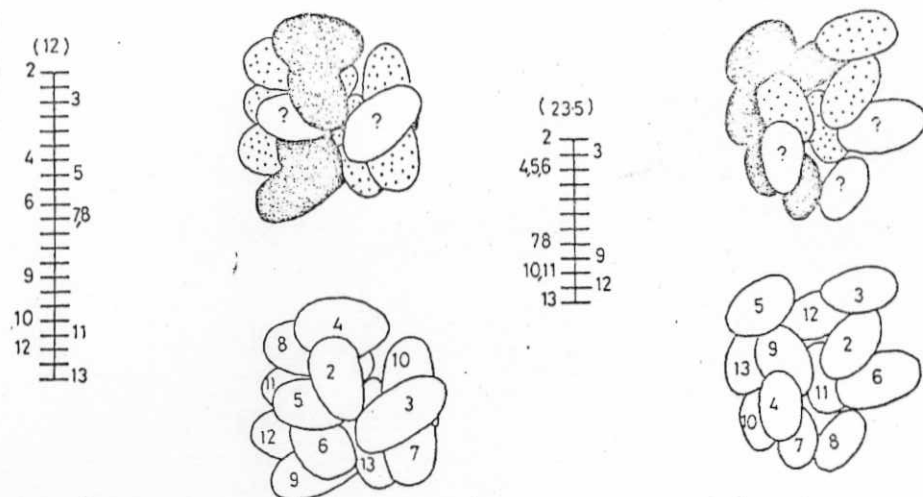
FIG. 8. Maps showing the distribution (when seen from above) of eggs with male (black) and female (dots) embryos. The number in brackets is the distance between the substrate surface and the top mid-point of the highest egg (egg 1 was removed for aging), and the vertical scale (5 mm divisions) shows the vertical distribution of eggs relative to each other. The nests took 85–86 days (left) and 70 days (right) to incubate. '?' indicates where embryos had died at a young age, and/or eggs were infertile.

(iv) The association between survivorship and T_i was the opposite to that between survivorship and T_f, if the five animals with T_i's in the 29.0–29.9°C category are considered anomalous.

(v) Minimum survivorship associated with T_i and T_j both corresponded with sex ratios strongly skewed towards females.

These results indicate that more animals selected nests in the T_i range 30.0–30.9°C than any other, and gained high survivorship and a nett sex ratio near 0.5. The normal distribution of temperatures selected around that mean resulted in most T_i's giving high survivorship, the exception being 34.0–34.9°C (female biased); in these, mortality probably reflects the effect of high temperature on young, rapidly developing embryos. Because T_i is independent of T_f, a range of T_i's could theoretically be utilized without compromising survivorship associated with T_f, but to shift by +1°C the normal distribution of T_i's selected would place even more animals in the critical range.

Few, if any, of the nests selected exceeded T_f values which were lethal to the advanced embryos: nests with the highest T_f's had maximal survivorship. In contrast, low survivorship tended to be associated with low T_f (mainly females). This trend is consistent with advanced embryos needing high temperatures to optimize growth and yolk utilization, both of which are impaired at low temperatures (unpublished data). It
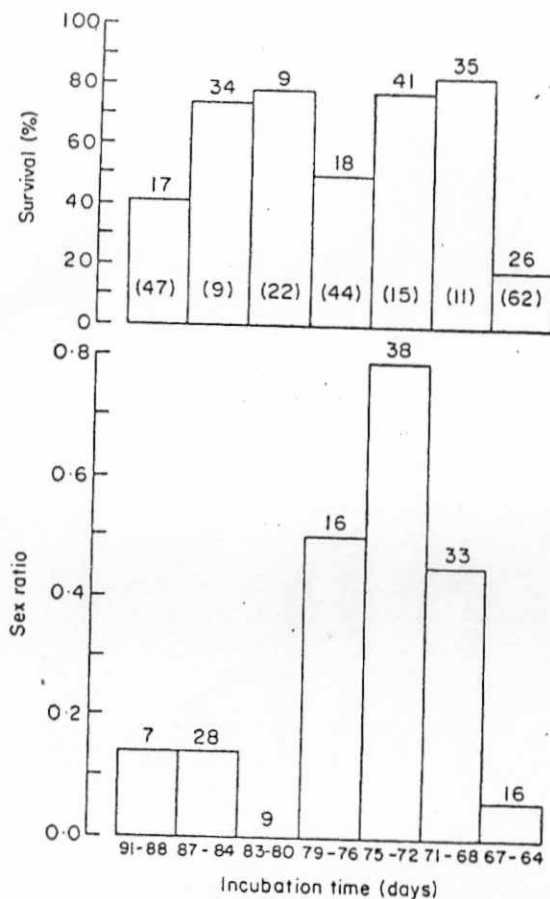
FIG. 9. The relationship between incubation time, sex ratio, and the percentage of viable eggs which survived in wild *C. johnstoni* nests. Upper: numbers are the numbers of viable eggs, and the percentage of viable eggs which contained advanced dead embryos (in parentheses). Lower: numbers are the numbers of individuals sexed.

is also consistent with nests being so selected that independent of T_i, they have a high T_f.

Temperature also affects survivorship of embryos through its influence on development rate, and thus time of hatching relative to the probability of there being a flood. From the nests studied 59 males and 67 females hatched successfully and were considered potential survivors (sex ratio = 0.47). As in *Graptemys* spp. (Vogt & Bull, 1982) there was a tendency for the fastest developing nests to hatch earliest, with males and high temperature females tending to hatch before lower temperature females. The greatest disparity in the hatching of the total complement of each sex was during 8–22 November (Fig. 11), which
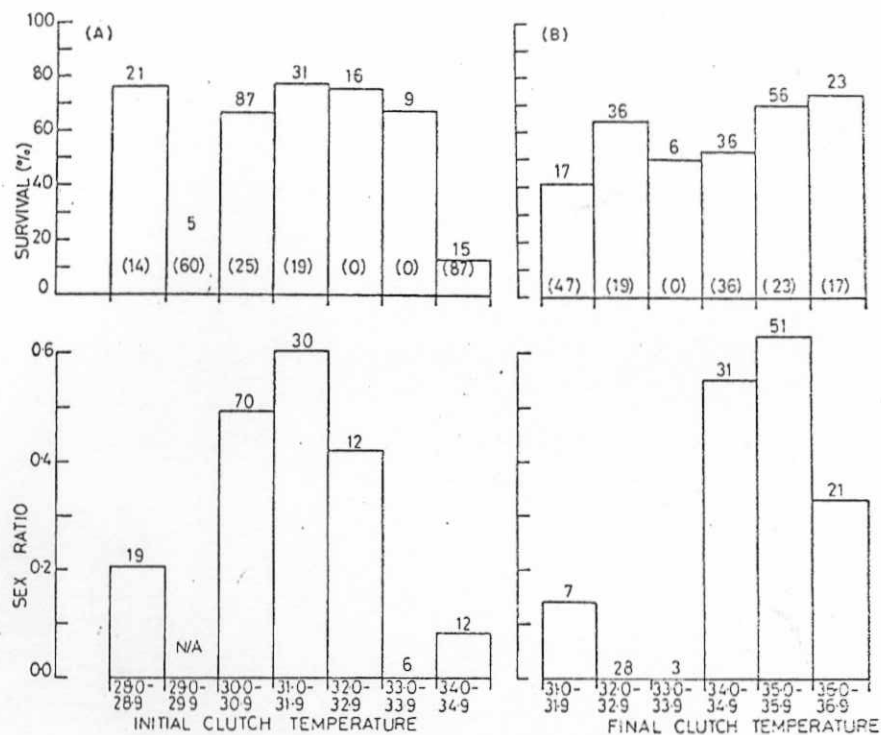

FIG. 10. The relationship between individual (A) and final (B) clutch temperatures, and the sex ratio and percentage survivorship of viable eggs. Upper: numbers are the number of viable eggs and the percentages of viable eggs which contained advanced dead embryos (in parentheses). Lower: numbers are the numbers of individuals sexed.
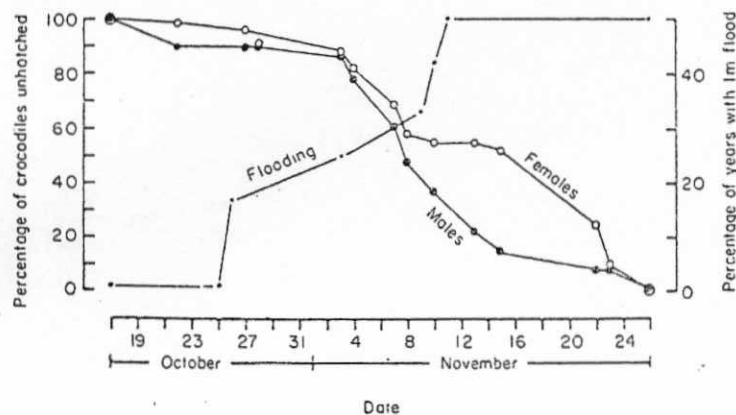

FIG. 11. The percentage of the total complement of male and female *C. johnstoni* from the field nests being monitored, which had hatched at different times, related to the increasing probability of there being a 1 m flood to inundate nests.

corresponded with an increased probability of flooding (based on 12 years' data).

The absolute height of clutches above water level at the time of laying in one part of the McKinlay River was 56 ± 18 cm ($N = 24$). This level varies within the system, as does absolute water height. Thus, when a 1 m water rise is recorded in one part of the study area, the actual rise in specific ponds varies around this recording. Nevertheless, such a water rise does inundate many nests, thereby drowning a greater proportion of females than males (no flood occurred during the study year).

Summarizing, the field results demonstrate:

(i) Nest temperatures affect *C. johnstoni* sex determination in the field in the same way that constant temperatures influence embryonic sex determination in laboratory incubators.

(ii) Nest temperatures affect embryo survivorship. There is a direct effect, which probably reflects the influence of temperature on developing embryos of different ages, and an indirect effect, mediated through development rates, the time of hatching and the probability of flooding.

(iii) The effects of nest temperature on sex determination and survivorship are not independent. Embryos most likely to die are females, and consequently, increased survivorship is likely to be associated with decreased sex ratios.

(iv) Within the range of temperatures where survivorship is not compromised, intitial and final nest temperatures are independent. However, the temperatures selected are normally distributed, and shifting the mean (for any reason) would shift that distribution. Thus, unless the thermal environment changed, a greater proportion of eggs would exceed lethal limits if the mean nest temperature was increased or decreased.

Geographic Variation

The time of hatching (Table III), the rate at which the total complement of hatchlings appeared (Table IV), and the sex ratio of hatchlings (Table V), varied between rivers. The following trends existed:

(i) Hatching in the south was later than in the north or central areas (Table III); nesting is also later (unpublished data).

(ii) The proportion of the total complement of hatchlings which appeared in the first week was higher in the south (40.4%; Table IV) than in the north (29.1%) or central (9.1%) areas.

(iii) The sex ratio of hatchlings was higher in the south (0.40) than in either the north (0.31) or central (0.31) areas.

In all rivers, sex ratio varied as a function of week of hatch (Table V).

TABLE III
The percentages of the total number of hatchlings from each river which hatched in different weeks

Area (no. of hatchlings)	River	No. hatchlings per river	Oct. 23–29	Oct. 30–Nov. 5	Nov. 6–12	Nov. 13–19	Nov. 20–26	Nov. 27–Dec. 3	Dec. 4–10	Dec. 11–17
North (795)	South Alligator	34	—	—	—	—	97.0	—	3.0	—
	Mary	629	—	8.9	19.4	39.9	5.4	23.1	3.3	—
	McKinlay	63	6.3	34.9	41.3	9.5	7.9	—	—	—
	Margaret	36	—	2.8	50.0	47.2	—	—	—	—
	Adelaide	33	—	—	—	30.3	66.6	3.0	—	—
Central (1858)	Finniss	337	—	—	22.9	43.0	22.3	8.3	3.6	—
	Reynolds	222	—	—	1.7	12.9	41.0	28.2	14.0	2.2
	Daly	1299	0.9	17.0	28.1	41.2	7.9	3.9	1.1	—
South (1916)	Roper	163	—	—	—	—	59.5	30.7	9.8	—
	Victoria	1134	—	—	1.7	12.9	41.0	28.2	14.0	2.2
	Wickham	235	—	—	2.1	2.1	13.6	44.3	26.4	11.5
	Wickham/Victoria	83	—	—	—	15.7	39.8	14.5	28.9	1.2
	East Baines	50	—	—	—	—	74.0	18.0	8.0	—
	West Baines	211	—	—	—	—	77.3	21.8	1.0	—
	E/W Baines	40	—	—	—	—	52.5	40.0	7.5	—
North	5 rivers	—	1.3	9.3	22.1	25.4	35.4	5.2	1.3	—
Central	3 rivers	—	0.3	5.6	18.2	35.4	18.3	20.4	1.6	0.2
South	7 rivers	—	—	—	0.5	4.4	51.1	28.2	13.7	2.1
North and Central	8 rivers	—	0.9	8.0	20.7	29.2	29.0	10.9	1.4	0.1
All areas (4569)	15 rivers	—	0.5	4.2	11.3	17.6	39.3	19.0	7.1	1.0

TABLE IV
The percentages of the total number of hatchlings from each river system which hatched in consecutive weeks relative to the first week of hatch in each area

Area	River	No. of hatchlings	Week of hatch relative to first hatchlings appearing						
			1	2	3	4	5	6	7
North	South Alligator	34	97.0	—	3.0	—	—	—	—
	Mary	629	8.9	19.4	39.9	5.4	23.1	3.3	—
	McKinlay	63	6.3	34.9	41.3	9.5	7.9	—	—
	Margaret	36	2.8	50.0	47.2	—	—	—	—
	Adelaide	33	30.3	66.6	3.0	—	—	—	—
Central	Finniss	337	22.9	43.0	22.3	8.3	3.6	—	—
	Reynolds	222	3.6	22.1	24.8	49.1	—	2.2	—
	Daly	1299	0.9	17.0	28.1	41.2	7.9	3.9	1.1
South	Roper	163	59.5	30.7	9.8	—	—	—	—
	Victoria	1134	1.7	12.9	41.0	28.2	14.0	2.2	—
	Wickham	235	2.1	2.1	13.6	44.3	26.4	11.5	—
	Wickham/Victoria	83	15.7	39.8	14.5	28.9	1.2	—	—
	East Baines	50	74.0	18.0	8.0	—	—	—	—
	West Baines	211	77.3	21.8	1.0	—	—	—	—
	E/W Baines	40	52.5	40.0	7.5	—	—	—	—
North	—	795	29.1	34.2	26.9	3.0	6.2	0.7	—
Central	—	1858	9.1	27.4	25.1	32.9	3.8	1.5	0.4
South	—	1916	40.4	23.6	13.6	14.5	5.9	2.0	—
North and Central	—	2653	21.6	31.6	26.2	14.2	5.3	1.0	0.1
All areas	—	4569	30.4	27.9	20.3	14.3	5.6	1.4	0.1

The sex ratio of hatchlings from different areas of the Northern Territory as a function of the time that first hatchlings appeared

Area	River	No. hatchlings sexed	Total sex ratio	Week of hatch relative to first hatchlings appearing						
				1	2	3	4	5	6	7
North	South Alligator	31	0.12	0.09	—	1.00*	—	—	—	—
	Mary	629	0.31	0.57	0.30	0.42	0.26	0.09	0.0	—
	McKinlay	60	0.25	0.25*	0.37	0.19	0.00*	—	—	—
	Margaret	36	0.44	1.00*	0.44	0.41	0.00*	—	—	—
	Adelaide	33	0.45	1.00	0.23	0.00*	—	—	—	—
Central	Finniss	337	0.29	0.45	0.23	0.28	0.21	0.17	—	—
	Reynolds	220	0.23	0.38*	0.23	0.20	0.24	—	0.00*	—
	Daly	1292	0.40	0.55	0.48	0.53	0.34	0.21	0.18	0.00
South	Roper	163	0.20	0.10	0.46	0.06	—	—	—	—
	Victoria	1134	0.34	0.32	0.44	0.41	0.26	0.27	0.00	—
	Wickham	235	0.36	1.00*	0.80	0.78	0.26	0.10	0.00	—
	Wickham/Victoria	83	0.25	0.38	0.36	0.25	0.43	0.00*	—	—
	East Baines	50	0.58	0.68	0.41*	0.00*	0.04	—	—	—
	West Baines	210	0.61	0.63	0.53	1.00*	—	—	—	—
	E/W Baines	40	0.48	0.80	0.13	0.00*	—	—	—	—
All North mean	< 5 rivers	—	0.31	0.55 (3)	0.31 (4)	0.34 (3)	0.26 (1)	0.09 (1)	0.00 (1)	—
All Central mean	< 3 rivers	—	0.31	0.50 (2)	0.31 (3)	0.34 (3)	0.26 (3)	0.19 (2)	0.18 (1)	0.00 (1)
All South mean	< 7 rivers	—	0.40	0.49 (6)	0.38 (5)	0.38 (4)	0.24 (3)	0.19 (2)	0.00 (2)	—
North and Central mean	< 8 rivers	—	0.31	0.53 (5)	0.33 (7)	0.34 (6)	0.26 (4)	0.16 (3)	0.09 (2)	0.00 (1)
Total mean	< 15 rivers	—	0.35	0.51 (11)	0.35 (12)	0.35 (10)	0.25 (7)	0.17 (5)	0.05 (4)	0.00 (1)

*Sample size < 10, in which case not included in totals.
Numbers in parentheses are the number of rivers included in each computation.

In most rivers, highest sex ratios occurred early in the hatching period, and as hatching progressed, sex ratios became more and more female-biased.

The South Alligator and Roper Rivers were notable exceptions. Sex ratios in the first week of hatch (0.09 and 0.10 respectively) were strongly female biased, even though 97% and 59.5% respectively of the total hatchling complement appeared in the first week (Table IV). The small number of animals collected reflects a paucity of hatchlings in these areas, and not a lack of catch effort.

As in the field nests (Fig. 11), variation in sex ratio and the time and rate of hatching was reflected in a disparity in the rate at which the total complement of males and females hatched (Fig. 12). The same pattern characterized most rivers (it was the opposite in the South Alligator and Roper), and indicates that as the probability of flooding increases (as it does throughout the period), the probability of female survivorship decreases at a greater rate than does that of males.

Age Structure Analysis

Sex ratio

Variation in cohort sex ratio (SR) could be examined using only seven year classes (Table II; Fig. 5). There was a highly significant trend for sex ratio to decrease (more females) with decreasing water levels at the times of nesting (August maximum water height: WH_{Aug}) (Fig. 13; $SR = 0.05 + 0.45\ WH_{Aug}$; $r^2 = 0.85$; $P = 0.003$), indicating that when water levels were low the nest sites selected (available?) had been more female-biased.

There was also a significant trend for sex ratio to decrease with increasing survival proportion from egg-laying to one year of age (SP_1) (Fig. 14; $SR = 0.60 - 4.14\ SP_1$; $r^2 = 0.56$; $P = 0.05$). Proportionally more females were associated with increased survivorship.

Multiple regression analysis predicting sex ratio from both WH_{Aug} and SP_1 explained a high proportion of the variation in sex ratio ($r^2 = 0.93$; $P = 0.005$), with both variables acting in the directions indicated on Figs 13 and 14 ($SR = 0.23 + 0.36\ WH_{Aug} - 1.92\ SP_1$; r^2 addition due to $SP_1 = 0.08$; $P = 0.09$). However, the relationship between SR and WH_{Aug} rendered the relationship between SR and SP_1 statistically insignificant; the relationship may be an artefact.

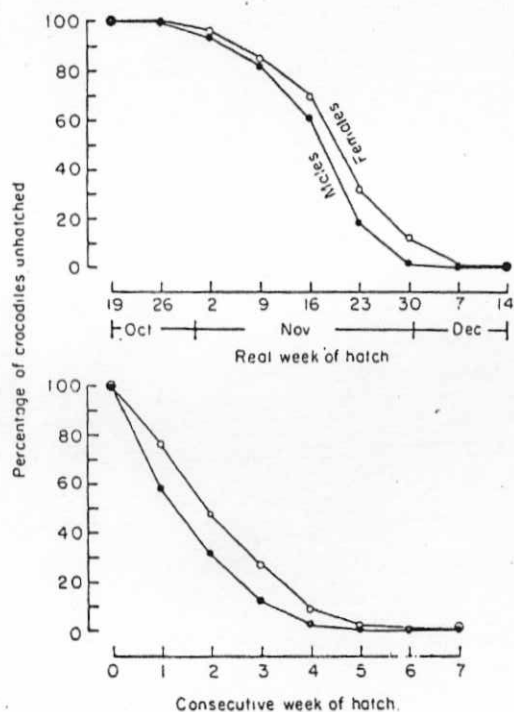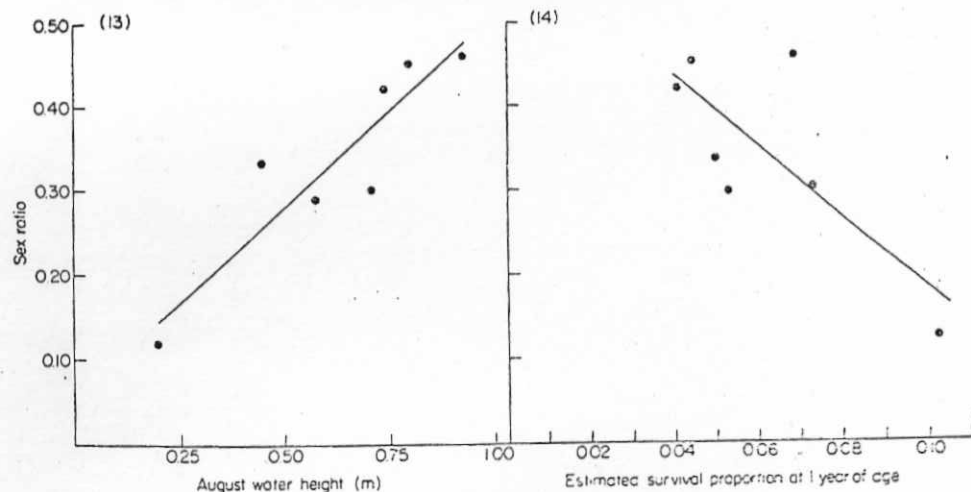Although tested, no significant relationship between sex ratio and

FIG. 12. The percentage of the total complement (4556) of male and female *C. johnstoni* examined in the geographic variation study, which hatched at different times (upper), and when related to the first week in which hatchlings appeared in each area (lower).

FIGS 13 & 14. (13) The relationship between recruit sex ratio and water height at the time of nesting, over seven years in the McKinlay river area. (14) The marginally significant trend ($P = 0.053$) between sex ratio and the number of one-year-olds (expressed as the estimated proportion of eggs laid which produced the one-year-old survivors), over seven years in the McKinlay River area.

monthly mean temperatures, nor the time of + 1 m floods, could be demonstrated. September maximum water height was highly correlated with WH_{Aug} and was thus similarly correlated with sex ratio, but no significant relationship between October or November water height (hatching) and either sex ratio or WH_{Aug} was apparent.

Survivorship

Using up to 15 years' data (Table II), an attempt was made to determine whether or not the variation in SP not already accounted for by age-specific mortality was attributable to environmental conditions during the incubation periods of each cohort (Fig. 5; age-specific mortality accounted for 75% of the variation in SP to consecutive years). The data on Fig. 5 were linearized by logarithmic transformation of SP ($\ln SP$), and multiple regression analysis used to predict $\ln SP$ from the major variable, age (or years; Y), plus combinations of the temperature, water height and rainfall data from the incubation periods of each year class.

The number of days in which it rained in October (RD) and the change in water level between nesting and hatching (October–August water heights; ΔWH) were highly correlated with each other, and were the only variables which accounted for a significant proportion of the unexplained variation in $\ln SP$. (October rain days: $\ln SP = -3.59 - 0.17Y + 0.33RD - 0.03RD^2$; $r^2 = 0.93$, $P < 0.001$; r^2 addition attributable to $RD = 0.17$; $P = 0.005$) (change in water height; $\ln SP = -3.59 - 0.17Y + 0.96\,\Delta WH - 0.43\,\Delta WH^2$; $r^2 = 0.90$; $P < 0.001$; r^2 addition attributable to $\Delta WH = 0.14$; $0.05 > P > 0.02$.)

In both cases, the relationship between RD, ΔWH and SP was parabolic. To demonstrate the trend graphically, the influence of age-specific mortality was removed (standardized) by plotting the relationship of SP_1 and ΔWH (Fig. 15; $SP_1 = 0.050 + 0.080\Delta WH - 0.048\Delta WH^2$; $r^2 = 0.65$; $0.05 > P > 0.01$).

This trend suggests that rain (water rises?) at or near hatching had enhanced survivorship (from egg-laying to one year of age), but that in the years where there was heavy rain (flooding?) survivorship had been reduced. It should be recognized that the seven years for which sex ratio and survivorship could be examined (Fig. 14) did not include the years with intense flooding on Fig. 15.

Decreased survivorship associated with heavy rains (Fig. 15) is probably a reflection of catastrophic nest inundation, and reduced survivorship of eggs. However, increased survivorship with rain and water level rises up to 1 m appears in direct contradiction to the known situation in the field, i.e. a + 1 m rise would inundate many eggs (the mean height of eggs above water level in one area was 56 ± 18 cm).
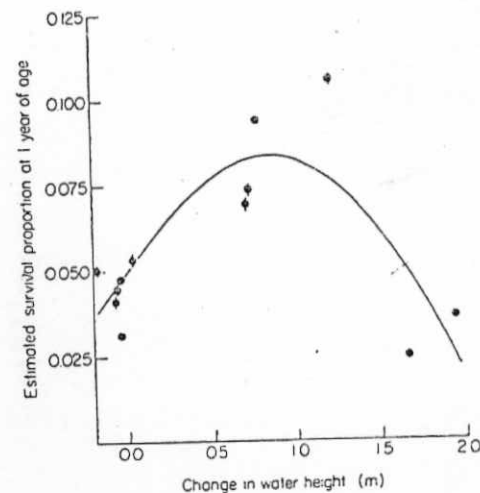
FIG. 15. The relationship between the number of one-year-old survivors (expressed as the proportion of eggs laid which produced them) and the change in water height between laying and hatching. The same parabolic trend exists with the number of rain days at or near hatching.

Two explanations, not mutually exclusive, suggest themselves, and both are dependent on the extent to which SP_1 reflects survivorship from eggs to hatching, or from hatching to one year of age (insufficient data are available to separate these two periods).

(i) Rains at the time of hatching may enhance survival of embryos destined to either die in eggs or hatch abnormally (as a result of desiccation and/or overheating), and this overcompensates for concurrent losses due to flooding.

(ii) Independent of survivorship from eggs to hatching, hatchling survivorship is greatly enhanced by early rains, the subsequent abundance of food, and availability of wet habitats. This survivorship overcompensates for embryo losses due to flooding.

Given the relationship between survivorship and high clutch temperatures at the time of hatching (Fig. 10), the former interpretation seems less likely than the latter.

Summarizing, the results of the age structure analysis demonstrate:

(i) A strong trend for sex ratio to decrease with water height at the time of egg-laying.

(ii) A weak trend for increased survivorship from egg-laying to one year old to be associated with increased survival of females, probably in the egg stage [(i) could render this insignificant].

(iii) Reduced survivorship, probably of eggs, with heavy rains at hatching.

(iv) Increased survivorship, probably of hatchlings rather than eggs, with some rain (flooding < 1 m) at the time of hatching.

DISCUSSION

Temperature-dependent Sex Determination

The mechanism by which incubation temperature determines sex in reptiles is unknown (Bull, 1980; Ferguson & Joanen, 1982, 1983), and it remains unclear whether temperature *per se* is an ultimate or proximate sex determining factor. The distinction is important, because results presented to date are consistent with sex being determined as a consequence of embryo development rates, the influence of temperature being pronounced because of its profound influence on development rate (at 26°C, *C. johnstoni* embryos develop 2.1 times more slowly than at 30°C; Webb, Buckworth & Manolis, 1983d; Webb, Buckworth, Manolis & Sack, 1983). If development rate and embryonic metabolic levels are indeed more proximally related to sex determination than is temperature, then factors such as the gaseous (Ackerman, 1980) and moisture (Packard, Packard, Boardman & Ashen, 1981; Morris *et al.*, 1983) environment of nests (which influence development rates) could also be expected to influence sex determination (Gutzke & Paukstis, 1983). Although perhaps minor variables compared to temperature, their existence could explain some experimental results.

When incubated at a constant temperature at or near the threshold temperature, there is a tendency for eggs of some clutches to be either male- or female-biased (Bull, Vogt & Bulmer, 1982; unpublished data on *C. johnstoni*). If temperature *per se* is the direct determiner of sex, then such results indicate a genetic predisposition to one sex or the other (Bull, Vogt & Bulmer, 1982). However, if development and metabolic rates are involved, such a finding could reflect minor inter-clutch variation in shell porosity, egg size or other factors likely to influence gas and moisture exchange; consequently, there are a number of pathways through which the variation could be environmentally rather than genetically determined.

A further implication is that shift experiments spanning the same temperature range (34–30°C; 30–26°C), or directed at the same base temperature (34°C and 32°–30°C), may not induce metabolic or development rate responses linearly related to temperature. Acclimation periods in which development rate stabilizes at a new temperature

could be expected to be proportional to the extent of exposure to the initial temperature, the temperature gradient of the switch, and the absolute temperatures involved.

Nevertheless, temperature clearly does have a profound effect on both development rate and sex determination, and is the most important variable involved.

The relationship between incubation temperature and sex in *C. johnstoni* is unusual. The species has two threshold temperatures, and it is the 'high' temperature females that appear limited by temperature-related survivorship constraints. In the few turtles with two thresholds (*Chelydra serpentina*, *Macroclemys* sp., *Kinosternon* sp., *Sternotherus odoratus*; Yntema, 1976, 1979; Bull, 1980; Vogt *et al.*, 1982; Wilhoft, Hotaling & Franks, 1983) males are bounded by females, as in *C. johnstoni*, but it is the production of 'low' temperature females that is constrained by temperature-related survivorship.

It may not be coincidental that among species with single threshold temperatures, turtles produce males at low temperatures and females at high temperatures, whereas crocodilians and lizards do the reverse (Bull, 1980; Mrosovsky, 1980; Miller & Limpus, 1981; Ferguson & Joanen, 1982, 1983; unpublished data on *C. porosus*). If the one mechanism of sex determination characterizes reptiles with TSD, then the potential of a two-threshold pattern may exist in all reptiles with TSD. Survivorship constraints may tend to eliminate low temperature female turtles and high temperature female crocodilians and lizards.

Differential development of the gonad in relation to other organs may be involved in the mechanism of TSD. The embryonic stage at which sex is determined itself depends on temperature (or development rate), and with the exception of *Chelydra serpentina* (Yntema, 1979), the relationship operates in the opposite direction to what would perhaps be expected. At higher incubation temperatures (when general embryonic development is enhanced), sex is determined in later embryonic stages (Bull & Vogt, 1981; Ferguson & Joanen, 1983). In *C. porosus*, for example (unpublished data), sex is determined at 20–25 days at 30°C (0.8 g embryo in an average egg), and 40–45 days (30°C equivalent ages; 7 g embryo) at 32°C.

In *A. mississippiensis* (M. W. J. Ferguson, pers. comm.) and *C. johnstoni* (this study), as incubation temperature is increased, so gonadal development appears more and more retarded in relation to the external morphology of the embryo. *Crocodylus johnstoni* females incubated at high temperatures appear to have, at hatching, an ovary severely retarded in relation to that of females incubated at 30°C (Fig. 3). Asynchrony in gonadal development opens a number of pathways that could lead to a

two-threshold pattern, especially if "female" is something of a sex-by-default and synchrony of development between the gonad and some other organ system is needed to induce "maleness". It also opens a number of pathways through which constraints on survival could operate. In *C. johnstoni*, the conditions resulting in "high" temperature females also lead to development so rapid that survival may be threatened. Selection against such extremes could result in the single-threshold pattern found in other crocodilians (low temperature females – high temperature males).

That selection can act on threshold temperatures is indicated by a comparison of turtle populations from the northern and southern United States (Bull, Vogt & McCoy, 1982). Threshold temperatures were slightly lower in the southern populations (about 1°C in *Chrysemys picta*), although ambient temperatures were significantly higher. This result would be expected if the higher ambient temperatures decreased the probability of surviving, so that sites which were cooler (at the time of sex determination) were needed in order to avoid lethal temperatures, even though these may occur at or near hatching. Without selection on the threshold temperature, a male bias (relative to northern populations) would be expected, and sex ratio selection would act to reduce the threshold, as was observed.

Sex Determination and Survivorship in the Field

The laboratory and field results with TSD complement each other quite closely, although under constant temperature incubation, few males were produced at any temperature. In this regard, *C. johnstoni* differs from *C. porosus* (unpublished data from the same incubators), *A. mississippiensis* (Ferguson & Joanen, 1982, 1983), and most turtles which have been examined (Bull & Vogt, 1979; Bull, 1980; Vogt & Bull, 1982) (all commonly give one sex or the other). The difference could reflect a stable versus a fluctuating incubation temperature (Wilhoft *et al.*, 1983), or perhaps the precision with which we could control incubation temperature. Additional studies are needed to clarify the issue. *Crocodylus johnstoni* clutch sex ratios in the field (Fig. 7) were also atypical when compared to other species; as a consequence of having two thresholds, rather than one, for sex determination, there was no pronounced bi-modality (Bull & Vogt, 1979; Bull, 1980; Ferguson & Joanen, 1982, 1983).

The temperatures of nests selected by females influenced both survivorship and sex ratio of the offspring. If it is accepted that surviving involves stronger selection than producing an optimal sex ratio, then it is quite possible that the range of nest temperatures selected by *C. johnstoni* is based primarily on survivorship considera-

tions. This hypothesis is testable, because it predicts that there would be no significant difference in the range of nest temperatures selected by two similar oviparous reptiles, one with genotypic and one with environmental sex determination.

When *C. johnstoni* nest temperatures are considered purely from the viewpoint of temperature-dependent survivorship, the species can be considered as having a preferred initial nest temperature (PT_i; 30.2 ± 1.6°C), which is 4–5°C below an initial lethal maximum temperature. An initial lethal minimum temperature exists, but is probably below the minimum temperatures available at the time of nesting in our particular study area. More important may be survival constraints associated with minimal temperatures delaying the time of hatching. If the distribution of temperatures around the PT_i reflects the variance or precision with which *C. johnstoni* of different sizes can or do select an optimal temperature, the consequences of raising or lowering PT_i are that a higher proportion of animals will surpass the inital lethal maximum, or die in floods.

That the PT_i corresponds with a sex ratio near 0.5 could be coincidental, but if not, it may indicate that sex ratio selection is constrained by survivorship considerations to the extent that synchronization represents the best compromise. If so, changes in PT_i associated with survivorship may be a prerequisite to changes in the threshold temperature/s.

Crocodylus johnstoni sex is determined up to a month after a nest (and thus nest temperature) has been selected, and accordingly the relationship between PT_i and the threshold temperatures must be predictive. Changes in time of nesting and the availability of nest sites could accordingly influence sex ratio, even though the same PT_i was chosen. For example, in a year with average water height, nests with a mean PT_i of 30.4°C may increase to 32.0°C, and a 0.5 sex ratio may result. However, if water levels were low at the time of nesting (Fig. 13), and the same PT_i only increased to 31.0°C (perhaps because the nest moisture levels, which affect temperature, remained more constant) a female bias would result, even though adults had passively nested in their normal manner.

If the relationships described above are correct, geographical variation in ambient temperatures and/or the rainfall/flood height patterns, should be reflected in significant sex ratio variation. Given that threshold temperatures tend to be conservative (Bull, Vogt & McCoy, 1982), PT_i may well vary substantially. The variation in sex ratio and time of hatching we found (Tables III, IV & V) were predictable on the basis of ambient temperatures.

Mean monthly 09.00 hours temperatures from the northern, central

and southern areas respectively are: June, 22.8°C, 20.8°C, 20.6°C; July, 21.5°C, 20.3°C, 19.7°C; August, 24.3°C, 22.6°C, 22.5°C; September, 26.9°C, 26.0°C, 26.4°C; October, 28.5°C, 28.2°C, 29.5°C; and November, 29.1°C, 28.9°C, 30.5°C. Temperatures are cooler in the south during the period when ovulation occurs in the north (June, July), and this could be expected to retard nesting (Joanen & McNease, 1979). Sex in southern *C. johnstoni* would be determined in October rather than September, when temperatures in any one area are higher. In addition, October and November temperatures in the south are higher than in the north and central areas, and thus it is not surprising that more males were produced.

There is generally less rainfall in the south (860 mm versus 1400 mm annually), and the wet season occurs up to two weeks later (although data with which flood régimes in different rivers could be compared realistically were unavailable).

It was concluded before data analysis (i.e. during the period when hatchlings were being collected), that the South Alligator and Roper Rivers had been subjected to intense illegal poaching. Subsequently, poachers were apprehended, although exactly where they had been operating was not ascertained. In both rivers, the first week of hatch was associated with a pronounced female bias (Table V), irrespective of high proportions of the total complement of hatchlings appearing (Tables III & IV). The total hatchling sex ratios in these rivers (0.12 and 0.20 respectively) were the lowest recorded.

In the McKinlay River area, a similar reduction in density occurred during the hunting period (1960–1963), and within the limits of the age structure accuracy, we tried to determine whether or not there was any indication of a sex ratio bias at that time. August water heights in those years were used to predict sex ratios, and even when the animals on either side of that period were lumped, more females were present than were predicted. In addition, sex ratios in the most recent years are well above the population mean (Tables I & II), although no pronounced sex-specific mortality is known. If sex ratio in the McKinlay River has changed since protection, it has been steadily increasing from a female bias at the time of protection.

When it is considered that the time of nesting results partly from social ordering of animals (large or old females nest first: Webb, Buckworth & Manolis, 1983d), reduced densities could allow survivors to nest earlier, when it was cooler, regardless of their age or size. Alternatively, survivors from an intense culling may be wary animals, perhaps located and nesting in more vegetated and secluded sites.

Theoretical Considerations

TSD and its consequences in terms of variable primary sex ratios raise a number of theoretical problems (Charnov & Bull, 1977; Bull, 1980, 1981a, b; Bulmer & Bull, 1982; Ferguson & Joanen, 1982, 1983). That a diploid species has TSD rather than GSD is not in itself inconsistent with sex ratio/sex allocation models of Fisher (1930) and later workers (see Charnov, 1982). But as TSD often results in offspring sex ratios that are consistently and markedly skewed from 0.5 at the end of parental investment, a new theoretical basis may be required. Furthermore, in *C. johnstoni*, *A. mississippiensis* (Ferguson & Joanen, 1982, 1983) and a number of freshwater turtles (Vogt & Bull, 1982), skewed secondary sex ratios in wild populations appear to result mainly from skewed primary sex ratios. Such relationships may be explicable under a number of classical models (reviewed by Charnov, 1982), but to pursue such possibilities in the absence of an acceptable hypothesis explaining TSD at the primary sex ratio level may be premature.

Charnov & Bull (1977) proposed that environmental sex determination *per se* would be more advantageous than GSD if sex-specific fitness was a function of the environment in which eggs were laid. If a particular nest environment was likely to produce a fit male and an unfit female, the mechanism would allow males to be selectively produced. This idea has been expanded in a number of theoretical treatments (Bull, 1981a, b; Bulmer & Bull, 1982), which also indicate that TSD may not be more primitive than GSD, but could evolve from it, under certain circumstances.

In turtles and crocodilians, sex-specific fitness consistent with the model of Charnov & Bull (1977) has proved difficult to demonstrate. With *A. mississippiensis* (Ferguson & Joanen, 1982, 1983) the rate of yolk utilization at different temperatures has been proposed as a possible advantage, because more yolk is associated with faster post-hatching growth rates, and it is more advantageous for females to grow and attain maturity rapidly than it is for males.

This relationship does not apply directly to *C. johnstoni*, because females result from both the highest and lowest incubation temperatures, male and female hatchlings grow at the same rates in the field (Webb, Buckworth & Manolis, 1983a), and the amount of yolk embryos contain at hatching is positively correlated with incubation temperature and thus negatively correlated with total development time (unpublished data). Fast developing *C. johnstoni* embryos (females and males) hatch with more yolk than slow developing ones (the same trend occurs in *C. porosus* where there are no "high" temperature females; unpublished

'data), which is the opposite to what occurs in *A. mississippiensis*. This interspecific difference could have its roots in the very rapid development of *A. mississippiensis* relative to *C. johnstoni* and *C. porosus* (64 rather than 85–95 days at 30°C), or it could reflect a bias associated with *A. mississippiensis* yolk masses being measured just prior to hatching. Regardless, the selective advantage to *A. mississippiensis* does not appear to exist in *C. johnstoni* or *C. porosus*.

Survivorship in *C. johnstoni* embryos is clearly sex-specific, because mortality is biased towards females. In areas prone to overheating, "low" temperature females could be expected to be more fit than either "high" temperature females or possibly males, whereas in flood-prone situations the reverse would be true. But these relationships represent consequences of having TSD, and do not constitute an evolutionary advantage for the mechanism.

A survivorship advantage consistent with the model of Charnov & Bull (1977) would exist if:

(i) Females were more likely to survive at high and low temperature extremes than males.

(ii) Females were less likely than males to have their reproductive potential compromised – perhaps through structural abnormalities.

We have no evidence indicating that either condition occurs and accordingly can present neither yolk mass nor survivorship advantages for *C. johnstoni* that are definitively consistent with Charnov & Bull's (1977) model. From a sex ratio point of view *C. johnstoni* would seem equally if not better adapted with GSD. However, if embryo survivorship at the physiological rather than ecological level is examined, a possible explanation for TSD in reptiles can be attained, which may be broadly consistent with the above model. Evidence comes from two sources, the reproductive strategies of reptiles with TSD, and the timing of sexual differentiation in the embryos of those species.

(1) TSD is common in two vertebrate groups (chelonians and crocodilians), both of which are highly aquatic. Eggs are typically laid in an environment (on land) which is separated from that in which adults spend most of their time (water), and the ability to select optimal nest sites may accordingly be constrained (relative to terrestrial animals nesting in a terrestrial environment). There is virtually no manipulation of eggs between laying and hatching (as occurs in most birds and some squamates), the embryonic stage at laying is young (compared to squamates but not birds) and development times in the field may be greatly prolonged (relative to squamates and birds). *Embryos* characterized by TSD are exposed to vagaries of the environment, over a large range of embryonic stages, without parental assistance, and for longer periods of time than is generally the case for related groups in which GSD predominates (squamates and birds).

(2) In embryos characterized by TSD, the time when part of the nephric system will be allocated to a gonad, and the type of gonad and ducts that will develop, both appear to be determined by the rate at which the embryos are themselves developing. The direction of timing is such that during rapid development, when nephric activity is presumably (and perhaps critically) maximized, gonadal development appears severely retarded.

If the latter facultative arrangement was primarily an adaptation towards enhancing embryo survivorship under extreme incubation conditions, and it was less readily attainable or unattainable with GSD, then the existence of TSD in groups where embryos are likely to incur environmental extremes would be explicable. TSD could be a somewhat unavoidable consequence of a mechanism which has been selected for, and maintained, for reasons other than those associated directly with sex determination.

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