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Gonadal cycles of the Western Australian Long-necked Turtles *Chelodina oblonga* and *Chelodina steindachneri* (Chelonia: Chelidae)

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Abstract

Seasonal changes of testis volume, spermatogenesis, follicular development, and oviducal eggs for preserved specimens of *Chelodina oblonga* and *Chelodina steindachneri* are reported. Testis cycles of both species show a typical temperate-zone chelonian pattern. The germinal epithelium is quiescent, during winter, spermatogonia start to multiply in spring, followed by spermatocytogenesis in late spring and summer. Spermiogenesis peaks in January and February in *C. steindachneri* and in April in *C. oblonga.* Spermiation starts during summer and continues until winter.

In *C. oblonga,* follicular enlargement starts during summer and continues until spring. Oviducal eggs were found in October and November, and up to three clutches may be laid between September and January. Females have a reproductive potential of 25-40 eggs per year.

Follicles of *C. steindachneri* start to enlarge in late spring. During summer and autumn, there is a stable phase without enlargement. Vitellogenesis is completed before ovulation in spring. Only one clutch of seven to eight eggs is laid per season. This pattern seems to be an adaptation to a relatively long period of aestivation.

Introduction

Few data are available on the gonadal cycles of Australian long-necked turtles. The south-eastern Australian species *Chelodina longicollis* has been the most extensively studied (Parmenter 1976, 1985, Chessman 1978), but data based on gonad histology are entirely lacking for this species. The only chelid turtle for which an accurate description of the gonadal cycle is available is *Emydura krefftii* (Georges, 1982, 1983). Legler (1985) discussed reproductive patterns in wide-ranging taxa of Australian chelid turtles, based mainly on nesting season, egg weight and incubation period, without giving details of annual gonadal cycles. However, data on *Chelodina oblonga* and *Chelodina steindachneri* were insufficient and these Western Australian species were excluded from Legler's comments.

Several authors have reported observations on nesting, egg laying, incubation and hatching of *Chelodina oblonga* (Burbidge 1967, Clay 1981, McCutcheon 1985, Nicholson 1975, Russ 1970). In *Chelodina steindachneri* even these data are very scarce and insufficient (Burbidge 1967). The aim of the present study

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is to describe the annual testicular and ovarian changes in these two long-necked turtles which are endemic to Western Australia. To avoid removal and killing of these protected animals from natural populations, this investigation was restricted to preserved specimens in the Western Australian Museum.

Material and Methods

The gonads of 32 *Chelodina oblonga* and 25 *Chelodina steindachneri* in the Western Australian Museum with known date of collection were examined. Only specimens which were killed immediately after collection were used. The geographic range of the two species in Figure 1 is based on all recorded locations of specimens in the Western Australian Museum. The locations of the specimens used in this study are shown additionally.

In females, ovarian follicles over 2 mm diameter were measured and classified as class 1 (< 3 mm), class 2 (3-7.9 mm), class 3 (8-12.9 mm in *C. steindachneri*, 8-13.9 mm in *C. oblonga*) and class 4, the largest size class of non ovulatory follicles (> 13 mm in *C. steindachneri*, > 14 mm in *C. oblonga*). Shelled eggs in the oviducts and corpora lutea were counted. Egg measurements are presented as means and standard deviations.

In males, one testis was removed and the volume determined by immersion in alcohol. Half the testis was paraffin embedded, sectioned (nominally 6 μm) and stained with Haematoxilin and Eosin for histological examination. The presence of sperm in epididymides and vasa deferentia was noted. The spermatogenetic stage was determined according to the criteria of Leblond and Clermont (1952).

Results

Chelodina oblonga

The males of *Chelodina oblonga* became sexually mature at about carapace length (CL) 14 cm. Two males of 13.1 and 14.0 cm CL showed immature testes, whereas two males of 13.7 and 14.1 cm CL had fully developed testes and sperm in the epididymides. All females examined had a carapace length over 21 cm and were mature.

Seasonal changes in testis volume and in the stage of the germinal epithelium are summarised in Figure 2. From November to January the epididymides were regressed and small and contained few spermatozoa. With the onset of spermiation spermatozoa entered the epididymides, which increased in diameter and remained filled with spermatozoa until November. The vasa deferentia contained sperm during the whole year and were maximally filled from May to November.

In females of *C. oblonga* follicular enlargement started during summer. In January, after the nesting season, the ovaries were regressed and only follicles of class 1 and 2 were present. However, a female showed follicles of all size classes in May, including a group of follicles near preovulatory size (14-15 mm

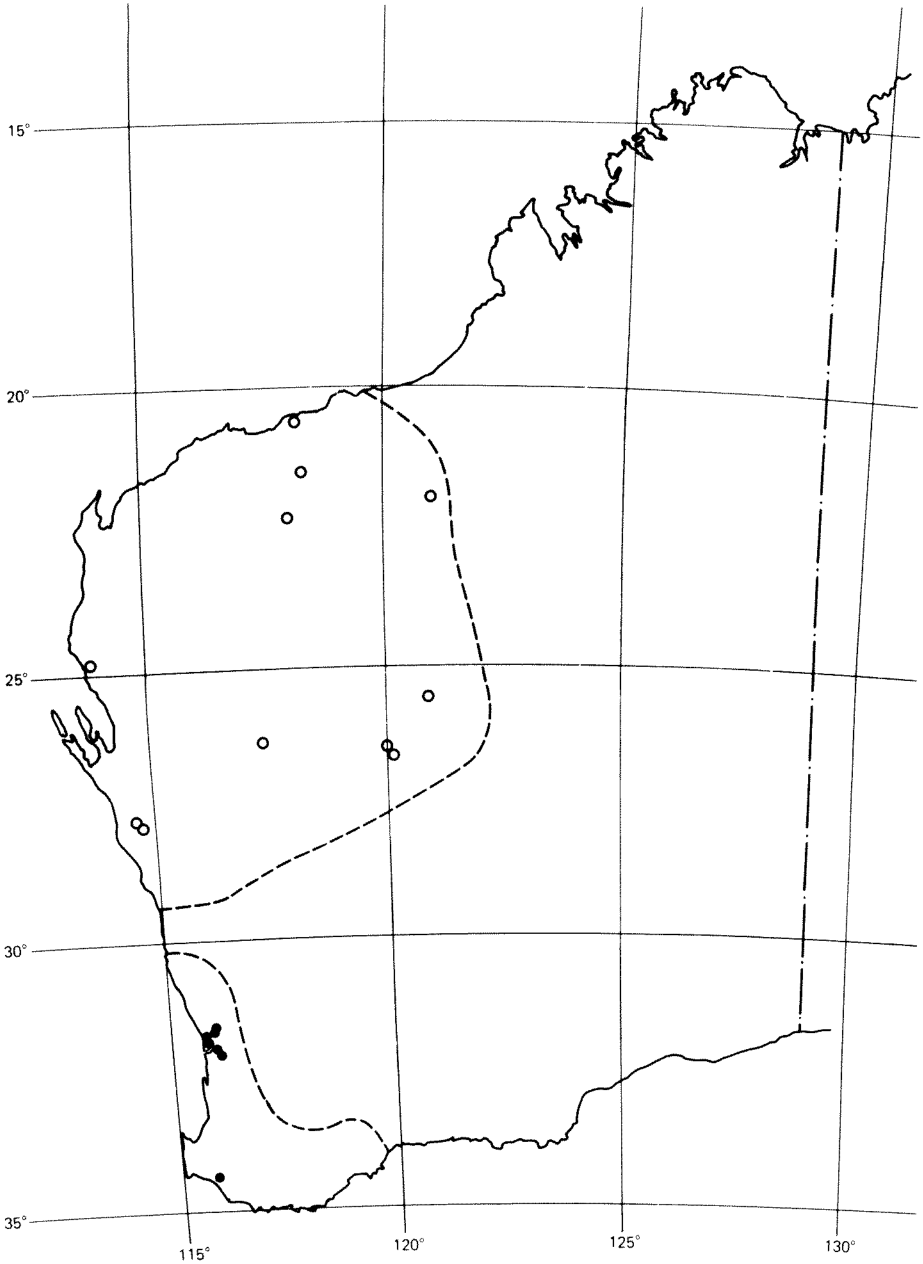

Figure 1 Distribution of *Chelodina oblonga* (●) and *Chelodina steindachneri* (○) specimens used in this study. Some localities have multiple specimens. Dotted lines indicate the ranges of the species.

diameter). During October all size classes of follicles were present together with oviducal eggs, indicating that follicular enlargement continued in spring (Table 1).

Three females had 13-16 eggs in the oviducts (Table 1). The eggs were elongate and differed slightly in size between females (Table 1). Egg sizes ranged from 31.0 to 34.4 mm in length and 20.2 to 24.0 mm in width. The yolk in the (preserved) eggs was rather spherical, with a length of 18.2 to 20.0 mm and width of 16.1 to 17.0 mm, which indicates the approximate size of the oocytes during ovulation.

Table 1 Seasonal occurence of oviducal eggs and ovarian follicles of various size classes for *Chelodina oblonga.*

Chelodina oblonga CL (cm)	date	number of enlarged follicles of class 1	2	3	4	number of eggs in oviducts	length of eggs (mm) mean ± SD	width of eggs (mm) mean ± SD
24.6	16 January	32	7	-	-	-	-	-
23.6	16 January	8	9	-	-	-	-	-
22.3	15 May	16	7	9	14	-	-	-
23.2	15 October	15	16	5	13	13	32.4 ± 0.88	21.8 ± 0.37
24.7	15 October	18	20	3	28	16	34.2 ± 0.38	23.8 ± 0.25
21.6	14 November	16	10	13	-	15	31.9 ± 0.53	20.8 ± 0.33

Chelodina steindachneri

The males of *C. steindachneri* became sexually mature at about 12 cm CL. A male of 10.1 cm CL showed immature testes, another male with 11.9 cm CL had fully developed testes and sperm in the epididymides. Although a female of 15.4 cm CL showed immature ovaries and oviducts, another female of 14.6 cm CL had mature ovaries with enlarged follicles and eggs in the oviducts.

Seasonal changes in testis volume and in the stage of the germinal epithelium are summarised in Figure 3. They closely paralleled the situation in *C. oblonga:* however, maximum testis volume was reached in January and February in *C. steindachneri* and April in *C. oblonga.* Spermiogenesis started during November, about one month earlier than in *C. oblonga.* The epididymides and vasa deferentia contained sperm during the whole year, with maximal proliferation from May to November.

Oviducal eggs were only found at the beginning of October: these females had only small numbers of follicles of classes 1 and 2 in the ovaries (Table 2). Follicular enlargement started in early summer and in January class 3 follicles could be found. During summer and autumn, however, the ovaries seemed to be in stable phase, judging from the lack of follicular enlargement. In March and June the same follicular classes were found as in January (Table 2). The largest follicles measured had a diameter of 12.0 mm in January, 12.5 mm in March and 12.0 mm in June. Therefore, a second phase of follicular enlargement may occur in late winter and spring.

The eggs of *C. steindachneri* are elongate and considerably smaller than eggs of *C. oblonga* (Table 2). Egg sizes ranged from 27.5 to 31.0 mm in length and 17.0-19.5 mm in width. The yolk in the preserved eggs was rather spherical, mean length and width being 17.0 and 15.0 mm.

Table 2 Seasonal occurence of oviducal eggs and ovarian follicles of various size classes for *Chelodina steindachneri.*

Chelodina steindachneri CL (cm)	date	number of enlarged follicles of class 1	2	3	4	number of eggs in oviducts	length of eggs (mm) mean ± SD	width of eggs (mm) mean ± SD
19.3	23 January	17	5	7	-	-	-	-
16.2	9 March	16	4	9	-	-	-	-
17.3	7 June	18	12	13	-	-	-	-
16.1	4 October	11	8	-	-	8	28.3 ± 0.23	18.8 ± 0.44
15.6	4 October	16	1	-	-	7	29.7 ± 0.43	18.6 ± 0.11
15.6	4 October	17	2	-	-	7	30.2 ± 0.72	19.1 ± 0.12
14.6	4 October	13	4	-	-	8	28.0 ± 0.53	17.1 ± 0.11

Discussion

In general the gonadal cycles of *Chelodina oblonga* and *C. steindachneri* are typical for temperate-zone chelonians (review: Moll 1979). Spermatogenesis occurs during the hot season, with peak enlargement of testes at the height of spermiogenesis. Testicular regression follows, paralleling the expulsion of spermatozoa into the epididymides. Females of most temperate-zone species start follicular enlargement in autumn, when temperatures are declining, and complete it in a second phase in spring. Those Australian chelids investigated have followed the same pattern (*Chelodina longicollis:* Chessman 1978, Parmenter 1976, 1985; *Emydura macquarii:* Chessman 1978; *Emydura krefftii:* Georges 1983). However, some peculiarities in the gonadal cycles of *Chelodina oblonga* and *Chelodina steindachneri* are notable.

Spermatocytogenesis and spermiogenesis occur over nine months of the year, from spring (September-October) to June. Though spermatogenesis is clearly cyclic, the periods of germinal quiescene are relatively short. The peak of testicular enlargement and spermiogenesis of the two species occurs at different times of the year: during early summer (January-February) in *C. steindachneri* and late summer/autumn (April-May) in *C. oblonga* (Figures 2, 3).

C. longicollis, which is found in a wide range of climatic conditions in eastern Australia, shows some differences between populations in the peak of testicular enlargement. In the Murray Valley the peak occurs in January-February (Chessman 1978), in Gippsland in Feburary-March (Chessman 1978), and near Armidale in March-April (Parmenter 1976, 1985). This seems to reflect changes from warmer to colder climatic conditions. The differences in the peak of spermiogenesis between *C. oblonga* and *C. steindachneri* may also be correlated with climate. *C. oblonga* inhabits the cooler parts of south-western Australia with good winter rains, whereas *C. steindachneri* is found in warmer country with scanty rains (mainly summer-autumn in north, mainly autumn-winter in south). However, the short-necked chelids *Emydura macquarii* and *E. krefftii* show peak testicular enlargement at about the same time, in March, although *E. macquarii* inhabits temperate south-eastern Australia and *E. krefftii* subtropical and tropical north-eastern Australia (Chessman 1978, Georges 1983).

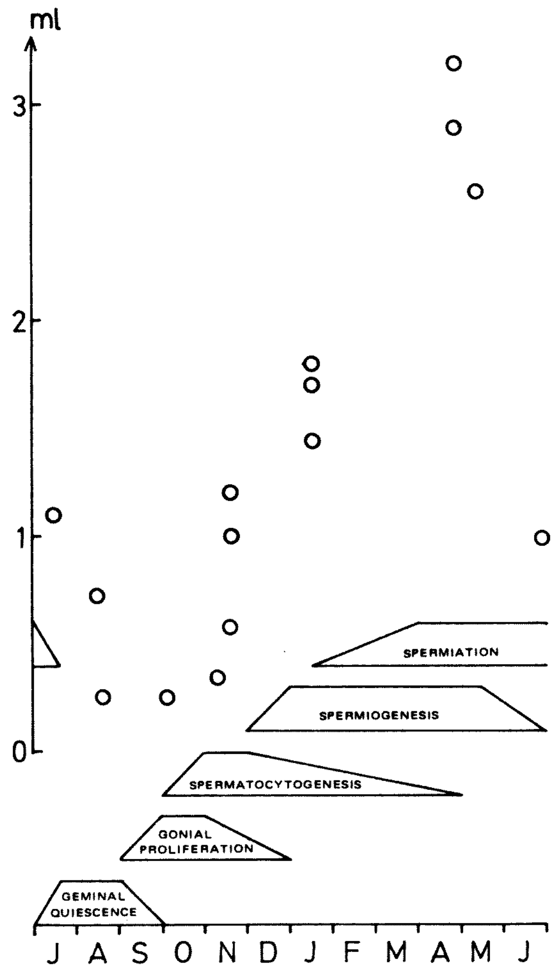

Figure 2 Testis volume (ml) of *Chelodina oblonga* versus month of collection. The scheme for the phases of the testis cycle indicates their timing and only approximately quantitative relationships between them.

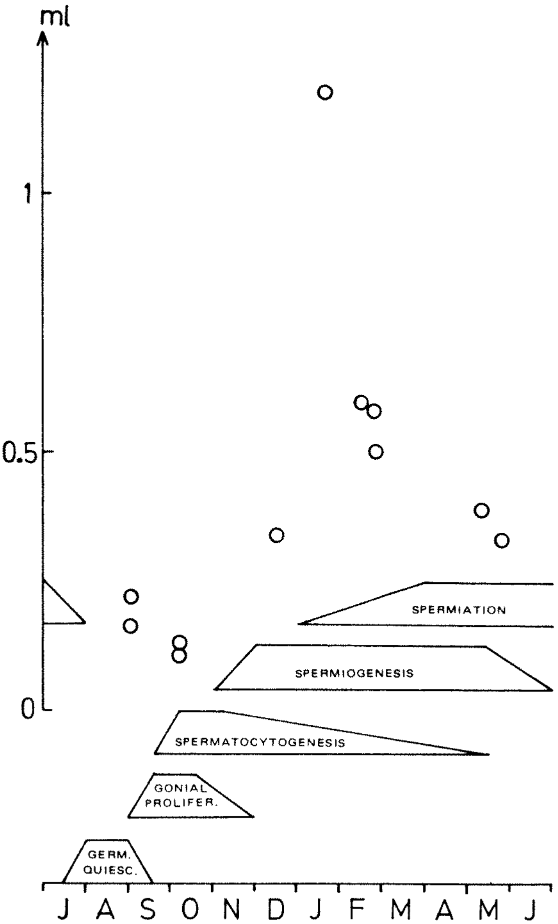

Figure 3 Testis volume (ml) of *Chelodina steindachneri* versus month of collection. Phases of the testis cycle as in Figure 2.

The annual ovarian cycle can be considered in four stages: follicular enlargement (resulting from yolk accumulation), ovulation and oviducal period, regression, and a stable phase normally during winter. In *C. oblonga* the ovaries regress during January after all eggs have been laid. Follicular enlargement starts in late summer or autumn, with some follicles reaching preovulatory size at the beginning of winter. Most chelonians of the temperate zone show a stable phase without follicular enlargement during winter. However, *C. oblonga* does not hibernate and it seems that some follicular enlargement continues during this time (Kuchling, unpublished). Ovulation starts in spring, presumably during September. Oviducal eggs have been found in October and November, and the presence of oviducal eggs together with different size classes of enlarged follicles indicates that three clutches of eggs may be laid per season. Clay (1981) reports two nesting periods for *C. oblonga,* one in spring (September to November) and one in summer (December to January). The present data are not sufficient to confirm this observation, but demonstrate that multiple clutches per season are quite normal in this species.

Clay (1981) found the largest clutch in *C. oblonga* to be 12 eggs. In the present study the three females with oviducal eggs all had larger clutches, the maximum being sixteen. According to the number of enlarged follicles, which may represent following clutches, the second and third clutches may be smaller. The average clutch size for the summer nesting period was significantly lower than that in spring in the study of Clay (1981).

The reproductive potential (the number of eggs produced by a female per year) can be estimated by combining the number of oviducal eggs with the number of enlarged follicles representing the following clutches of a single season. This gives only a rough approximation, for some of the follicles may become atretic (Georges 1983). For one female of *C. oblonga* in this study the maximum reproductive potential is estimated at 47 eggs, but normally females of the medium size studied here may lay 25 to 40 eggs per year. It is known that egg numbers may vary in a species, with larger females often producing larger clutches (Moll 1979). Resource availability of energy and nutrients, which may differ between populations, may also influence the reproductive output of turtles (Georges 1983, Parmenter 1985).

In *C. steindachneri* the nesting time is unknown. Burbidge (1967) suggests that the eggs are laid in spring (September to October). This is confirmed by the present data. No indication could be found for multiple clutches. The period of ovarian regression seems to be short in *C. steindachneri.* Follicular enlargement starts in late spring or early summer. Interestingly there is a stable phase of the ovaries during summer and autumn, with no follicular enlargement between January and June. Vitellogenesis is completed before ovulation in spring. No comparable pattern of the ovarian cycle has been reported in other chelonians. The pattern in *C. steindachneri* may be an adaptation to the unpredictable rainfall of the dry country it inhabits. In most of the range of *C. steindachneri* the annual rainfall is between 200 and 250 mm. It is highly likely that this species is physiologically better adapted for surviving arid conditions than any other Australian turtle (not all species have been studied), its habitat usually being dry for many months (Burbidge 1967). It may be an advantage to start the energy input of vitellogenesis for the eggs to be laid in the next season as early as possible, before aestivation becomes necessary. The long period of aestivation may also be the reason that only one clutch is produced per season. It seems impossible for *C. steindachneri* to extend reproductive costs over as long periods as in species with multiple clutches. The only other Australian turtle with an extended period of aestivation, *Pseudemydura umbrina*, also produces only one clutch per year (Burbidge 1981).

Reproductive potential in *C. steindachneri* is 7-8 eggs per year and considerably smaller than in *C. oblonga.* Due to the small size of *C. steindachneri* (this species is the smallest long-necked turtle) it is difficult to imagine a female containing more eggs. In comparison *C. longicollis* has a mean clutch size of 10-14 eggs, with a maximum of 24, and may produce one to three clutches per season (review: Parmenter 1985).

Egg measurements of *C. oblonga* in this study are in the range of the data reported by Clay (1981) for this species. The eggs are considerably smaller than the measurements indicated by Legler (1985) for the *C. expansa* group (mean length 39.2 mm, mean width 27.6 mm). Egg measurements of *C. steindachneri* correspond closely to the egg size reported for *C. longicollis* (Parmenter 1976, Chessman 1978, Legler 1985).

Legler (1985) proposed to divide the present genus *Chelodina* and to include *C. oblonga* in the *C. expansa*-group and *C. steindachneri* in the *C. longicollis* group. He suggested that reproductive patterns in Australian chelids do not change over wide geographic ranges within the genera he defined. The present study does not confirm Legler's presumption. The reproductive pattern of *C. oblonga* indicates that this species may not be closely related to the *C. expansa* group, as proposed by Legler, but confirms the view of Burbidge *et al.* (1974) who placed *C. oblonga* in a seperate group, differing equally from both the *C. expansa* group and the *C. longicollis* group. Differences of the reproductive pattern of *C. steindachneri* from that of the *C. longicollis* group may reflect adaptation to specific environmental and climatic conditions.

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