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Reproduction of the Australian freshwater turtle *Emydura krefftii* (Chelonia: Chelidae)

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(With 2 plates and 4 figures in the text)

A study of the reproduction of Krefft's river tortoise, *Emydura krefftii*, was conducted in the perched dune lakes of Fraser Island, Queensland. Mature male specimens exhibit a post-nuptial pattern of spermatogenesis typical of temperate-zone turtles elsewhere, with a peak in spermatogenic activity in autumn and a cessation of activity during the breeding season in spring and early summer. The spermatogenic cycle is paralleled by seasonal variation in testicular weight (standardized for body size) and in the diameter of the seminiferous tubules. Sperm are abundant in the epididymal canals throughout the year. Mating was observed in autumn, late winter and spring.

Females have a cyclic reproductive pattern, with distinct phases of follicular enlargement, ovulation and oviducal period, and quiescence. Yolk begins to accumulate in the ovaries in late summer, and the accumulation continues unabated through the winter, presumably by the transfer of material from fat stores to the ovaries. Ovulations occur from late winter to mid-summer. Atresia of follicles that fail to ovulate was demonstrated histologically.

Emydura krefftii lay up to three clutches of hard-shelled ellipsoid eggs per season. Each clutch contains between four and 10 eggs; the number is strongly correlated with maternal body size. Reproductive potential ranges from 12 eggs per annum for a female that has recently matured (carapace length *c.* 150 mm), to 30 eggs per annum for a full-sized female (length *c.* 250 mm). Selected life-history traits of *Emydura krefftii* are discussed in the context of findings for other populations of the species and for other species of freshwater turtle.

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Introduction

The reproduction of chelid turtles has received more attention than possibly any other aspect of their biology. Goode (1965, 1966, 1967) and Goode & Russell (1968) described the nesting, natural and artificial incubation, and embryological development of the Australian *Chelodina longicollis, C. expansa* and *Emydura macquarii.* Vestjens (1969), Parmenter (1976) and Chessman (1978) conducted comprehensive studies of the reproduction of *Chelodina longicollis* at various latitudes, and the latter author also described the reproduction of *Emydura macquarii.* Legler & Cann (1980) provided data on the eggs, incubation times and annual reproductive potential for *Chelodina longicollis, Elseya dentata, E. latisternum, Emydura krefftii* and *Rheodytes leucops* from Queensland, and Burbidge (1981) described the reproduction of the relict species *Pseudemydura umbrina* from south-western Australia. South-American chelid turtles have been less studied, and much of what is known of their reproductive biology has resulted from the work of Medem (1960, 1966, 1973).

Despite these studies, the reproduction of turtles in the family Chelidae is poorly known when compared to that of cryptodiran families of the Northern Hemisphere—many chelid species have received scant attention, and much information remains unpublished. One species in particular, *Emydura krefftii* is abundant and widespread in Queensland where it inhabits large rivers and the larger waterholes and billabongs of their floodplains (Cogger, 1975), yet there are no detailed accounts of its reproduction. This paper describes the reproductive cycles and annual reproductive potential of *Emydura krefftii,* and provides what appears to be the first published description of the gonad cycles of a chelid turtle.

The study was conducted in an unusual environment for *Emydura krefftii*—the perched dune lakes of Fraser Island. These lakes are noted for their low nutrient levels, low productivity, and depauperate biota (Bayly, 1964; Bayly *et al.*, 1975; see below). In this paper, the reproductive characteristics of *Emydura krefftii* on Fraser Island are compared to available data for populations on the mainland, and differences are explained in terms of differences in the productivity of the two environments.

Materials and methods

Study area

Fraser Island is situated off the coast of Queensland between the latitudes 24°40′ and 25°51′S, and longitudes 152°55′ and 153°20′E (Fig. 1). The island is composed almost entirely of siliceous sands and, with an area of 1600 km², is the largest sand island in the world. Contrary to what one would expect of a land mass composed of a material as porous as sand, the island is well endowed with freshwater lakes; they occur in topographic depressions where fines and organic matter have accumulated to form impermeable basements (Whitehouse, 1968), or where impermeable layers of peat and sand come close to the surface (James, 1977). The dune lakes are often "perched" high above the regional water table. They are oligotrophic, with very dilute (average salinity *c.* 40 mg/l) acidic waters (pH = 4·0–6·0) containing high proportions of allochthonous organic material (Bayly, 1964; Bayly *et al.*, 1975). Their brown colouration severely limits penetration of sunlight (Bayly, 1975), restricting photosynthetic growth to surface waters. Limited photosynthesis, coupled with low concentrations of nutrients, results in low secondary productivity, and perhaps also in low biotic diversity. Macrophytic plants are represented by only a few species. The invertebrate fauna of the perched dune lakes is low in both diversity and numbers, when compared with other freshwater lakes (Timms, 1973). The lakes are unable to maintain large aggregations of waterfowl typical of more productive water bodies on the mainland (Kikkawa *et al.*, 1979), and many fish have been unable to invade the lakes because they

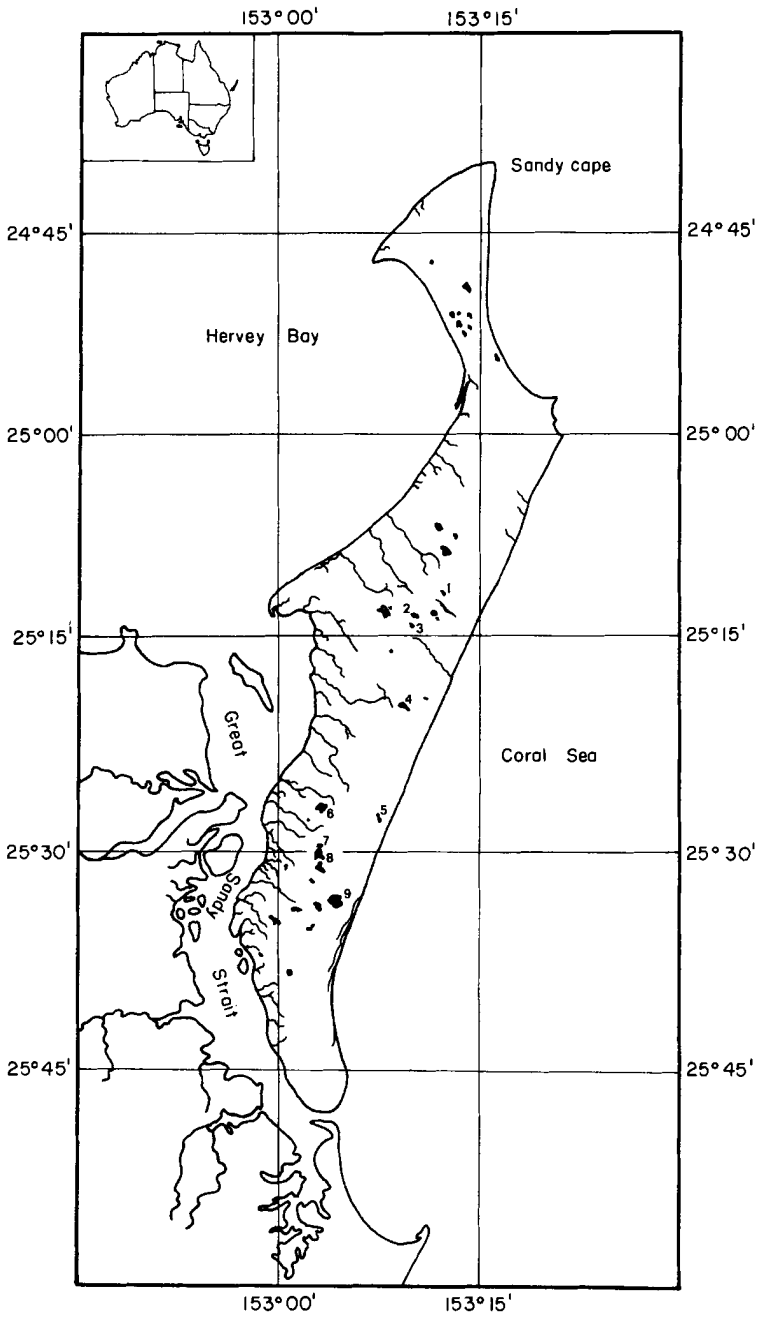


FIG. 1. A map of Fraser Island showing the position of the island in relation to the Australian mainland and the lakes from which specimens of *Emydura krefftii* were collected. The Lakes are numbered: 1, Allom Lake; 2, Lake Coomboo; 3, Hidden Lake; 4, Lake Garawongera; 5, Wabby Lakes; 6, Lake McKenzie; 7, Jennings Lake; 8, Lake Birrabeen; 9, Lake Boemingen.

have never been connected to the ocean. Those fish that are present are small (*Rhadinocentrus ornatus, Hypseleotris klunzingeri*) and have presumably been introduced as eggs on the feet of birds. In summary, the habitat of Fraser Island populations of *Emydura krefftii* is deficient in nutrients, of low productivity and of low biotic diversity when compared with the more usual habitats of the species on the mainland.

Fraser Island has a typical subtropical climate with hot wet summers (November–February) and cool dry winters (May–August). The Sandy Cape Meteorological Station, at the northern tip of the island, receives an average annual rainfall of 1278 mm (as of 1978), with a maximum monthly average of 169 mm in January and February and a minimum of 55 mm in September. January and February are the hottest months, each with an average daily maximum of 28·6°C and an average minimum of 22·0°C and 22·1°C, respectively. The coldest month is July, with a mean daily maximum of 20·7°C and a mean minimum of 14·0°C. Water temperatures (depth 1·0 m, Lake Coomboo) range seasonally between 17°C and 30°C.

General

Samples of *Emydura krefftii* were collected from various dune lakes on Fraser Island between March 1977 and April 1980. The samples, combined over the 4 years, were taken in all months. The turtles were collected with hoop traps (Legler, 1960*a*) baited with bread, and were killed, and examined as soon as possible after collection, usually within 1 week. The animals were killed by intra-cranial injection of absolute alcohol, and their body weights (± 5 g) and maximum carapace lengths (±0·1 mm) were recorded.

Twenty-nine mature males (carapace lengths >106 mm) were dissected fresh and the colouration of their testes and epididymes was noted. Fluid from the testes, epididymes and vasa deferentia was examined under a light microscope to determine the abundance of spermatozoa (recorded as absent, scarce, common, abundant, or very abundant) and whether or not the sperm were motile. Males with sperm in their epididymes were considered mature. Testes and epididymes were fixed in Bouin's Fluid, and the testes were later weighed (±0·1 g) and measured (±0·1 mm). An index to testes "size" was calculated for each animal as the combined weight of both fixed testes expressed as a percentage of total body weight. Standardization of testes weight was considered necessary because it is known to be strongly correlated with body size in some species of turtle (see Gibbons, 1968). The tissues were imbedded in wax, sectioned (nominally 6 μm), and dyed with Mayer's haematoxylin and precipitated eosin. The sections were examined under a light microscope, and the relative abundance of each spermatogenic stage (cell type) was assessed qualitatively. Spermatogenic stages were identified with the aid of papers on mammalian spermatogenesis (Leblond & Clermont, 1952; Clermont & Perey, 1957), and by referring to published photomicrographs of turtle testes (Risley, 1938; Moll, 1979). Thirty seminiferous tubules from each pair of testes were measured using a calibrated eyepiece, and mean tubule diameter was calculated for each pair. Histological examination of the testes of 2 of the 29 mature males revealed little spermatogenic activity at a time when spermatogenesis in other individuals was at a peak. They were considered to be non-breeding individuals, possibly senescent, and data taken from them were excluded from the analysis.

Ovaries were removed from 30 females (carapace length >150 mm). Corpora lutea on each ovary, if present, were counted, and each ovary was weighed (±0·1 g) before preservation in 10% formalin. An index to ovary "size" was calculated for each animal as the combined weight of both ovaries expressed as a percentage of total body weight. Ovarian weight was standardized in this fashion because reproductive output of female *Emydura krefftii* is strongly correlated with body size (see Results). Once the ovarian tissue had hardened in the fixative, the oocytes were removed and their diameters measured to the nearest millimetre by means of a gauge (Linex 1116) containing a graduated series of holes (after Moll & Legler, 1971). Follicles were then classified according to their degree of development. Those with oocytes less than 1 mm in diameter were abundant on all ovaries, but were not studied. Follicles containing oocytes with diameters between 1 mm and 3 mm inclusive, were designated "germinal fol-

licles". They were white in colour, presumably lacking yolk. "Pre-ovulatory follicles" were those that contained oocytes with diameters equal to the diameters of the species' egg-yolks (14–15 mm, $n = 10$). All follicles that were yellow (probably indicating that they were acquiring yolk) but which had not yet reached pre-ovulatory size, were designated "developing follicles". One oocyte from a developing follicle and 2 oocytes that appeared to be degenerating were imbedded in wax, sectioned (nominally 6 μm), dyed with haematoxylin and eosin, and viewed and photographed through a light microscope.

Corpora lutea, when present, were removed from the ovaries and measured to the nearest 0·1 mm with vernier calipers. Oviducal eggs if present, were removed at the time of dissection, weighed, measured, and then fixed in 10% formalin. They were later opened and intact yolks were weighed and measured.

Subcutaneous fat was removed from the inguinal pockets of 13 males and 20 females, and was weighed to the nearest 0·1 g.

The females were all considered mature because their ovaries contained developing follicles, corpora lutea, or atretic follicles. Five of the females had small ovaries that contained no developing follicles at a time when the ovaries of others were clearly developing. One had rudimentary oviducts and was probably incapable of breeding. Small orange-coloured atretic follicles were present on the ovaries of the other four, indicating that they had bred in the past. These five turtles were considered to be non-breeding individuals, and data taken from them were excluded from most of the analysis.

Results

Male reproductive cycle

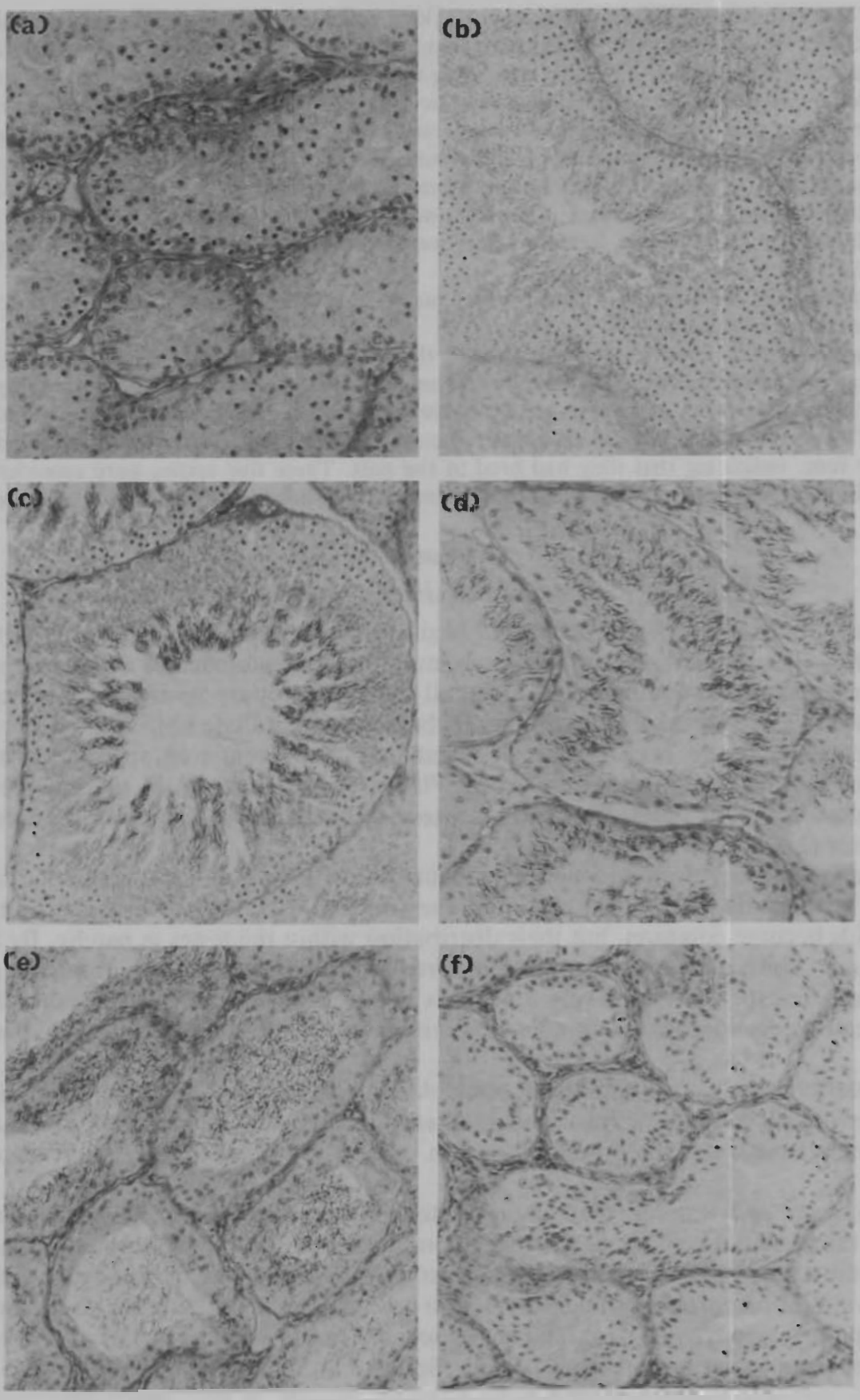
The germinal cells of *Emydura krefftii* begin to proliferate early in the austral summer (November; Plate I(a)) when spermatogonia multiply and leptotene and zygotene spermatocyte stages appear. As summer progresses, all stages of primary spermatocytes increase in abundance to reach a peak in late summer (February, March; Plate I(b)). At this time secondary spermatocytes are occasionally observed and undifferentiated spermatids are very abundant. During Autumn (March, April; Plate I(c)) the numbers of spermatogonia and spermatocytes decline, and the entire process of germinal proliferation is finished by mid-winter (June; Plate I(d)).

Spermiogenesis begins in mid-summer (January, February; Plate I(b)), soon after undifferentiated spermatids first appear. Later in the summer (February, March), differentiating spermatids become common, but their distribution within the testes is patchy. By autumn (late March, April; Plate I(c)), spermiogenesis replaces proliferation as the dominant process: undifferentiated spermatids are very numerous and other spermatid stages are abundant and widespread. Spermiogenesis continues into winter and is virtually complete by the end of June.

Spermiation begins in autumn (late March, April), although mature spermatids, embedded in the Sertoli cell cytoplasm, are abundant as early as February. Spermiation continues until mid-winter (June, July; Plate I(e)) and sperm remain abundant in the tubule lumen until spring.

In spring and early summer (September, October) the testes of *Emydura krefftii* are quiescent and the germinal cells show no signs of proliferating (Plate I(f)). Spermatogonia are the only germinal cells present and they are greatly outnumbered by Sertoli cells. The tubule lumen is almost completely occluded by Sertoli cell cytoplasm and contains no spermatozoa.

The spermatogenic cycle described above seemed to vary slightly with body size. Larger males appeared to begin spermatogenesis earlier, and to finish marginally later than smaller males.


(a)
(b)
(c)
(d)
(e)
(f)

Testes index and mean diameter of the seminiferous tubules both underwent a seasonal cycle (Fig. 2). The testes, and their tubules, were smallest when the testes were sexually quiescent in the spring (September, October), and also during the early phases of germinal proliferation (November, December). At these times the testes were yellow and compact. In mid-summer (January), the size of the testes and the diameter of the seminiferous tubules

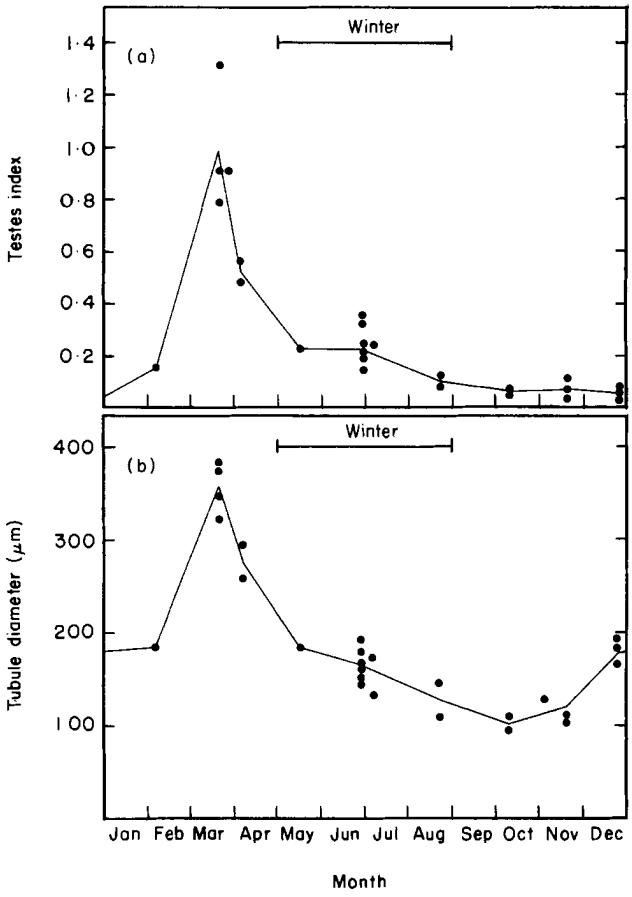


FIG. 2. Seasonal variation in the testes index (a) and in the diameter of the seminiferous tubules (b) for mature male specimens of *Emydura krefftii*. The continuous lines demonstrate general trends. Testes index was calculated as the weight of both testes expressed as a percentage of total body weight.

PLATE I. Selected photomicrographs illustrating seasonal cycle of spermatogenesis in *Emydura krefftii*. Scale ×205. (a) Late November. Proliferation of the germinal epithelium has begun. Spermatids are not yet present, and tubule lumina are occluded by Sertoli cell cytoplasm. (b) February. Germinal proliferation is at a peak. Spermatocytes are by far the predominant stage, though spermatids at all stages of differentiation are present. (c) Early April. Spermiogenesis is at a peak. Spermatocytes are much less numerous than in (b), and spermatids at all stages of differentiation predominate. (d) Late June. Proliferation and spermiogenesis are complete. Spermatogonia and mature spermatids are the predominant stages remaining. (e) Late August. The germinal epithelium is quiescent. Spermiation is complete but sperm are still abundant in the tubule lumina. (f) October. The testes are quiescent. Few sperm remain and the tubule lumina are occluded by Sertoli cell cytoplasm.

increased rapidly and reached a peak in autumn (April) coincident with the peak in spermiogenesis. The testes became vascularised and flesh-coloured. The winter months saw a steady decline in both testes size and tubule diameter, until both reached their minimum size again in the following spring.

Sperm were present in the epididymes of mature males throughout the year, but they varied in abundance from season to season. Fewest sperm were found in the epididymes, together with unidentified cellular material, in mid-summer (December, January). By autumn (April), when spermiogenesis and spermiation became the dominant processes, the epididymes were white and partially distended, indicating that sperm move from the testes to the epididymes soon after they are released into the tubule lumen. The epididymes became engorged by May and remained so until November. Since sperm were abundant in the seminiferous tubules until August, they were probably passing from the testes to the epididymes throughout the winter. Sperm were almost entirely immotile while in the testes, becoming motile only upon entering the epididymes.

Mating activity was observed in autumn, late winter and spring.

Female reproductive cycle

The ovaries of *Emydura krefftii* are quiescent in mid-summer (December, January). Most ovarian follicles were small, and few exceeded 5 mm in diameter (Table I). This period of quiescence indicates that follicles which develop in one year are not retained into the next.

Follicles begin to develop in late summer (February) and continue to enlarge well into winter until they reach pre-ovulatory size. Pre-ovulatory follicles first appear in early June, but smaller follicles continue to develop and may reach pre-ovulatory size as late as October or November (Table I). These observations indicate that follicular development continues throughout winter, which explains the continued increase in the ovarian index as winter progresses (Fig. 3). A steady decline in the weight of the inguinal fat bodies (expressed as a percentage of body weight) coincided with the period of follicular development in breeding females. This steady decline with time (March–December, in days) was significant for both males ($r_s = -0{\cdot}68$, $n = 13$, $P < 0{\cdot}05$) and females ($r_s = -0{\cdot}65$, $n = 15$, $P < 0{\cdot}01$). The correlation for females was considerably poorer when non-breeding females were included in the analysis ($r_s = -0{\cdot}47$, $n = 20$, $P < 0{\cdot}05$), despite an increase in sample size from 15 to 20.

Presence of oviducal eggs (Table I) was taken as evidence of recent ovulations. Ovulation can occur as early as August and as late as December, but more usually occurs in spring and early summer (September, October, November). Females lay up to three clutches in a single summer, so evidence of ovulations can be observed at any time within a four month period.

After ovulation the collapsed follicles become glandular corpora lutea that can be distinguished from other ovarian structures by their cup-like shape and translucent white colouration (after fixation). The ovaries of gravid females possessed corpora lutea in numbers equal to or greater than the number of eggs in the oviducts. The corpora lutea were of various sizes, but could usually be grouped into one, two or three distinct categories. The category containing the largest corpora lutea, with one exception, corresponded in number with the number of eggs in the oviducts. It was therefore assumed that these large corpora lutea had given rise to the eggs during recent ovulations. One female contained four exceptionally thin-shelled eggs which, presumably, had recently entered the oviducts. The four fresh corpora

TABLE 1

Seasonal occurrence of oviducal eggs, corpora lutea and ovarian follicles of various sizes for ***Emydura krefftii.*** *Follicles less than 1 mm in diameter were abundant on all ovaries but were not studied. Each rectangle encloses follicles, corpora lutea or oviducal eggs thought to be associated with a clutch of eggs that was past or pending at the time of dissection. Asterisks mark atretic follicles. Data from non-breeding mature females are omitted*

Ref.	Month	Ovarian follicles (Diameter in mm) 1–3	4	5	6	7	8	9	10	11	12	13	14	15	16	Corpora lutea Old	Medium	Fresh	Oviducal Eggs
EK11D	Jan	33																	
EK13D	Feb	67	1	2	1														
EK33D	Feb	44	2		2	2													
EK34D	Feb	37	4																
EK57D	Mar	—		3	2														
EK58D	Mar	—	5	7	2														
EK59D	Mar	—	8		[2	2		2]											
EK60D	Mar	—	9	5		[1	3	1	1]										
EK35D	Mar	28	9	4															
EK36D	Mar	94	10	15															
EK02D	May	60	9	1	3	2	1		[4	2	3]								
EK03D	May	52	3	2	2	3	1	1		[2	2	6]							
EK15D	May	62	4	4	2	2		[2	4]										
EK39D	Jun	33	5	1	1	3	2	1	1				[6]						
EK40D	Jun	61	13	5	2	3	3					■							
EK17D	Jun	53	5			2	3			[2	2	1]							
EK20D	Aug	36	2					[1		1	3]							4	[4]
EK04D	Sep	40	5	1		1	1	1	3					[5]					
EK26D	Oct	80	1	2	1						[2	6]						9	[9]
EK27D	Oct	67	4				2	1		[1	2	1	1	2]		[7]		10	[9]
EK06D	Oct	46	1	1	1		1					[1	4]					5	[5]
EK09D	Nov	65		1		1	1									[4]	[4]	4	[4]
EK30D	Nov	37		4												[7]			
—	Nov					----X-Rayed----													[4]
—	Nov					----X-Rayed----													[7]
EK51D	Dec	59	1				1	1											
EK52D	Dec	82			1*					2*						[+]		6	[6]

*Atretic follicles.

lutea associated with these eggs had a mean maximum diameter of 7·2 mm (±0·1). The average maximum diameter of corpora lutea known to be associated with oviducal eggs was 5·9 mm (±0·1, $n=34$). Since smaller corpora lutea were associated with hard-shelled eggs, the data indicate that the corpora lutea reduce in size while eggs are still in the oviducts. This reduction in size probably results from consolidation of the spent follicle into a functional corpus luteum rather than from atrophy.

Regression of corpora lutea follows the completion of egg-laying. After egg-laying, corpora lutea ranged between 5·6 mm and 2·6 mm in diameter (mean diameter 4·0 mm ±0·3, $n=15$), and they were much smaller than that of fresh corpora lutea and significantly smaller than those associated with oviducal eggs (t-test, $P<0·05$). This information, together with the fact

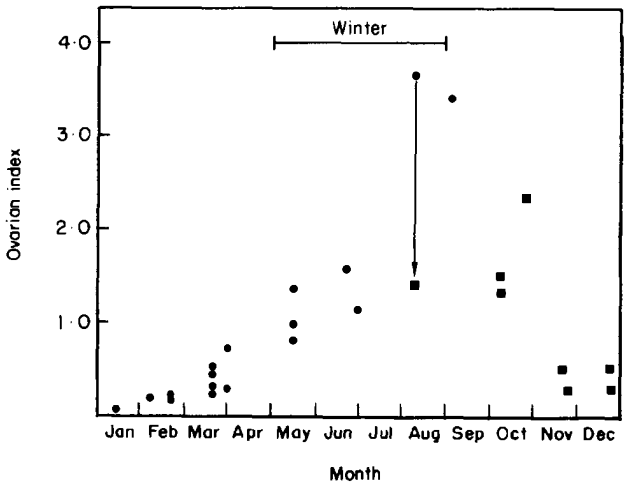


FIG. 3. Seasonal variation in ovarian index (weight of both ovaries expressed as a percentage of body weight) for 24 mature female specimens of *Emydura krefftii*. Data from non-breeding females are omitted. Sixteen specimens were dissected before ovulation (●) and the remainder (■) after ovulation. The arrow represents the estimated drop in ovarian index as a result of ovulations in EK20D (weight of eggyolk: 2·31 ±0·13 g, $n=14$).

that no corpora lutea were observed on the ovaries after December (Table I), suggests that the corpora lutea degenerate shortly after the eggs are laid.

Ovarian follicles remaining after the breeding season also appear to degenerate. One specimen of *Emydura krefftii* (EK52D), dissected in late December, contained three moderately large ovarian follicles (Table I) that appeared to be undergoing follicular atresia. Instead of the normal homogeneous yellow colouration, the three oocytes had much of their surfaces covered with mauve blotches. The smallest of the three follicles showed most pronounced blotching, and its surface was pitted. Histological examination confirmed that they were degenerating (Plate II). Follicles that had completed this process of atresia appeared on nearly all of the mature ovaries examined. They were small spherical bodies, less than 4 mm in diameter, that could be distinguished from normal follicles (yellow) by their dark orange colouration.

After release from the ovary, it is generally accepted that turtle ova pass into the coelom before being taken up by the oviduct's infundibulum. Occasionally an ovum may find its way into the contralateral oviduct, an event referred to as trans-coelomic migration (Tinkle, 1959). Table II compares numbers of fresh corpora lutea with numbers of oviducal eggs for gravid specimens of *Emydura krefftii*. Of the six gravid females, four showed evidence of trans-coelomic migration. In all four cases, the nett effect of the transfers was to more evenly distribute the ova between the oviducts.

It is not known how long eggs remain in the oviducts of *Emydura krefftii*. Estimates of the oviducal period are usually obtained by comparing the periods of ovulation and oviposition for the population (Edgren, 1960; Legler, 1960*b*; Gibbons, 1968), but such estimates are accurate only for species that lay single clutches and that have a very brief nesting season. Nesting was not observed in the present study, but judging from the period in which eggs were found in the oviducts (Table I), the nesting season probably ranges from early September to early January.

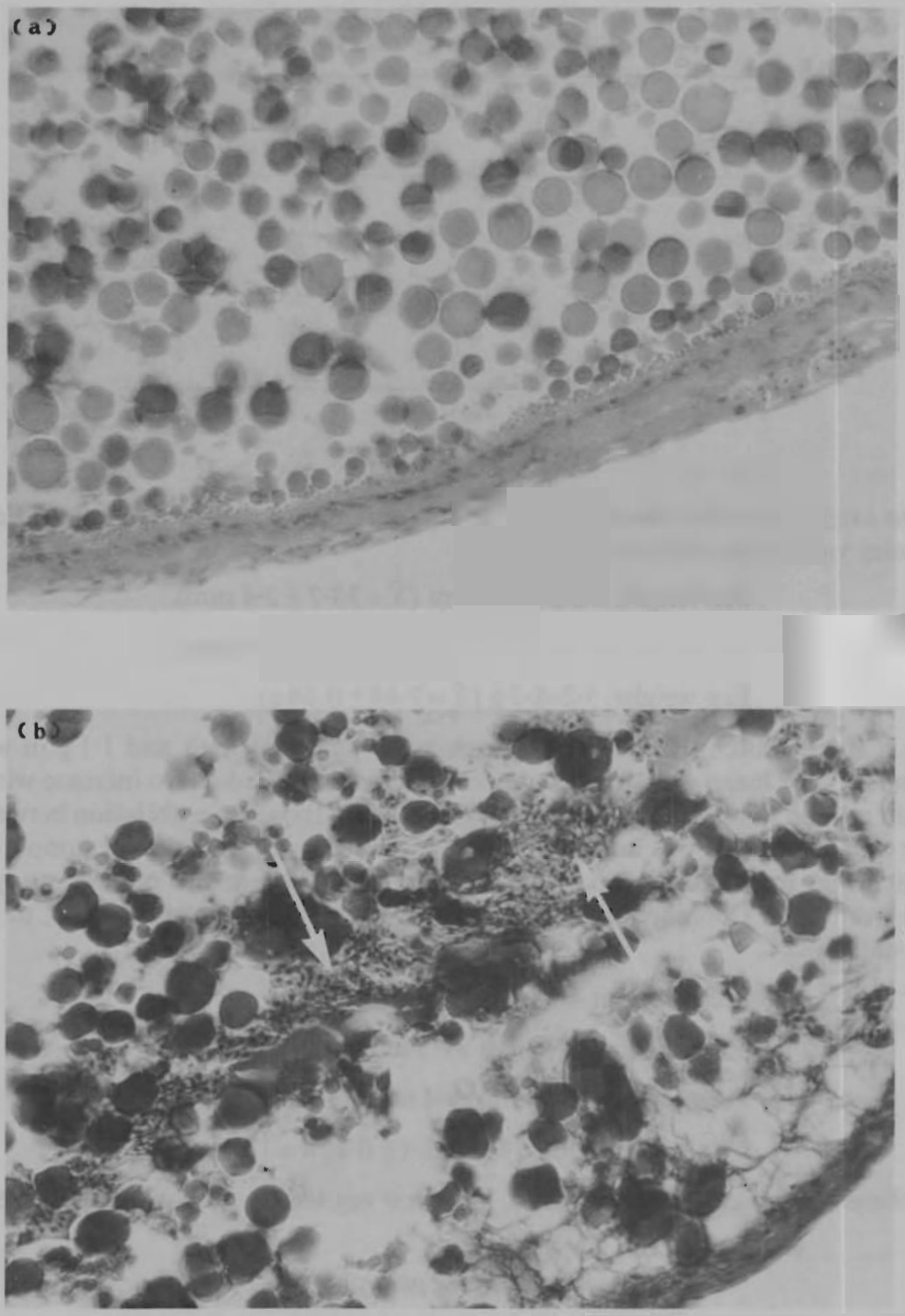


PLATE II. Photomicrographs of a section of a developing oocyte (a), and of a section of an atretic oocyte (b). The developing oocyte (6·0 mm in diameter) was taken from a specimen of *Emydura krefftii* just before the breeding season (July), whereas the atretic oocyte (6·5 mm diameter) was from a specimen taken at the end of the breeding season (December). Note the extensive vascular invasion of the atretic oocyte; nucleated erythrocytes are prominent in the blood vessels (arrow).

TABLE II

Corpora lutea and oviducal eggs counted in gravid ***Emydura krefftii.*** *Specimens marked with an asterisk show evidence of trans-coelomic migration. Note that reciprocal transfers go undetected*

Reference	Corpora lutea		Oviducal eggs	
	Left	Right	Left	Right
EK06D	2	3	2	3
EK09D	2	2	2	2
EK20D*	1	3	2	2
EK26D*	3	6	5	4
EK27D*	2	8	4	5
EK52D*	2	4	3	3

Eggs and hatchlings

Emydura krefftii lay white, hard-shelled (calcareous), ellipsoid eggs. A total of 42 eggs from seven females yielded the following statistics.

Egg length: 28·8–37·0 mm ($\bar{X}=33{\cdot}7\pm2{\cdot}4$ mm).

Egg width: 17·5–20·9 mm ($\bar{X}=19{\cdot}3\pm1{\cdot}4$ mm).

Egg weight: 5·2–8·7 g ($\bar{X}=7{\cdot}44\pm0{\cdot}14$ g).

Eggs from a single clutch differed by as much as 6·7 mm in length and 1·1 g in weight. A general tendency for mean egg weight (calculated for each individual) to increase with female body weight proved significant ($r_s=0{\cdot}78$, $n=7$, $P<0{\cdot}05$), though a correlation between mean egg weight and clutch size was not significant, probably owing to the small sample size.

Five hatchlings from Allom Lake (Fig. 1) were examined in the present study and 10 hatchlings from Lake Coomboo were measured by David Barry (pers. comm.), to yield the following statistics.

Mean weight 4·6 g ($\pm0{\cdot}1$, $n=5$).

Mean carapace length 29·5 mm ($\pm0{\cdot}3$, $n=15$).

Mean carapace width 26·4 mm ($\pm0{\cdot}3$, $n=15$).

Mean shell depth 14·5 mm ($\pm0{\cdot}2$, $n=15$).

The hatchlings, which could not be sexed, had their egg teeth intact when measured.

Reproductive potential

Two of six gravid females possessed greater numbers of corpora lutea than eggs in the oviducts (Table I). Since corpora lutea do not persist for long after egg-laying, those present in excess of the number of eggs were assumed to be associated with clutches laid earlier in the same season. For example, the ovaries of one individual (EK09D) possessed three distinct sets of corpora lutea: four fresh ones (corresponding to its four oviducal eggs), four of inter-

mediate size, and a further four small ones. This female appears to have produced three clutches, each of four eggs. The ovaries of another individual (EK27D): Table I) possessed ten large, vascular corpora lutea and seven smaller ones. It is reasonable to assume that nine of the ten large corpora lutea formed when the nine eggs in the oviducts ovulated (the additional ovum was either lost during coelomic migration and resorbed, or its corpus luteum was late to regress and rightly belongs with the smaller seven). The remaining seven older (smaller) corpora lutea must therefore belong to a previous clutch.

Females possessing pre-ovulatory follicles after having already produced a clutch of eggs, provided additional evidence for multiple clutches. One female (EK06D), with five hard-shelled eggs in her oviducts, possessed four follicles of pre-ovulatory size and one follicle approaching pre-ovulatory size (Table I). Another (EK27D), which had already produced two clutches, still had three follicles of pre-ovulatory size and a further three approaching that size. Yet another (EK26D), with nine eggs in the oviducts, had eight follicles close to pre-ovulatory size. Since follicles of pre-ovulatory size were not present in females dissected after the breeding season, it is reasonable to suggest that enlarged follicles on the ovaries of these three females represented future clutches. Consistent with this suggestion is the observation that the female known to have produced three clutches (EK09D) possessed no follicles large enough to indicate that a future clutch was to be laid (Table I).

These observations suggest that two females (EK09D, EK27D) would have laid three clutches for the year, and that two (EK06D, EK26D) would have laid two clutches. The remaining two gravid females showed evidence of only one clutch—but one of these females was examined very early in the season (EK20D, August 10) at which time four months remained to develop further clutches, and the other was examined very late in the season

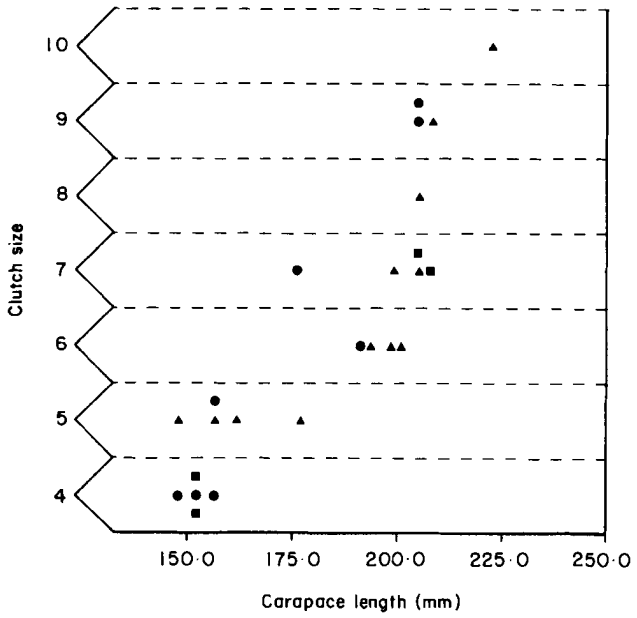


FIG. 4. Clutch size as a function of body length for *Emydura krefftii*. Clutch size was estimated from counts of oviducal eggs (●), corpora lutea (■), and pre-ovulatory follicles (▲).

(EK52D, December 24) when evidence of previous clutches may have been lost. The ability to produce several clutches per season appears to be independent of size, since both small (EK09D: carapace length 152 mm) and large turtles (EK27D: 205 mm) showed signs of producing three clutches.

Clutch size and carapace length of *Emydura krefftii* were positively correlated ($r=0{\cdot}86$, $n=24$, $P<<0{\cdot}01$; see Fig. 4). Thus it is of little use to calculate a mean clutch size for this species, since the mean value would be strongly influenced by the size distribution of females examined. Instead, clutch size for the species is best summarized by the predictive regression.

$$\text{Expected clutch size} = 0{\cdot}062 \text{ carapace length} - 5{\cdot}16,$$

$$(r^2 = 0{\cdot}74,\ n = 24,\ \text{length in mm}).$$

Broadly speaking four eggs is the minimum clutch size, produced by females of about 150 mm in length, and the clutch size increases by one egg for each 16 mm increase in carapace length. The maximum clutch size is 10 eggs.

The reproductive potential of *Emydura krefftii* on attaining maturity is about 12 eggs per annum—three clutches each of four eggs. As the turtles grow, their reproductive potential increases with the maximum of about 30 eggs per annum, reached in a turtle 250 mm long. These generalisations need to be interpreted cautiously however, because growth rates are highly variable and appear to stabilize at markedly different maximum sizes (Georges, 1982*a*).

Discussion

Lofts (1968) and Moll (1979) have described the classical pattern of postnuptial spermatogenesis exhibited by most temperate-zone turtles. Testicular activity peaks in late summer and is usually complete by the end of autumn. Sperm spend the winter in the epididymal canals and are available to fertilize eggs in the following spring. The testes usually remain quiescent during the breeding season in spring and early summer. This pattern, which contrasts strongly with the pre-nuptial pattern normal in homeotherms (Lofts, 1968), was first established for temperate-zone turtles by Risley (1938) and later confirmed for species belonging to the Emydidae (Lofts & Boswell, 1961; Ernst, 1971; Moll & Legler, 1971), the Chelydridae (Dobie, 1971; White & Murphy, 1973), the Trionychidae (Lofts & Tsui, 1977), and the Kinosternidae (Mahmoud & Klicka, 1972; Christiansen & Dunham, 1972; Iverson, 1978). The results of the present study, and of unpublished works by Parmenter (1976) and Chessman (1978), establish that the pattern of spermatogenesis of Australian temperate-zone chelids does not differ in any important respect from that of the cryptodiran turtles of the Northern Hemisphere.

Testes weight (expressed as a percentage of body weight) and mean diameter of the seminiferous tubules both underwent a cycle that corresponded with the seasonal cycle of spermatogenic activity in *Emydura krefftii*. Variation in standardized testes weight might be used as a reliable indication of peaks and lulls in spermatogenic activity of chelid turtles, when histological examination of the testes is not feasible. However, the epididymes of mature male specimens of *E. krefftii*, like those of *E. macquarii* and *Chelodina longicollis* (Parmenter, 1976; Chessman, 1978), contain substantial quantities of sperm in all months. While presence of sperm in the epididymes is a reliable indication of sexual maturity, it does

not indicate the state of spermatogenic activity—though it has sometimes been assumed to do so in other species of freshwater turtle (Dobie, 1971).

The ovarian cycle of *E. krefftii* is also broadly similar to that in other freshwater turtles of the temperate zones. The cycles usually involve distinct phases of follicular enlargement, ovulation and oviducal period, and quiescence, though in colder regions the quiescence phase may be ill-defined because large follicles persist from one year to the next (e.g. *Chrysemys picta* in Nova Scotia; Powell, 1967). Turtles of the temperate zones usually lay the first clutch of the breeding season in the spring. They prepare for spring ovulations in a variety of ways. For example, *Macroclemys temmincki* and *Chelydra serpentina* complete their follicular enlargement in the autumn, before hibernation in the winter (Dobie, 1971; White & Murphy, 1973), whereas *Kinosternon flavescens* and *Clemmys caspica* do not complete follicular enlargement until just before ovulation in the spring (Christiansen & Dunham, 1972; Lofts & Boswell, 1961). Similar variation occurs among Australian species. Chessman (1978) noted that *Chelodina longicollis* complete most of their follicular enlargement in autumn, whereas most of the follicular enlargement of *Emydura macquarii* occurs in spring. Both species were studied in southern regions of the continent (Victoria), and follicular development was arrested in the cold winter months. In contrast, enlargement of the ovarian follicles of *E. krefftii*, at the lower latitudes of Fraser Island, began in late summer and continued unabated through the winter. This continuation of follicular enlargement in winter is difficult to explain, because, although *E. krefftii* does not hibernate, food became scarcer and activity, digestive rate and digestive efficiency are considerably reduced in winter—so much so that growth in body size ceases completely (Georges, 1982*a*). The coincidental depletion of fat stores in winter may indicate that growth of the ovaries in the colder months depends on a transfer of material from the fat stores to the ovaries, a process known to occur in other reptiles (Hahn & Tinkle, 1965; Hahn, 1967). The significant negative correlation between relative fat-body weight and time of year (February to November) for breeding female *E. krefftii*, was considerably weakened when non-breeding females were included in the analysis. Since the winter months can be expected to exert a similar energetic burden on both breeding and non-breeding females, this finding supports the proposal that depletion of fat stores, as the year progresses, is because of reproductive expenditure. The similar depletion of the fat stores of males cannot be explained because lipid cycles of the testes were not investigated in the present study.

After ovulation the spent follicles consolidate into corpora lutea, which reduce in size while the eggs are in the oviducts, a reduction attributed to consolidation rather than to degeneration. Klicka & Mahmoud (1972, 1973) demonstrated that the reptilian corpus luteum is capable of synthesising progesterone, and corpora lutea appear to be the major source of this hormone following ovulation in turtles (Callard, Lance *et al.*, 1978). Progesterone prevents ovulation in *Chrysemys picta* (Klicka & Mahmoud, 1977), by suppressing ovarian growth rather than by directly inhibiting ovulation (Callard, Doolittle *et al.*, 1972). Callard, Lance *et al.* (1978) concluded that progesterone may induce the regression of the ovaries after ovulation, though in species that lay more than one clutch of eggs per year a more likely function would be to suppress development and ovulation of one clutch until the previous one has been laid (Moll & Legler, 1971). Since progesterone is known to retard contractions of smooth muscle in the turtle oviduct (Callard & Hirsch, 1976), the hormone may also prevent premature expulsion of the eggs from the oviducts, thereby exerting control on the timing of egglaying. Cox & Marion (1978) have suggested that the corpora lutea play

a role in albumin secretion, shell-membrane formation and eggshell calcification. The corpora lutea of *Emydura krefftii* regress rapidly after egglaying, a finding which is consistent with their supposed functions.

The fate of enlarged follicles that do not ovulate is often less clear. Several authors have identified atretic follicles from their distinctive colouration (Legler, 1960*b*; Dobie, 1971; Plummer, 1977) and poor blood supply (Swingland & Coe, 1978). Others have found enlarged follicles on the ovaries at the end of the breeding season, whereas ovaries examined later in the year have no enlarged follicles (Dobie, 1971; Parmenter, 1976; Chessman, 1978; Iverson, 1980). Few studies have included histological preparations of follicles suspected to be degenerating (but see Altland, 1951). Histological examination of atretic follicles described here (two of them 11 mm in diameter) confirm previous reports that enlarged follicles can degenerate rather than developing further and ovulating. Clearly, care must be exercised when using a cluster of follicles as evidence of a pending clutch of eggs.

The clutch size of *Emydura krefftii* was strongly correlated with maternal body size, and similar correlations have been reported for many other freshwater turtles (Dobie, 1971; Ernst, 1971; Moll & Legler, 1971; Cox & Marion, 1978; Iverson, 1978; Plummer, 1977; Vogt, 1980; Alho & Padua, 1982). Gibbons (1982) was able to partition the effects of age and body size on clutch size, and found that observed differences in clutch size can be explained in terms of differences in maternal body size alone. Body size appears to limit the number of eggs per clutch: as the female grows, her capacity for eggs increases, and clutch size can increase accordingly. Early reproduction, even though the full annual reproductive potential cannot be achieved until later in life, is probably of great advantage to a long-lived, slow-growing vertebrate.

The results show that *Emydura krefftii,* on Fraser Island, can lay up to three clutches of eggs per year. Christiansen & Moll (1973) proposed that a chief advantage of multiple clutches is to spread the annual reproductive effort in time and space and thus prevent predators from destroying an entire year's reproductive effort by finding a single nest. Multiple clutches may also be a strategem that lessens the effects of freak periods of inundation or desiccation. In view of the inter-relationship between body size and clutch size described above, partitioning of the annual reproductive effort into two or more small clutches would also allow a greater reproductive output to be achieved without a corresponding increase in body size. Hence a higher reproductive output can be achieved much earlier in life.

Christiansen & Moll (1973) report a latitudinal trend in the number of clutches laid per female per year. The American *Chrysemys picta* in the north-eastern portion of their range lay fewer clutches, each with more eggs, while in the south-west, they lay more clutches each with fewer eggs. A similar trend is not yet clear among Australian forms, probably because the effects of latitude are confused by the effects of altitude. Legler & Cann (1980) report that multiple clutches are the rule for all species at the latitude of the Fitzroy River (23°10′; 40 m) and the present study shows that multiple clutches are usual for *Emydura krefftii* on Fraser Island (24°40′ to 25°51′; 0–120 m). Parmenter (1976) found that second clutches were not common for *Chelodina longicollis* on the New England Tableland (30°31′; *c.* 1000 m), and Vestjens (1969) reported that this species lays only one clutch per year in the Australian Capital Territory (35°17′; 550 to 600 m), although he did not include the data upon which he based his conclusion. However, at the higher latitude of the Murray River Valley (34°28′ to 36°08′; 75–105 m), Chessman (1978) found that both *Emydura macquarii* and *Chelodina longicollis* can produce up to three clutches of eggs per year. In Gippsland (38°08′; 10 to

30 m), the southern range limit for Australian freshwater turtles, Chessman found that *C. longicollis* could produce at least two clutches of eggs per year. Clearly, more work is required to determine the effects of latitude on the number of clutches produced per year by chelid turtles.

Table III shows a comparison of selected life history parameters for Fraser Island and mainland populations of *E. krefftii*. The turtles lay fewer, smaller eggs per clutch, have a much lower reproductive potential, and reach smaller maximum sizes on the island than on the mainland. Clutch size of freshwater turtles is determined largely by the influence of two proximal factors: (1) maternal body size, which sets the maximum number of eggs per clutch; and (2) resource availability, which permits a female to realize her reproductive potential only under favourable resource conditions (Gibbons *et al.*, 1982). The substantially smaller clutch sizes and lower reproductive potential of the Fraser Island turtles may simply represent their inability to accumulate sufficient energy and nutrients to match the reproductive output of turtles in more productive mainland waters. If so, the Fraser Island populations may persist only because of their ability to draw upon a wide variety of available foods (Georges, 1982*b*) and because they have few predators and competitors in the dune lakes.

The small size of eggs laid on Fraser Island (Table III) is probably a direct result of smaller maternal body sizes, since egg size and maternal body size were positively correlated. The smaller maximum maternal size of turtles on the island is less easily explained. The low productivity of the dune lakes has had a dampening effect on the turtles' growth rates (Georges, 1982*a*), and slower growth may have resulted in smaller body sizes. However, an alternative explanation is available. Moll (1979) reported a positive correlation between the maximum recorded carapace length and the mean or usual clutch size of 109 species of turtle. It appears that growth to a size greater than that required to contain the maximum attainable

TABLE III

A comparison of selected life history parameters for mainland and Fraser Island populations of ***Emydura krefftii****. Parameters relating to reproductive potential are defined by Legler & Cann (1980: Table 5); they were calculated for the Fraser Island populations from the October sample of Table I*

Parameter	Fraser Island	Mainland
Egg weight	7·44 ± 0·14 g (n = 42)	9·75 ± 0·37 g* (n = 82)
Clutch size	Max. 10 eggs	Mean 16·4 eggs*
Minimum reproductive potential	10–23 (n = 3)	29–66* (n = 5)
Maximum reproductive potential	12–26 (n = 3)	35–75* (n = 5)
Maximum maternal carapace length	246 mm (n = 728)	281 mm† (n = 98)

*Legler & Cann (1980).
†Burnett River, Qld.

clutch is disadvantageous—females that use the surplus energy to increase reproductive output instead would have a selective advantage. *Emydura krefftii* on Fraser Island are a distinct morph, differing from mainland varieties in colouration and morphology (Georges, 1982*a*), differences which may indicate a long separation from mainland populations. A low reproductive output caused by energetic constraints, and over many generations, may have resulted in selection for smaller body sizes.

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