CHAPTER 50

The Effects of Incubation Temperature on Sex Determination and Embryonic Development Rate in *Crocodylus johnstoni* and *C. porosus*

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THE temperature at which eggs are incubated influences sex within the embryos of crocodilians (Ferguson and Joanen 1982, 1983; Webb *et al.* 1983b; Webb and Smith 1984; Hutton 1984; Ferguson 1985) and some other reptiles without sex chromosomes (for example see: Bull 1980; Bull and Vogt 1979; Bull *et al.* 1982b; Hou Ling 1985; Limpus *et al.* 1984, 1985; McCoy *et al.* 1983; Miller and Limpus 1981; Mrosovsky 1980; Mrosovsky and Yntema 1980; Raynaud and Pieau 1985; Standora and Spotila 1986; Tokunaga 1985, 1986; Vogt and Bull 1982; Vogt *et al.* 1982; Webb *et al.* 1986; Yntema 1976). The moisture environment of incubation may also affect sex (Gutzke and Paukstis 1983) and some clutches incubated near the temperature which gives 50% males and 50% females may have a bias towards one sex or the other (Bull *et al.* 1982a).

The temperature of egg incubation also influences post-hatching patterns of crocodilian thermoregulation (Lang Chapter 30), growth and survivorship (Joanen *et al.* Chapter 51). It remains unclear whether sex and post-hatching performance are affected by incubation temperature independently, or whether the two are associated with each other in some causal fashion. Regardless, the relationships have obvious application to the crocodile farming industry (Joanen *et al.* Chapter 51), and to various theoretical issues raised by the existence of environmental sex determination in reptiles (Bull 1981a, b; Bull *et al.* 1982a, b; Bulmer and Bull 1982; Ferguson and Joanen 1982, 1983; Mrosovsky 1980; Webb and Smith 1984).

In reality, embryonic development rate may be more closely related to sex and post-hatching performance than is incubation temperature *per se* (Webb and Smith 1984). Temperature profoundly influences development rate, but at any one temperature, development rate can also be influenced by the moisture (Packard and Packard 1984) and gaseous (Ackerman 1981) environments of incubation. As emphasized by Miller (1985), the "temperature, hydric environment, and gas exchange act synergistically to influence embryonic metabolism".

This chapter presents data on the effects of incubation temperature on both the rate of embryonic development and sex determination in *Crocodylus johnstoni* (the Australian freshwater crocodile) and *C. porosus* (the saltwater or estuarine crocodile). Specific areas addressed are:

1. Variation in the stage of embryonic development at laying;
2. Quantification of the relationship between days of incubation at 30°C (under *Method A* incubation conditions; see later) and embryo size;
3. Derivation of formulae which allow any embryo to be given a 30°C age equivalent, independent of real age. This is referred to as an embryo's Morphological Age in days (Ferguson 1985; also see Chapter 45) at 30°C with *Method A* incubation (MA_{30A} in days);
4. Quantification of the relationship between incubation temperature, hatchling mass and residual yolk mass;
5. Quantification of the relationship between sex and incubation temperature, embryonic development rate and total incubation time; and,
6. Identification of the embryological stages at which "significant" sex determining events occur, as revealed by "switch experiments". Eggs are incubated at one temperature (giving one sex) and switched to another temperature (giving the opposite sex) after different periods of incubation at the first temperature (see Yntema 1979; Bull and Vogt 1981; Yntema and Mrosovsky 1982).

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The results demonstrate a variety of effects of incubation temperature on the embryos and hatchlings of both species, and shed some new light on the mechanism of temperature-dependent sex determination [which remains unknown (Raynaud and Pieau 1985; Standora and Spotila 1985)]. From a theoretical point of view, temperature-dependent sex determination in reptiles would be explicable in terms of Charnov and Bull's (1977) model, if the potential for post-hatching growth also varied with the incubation environment, as appears to be the case in *Alligator mississippiensis* (Joanen *et al.* Chapter 51).

METHODS

Eggs

Clutches of both species were obtained soon after laying (usually within two days), and at least one egg from each clutch was sacrificed to determine the stage of development of embryos at the time incubation started. All eggs were measured, weighed, individually numbered and placed horizontally in the incubators such that the orientation of the egg in the nest was maintained as far as practical in the incubators.

Incubation Methods

Most eggs were incubated in "Labec" water-jacketed laboratory incubators with glass observation doors, although during some experiments "Qualtex" and "Labmaster" incubators were also used. All incubators were maintained in an air-conditioned room in which ambient temperature ranged from 18-22°C. Incubators were covered with polystyrene sheets to improve insulation. Temperatures were measured with a "Yellowsprings" model 46 tele-thermometer and 400-Series thermistor probes calibrated against a certified thermometer. Absolute temperatures reported are accurate to within 0.1°C. The accuracy with which a desired mean incubation temperature was maintained was ± 0.05°C to ± 0.10°C (SE), and the extent of variation throughout the incubation period was ± 0.20°C to ± 0.30°C (SD).

Two methods of incubation were used:

Method A

Most early results, and the standard series of embryos for both species, were obtained using this method. Eggs were placed in plastic trays (31 × 25 × 12 cm high) and each tray was placed in a sealed plastic bag. The lower 4 cm of each tray was filled with pelletted polystyrene beneath a wire gauze platform taped to the tray sides. This lower compartment was filled with enough water to force the pellets against the wire (for support); the water also served to maintain high (but unmeasured) humidity within the bags. For seating the eggs, a sealed plastic bag containing moist sand (approximately 5% water by weight) was positioned on the gauze and spread such that it was about 1 cm deep. Eggs were positioned in a single layer on the plastic within a shallow dimple made in the enclosed sand. A temperature probe was positioned among the eggs in each box.

Within each incubator fans circulated air for twenty minutes each twenty minutes. During the later stages of development each bag was opened and flushed daily and gas measurements indicated partial pressures of oxygen and carbon dioxide did not reach limits likely to be criticial (see Whitehead Chapter 47). Temperatures were measured at least daily and to control temperature increases resulting from the production of metabolic heat, eggs of different ages were shifted between boxes and the incubator thermostat adjusted down.

Method B

Most later results were obtained using this method. Only the "Labec" water-jacketed incubators were used. Eggs were laid on metal racks in the central area of the incubator and were surrounded by air. A temperature probe sealed in a container of water (20 cm^3) was positioned in the centre of the eggs. One or two trays of water were placed in each incubator and air was continually bubbled through the water with aquarium pumps. Temperature gradients among the exposed eggs were in the vicinity of 0.1°C.

Regardless of incubation method, when embryos were due to hatch, individual eggs were placed in small plastic bags in which numerous holes had been punched. This ensured that hatchlings were allocated to their respective eggs when more than one hatched at a time.

Embryos

Whenever embryos were removed for preservation the egg contents were separated and frozen for later analysis (Manolis *et al.* Chapter 46). Embryos were preserved in 10% buffered formalin and were assigned an Australian Museum field tag number. An injection of "Nembutal" was used to kill older embryos and any hatchlings preserved.

REFERENCE SERIES

Crocodylus johnstoni

Because of the small clutch size [13.2 ± 3.2 eggs (SD); Webb *et al.* 1983b], multiple clutches were used to obtain a reference series using *Method A* incubation at 30°C. Up to five embryos were removed from eggs at five day intervals and usually two at two or three day intervals in between.

This same series of eggs formed the basis of the egg chemistry data reported by Manolis *et al.* (Chapter 46) and is being used to describe the effects of incubation temperature on gonad histology. Additional eggs from the same clutches were incubated (*Method A*) at 28°C, 29°C, 31°C, 32°C, 33°C and 34°C so that representative embyros at other temperatures could be obtained throughout incubation. Some of these eggs were also used to measure metabolic rates (Whitehead Chapter 47).

Crocodylus porosus

Eggs from a single clutch [121.7 ± 3.7 g (SD); N = 46)] were used to establish the rate at which embryos developed under *Method A* conditions at 30°C. Embryos were preserved each five days throughout the incubation period. [The metabolic rates of these eggs were also measured (Whitehead 1987)]. Additional eggs from the same clutch were used to obtain representative embryos at 28°C, 29°C, 31°C, 32°C and 33°C.

Morphological Ages at 30°C (MA_{30A})

Morphological age, for the purposes of the present study, is defined as the age (in days) that an embryo would be if it had been incubated at 30°C under the environmental conditions of *Method A*. We assigned MA_{30A}'s originally by physically matching embryos against the appropriate reference series and extrapolating between reference embryos when necessary.

However, embryo dimensions proved to be extremely good predictors of MA_{30A}, and thus formulae for predicting MA_{30A} were derived and are now used regularly. When this same approach was used previously (Webb *et al.* 1983b), it was assumed that embryo size needed to be scaled to egg size throughout incubation. The formulae for predicting MA_{30A} from embryo measurements (mostly head length) therefore relied on the measurement expressed as a ratio of egg length. This approach was confusing to some (Miller 1985), but perhaps more important, introduced an error. Scaling of embryo size to egg size occurs mainly during the rapid growth phase of embryos during the last third of incubation. Thus embryo measurements do not need to be scaled to egg size during the first half to two-thirds of development.

Development Rate Coefficients

From the constant temperature experiments above, series of embryos were obtained which had known real ages (RA in days) and equivalent ages at 30°C incubation (predicted MA_{30A} in days). One was plotted against the other for each temperature and regression analyses were used to derive coefficients by which MA_{30A}'s could be predicted from RA's at each temperature. These coefficients are essentially development rate coefficients (DRC's), and they indicate how many days development at a particular temperature is the equivalent of one day's development at 30°C. The utility of the coefficients lies in the ability to predict how many days of incubation at any temperature will be required to reach a particular stage of development (MA_{30A}).

Sex and Incubation Temperature

The relationship between constant incubation temperature and sex was examined in *C. johnstoni* and *C. porosus* incubated under *Method A* and *Method B* conditions. Hatchlings of both species can be reliably sexed by the extent of differentiation of the cliteropenis at the time of hatching (Webb *et al.* 1984), and in these experiments, sexing was carried out at least twice on every individual, with reference only to its number; it could not be biased by any "expected" result. However, the sex of many individuals was also determined by examining gonad histology. This "confirmation" was specifically carried out on any outlying individuals, to ensure, for example that the production of one female in a batch of males, in any one experiment, was not a sexing artefact.

Unlike *Alligator mississippiensis* (Ferguson and Joanen 1982, 1983), the highest incubation temperatures with both *C. johnstoni* and *C. porosus* produce females. We thus refer to high temperature and low temperature females to make a broad distinction between the two, although with *C. johnstoni* incubated at constant temperature, females are produced at all temperatures so far tested.

Switch Experiments

Switch experiments were conducted to determine the embryological stages of development at which "significant" sex determining events occur. Eggs were incubated at a temperature which would give a known sex ratio (if incubation was completed at that temperature), and were switched to a temperature giving a different sex ratio after varying periods of incubation at the first temperature. With prior knowledge about what each constant temperature could be "expected" to give, any point at which deviations from the expected result occurred, was considered a significant sex determining event (SSDE). Through the use of the development rate coefficients derived above, the SSDE's could be given an MA_{30A}, thereby allowing the effects of the switches to be viewed on a time scale independent of varying development rates.

Given the wide range of possible switches and periods of exposure required, the approach taken was to shift single eggs, or pairs of eggs, from one temperature to another at each time. Once an approximate SSDE was detected, additional eggs were concentrated around that point the following

year if possible. Availability of eggs has meant that sample sizes are generally small in these experiments.

Statistical Methods

Statistical methods generally follow SPSS Inc. (1983), Zar (1974) and Bailey (1974). The error value given with regression formulae is the standard error of estimate (SEE), and that given with means is either the standard error (SE) or standard deviation (SD), depending on whether the precision of the mean or extent of variability respectively, are under consideration.

RESULTS

Standardizing Time of Laying

To quantify the relationship between development rate and incubation temperature with *C. johnstoni*, clutches from different females, laid at different times and at slightly different stages of embryonic development, needed to be pooled. To align these clutches with each other, and thereby reduce variation attributed to embryonic stage of development at laying, a theoretical mean embryonic stage at laying was derived.

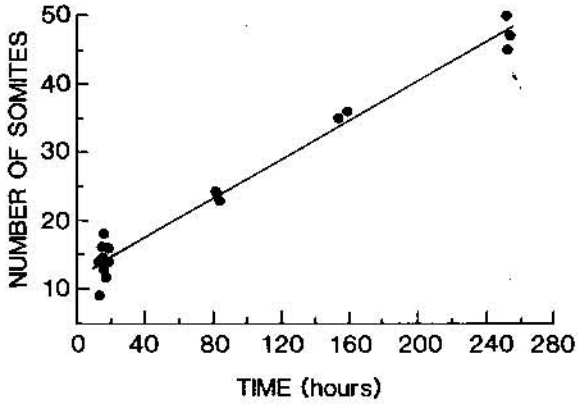

Fig. 1. The relationship between numbers of somites and age in hours for *C. johnstoni* embryos incubated at 30°C (*Method A*). Laying time was assumed to be midnight on the night eggs were laid. The line is the linear regression of best fit.

A sample of *C. johnstoni* embryos retrieved from eggs known to be laid the previous night were assigned an age in hours which was the difference between midnight of the night of laying and the time the embryos were removed from eggs and preserved. Additional embryos incubated at 30°C for up to 10 days were given a similar age in hours using the same method. Somite counts (which included partially formed somites) were then plotted against age in hours (Fig. 1), and regression formulae were derived for predicting age in hours (A) from somites (S), and somites from age in hours:

(1) $A = -78.01 + 6.823S \pm 15.0\,h\ (N=17; r^2=0.98)$

(2) $S = 11.9 + 0.14A \pm 2.2$

Formula (1) was then used to calculate an age for each clutch (one or two embryos from each clutch were sacrificed for somite counts). Time of laying could thus be assigned to each clutch, based on the assumption that all clutches were laid with the embryos at the same stage of development [11.9 ± 2.3 somites; formula (2)]. In reality, this growth in the immediate post-laying period is probably neither linear nor occurs at the same post-laying rate, but for our purposes, it was simply a means of aligning clutches to an equivalent starting point, independent of the stage of development of the embryo when it was found. If this correction was not applied initially, a constant bias of up to ± 3 days occurred throughout development.

The coefficients of formulae (1) and (2) indicate that it takes approximately 6.8 hours at 30°C for one somite to form.

Within clutch variation in stage of development was up to two somites in one *C. johnstoni* clutch examined and was generally less than two somites in pairs of embryos examined from any one clutch. In comparison to between clutch variation it is minor and has been ignored in the results presented here.

Equivalent data for standardizing *C. porosus* stage of development at laying are as yet unavailable, as it is much more difficult to obtain clutches on the day after laying. However, those that have been obtained (N = 7) indicate that laying generally occurs at an advanced stage relative to *C. johnstoni* (about 6 somites more). The rate at which the somites develop at 30°C is similar, as is the relationship between size and age at 30°C (age is displaced by about 1.8 days for at least the first third of incubation because of the difference in the number of somites at laying). Accordingly, we have assumed a mean theoretical laying stage of 18 somites and a similar rate of somite formation (6.8 h per somite), giving the following approximate formulae:

(3) $A = -122 + 6.8S$

(4) $S = 18 + 0.14A$

Reference Series

The approximate relationship between the embryological stages defined by Ferguson (1985, Chapter 45) and age in days at 30°C with *Method A* incubation (MA_{30A}) for both species is on Table 1.

For the youngest embryos of both species, embryological stages and the formulae relating somite number to age [formulae (1) and (3)] are the best indicators of MA_{30A}. Formulae for predicting an approximate MA_{30A} in days from head length (measured on embryos preserved in 10% buffered formalin) for both species are given below. For *C. johnstoni*, data were derived from different clutches, with different egg sizes, and the formulae refer to an "average" reference series. With *C. porosus*, they

Table 1. The approximate relationship between the embryo stages defined by Ferguson (1985; see Chapter 45) and days of incubation at 30°C, for *C. johnstoni* and *C. porosus* incubated under *Method A* conditions. Means are presented where ranges of days are involved.

Ferguson Stage	*C. johnstoni* (days)	*C. porosus* (days)
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	9
9	10	11
10	11	12
11	13	13
12	14	14
13	16	15
14	18	17
15	20	20
16	22	22
17	26	24
18	28	28
19	30	29
20	34	34
21	38	39
22	41	45
23	55	56
24	60	–
25	72	70
26	81	–
27 (yolk going in)	83.5	–
28 (yolk just in)	86	90
28 (hatch)	90	94

were derived from one clutch, and the formulae align embryos precisely to that one reference series. Periods of non-linearity in the relationship between head length and age due to allometric growth (for example where the snout extends at a faster rate than the remainder of the head), are accounted for in the *C. porosus* formulae.

With both species, embryo size scales to egg size after about 60 days MA_{30A}, when the chorioallantois completely surrounds the inner surface of the eggshell membrane. Up until that stage, formulae for predicting MA_{30A} (in days) from head length (HL in millimetres) are:

Crocodylus johnstoni

	Head Length Range (mm)	
(5)	1.4- 9.0	$MA_{30A} = +2.85 + 2.25HL$
(6)	9.1-23.5	$MA_{30A} = +3.66 + 1.91HL$
(7)	23.6-28.5	$MA_{30A} = -4.21 + 2.25HL$

Crocodylus porosus

	Head Length Range (mm)	
(8)	<2.3	$MA_{30A} = -10.0 + 14.55HL - 3.21\,HL^2$
(9)	2.4-11.0	$MA_{30A} = -1.13 + 4.207HL - 0.363HL^2 + 0.0177HL^3$
(10)	11.1-24.5	$MA_{30A} = -80.23 + 18.415HL - 1.03HL^2 + 0.0203HL^3$
(11)	24.6-30.0	$MA_{30A} = -83.53 + 10.99HL - 0.306HL^2 + 0.0033HL^3$

To simplify the assignment of standard ages (MA_{30A}'s) to *C. johnstoni* and *C. porosus* embryos within the first two-thirds of their development, the above formulae were used to compute the relationships on Table 2.

Table 2. The relationship between embryo head length and the age of the reference series of embryos incubated at 30°C *Method A* (MA_{30A}) for both *C. johnstoni* and *C. porosus* up until 60 days MA_{30A}. After this stage of development embryo size begins to scale to egg size. Measurements refer to embryos preserved in 10% buffered formalin.

Head Length (mm)	Age in Days *C. johnstoni*	*C. porosus*
1	0-2	1.3
2	7.3	6.3
3	9.6	8.7
4	11.8	11.0
5	14.1	13.0
6	16.3	14.9
7	18.6	16.6
8	20.8	18.4
9	21.9	20.2
10	22.7	22.3
11	24.6	24.8
12	26.5	27.5
13	28.4	29.7
14	30.3	31.4
15	32.2	32.8
16	34.1	33.9
17	36.0	34.9
18	38.0	35.9
19	39.9	37.1
20	41.8	38.5
21	43.7	40.3
22	45.6	42.5
23	47.5	45.4
24	49.7	49.1
25	51.9	51.5
26	54.2	53.4
27	56.4	55.1
28	58.7	56.7
29	–	58.3
30	–	59.9

When embryo size begins to scale to egg size, predictions of MA_{30A} from embryo size alone will be biased in the same direction to egg size. The head length of hatchlings is scaled directly to egg size in both species. The relationships on Tables 3 and 4 allow egg size to be accounted for when giving older embryos an approximate MA_{30A}.

DEVELOPMENT RATE COEFFICIENTS

Deriving Coefficients

Incubation of *C. johnstoni* embryos at different temperatures was usually initiated within one to three days of the predicted laying time (derived above), and to calculate incubation days it was

Table 3. Data for predicting the approximate MA_{30A} for *C. johnstoni* embryos with head lengths greater than 28.5 mm (60 days MA_{30A}). In the later stages of development embryo size scales to egg size. Mean egg length for the 30°C reference series was 69.9 mm.

Head Length (mm)	Egg Length (mm) 50.0	55.0	60.0	65.0	70.0	75.0	80.0
	Morphological Age in Days						
28.6	60	60	60	60	60	60	60
30	69	67	66	65	65	64	64
31	74	72	70	69	68	67	66
32	80	76	74	72	71	70	69
33	86	81	78	75	74	72	71
34	92	86	82	79	77	75	74
35	97	91	86	82	80	78	76
36	–	95	90	86	83	80	78
37	–	–	94	89	86	83	81
38	–	–	–	93	89	86	83
39	–	–	–	96	92	89	86
40	–	–	–	–	95	91	88
41	–	–	–	–	98	94	91
42	–	–	–	–	–	97	93
43	–	–	–	–	–	–	96

Table 4. Data for predicting the approximate MA_{30A} in days for *C. porosus* embryos with head lengths greater than 30.0 mm (60 days MA_{30A}). In the later stages of development embryo size scales to egg size. Mean egg length for the reference series incubated at 30°C was 82.3 mm.

Head Length (mm)	Egg Length (mm) 65.0	70.0	75.0	80.0	82.3	85.0	90.0
	Morphological Age in Days						
30	60	60	60	60	60	60	60
32	65	65	64	63	63	63	62
34	71	70	68	67	66	66	65
36	78	75	73	72	70	70	69
38	85	81	79	76	73	73	73
40	94	87	84	80	78	78	77
42	–	95	89	86	83	82	81
44	–	–	96	90	89	87	85
46	–	–	–	96	96	91	88
48	–	–	–	–	100	97	92
50	–	–	–	–	–	–	98

assumed that embryos had been exposed to the experimental temperature regimes from the predicted time of laying rather than from the time they were actually placed in the incubators.

Embryos preserved after varying periods of exposure at different temperatures were assigned an MA_{30A} from the formulae given above. Regression analyses were then used to derive predictive relationships between the assigned morphological ages (MA_{30A}) and the real ages (RA) for each temperature.

As zero days real age (the standardized laying time) was the same as zero days MA_{30A}, the regression analyses were forced through the zero origin (SPSS Inc. 1983). Thus in the regression formula ($MA_{30A} = a + b$RA), the constant a becomes zero and the slope b alone can be used to predict MA_{30A} from RA (real age in days). The slopes of the lines on Figures 2-4 can thus be considered as temperature specific *development rate coefficients*; they

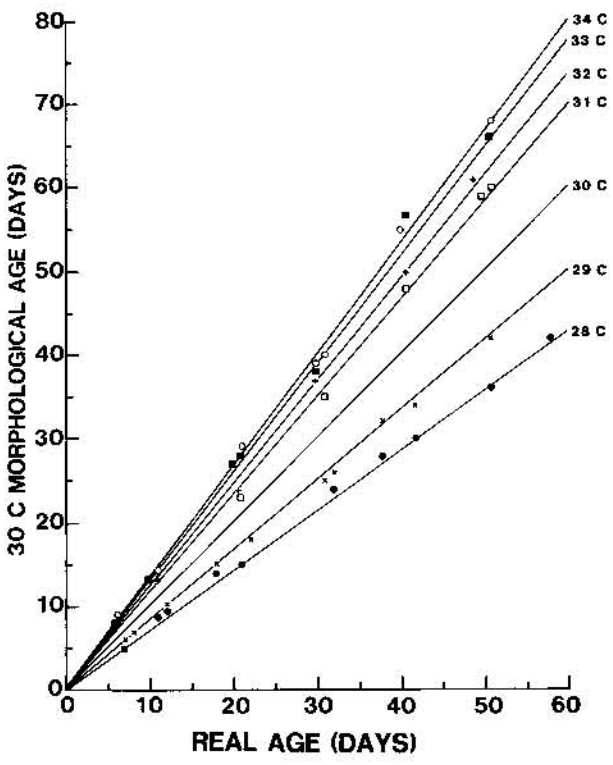

Fig. 2. The relationship between real age and standardized age (MA_{30A}) for *C. johnstoni* embryos incubated at different temperatures under *Method A* conditions. Lines are the regression lines of best fit forced through the origin. The eggs were from mixed clutches.

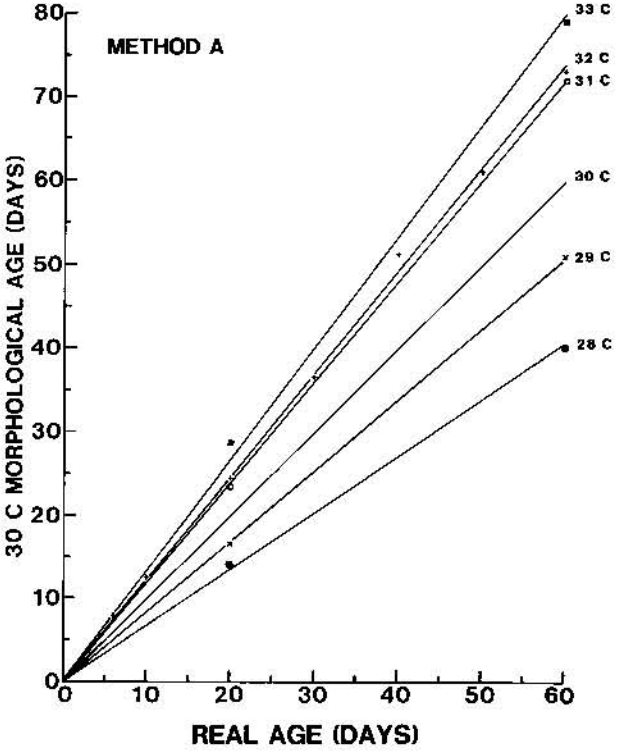

Fig. 3. The relationship between real age and standardized age (MA_{30A}) for *C. porosus* embryos incubated at different temperatures under *Method A* conditions. Lines are the regression lines of best fit forced through the origin. The eggs were from a single clutch.

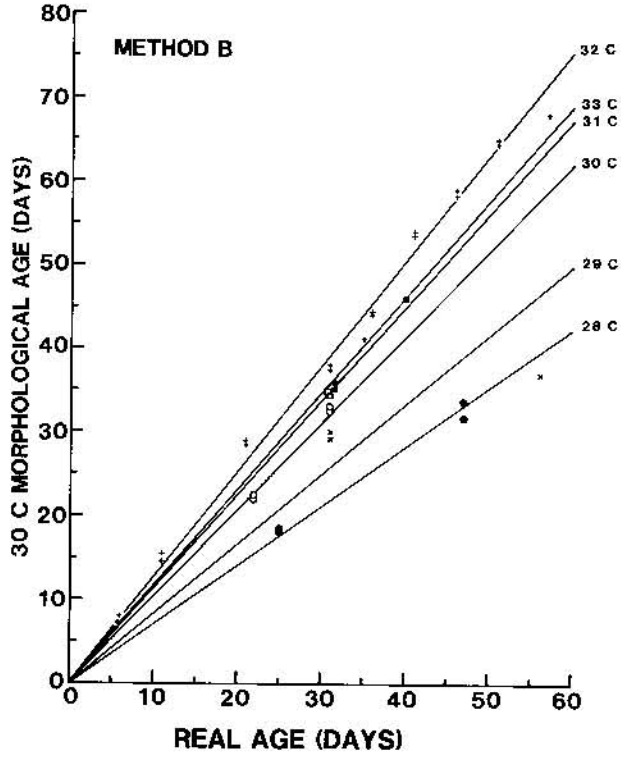

Fig. 4. The relationship between real age and standardized age (MA_{30A}) for *C. porosus* embryos incubated at different temperatures under *Method B* conditions. Lines are the regression lines of best fit forced through the origin. The eggs were from different clutches.

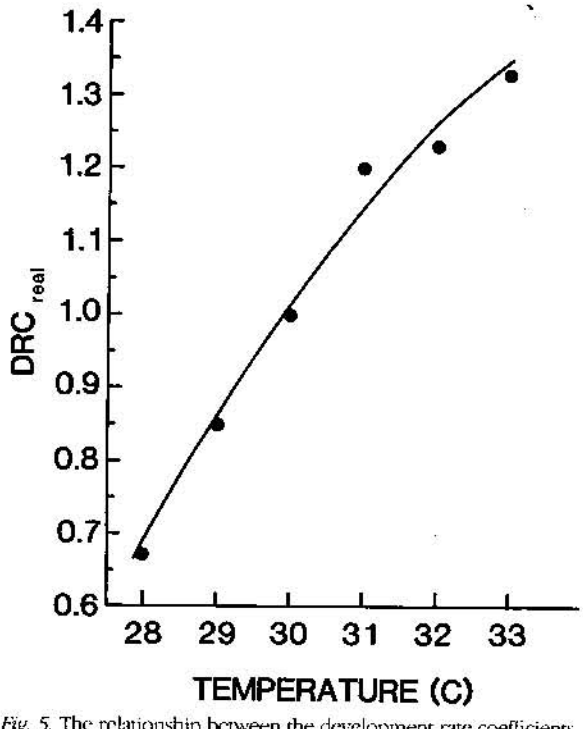

Fig. 5. The relationship between the development rate coefficients (DRC_{real}) standardized against 30°C and incubation temperature for *C. johnstoni* incubated under *Method A* conditions. The curve is the polynomial regression of best fit as described by formula (13) in the text, and can be used for predicting DRC's at any temperature (DRC_{pred}). One day of development at 32°C was the equivalent of 1.23 days development at 30°C (using DRC_{real}).

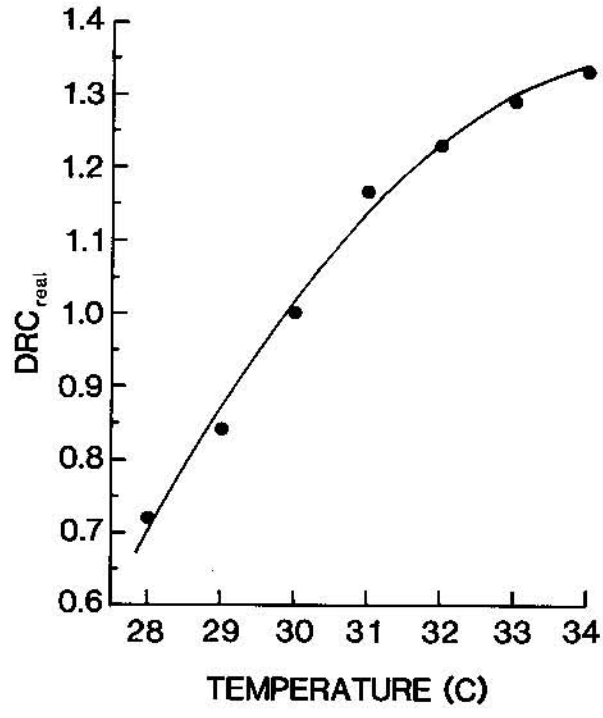

Fig. 6. The relationship between the development rate coefficients (DRC_{real}) standardized against 30°C and incubation temperature for *C. porosus* incubated under *Method A* conditions. The curve is the polynomial regression of best fit as described by formula (14) in the text, and can be used for predicting DRC's at any temperature (DRC_{pred}). One day of development at 32°C was the equivalent of 1.23 days development at 30°C (using DRC_{real}).

describe the number of days of development at 30°C *Method A* which is the equivalent of one day of development at the other temperatures.

Method A

For *Method A* incubation, data were obtained for *C. johnstoni* (Fig. 2) and to a lesser extent, for *C. porosus* (Fig. 3). The slopes of these lines (the development rate coefficients; DRC's) were plotted against temperature (Figs 5 and 6) and formulae (13 and 14) were derived for predicting DRC's at any temperature (T in degrees centigrade) under *Method A* conditions:

Crocodylus johnstoni

$$(13)\quad DRC_{pred} = -14.6037 + 0.90919T - 0.01295T^2 \pm 0.0271\ (r^2 = 0.991)$$

Crocodylus porosus

$$(14)\quad DRC_{pred} = -17.54665 + 1.08985T - 0.0157T^2 \pm 0.0321\ (r^2 = 0.990)$$

Thus real (DRC_{real}) and predicted (DRC_{pred}) values are available for some temperatures, and just predicted values for others (Tables 5 and 6).

Table 5. Development rate coefficients (DRC) for predicting real ages from MA_{30A} ages for *C. johnstoni* embryos incubated at different temperatures under *Method A* conditions. The coefficients are the slopes of the regression lines on Figure 2. Predicted values are from the curves relating development rate coefficients to temperature (Fig. 5).

Temperature (°C)	DRC_{real}	DRC_{pred}	Diff.	%Diff.
27.0	–	0.502	–	–
28.0	0.720	0.699	−0.021	−2.9
29.0	0.837	0.870	+0.033	+3.9
30.0	1.000	1.015	+0.015	+1.5
31.0	1.168	1.134	−0.034	−2.9
31.5	–	1.184	–	–
32.0	1.227	1.228	+0.001	+0.1
32.5	–	1.264	–	–
33.0	1.290	1.295	+0.005	+0.4
34.0	1.333	1.336	+0.003	+0.2

Table 6. Development rate coefficients (DRC) for predicting real ages from MA_{30A} ages for *C. porosus* embryos incubated at different temperatures under *Method A* and *Method B* conditions. The coefficients are the slopes of the regression lines on Figures 3 and 4. Predicted values are from the general curves relating development rate to temperature on Figures 6 and 7.

Temperature (°C)	DRC_{real}	DRC_{pred}	Diff.	%Diff.
		Method A		
27.0	–	0.434	–	–
28.0	0.670	0.660	−0.010	−1.5
29.0	0.851	0.855	+0.004	+0.5
30.0	1.000	1.019	+0.019	+1.9
31.0	1.198	1.151	−0.047	−3.9
31.5	–	1.205	–	–
32.0	1.231	1.252	+0.021	+1.7
32.5	–	1.290	–	–
33.0	1.329	1.321	−0.008	+0.6
34.0	–	1.359	–	–
		Method B		
27.0	–	0.414	–	–
28.0	0.705	0.670	−0.035	−5.0
29.0	0.830	0.875	+0.045	+5.4
30.0	1.036	1.030	−0.006	+0.6
31.0	1.124	1.134	+0.010	+0.9
31.5	–	1.166	–	–
32.0	1.261	1.187	−0.074	−5.9
32.5	–	1.194	–	–
33.0	1.157	1.189	+0.032	+2.8
34.0	–	1.140	–	–

The relationship between DRC's and temperature in both species followed similar patterns (Figs 5 and 6), and in both species 31°C was associated with faster development than would have been predicted from temperature alone. This result could be fortuitous, but it may also be indicating that under *Method A* conditions, development at 31°C is associated with a slight degree of temperature independence.

The limits of constant temperature incubation at which survivorship is severely compromised are about 27-28°C and 34°C with both species (Webb *et al.* 1983b; additional unpublished data).

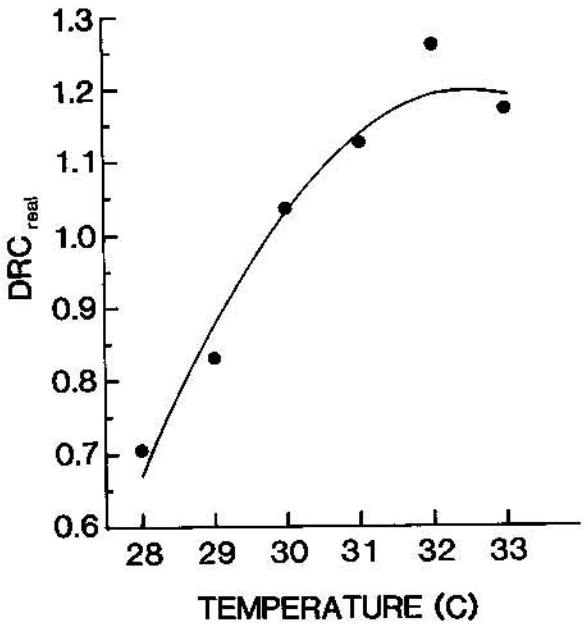

Fig. 7. The relationship between the development rate coefficients (DRC_{real}) standardized against 30°C and incubation temperature for *C. porosus* incubated under *Method B* conditions. The curve is the polynomial regression of best fit as described by formula (15) in the text, and can be used for predicting DRC's at any temperature (DRC_{pred}). One day of development at 32°C was the equivalent of 1.26 days development at 30°C (using DRC_{real}).

Method B

For *C. porosus*, a set of coefficients for *Method B* (Fig. 7) were derived (Table 6) against the same *Method A* reference series. They were based on small sample sizes, and included only embryos up to 60 days MA_{30A}, as with the *C. porosus Method A* coefficients. The formula for predicting *C. porosus Method B* DRC's is:

$$(15)\quad DRC_{pred} = -25.6851 + 1.6519T - 0.02538T^2 \pm 0.0572\ (r^2 = 0.956)$$

The *Method B* coefficients were more variable than those for *Method A*, and they indicated that in the younger stages, development at 29°C and 33°C was retarded, whereas at 32°C it was greatly enhanced. The *Method B* DRC_{real} at 30°C was 1.036, indicating that at 30°C, development with *Method B* was about 4% faster than with *Method A*.

It is unknown whether the variation between the measured development rate coefficients (DRC_{real}) and the ones predicted from the curves on Figures 5, 6 and 7 (DRC_{pred}) is random noise or whether it indicates biologically meaningful deviations from a general relationship between development rate and temperature. Thus it is unclear as to whether DRC_{real}'s or DRC_{pred}'s should be used for predicting the timing of events. In most cases we present both and use DRC_{real}'s when possible.

Using the Development Rate Coefficients

The DRC_{real}'s (and DRC_{pred}'s) can be used to predict approximate real ages, MA_{30A}'s and embryo stages from each other during the first two-thirds of incubation. For example, if one wanted two *C. johnstoni* embryos at say 28 days MA_{30A} (stage 18 of Ferguson 1985), even though one egg was being incubated at 28°C *Method A* and the other at 32°C *Method A*, the real time in days required at each temperature using DRC_{real}'s can be predicted: 28/0.72 = 39 days at 28°C; and, 28/1.227 = 23 days at 32°C.

With *C. porosus*, the difference between the DRC's for *Methods A* and *B* were maximized at 33°C (Table 6). With *Method A*, development rate at 33°C is reaching a peak (1.329; Fig. 6) whereas with *Method B*, it appears to be over the peak (1.157; Fig. 7) and is thus retarded. In practical terms, this means the difference between obtaining a 60 day MA_{30A} embryo at 33°C incubation in 45 days with *Method A* (60/1.329) and 52 days (60/1.157) with *Method B*. At other temperatures, the difference is not as great. For example, at 32°C a 60 day MA_{30A} embryo would take 49 days (60/1.231) with *Method A* and 48 days (60/1.261) with *Method B*.

We have not as yet derived *Method B* DRC's for *C. johnstoni*, but assume that development will tend to be slightly faster, with the possible exception of the highest incubation temperatures.

The Later Stages of Development

Development at different temperatures can be reasonably well modelled by straight lines during the early phases of incubation (Figs 2-4), which means that the errors likely to be incurred by using the DRC's will be minor. However, there *are* deviations from linearity during these early phases, just as in turtles (Ewert 1985), which result from temperature effects being more pronounced on some stages of embryonic development than on others. If more precise "aging" was required, these would need to be quantified more precisely, using larger series of embryos at each temperature.

During the later stages of development, there appears generally to be a greater degree of temperature independence of development in some species (Yntema 1968, 1978; Ewert 1985). However, such effects could be compounded by both metabolic heat production and variation in the stage of hatching. Metabolic heat production reaches a peak after about 90% of incubation in *C. johnstoni* and *C. porosus*, and then declines (Whitehead Chapter 47). This can raise egg temperature within some experimental incubators, thereby enhancing development. In addition, yolk internalization and hatching do not appear to occur at the same stage of embryonic development (the crocodilian embryo that hatches at 33°C is younger relative to the embryo that hatches at 30°C), and thus may not be comparable as equivalent stages for assessing development rate.

The extent to which DRC's derived in the first two-thirds of incubation applied through to hatching was investigated with *C. johnstoni* and *C. porosus*.

1. *Crocodylus johnstoni*

At 30°C *(Method A)*, the time relations of the final stages of incubation for *C. johnstoni* are in Table 7. Yolk is internalized at about 82-83 days at 30°C, and we used the mean (82.5 days) and the DRC_{real} and DRC_{pred} (Table 5) to predict the times at which this event should occur at other temperatures (82.5/DRC days), *if* the DRC's applied to the total development period. These results were then compared with limited data on the real times at which this event occurred at different temperatures (Table 8).

Table 7. Time relations of yolk internalization and hatching for *C. johnstoni* eggs incubated at 30°C *(Method A)*.

Age in days	Opened or hatched	Egg No.	Notes
80	O	2.13	Yolk completely external
80	O	6.13	Yolk completely external
80	O	7.12	Yolk completely external
80	O	10.9	Yolk completely external
82	O	6.15	Yolk partly internalized
83	O	6.16	Yolk partly internalized
85	O	4.7	Yolk internalization just complete
85	O	6.14	Yolk internalization just complete
87	H	2.15	Yolk internalization just complete
88	O	4.8	Internal yolk
89	H	37.7	Internal yolk
91	H	37.9	Internal yolk
91	H	37.11	Internal yolk
92	H	18.5	Internal yolk

Table 8. Limited data on the days of incubation until yolk internalization in *C. johnstoni* incubated at different temperatures, compared to predicted times. Fast development (33°C and 34°C) appears to be related to premature internalization of yolk.

Temperature (°C)	N	Real Days	Prediction Errors (% real days) DRC_{real}	Prediction Errors (% real days) DRC_{pred}
29.0	3	97.3	−2.5	+1.8
30.0	2	82.5	–	–
31.0	1	70	−0.9	−4.0
33.0	1	60	−6.7	−6.2
34.0	1	57	−8.6	−8.4

At 33-34°C the embryos internalizing yolk were smaller (relative to egg size) than those at 30°C and had relatively large amounts of residual yolk. Deviations from the predicted values are partly due to earlier yolk internalization, and do not necessarily represent a marked deviation from the rates of development established in the younger embryos.

The total incubation times of *C. johnstoni* incubated under both *Methods A* and *B* are on Table 9. At 30°C *(Method A)*, mean total incubation time to

Table 9. Measured incubation times to hatching (in days) for *C. johnstoni* embryos incubated at different temperatures with both *Method A* and *B*. Predicted total incubation times are the 30°C mean (90.0 days) divided by DRC_{real} and DRC_{pred} (see text).

Temperature (°C)	Method	N	Mean	SD	SE	Range
28.0	DRC_{real}		125.0			
	DRC_{pred}		138.8			
	A	1	123	–	–	–
29.0	DRC_{real}		107.5			
	DRC_{pred}		103.4			
	A	8	99.0	1.2	0.4	97-101
	B	7	106.1	1.8	0.7	104-109
30.0	DRC_{real}		90.0			
	DRC_{pred}		88.7			
	A	5	90.0	2.0	0.9	87-92
	B	23	89.0	2.6	0.5	84-94
31.0	DRC_{real}		77.1			
	DRC_{pred}		79.4			
	A	7	80.1	2.3	0.9	77-83
	B	17	76.2	1.4	0.4	74-78
31.5	DRC_{pred}		76.0			
	B	27	74.4	1.3	0.3	73-77
32.0	DRC_{real}		73.4			
	DRC_{pred}		73.3			
	A	9	76.3	3.2	1.1	73-83
	B	14	69.3	0.8	0.2	68-71
32.5	DRC_{pred}		71.2			
	B	6	69.8	1.5	0.6	68-72
33.0	DRC_{real}		69.8			
	DRC_{pred}		69.5			
	A	8	67.9	1.7	0.6	66-71
	B	11	68.6	3.1	1.0	65-76
34.0	DRC_{real}		67.5			
	DRC_{pred}		67.4			
	A	6	64.3	1.5	0.6	62-66

pipping of the shell (considered as "hatching" here) was 90.0 ± 0.9 days (SE; N = 5), and predicted times to hatching were calculated from this using both DRC_{real} and DRC_{pred}.

At 30°C, 31°C and 32°C, *Method B* incubation resulted in slightly reduced total incubation times (faster development) than with *Method A*. At 33°C, the mean incubation time with *Method B* was slightly longer than with *Method A*, however within the small sample size this was attributable to one individual which took 76 days; five days longer than any other 33°C animal (with this animal deleted, the mean incubation time was 67.9 days, the same as for *Method A*). Development at 29°C *Method A* was 4-8 days faster than predicted, and seven days faster than with *Method B* incubation. These particular eggs were manipulated frequently during the later stages of incubation (for metabolic rate determinations), and hatching may have been accelerated.

Predicted total incubation times using both DRC_{real} and DRC_{pred} were generally within the range of recorded incubation times, although at higher temperatures hatching occurred earlier than predicted. From a practical point of view, the DRC's standardized against incubation at 30°C *(Method A)* are reasonably good predictive tools for *C. johnstoni* incubated under both methods.

2. *Crocodylus porosus*

Equivalent data for *C. porosus* are in Table 10, where DRC's for *Method A* are compared to known data for *Method A* incubation times, and DRC's for *Method B* are compared with known data for *Method B* incubation times.

Total incubation times with *Method B* were less than those with *Method A* at the four temperatures for which data can be compared (30°C, 31°C, 32°C and 33°C) which suggests slightly faster development with *Method B*.

The difference between the predicted times and the observed times of hatching is an indication of how the relationship between development rate at any temperature and that at 30°C established in the first two-thirds of incubation (Figs 6 and 7) was maintained through to hatching. With *Method A*, at 31°C, 32°C and 33°C, predictions underestimated total incubation times (Table 10); the final stages of incubation were prolonged relative to what occurred at 30°C, and sometimes substantially (eight days at 32°C).

Table 10. Measured incubation times to hatching (in days) for *C. porosus* embryos incubated at different temperatures with both *Method A* and *B*. Predicted total incubation times are the 30°C *Method A* mean (93.6 days) divided by DRC_{real} and DRC_{pred} for both Methods.

Temperature (°C)	Method	N	Mean	SD	SE	Range
			Method A			
28.0	DRC_{real}		139.7			
	DRC_{pred}		141.8			
	A	1	132	–	–	–
30.0	DRC_{real}		93.6			
	DRC_{pred}		91.9			
	A	7	93.6	1.7	0.7	92-97
31.0	DRC_{real}		78.1			
	DRC_{pred}		81.3			
	A	2	86.5	–	–	86-87
32.0	DRC_{real}		76.0			
	DRC_{pred}		74.8			
	A	9	83.0	1.5	0.5	80-84
33.0	DRC_{real}		70.4			
	DRC_{pred}		70.9			
	A	2	78.0	–	–	77-79
			Method B			
29.0	DRC_{real}		112.8			
	DRC_{pred}		107.0			
	B	19	106.4	2.9	0.7	100-112
30.0	DRC_{real}		90.4			
	DRC_{pred}		90.0			
	B	59	91.8	3.1	0.4	88-98
31.0	DRC_{real}		83.3			
	DRC_{pred}		82.5			
	B	16	83.8	1.9	0.5	81-87
32.0	DRC_{real}		74.2			
	DRC_{pred}		78.9			
	B	59	80.1	2.1	0.3	75-87
33.0	DRC_{real}		80.9			
	DRC_{pred}		78.7			
	B	25	75.0	0.7	0.1	74-78

The situation with *Method B* is interesting, because development in the younger stages at 32°C was faster than at 33°C (Fig. 7), and gave higher DRC's. Yet, the 33°C animals hatched before the 32°C animals. This result is not an artefact of the reference series, because when 32°C and 33°C embryos from the same clutch were examined on the same days, the 32°C embryos were larger and more advanced; development at 32°C was proceeding faster than at 33°C during the early stages. Yet the 32°C animals hatched six days later than predicted on their early development rates (the DRC_{real}), and the 33°C animals hatched six days earlier than predicted (these were reduced to one and three days respectively if DRC_{pred} rather than DRC_{real} were used).

When the results for *C. porosus* are taken together, there seems to be a greater disparity between early and late development rates, at different temperatures, than there does in the smaller eggs of *C. johnstoni*.

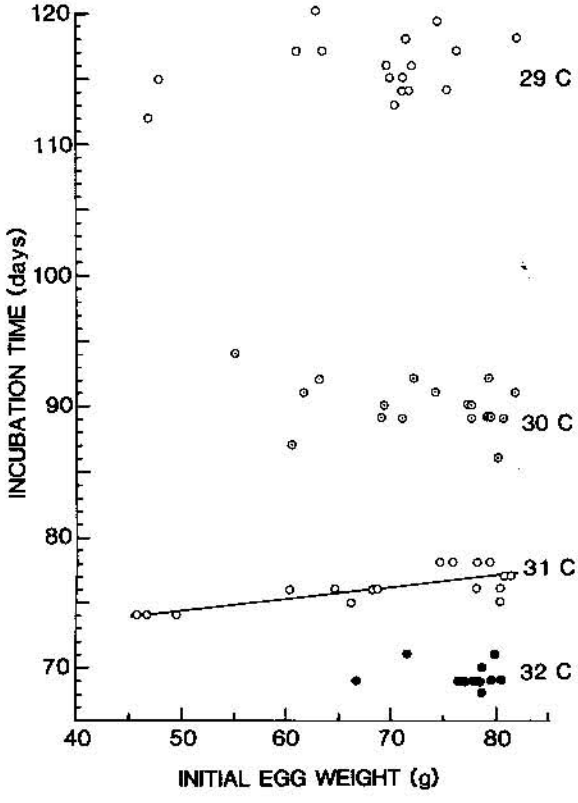

Fig. 8. The relationship between initial egg weight and total incubation time for *C. johnstoni* incubated at 29°C, 30°C, 31°C and 32°C under *Method B* conditions. At 31°C the slope of the regression line was significant, although no such trend was apparent in the more limited data at other temperatures.

Effects of Egg Size on Total Incubation Time

The relationship between total incubation time and egg size (weight) was investigated using *C. johnstoni* incubated under *Method B* conditions (Fig. 8). No significant relationship was detected at 29°C, 30°C or 32°C, but at 31°C incubation time (I, in days) increased with increasing egg weight (E, in grams):

(16) $I = 70.9 + 0.096E \pm 1.2$ days ($r^2 = 0.52$; $0.002 > p > 0.001$)

Given the limited data, it is possible that the same trend exists but was not detected at the other temperatures. However, the non-significant slope of the 30°C data was negative, indicating that if anything, there was a tendency towards longer total incubation times in smaller eggs.

The Effects of Temperature on Hatchling Size and the Amount of Residual Yolk

Using data from *C. johnstoni* hatchlings (N = 45) incubated at different temperatures (28-34°C; *Method A*) and sacrificed at the time of hatching, multiple regression analyses were used to predict total hatchling weight (H+Y in grams), yolk weight (Y in grams) and yolk-free hatchling weight (H in grams) from initial egg weight (E in grams) and incubation temperature (T in degrees C). The results indicate the direction and magnitude of incubation temperature and egg size effects. The mean egg weight in this experiment was 76.73 g.

(17) $H+Y = 25.907 + 0.514E - 0.576T \pm 2.6$ g (total $r^2 = 0.813$; r^2 attributable to E $= 0.783$, $p \ll 0.001$; r^2 attributable to T $= 0.030$, $0.025 > p > 0.01$)

The r^2 values for formula (17) indicate that 78% of the variation in H+Y weight could be attributed to egg weight; large eggs produced heavy hatchlings. Temperature accounted for only 3.0% of the variation in H+Y weight, in the direction of higher incubation temperatures giving smaller H+Y's at a rate of about 0.58 g per degree centigrade.

(18) $Y = -43.901 + 0.178E + 1.295T \pm 2.59$ g (total $r^2 = 0.603$; r^2 attributable to E = 0.289, $p \ll 0.001$; r^2 attributable to T = 0.314, $p \ll 0.001$)

The r^2 values for formula (18) indicate that 29% of variation in the weight of residual yolk could be attributed to egg size; larger eggs gave hatchlings with more residual yolk. Incubation temperature accounted for 31% of the variation in yolk weight, in the direction of increased yolk weight with higher temperatures, at a rate of about 1.03 g per degree centigrade.

(19) $H = 71.427 + 0.332E - 1.916T \pm 4.2$ g (total $r^2 = 0.542$; r^2 attributable to E = 0.243, $p \ll 0.001$; r^2 attributable to T = 0.299, $p \ll 0.001$)

The r^2 values for formula (19) indicate that 24% of the variation in hatchling weight (without the yolk) could be attributed to egg size; larger eggs gave larger hatchlings. Incubation temperature

accounted for 30% of variation in hatchling weight, in the direction of decreasing weight with increasing temperatures, at about 1.92 g per degree centigrade.

When these results are summarized, they indicate that large eggs tend to produce large hatchlings with large amounts of internalized yolk. However, independent of egg size, high incubation temperatures tend to give small hatchlings with large amounts of internalized yolk, whereas low incubation temperatures tend to give large hatchlings with small amounts of internalized yolk. These trends are independent of sex, as with *C. johnstoni* incubated at constant temperature, few males result at any temperature. The extent of these effects of incubation temperature (or embryonic development rate) in an average size egg (68.2 g; Webb *et al.* 1983b) are quite substantial (Table 11).

Table 11. The effect of incubation temperature *(Method A)* on *C. johnstoni* hatchling weight and residual yolk weight, as predicted for the average sized egg in the population (68.2 g) using formulae 17, 18 and 19. As the majority of *C. johnstoni* incubated at all temperatures are females, such changes are probably independent of sex.

Temperature (°C)	Yolk (g)	Hatchling (g)	Hatchling+yolk (g)
28.0	4.5	40.4	44.8
29.0	5.8	38.5	44.3
30.0	7.1	36.6	43.7
31.0	8.4	34.7	43.1
32.0	9.7	32.8	42.5
33.0	11.0	30.8	42.0
34.0	12.3	28.9	41.4

Table 12. Data on the relationship between constant incubation temperature and sex in *C. johnstoni* incubated under different conditions. Data presented by Webb and Smith (1984) summarizes preliminary results, in which temperatures (means, SD's and gradients within the incubators) were not as precisely controlled as in later experiments and a variety of incubation techniques were used. Sex ratio (SR) is the proportion of males.

Temperature	Webb and Smith (1984)			*Method A*			*Method B*		
(°C)	M	F	SR	M	F	SR	M	F	SR
26.0	0	12	0.00	–	–	–	–	–	–
28.0	0	15	0.00	0	4	0.00	–	–	–
29.0	–	–	–	0	12	0.00	0	19	0.00
30.0	0	162	0.00	0	23	0.00	0	25	0.00
31.0	3	20	0.13	0	9	0.00	2	14	0.13
31.5	–	–	–	–	–	–	7	24	0.23
31.7	–	–	–	5	15	0.25	–	–	–
32.0	5	25	0.17	4	9	0.31	0	14	0.00
32.5	–	–	–	6	20	0.23	0	6	0.00
33.0	5	20	0.20	0	12	0.00	0	15	0.00
34.0	0	41	0.00	0	9	0.00	–	–	–

Sex, Incubation Temperature, Development Rate and Total Incubation Period

Crocodylus johnstoni

Data on the relationship between constant incubation temperature and sex for *C. johnstoni* are summarized on Table 12. The preliminary published results (Webb and Smith 1984) indicated three important trends: firstly, exclusively females were produced at the highest and lowest incubation temperatures at which survival was possible; secondly, no constant temperature tested produced 100% males; and thirdly, the mean total incubation times of wild nests producing mostly males in the field (72-74 days) was similar to the incubation times under constant temperature that produced the highest proportions of males.

With incubation temperature more precisely controlled (Table 12; *Methods A* and *B*), the general trends between sex ratio and incubation temperature were confirmed. The range of temperatures producing males was reduced further (33.0°C appears to produce exclusively females) and there appeared to be a difference between the two methods with regard to which temperature produced the peak of males. With *Method A*, the peak was at 32°C (none were produced at 31°C), whereas with *Method B* it was at 31.5°C (none were produced at 32°C). This difference could be an artefact of the small sample sizes, but it could equally reflect differences in development rate between the two incubation methods.

Table 13. The relationship between embryonic development rate during the first two-thirds of incubation (DRC_{real}), total incubation time and sex ratio (proportion of males) in *C. johnstoni* incubated under *Methods A* and *B* at different temperatures.

DRC_{real}	Incubation Time (days)	Sex Ratio	Temperature (°C) (Method)
0.720	123.0	0.00	28.0 (A)
–	106.4	0.00	29.0 (B)
0.837	99.0	0.00	29.0 (A)
0.970	89.0	0.00	30.0 (B)
1.000	90.0	0.00	30.0 (A)
1.168	80.1	0.00	31.0 (A)
1.227	76.3	0.31	32.0 (A)
–	76.2	0.13	31.0 (B)
1.204	75.9	0.25	31.7 (A)
1.238	74.4	0.29	31.5 (B)
1.264	72.0	0.23	32.5 (A)
–	69.8	0.00	32.5 (B)
–	69.3	0.00	32.0 (B)
–	68.6	0.00	33.0 (B)
1.290	67.9	0.00	33.0 (A)
1.333	64.3	0.00	34.0 (A)

Notwithstanding the small sample sizes, when the data on development rate in younger embryos (the DRC_{real}'s), total incubation time, sex ratio and both incubation temperature and method were aligned according to total incubation time (Table 13) it can be seen that males were restricted to a reasonably small band of both DRC_{real}'s (1.204-1.264) and mean total incubation times (72.0-76.4), and that these two parameters were better predictors of "maleness" than were incubation temperatures *per se*.

Table 14. Data on the relationship between constant incubation temperature and sex in *C. porosus* incubated under *Method A* and *B* conditions. Sex ratio (SR) is expressed as the proportion of males.

Temperature	Method A			Method B		
(°C)	M	F	SR	M	F	SR
28.0	0	4	0.00	-	-	-
29.0	–	1	–	0	26	0.00
30.0	0	9	0.00	0	61	0.00
31.0	1	1	0.50	2	15	0.12
32.0	10	1	0.91	52	9	0.85
33.0	4	0	1.00	1	25	0.04

Crocodylus porosus

The sometimes limited data available for *C. porosus* incubated at precise incubation temperatures are summarized in Table 14. In contrast to *C. johnstoni*, some constant incubation temperatures gave close to 100% males. The most puzzling result was that 33°C *Method B* gave 96% females, which is consistent with "high temperature females", as in *C. johnstoni*. Yet as referred to above and as can be seen in Table 15, the rate of development of these embryos during the early stages of development (DRC_{real} = 1.157) was very low. It is thus unclear whether these are "high temperature females" in the same sense as those of *C. johnstoni*, which have both rapid early development and short total incubation times (Table 13).

Table 15. The relationship between embryonic development rate during the first two-thirds of incubation (DRC_{real}), total incubation time and sex ratio (proportion of males) in *C. porosus* incubated under *Methods A* and *B* at different temperatures.

DRC_{real}	Incubation Time (days)	Sex Ratio	Temperature (°C) (Method)
0.670	132.0	0.00	28.0 (A)
0.830	106.4	0.00	29.0 (B)
1.000	93.6	0.00	30.0 (A)
1.036	91.8	0.00	30.0 (B)
1.198	86.5	0.50	31.0 (A)
1.124	83.8	0.12	31.0 (B)
1.231	83.0	0.91	32.0 (A)
1.261	80.1	0.85	32.0 (B)
1.329	78.0	1.00	33.0 (A)
1.157	75.0	0.04	33.0 (B)

Switch Experiments

Switch experiments were undertaken to identify the real ages, MA_{30A}'s and embryological stages at which "significant" sex determining events (SSDE's) occurred at different incubation temperatures. In all cases sample sizes were small, and as a consequence, the assigning of SSDE's is approximate, but sufficient to identify trends. Results for any particular switch may have come from two or more years.

A number of different types of SSDE are recognized:

1. *Final Transition.* Where there is a transition from the sex expected from constant incubation at the second temperature, to the sex expected from constant incubation at the first temperature. Based on the results of pulse experiments by Yntema (1979), Bull and Vogt (1981) and Yntema and Mrosovsky (1982), sex is probably irreversibly determined (at the initial temperature) by the time of the *final transition.*
2. *Early Transition Stage.* With *C. johnstoni*, there was sometimes an earlier transition resulting in a sex ratio that could not be predicted from the results of constant temperature incubation at either the initial or final temperatures.
3. *Sensitive Stages.* Switches spanning a wide range of developmental stages often resulted in essentially one sex being produced, with the occasional animal giving the opposite sex. The minority giving the opposite sex could reflect random variation, however, they could equally be indicating stages of development at which sex determination is particularly sensitive to an increase or decrease in incubation temperature.
4. *Early Sensitive Stages.* Early shifts from an initial to a final temperature develop almost exclusively at the final temperature, and could thus be expected to have sex ratios equivalent to those at constant (final) temperature. However with *C. johnstoni*, incubation for four days at an initial temperature was sometimes sufficient to cause marked deviations from what would be expected from constant incubation at the final temperature. We have identified these as *early sensitive stages.*

Crocodylus johnstoni

The switch experiments with *C. johnstoni* (Fig. 9; Table 16) were all undertaken with *Method B* incubation. The results for constant temperature incubation at each of the switch temperatures used are in Tables 12 and 13.

29°C to 32°C

Constant temperature incubation at 29°C and 32°C *(Method B)* could be expected to give low temperature females and high temperature females respectively (Table 12).

SSDE-1. After only five days incubation at 29°C before being switched to 32°C, the exclusively female producing influence of 32°C was impaired, and mainly males resulted. SSDE-1 is thus four days or earlier and appears to be an early sensitive stage.

SSDE-2. Two eggs incubated for 15 and 17 days at 29°C before being switched to 32°C produced females, when eggs switched before and after

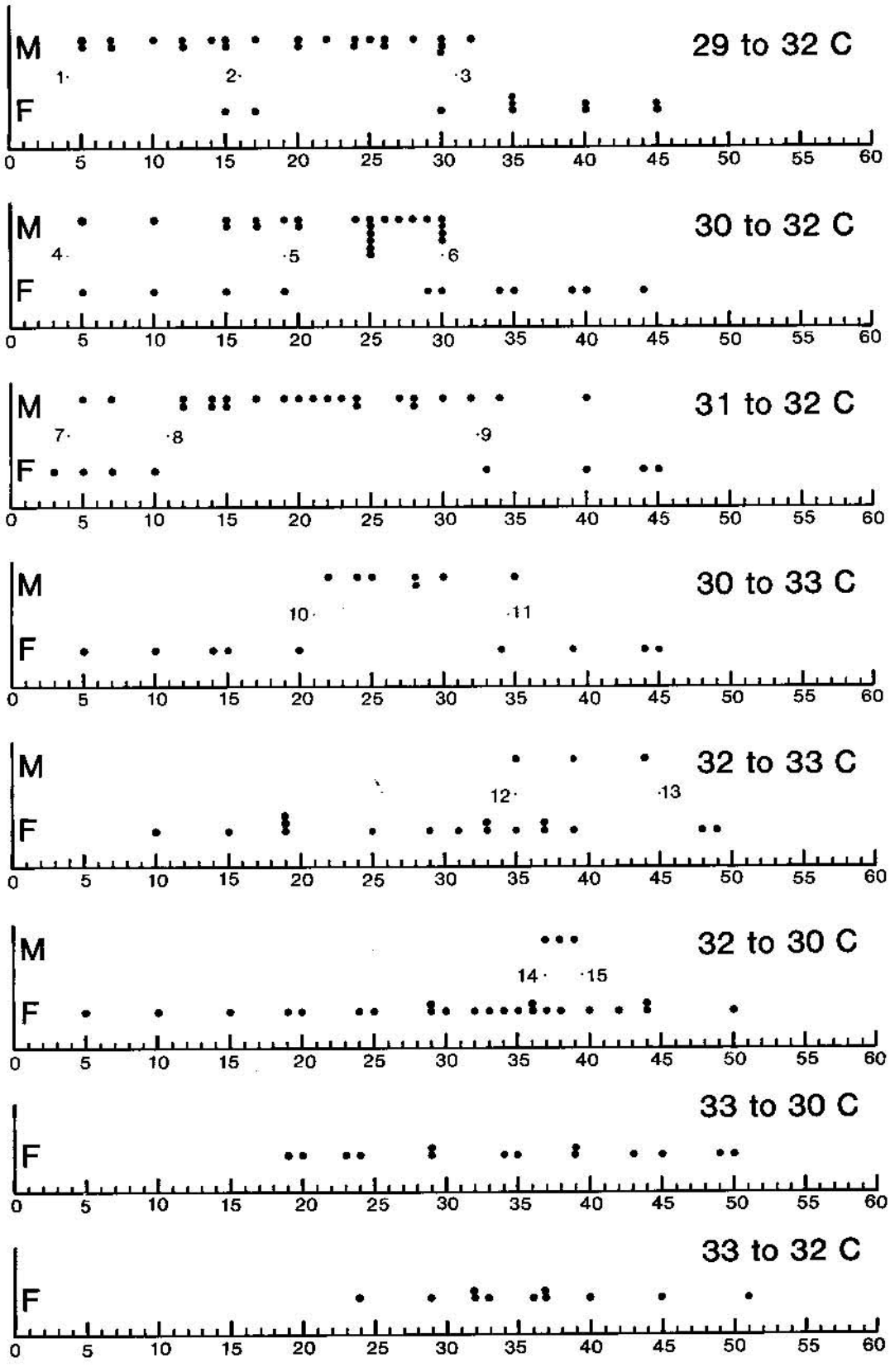

Fig. 9. The results of shift experiments with *C. johnstoni* eggs. Incubation was initiated at one temperature, and after varying periods of exposure individual eggs (closed circles) were shifted to a second temperature at which incubation was completed. Hatchlings were sexed and sexes allocated to the real ages at which the shifts had been implemented. Numbers refer to possibly "significant" sex determining events (see text).

produced males. Although possibly insignificant, about 16 days could be a stage of development at which sex determination is particularly sensitive to an increase in incubation temperature.

SSDE-3. After approximately 31 days incubation at 29°C before a switch to 32°C, female sex is fixed and further switches to 32°C no longer produce males. Thus at about 31 days at 29°C there is a final transition from one influence to another.

30°C to 32°C

Constant temperature incubation at 30°C and 32°C *(Method B)* could be expected to give low temperature females and high temperature females respectively (Table 12).

SSDE-4. The exclusively female producing influence of 32°C was impaired by five days incubation at 30°C, and both sexes resulted, suggesting an early sensitive stage of about four days or earlier.

SSDE-5. Switches from 30°C to 32°C after approximately 19 days incubation at 30°C gave exclusively males, indicating that this stage represents an early transition from one influence to another.

SSDE-6. After approximately 30 days incubation at 30°C, there appears to have been a cessation of the male producing influence of the switch to 32°C, and the start of the female influence of constant 30°C; a final transition between the two temperature influences.

31°C to 32°C

Constant temperature incubation at 31°C *(Method B)* could be expected to give males and females (Table 12), whereas incubation at 32°C *(Method B)* could be expected to give exclusively high temperature females.

SSDE-7. The exclusively female producing influence of 32°C was impaired after four days incubation at 31°C, and both sexes resulted, suggesting that this is an early sensitive stage.

SSDE-8. Switches from 31°C to 32°C after approximately 11 days incubation at 30°C gave exclusively males, indicating that this stage may be an early transition between two influences.

SSDE-9. After approximately 32.5 days incubation at 31°C before a switch to 32°C, there appears to have been a cessation of the exclusively male producing influence of the switch to 32°C; a final transition to the mixed sexes to be expected from constant 31°C.

30°C to 33°C

Constant temperature incubation at 30°C and 33°C *(Method B)* could be expected to give low temperature females and high temperature females respectively (Table 12).

SSDE-10. The exclusively female producing influence of 33°C was impaired after 21 days incubation at 30°C, and males resulted.

SSDE-11. After approximately 34.5 days incubation at 30°C before a switch to 33°C, there appears to have been a final transition from the male producing influence of the early switches to the exclusively female influence of 30°C.

32°C to 33°C

Constant temperature incubation at 32°C and 33°C *(Method B)* could be expected to give high temperature females (Table 12).

SSDE-12. The exclusively female producing influence of 32°C and 33°C was interrupted after 35 days incubation at 32°C, and some males resulted, suggesting this may be the start of a sensitive stage.

SSDE-13. After approximately 45 days incubation at 32°C, there appears to have been a cessation of the male producing influence of the switches to 33°C; a final transition to the sex (femaleness) committed at 32°C.

32°C to 30°C

Constant temperature incubation at 32°C and 30°C *(Method B)* could be expected to give high temperature females and low temperature females respectively (Table 12).

SSDE-14. The exclusively female producing influences of 32°C and 30°C were interrupted after 37 days incubation at 32°C, and some males resulted.

SSDE-15. After approximately 39.5 days incubation at 32°C, there appears to have been a cessation of the male producing influence of the switch to 33°C and a final transition to the exclusive female influence of 32°C.

33°C to 30°C and 33°C to 32°C

Constant temperature incubation at 30°C, 32°C and 33°C *(Method B)* could be expected to give exclusively females, which is consistent with the results obtained.

Summary of Crocodylus johnstoni Switch Results

The SSDE's identified on Figure 9 are summarized in Table 16. Approximate development rate coefficients for *Method B* incubation of *C. johnstoni* were derived by extrapolating between the values on Table 13, such that approximate MA_{30A}'s and embryonic stages (after Ferguson 1985; see Table 1) could be estimated for each SSDE. The following generalizations can be made:

1. Under constant temperature incubation, males are rarely produced at any temperature (Table 12), just as they are rarely produced in switches

Table 16. A summary of the real ages at the initial temperature, MA_{30A}'s and embryonic stages (after Ferguson 1985) at which significant sex determining events (SSDE's) occurred during switch experiments with *Crocodylus johnstoni* under *Method B* conditions. "% Incub." is the real days to the shift expressed as a percentage of the mean total incubation time at the initial temperature with *Method B* incubation (see Table 9).

SSDE No.	Switch (°C)	Real Age	MA_{30A}	%Incub.	Stage	Notes
1	*29*-32	4	3	4%	3	Early sensitive stage
4	*30*-32	4	4	5%	4	Early sensitive stage
7	*31*-32	4	5	5%	5	Early sensitive stage
2	*29*-32	16	13	15%	11	Possible sensitive stage
5	*30*-32	19	18	21%	14	Early transition stage
10	*30*-33	21	20	24%	15	Early transition stage
8	*31*-32	11	13	14%	11	Early transition stage
12	*32*-33	35	45	51%	22	Possible sensitive stage
14	*32*-30	37	48	53%	22-23	Possible sensitive stage
3	*29*-32	31	25	29%	17	Final transition
6	*30*-32	30	29	34%	18	Final transition
11	*30*-33	34.5	34	39%	20	Final transition
9	*31*-32	32.5	39	43%	21	Final transition
13	*32*-33	45	58	65%	23-24	Final transition
15	*32*-30	39.5	51	57%	23	Final transition

from a high to a low temperature (Fig. 9). In contrast; switches from a low to a higher temperature commonly produce males.

2. Switches from 29°C, 30°C and 31°C to a higher temperature (32°C) as early as 3-5 days (MA_{30A}) may be sufficient to promote "maleness" at a constant temperature (32°C) which normally produces exclusively females.
3. Because none of the temperatures produced exclusively males, none of the final transition stages could be used to identify when "maleness" was determined.

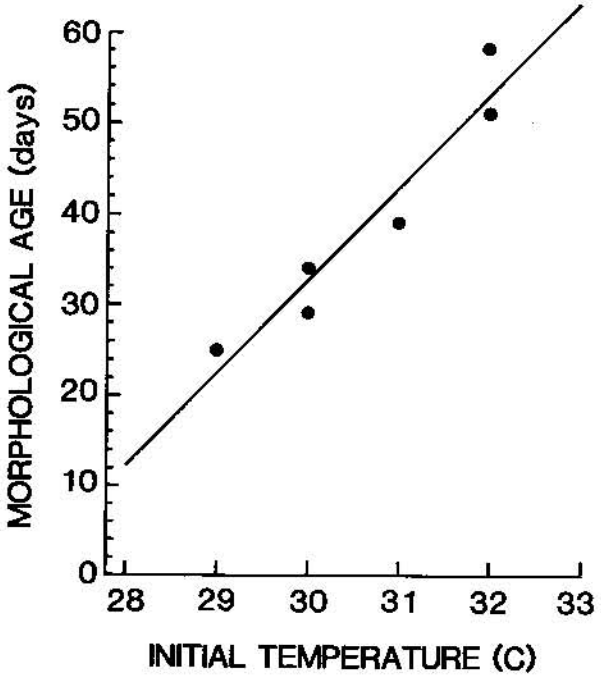

Fig. 10. The approximate MA_{30A}'s at which final transition stages occurred during switch experiments with *C. johnsoni*. These could represent when femaleness was irreversibly determined at the initial temperatures. The regression line is described by formula (20) in the text.

4. The final transition stages (Table 16), which appear to reflect the onset of "femaleness" (high and low temperature females) increased with increasing initial temperature (T). If the relationship is biologically significant, the MA_{30A} final transition stages (Fig. 10) can be predicted from the regression equation:

(20) $MA_{30A} = -272.9 + 10.18T \pm 4.0$ days
$(N = 6; r^2 = 0.92; 0.005 > p > 0.001)$

However, it is possible that SSDE's identified in shifts up to 32°C and 33°C from lower temperatures are a completely separate entity to those from 32°C to either 30°C or 33°C, in which case the linear relationship described above could be misleading.

Crocodylus porosus

Switch experiments with *C. porosus* (Fig. 11; Table 17) were undertaken with both *Methods A and B* incubation. The results for constant temperature incubation at each of the switch temperatures are in Tables 14 and 15.

28°C to 32°C

Constant temperature incubation at 28°C with both *Methods A* and *B* could be expected to give 100% females (Tables 14 and 15), whereas constant 32°C with both methods could be expected to give at least 85% males. The pattern of sexes resulting from the switches (Fig. 11) is much as would be expected: the early switches take on the sex of the final temperature (males) and the later switches the sex of the initial temperature (females).

SSDE-1. Animals switched to 32°C after 20 days of incubation were females, whereas animals switched before and later were males; 20 days incubation at 28°C *(Methods A* and *B)* may be a sensitive stage.

SSDE-2. The male inducing effect of 32°C ceased after about 24.5 days incubation at 28°C, and there appears to be a final transition to the sex (femaleness) committed at 28°C.

29°C to 32°C

Constant temperature incubation at 29°C with both *Methods A* and *B* could be expected to give 100% females (Tables 14 and 15), whereas constant 32°C with both methods could be expected to give at least 85% males. The pattern of sexes resulting from the switches (Fig. 11) is as would be expected.

SSDE-3. An animal switched to 32°C after 21 days of incubation was a female, whereas animals switched before and later were males; 21 days incubation at 29°C *(Methods B)* may be a sensitive stage.

SSDE-4. After about 25.5 days at 29°C, there appears to have been a final transition to the sex (femaleness) committed at 29°C.

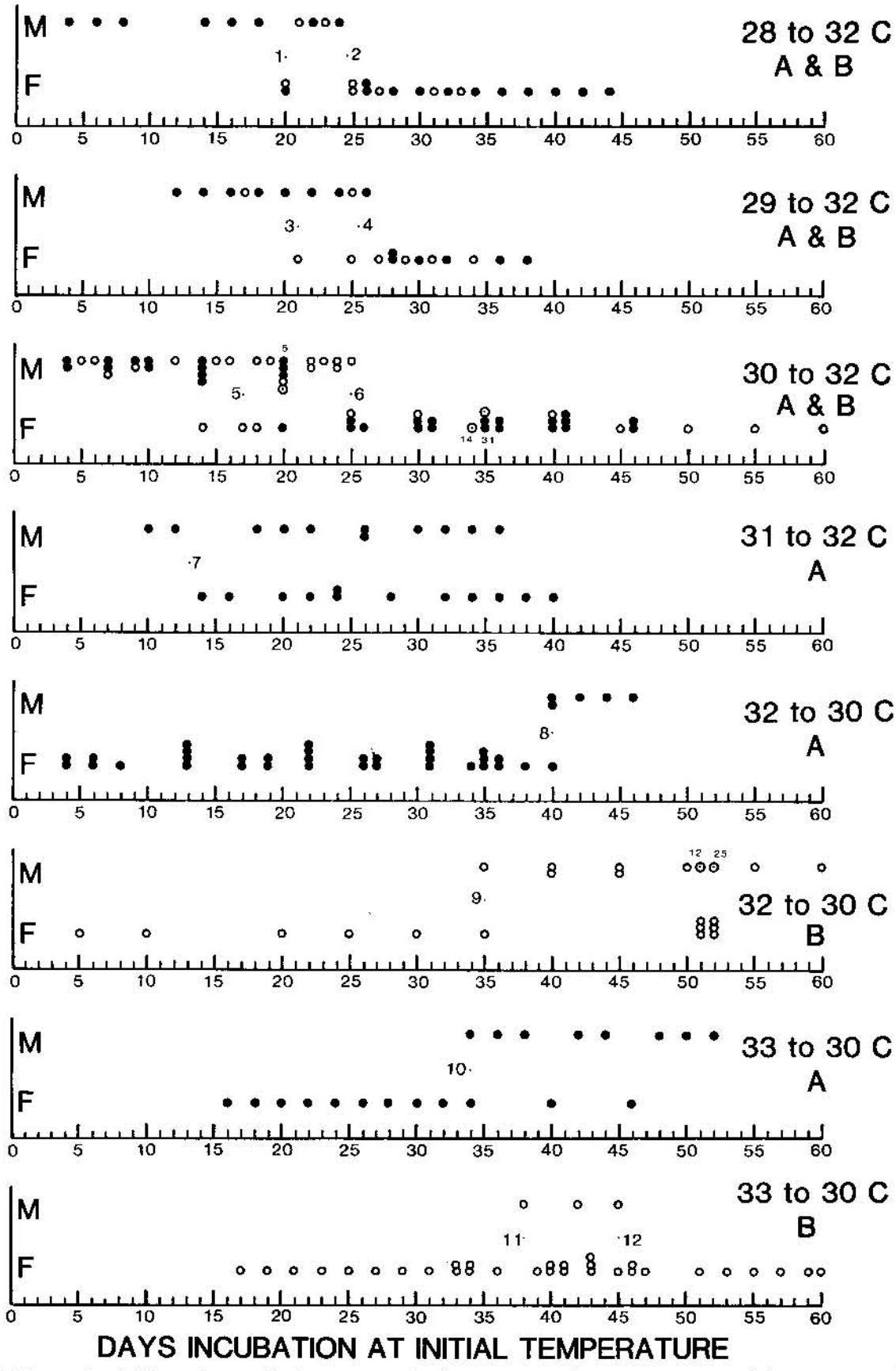

Fig. 11. The results of shift experiments with *C. porosus* eggs. Incubation was initiated at one temperature, and after varying periods of exposure individual eggs (open and closed circles) were shifted to a second temperature at which incubation was completed. Hatchlings were sexed and sexes allocated to the real ages at which the shifts had been implemented. Centrally situated numbers refer to possibly "significant" sex determination events (see text); open circles with central dots and associated numbers refer to larger sample sizes.

30°C to 32°C

Constant temperature incubation at 30°C with both *Methods A* and *B* could be expected to give 100% females (Tables 14 and 15), whereas constant 32°C with both methods could be expected to give at least 85% males. The pattern of sexes resulting from the switches (Fig. 11) is as would be expected.

SSDE-5. Eggs switched to 32°C after 14 to 20 days incubation at 30°C gave both males and females, whereas those switched earlier and later gave exclusively males; the mean of 17 days at 30°C may be a sensitive stage for sex determination.

SSDE-6. The male inducing effect of 32°C ceased after 25 days incubation at 30°C, which appears to be a final transition to the sex (femaleness) committed at 30°C.

31°C to 32°C

Constant temperature incubation at 31°C with both *Methods A* and *B* could be expected to give males and females (Tables 14 and 15), whereas constant 32°C with both methods could be expected to give at least 85% males, although early switches to 32°C from 28°C, 29°C and 30°C gave exclusively males. The pattern of sexes resulting (Fig. 11) is difficult to interpret, and the SSDE identified could be an artefact.

SSDE-7(?). In switches from other low temperatures to 32°C, only males resulted initially, and thus the first females in the shifts from 31°C to 32°C represent a deviation from the 32°C males: 13 days at 31°C could represent a transition to the sex ratios to be expected from constant 31°C.

32°C to 30°C

Constant temperature incubation at 32°C with both *Methods A* and *B* could be expected to give at least 85% males, whereas 30°C could be expected to give 100% females (Tables 14 and 15). The pattern of sexes resulting from the switches (Fig. 11) is much as would be expected. However, because of differences in development rate between *Methods A* and *B*, results for both methods are presented separately.

SSDE-8. With *Method A* incubation, the female inducing effect of 30°C ceased after 40 days at 32°C, which appears to be a final transition to the sex committed at 32°C.

SSDE-9. With *Method B* incubation, the female inducing effect of 30°C ceased after 35 days at 32°C, which appears to be a final transition to the sex committed at 32°C. A large sample of eggs switched at 51-52 days (N = 43) gave 86% males, which is consistent with the sex ratio expected at constant 32°C with *Method B* (85% males; Table 14).

33°C to 30°C

Constant temperature incubation at 33°C with *Method A* gave males, but with *Method B* gave mainly (96%) females (Table 14), and thus the results for *Methods A* and *B* are presented separately. The pattern of sexes resulting from the switches with both methods (Fig. 11) are much as would be expected: the early switches took on the sex of the final temperature (females for both methods) and the later switches resulted in mainly males for *Method A* and females for *Method B*.

SSDE-10. With *Method A*, the exclusively female producing effect of 30°C incubation ceased after 34 days at 33°C, which could represent a final transition to the sex ratio committed at 33°C.

SSDE-11. With *Method B*, the exclusively female producing effect of 30°C incubation had ceased after about 38 days at 33°C and some males resulted; it may be the start of a sensitive stage.

SSDE-12. With *Method B* incubation there appears to be a transition to the almost exclusively female producing influence of 33°C *(Method B)* at 45 days.

Summary of Crocodylus porosus Switch Results

The SSDE's identified on Figure 11 are summarized in Table 17 with information on the MA_{30A}'s and embryonic stages (after Ferguson 1985; see Table 1) that apply to them. The following generalizations can be made:

1. Relative to *C. johnstoni*, switches with *C. porosus* resulted in reasonably clear and predictable allocations of sexes; early switches adopted the sex of the second temperature, and later switches the sex of the first temperature. Accordingly, final transition stages were more clearly defined.
2. As in *C. johnstoni*, the final transition stages (Table 16) increased with increasing initial temperature (T), and if the questionable SSDE-7 (31°C) is deleted, the MA_{30A} final transition stages (Fig. 12) can be predicted from the regression equation:

$$(21)\quad MA_{30A} = -180.7 + 7.0T \pm 4.0 \text{ days} \qquad (N = 7; r^2 = 0.94; 0.005 > p > 0.001)$$

The 31°C final transition stages identified in shifts up to 32°C may be biologically significant, and the SSDE's in upward shifts may be a completely separate entity to those identified in shifts from 33°C and 32°C to lower temperatures. If so, the linear relationship described above could be misleading.

The Effects of Switch Experiments on Total Incubation Time

The effects of switch experiments on total incubation time (to hatching) are exemplified on Figures 13 and 14. In both cases, the results are much as

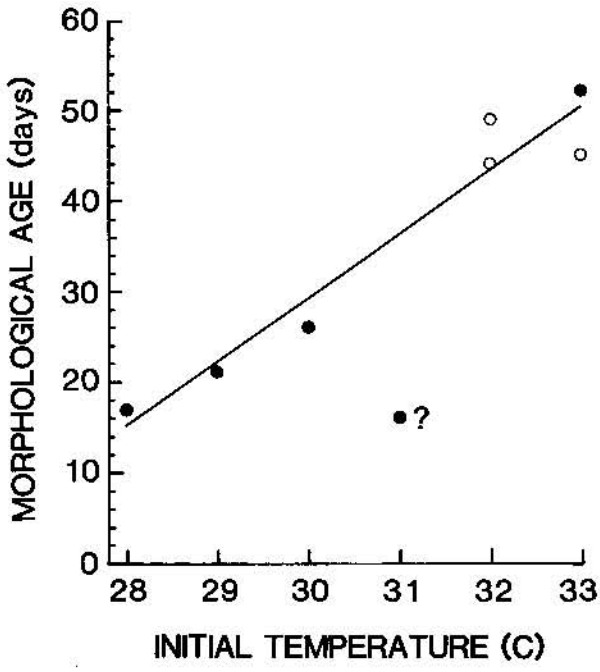

Fig. 12. The approximate MA_{30A}'s at which final transition stages occurred during switch experiments with *C. porosus*. These could represent when femaleness (closed circles) and maleness (open circles) were irreversibly determined. The possible transition at 31°C was omitted from the regression analysis [see formula (21) in the text].

would be predicted on the basis of the relationship between development rate and temperature. In the 32°C to 30°C switches (Fig. 14), it is perhaps noteworthy that the small number of females produced from switches at 51 and 52 days (which was after the final transition stage) tended to have longer incubation times than the males which predominated at that switch.

A General Summary of Switch Experiment Results

Although the results of the switch experiments varied greatly between the two species, there were similarities in the embryonic stages at which significant sex determining events occurred, and these could have more general application to other species (Table 18).

In *C. johnstoni*, a species in which no constant temperature seems to give a preponderance of males, switches at very early stages of incubation had a major influence on sex. In *C. porosus*, no equivalent early sensitive periods were detected.

In *C. porosus*, the final transition stage at 28°C was stage 14 (of Ferguson 1985), and no equivalent data are available for *C. johnstoni*. In both species, 29°C incubation was associated with final transition stages of 15-17, and at 30°C, stages 17-20. The situation at 31°C is unclear, and appears to be considerably later in *C. johnstoni* (stage 21) than in *C. porosus* (stage 13-14). The difficulty with 31°C is that it is a temperature which can produce both sexes with *C. porosus* and *C. johnstoni* (under *Method B* incubation). In all the above cases the direction of the final transition stage is from males to females, and thus these stages could represent the embryonic stage at which femaleness is committed at the different initial temperatures.

At 32°C and 33°C, regardless of whether the switch was to a higher or lower temperature, final transition stages occurred between stage 22 and 24. With *C. johnstoni* and *C. porosus* incubated at 33°C *Method B*, these appear to be the embryonic stages of development at which high temperature femaleness is committed. With *C. porosus* incubated at 32°C and 33°C *Method A*, they may represent the stages at which maleness is committed.

Table 17. A summary of the real ages at the initial temperature, MA_{30A}'s and embryonic stages (after Ferguson 1985) at which significant sex determining events (SSDE's) occurred during switch experiments with *Crocodylus porosus*. "*" indicates where a mean development rate coefficient (for *Methods A* and *B*) has been used to derive the MA_{30A}. "% Incub." is the real days to the shift expressed as a percentage of the mean total incubation time at the initial temperature of incubation (see Table 10).

SSDE No.	Switch (°C)	Real Age	Method	MA_{30A}	%Incub.	Stage	Notes
1	*28*-32	20	A and B	14*	15%	12	Possible sensitive stage
3	*29*-32	21	B	17	20%	14	Possible sensitive stage
5	*30*-32	17	A and B	17*	18%	14	Possible sensitive stage
11	*33*-30	38	B	44	51%	22	Possible sensitive stage
2	*28*-32	24.5	A and B	17*	19%	14	Final transition
4	*29*-32	25.5	A and B	21*	23%	15-16	Final transition
6	*30*-32	25	A and B	26*	27%	17-18	Final transition
7?	*31*-32?	13	A	16	15%	13-14	Possible final transition
8	*32*-30	40	A	49	48%	22	Final transition
9	*32*-30	35	B	44	44%	22	Final transition
10	*33*-30	34	A	45	44%	22	Final transition
12	*33*-30	45	B	52	60%	23	Final transition

Using the MA_{30A}'s on Tables 16 and 17, it can be shown that femaleness at 28°C with *C. porosus* is committed at 19% of incubation. At 29°C and 30°C, femaleness is committed at 23-27% of incubation in *C. porosus*, and 29-39% of incubation in *C. johnstoni*. The situation at 31°C is confounded by mixed sexes, but appears to be about 43% in *C. johnstoni* and 15% in *C. porosus*. In contrast to these results femaleness in *C. johnstoni* at 32°C does not appear to be committed until 57-65% of incubation and high temperature femaleness with *C. porosus* (33°C *Method B*) at 60% of incubation. Maleness with *C. porosus* at 32°C and 33°C is committed at 44-48% of incubation.

Table 18. The relationship between initial temperature, the direction of the shift, and the numbered embryonic stages (after Ferguson 1985) at which significant sex determining events were identified.

Temperature		Stages Identified	
(°C)	Direction	*C. johnstoni*	*C. porosus*
28.0	increase	– –	12,14
29.0	increase	3,11,18	14,15-16
30.0	increase	4,14,15,18,20	14,17-18
31.0	increase	5,11,21	13-14
32.0	increase	22,23-24	– –
32.0	decrease	22-23,23	22,22
33.0	decrease	– –	22,22,23

DISCUSSION

Development Rates

Deriving a methodology for predicting between embryological stages, real ages and relative ages (MA_{30A}), under specific incubation conditions, was a central aim of the present study. It allowed embryological stages at which significant sex determining events occurred (identified through the switch experiments), to be described in terms of the two time scales and the numbered stages of Ferguson (1985). This meant that the results could be analysed as continuous measures (age as MA_{30A}'s) where appropriate. From a practical point of view, the methodology allows the incubation times needed to reach a particular embryonic stage to be predicted, regardless of temperature. For the future, it means that examinations at the histological and histochemical levels, which will hopefully lead to a better understanding of the sex determining mechanism, can proceed within a framework of basic information on development rate.

Our use of development rate coefficients, derived from linear regressions, was sufficient for the general indices of development rate that we required. However, with larger series of embryos at each temperature, it should be possible to model more precisely the relationships between real age and relative age, and account for specific stages where the relationship between development rate and temperature is non-linear (Yntema 1960; Miller 1985; Ewert 1985).

Total incubation time and percentage of total incubation time are both indices of development rate amenable to quantitative analyses, which could be used in preference to our approach (development rate coefficients), but there are some potential problems with both of them:

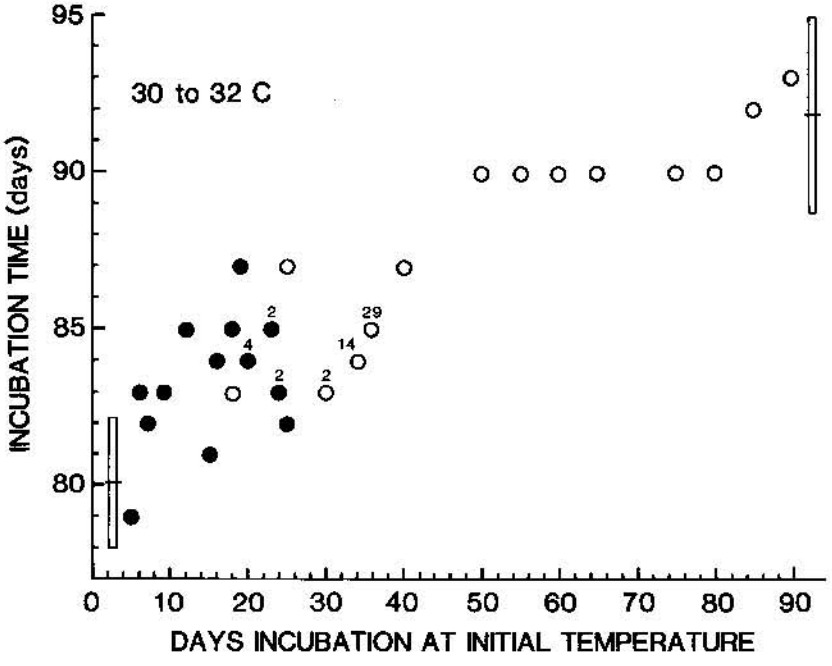

Fig. 13. The relationship between days of incubation at the initial temperature and total incubation time in *C. porosus* incubated under *Method B* conditions and switched from 30°C to 32°C. Horizontal bars and boxes represent the mean ± 1 standard deviation for incubation times at constant temperature (from Table 10). Where more than one value was available for a particular switch, means are presented; numbers are the sample sizes of means.

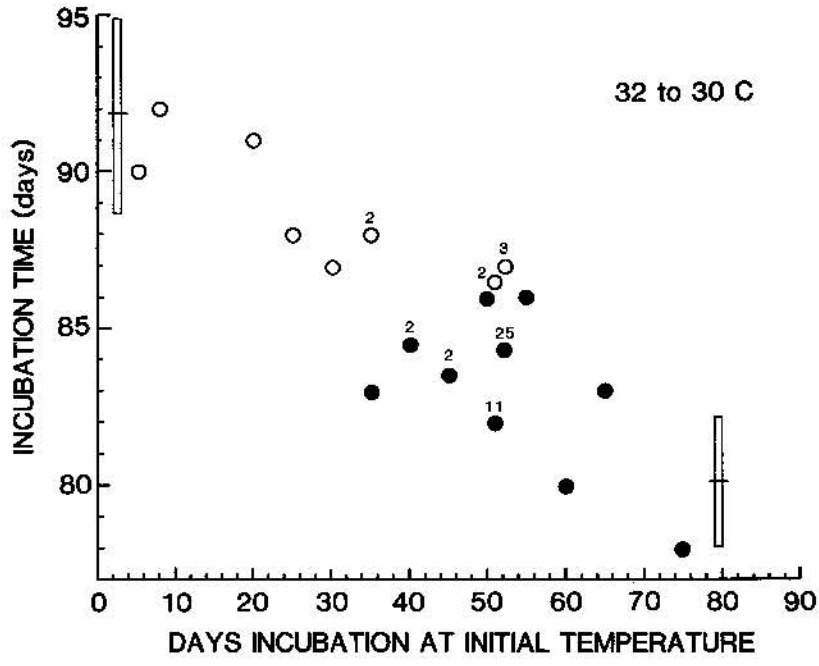

Fig. 14. The relationship between days of incubation at the initial temperature and total incubation time in *C. porosus* incubated under *Method B* conditions and switched from 32°C to 30°C. Horizontal bars and boxes represent the mean ± 1 standard deviation for incubation times at constant temperature (from Table 10). Where more than one value was available for a particular switch, means are presented; numbers are the sample sizes of means.

1. Total incubation time ignores changes in development rate between the early and late stages of development. For example, the retarded development which occurred with *C. porosus* incubated at 33°C *Method B* (Table 15), and which may have been sufficient to commit "femaleness" to the embryos, would not have been detected by using total incubation time alone.

2. Percentage of incubation time is potentially confounded by embryos not necessarily hatching at the same stage of development. At 34°C, *C. johnstoni* embryos internalize their yolk (Table 8) and hatch (Table 9) at a relatively early stage, giving small hatchlings with large amounts of residual yolk (Table 11). Fifty percent of total incubation time at 34°C is not necessarily the same embryonic stage as 50% of total incubation time at 28°C.

One of the areas of concern we have about the approach we took is that the standard temperature chosen (30°C) should perhaps be within what appears to be the "optimal" incubation temperature range (31-32°C). If 30°C was sub-optimal, and development in the later stages was relatively enhanced because of it (in order to hasten hatching and thus escape from sub-optimal conditions), then the development rate coefficients derived from a 30°C standard series would underestimate the time of hatching at more optimal temperatures [as occurred here with *C. porosus* (Table 10), but not with *C. johnstoni* (Table 9)].

Sex Determination

Incubation temperature has a profound influence on sex determination, rate of embryonic development, hatchling size and total incubation time in both *C. johnstoni* and *C. porosus*. Thus although both species have "temperature-dependent sex determination", it is unclear whether temperature *per se* is an ultimate or proximate sex determining factor. The sex determining mechanism, which remains unknown (Standora and Spotila 1985), could be responding to either the overall rate of embryonic development, the rate of growth of specific embryonic tissues at a particular stage of development, the supply of nutrients available to an embryo or specific set of tissues at a particular stage, or to some other factor or group of factors which are strongly temperature dependent, but which may also be influenced by other aspects of the incubation environment.

In the results presented here, *Method A* and *Method B* incubation, at the same constant temperatures, resulted in different embryonic development rates, different total incubation times and different allocations of sex ratios to any particular constant temperature. We do not know which aspects of the incubation environment were responsible for this variation between methods, and both the moisture (Gutzke and Paukstis 1983) and gaseous environments of incubation could be involved. However, by uncoupling incubation temperature from both development rate and total incubation time, we were able to demonstrate (Tables 13 and 15) that

development rate and total incubation time were as good if not better predictors of sex ratio than was temperature itself.

Development rate and total incubation time appear to have become uncoupled from each other with the incubation of *C. porosus* eggs at 33°C *Method B* (Table 15). There was retarded development during the early stages of embryogenesis, but no obvious change in total incubation time (Table 15). The retarded early development placed the embryos within the development rate range that produced females. Thus the "high temperature" *C. porosus* females may not be equivalent to the "high temperature" *C. johnstoni* females which result from both rapid development and short total incubation times.

Together, the results support the hypothesis that the mechanism of sex determination is more closely related to embryonic development rate during the first half of incubation than it is to either incubation temperature or total incubation period *per se*. Thus variation in the incubation temperature which produces 50% of each sex ["threshold" temperature of Bull (1980); pivotal temperature of Mrosovsky and Yntema (1980); and the "sex determining temperature, 50% female (SDT_{50})" of Limpus *et al.* (1983)] could be expected from different incubation techniques and perhaps even from minor variation in the structure of the eggshell and eggshell membrane of different eggs (see Whitehead Chapter 47).

The relationship between sex and constant incubation temperature in *C. johnstoni* may be reflecting some hitherto unknown aspects of the sex determining mechanism. With *C. johnstoni* few males were produced at any constant temperature, yet in the wild, nearly 100% males come from nests taking 72-74 days to incubate (Webb and Smith 1984) [at constant temperature such incubation times correspond to about 32°C (Table 9)]. The small, shallow nests of *C. johnstoni* are exposed to considerable daily fluctuations in temperature (Webb *et al.* 1983b; Anthony Smith, additional unpublished data) and mean nest temperatures increase throughout the incubation period (Webb and Smith 1984). Thus "maleness" in this species would appear to need either daily fluctuations in temperature (embryonic development rate?), or steadily increasing temperatures (rates of embryonic development).

The results of the switch experiments with *C. johnstoni* are consistent with this view. Switches from a low to a higher temperature as early as five days, resulted in a preponderance of males. Yet switches from a high to a lower temperature rarely resulted in males. Thus the sex determining mechanism in *C. johnstoni* seems to have become dependent on both the mean and variability of incubation temperatures in the wild.

In contrast to this situation, the sex determining mechanism of *C. porosus* and *A. mississippiensis* (Ferguson and Joanen 1982, 1983) does not appear to depend on either the daily fluctuations in nest temperature, nor on a steadily increasing temperature throughout development. These two species both have large mound nests with reduced daily fluctuations in temperature (Joanen 1969; Webb *et al.* 1977) and relative to *C. johnstoni* (Webb and Smith 1984), no pronounced increase in mean nest temperature throughout the incubation period (Webb *et al.* 1977; Joanen 1969).

The influence of fluctuating nest temperatures on sex determination in the small, shallow nests of many turtles could perhaps be expected to follow the *C. johnstoni* rather than *C. porosus* model. However, although the temperatures experienced in a fluctuating incubation environment have a dramatic effect on the sexes produced among turtles (Wilhoft *et al.* 1983), constant temperature incubation readily produces both sexes.

In the absence of histological and histochemical data, the results of our switch experiments are difficult to interpret in any depth. One of the first questions which needs to be answered is whether or not the final transition stages identified, which correspond broadly to the *primary sensitive periods* of Bull and Vogt (1981), are indeed stages at which sex is determined irreversibly at the initial temperature [this appears to be the case in turtles (Yntema 1979; Bull and Vogt 1981; Yntema and Mrosovsky 1982)].

That some sensitive stages identified in switch experiments can be stages at which sex is set on a path, but is irreversibly fixed much later, is perhaps exemplified by the "early sensitive periods" identified in the switches to 32°C from 29°C, 30°C and 31°C [with *C. johnstoni* (Table 16)]. At these early stages of development (stages 3 to 5 of Ferguson 1985) gonadal tissue is at most a primordium (it cannot be recognized in stained sections; unpublished data), yet a 1-3°C increase in temperature is sufficient to commit the embryo to becoming a male if the final incubation regime is maintained at a constant high temperature (32°C).

If growth of the tissues composing the gonadal primordium at this early stage was greatly stimulated by the switch (and subsequent 32°C incubation), the result could be consistent with Mittwoch's (1983) proposal that the rate of growth of gonadal tissue is implicated in sex determination. The result also suggests that storing and transporting eggs at a very low temperature, in order to retard development before initiating experiments aimed at elucidating the relationship between incubation temperature and sex (Limpus *et al.* 1985), may introduce an additional bias.

If sex is irreversibly fixed at the final transition stages identified here, then the embryonic stages at which sex is irreversibly committed appear to be early at low incubation temperatures, late at high incubation temperatures, and to be somewhat independent of sex itself (Figs 10 and 12).

Theoretical Considerations

The selective advantages, if any, of some reptiles having temperature (environmental?) dependent sex determination, whereas the majority have their sex determined by genotypic means, remain obscure. We considered it possible (Webb and Smith 1984) that the relationship between sex and incubation temperature could be a consequence of a mechanism for enhancing embryo survivorship. This would require the timing of gonadal development to be constrained by demands being made upon the mesonephric kidney under different incubation regimes, while the stimulus for producing one sex or the other was independent of those constraints. The results of the present study shed little light on that possibility.

If we assume that such survivorship constraints are implicated in the existence of temperature-dependent sex determination in some reptiles and not in others, then Charnov and Bull's (1977) model would seem to be a particularly useful starting point. They proposed that environmental sex determination would be advantageous in situations where sex specific fitness was a function of the nest environment. If a particular nest environment was likely to produce a fit male and an unfit female (or vice versa), the mechanism would allow fit males (or females) to be selectively produced.

Ferguson and Joanen (1982, 1983) felt that hatchling "fitness" in *A. mississippiensis* could be reflected in the amount of residual yolk that embryos contained at hatching, as this may favour post-hatching growth of one sex more than the other. As pointed out previously (Webb and Smith 1984), there are a number of problems with that scenario. Firstly, their finding of more yolk in the lower temperature animals (the opposite of what was found here) may be an artefact of examining yolk weights at the same "real time" at each temperature rather than at the point of hatching. Secondly, in *C. porosus* and *C. johnstoni*, hatchlings with the most yolk (highest incubation temperatures) and with the least yolk (lowest incubation temperatures) are both females. Thirdly, the most "fit" *A. mississippiensis* in terms of growth and survival (Joanen *et al.* Chapter 51) are now known to be those from medium incubation temperatures, which could be expected to have medium amounts of yolk.

Hatchling size and the amount of residual yolk contained at hatching both appear to be a consequence of development rate in crocodilians (Table 11) and some turtles (Webb *et al.* 1986). By varying the time of hatching, and thus the amount of yolk converted to embryonic tissue prior to hatching, embryos appear to be optimising their chances of surviving to hatching. Extremes of incubation environment give abundant yolk and small body sizes, or little yolk and large body sizes, but in both cases post-hatching performance is severely compromised (Joanen *et al.* Chapter 51). Thus selection in either direction (to produce hatchlings with large body size and/or large amounts of residual yolk) is unlikely, because it does not produce fit hatchlings, regardless of appearances.

Yet the demonstrated relationship between post-hatching performance and incubation conditions in *A. mississippiensis* (Joanen *et al.* Chapter 51) could indeed be related to the selective advantages of having temperature-dependent sex determination, as suggested by Ferguson and Joanen (1982, 1983) and Joanen *et al.* (Chapter 51). It would require that the "growth potential" apparently endowed in embryos by the incubation environment (Joanen *et al.* Chapter 51) have a distinct physiological basis. For example (Jean Joss, pers. comm.), the rate of growth hormone production could be irreversibly set in the embryos as a function of incubation environment (or embryonic development rate).

Adult male crocodilians are bigger than females, and large size is an important prerequisite for dominance. Temperature-dependent sex determination in crocodilians could function to ensure that "maleness" was allocated only to embryos which had the growth potential to become large, reproductively successful males. The default option would be females, because even small females can reproduce successfully. Thus at incubation temperatures that were "too high" or "too low", and where growth potential was likely to be compromised or there was an increased probability of abnormalities likely to affect post-hatching growth (Webb *et al.* 1983c; Ferguson 1985), femaleness would predominate. It may not be coincidental that the temperature range giving males in *C. johnstoni* and *C. porosus* (31-32°C) is similar to the range at which maximum post-hatching growth was recorded in *Alligator mississippiensis*.

For the hypothesis to hold with turtles, where females are often larger than males, one would predict that the optimal temperatures for "growth potential" would tend to correspond with females rather than males. There are insufficient data available on growth rates of turtles incubated under constant temperature conditions to test this. However, the most common products of temperature-dependent sex determination in turtles are high temperature females and males, whereas in crocodilians it is males and low temperature females. It would thus not be surprising if the females rather than males were endowed with enhanced "growth potential".

Crocodile Farming

Crocodile farming ventures are becoming increasingly involved in the artificial incubation of eggs and the raising of young for release schemes (see Whitaker Chapter 7), the production of both "domestic" breeding stock, and for the production of skins and meat. Thus, the extent to which growth, survival and sex can be controlled by altering artificial incubation conditions has obvious application in both the farming and management of crocodilians.

The optimal constant incubation temperature for incubating crocodilian eggs appears to be 31°C to 32°C. With *A. mississippiensis*, the "optimal" hatchlings for raising were produced from incubation temperatures around 31°C (Joanen *et al.* Chapter 51). This temperature under *Method A* conditions also corresponded with where *C. porosus* and *C. johnstoni* had a little independence from the influence of temperature on development rate (Figs 5 and 6), and where there was a significant relationship between egg size and total incubation time (Fig. 8). It is also a temperature close to the threshold for producing males and females (Tables 12 and 14), and is a common mean incubation temperature amongst wild crocodilian nests in general (Magnusson *et al.* 1984).

If 31°C is indeed an "optimal" incubation temperature, then it raises some questions about judging hatchling "fitness" from appearances. Hatchlings produced from low incubation temperatures tend to be large, have little remaining residual yolk (Table 11) and generally look "good"; yet their post-hatching performance may be compromised. Similiarly, hatchlings from high incubation temperatures have abundant residual yolk (at the expense of body size), and may also have their post-hatching performance compromised.

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