6.0 REPRODUCTION

6.1 INTRODUCTION

The reproduction of Australian skinks has received more attention than any other aspect of their ecology. The aim of the present chapter is to add to this knowledge (i) by describing the reproductive cycles of female and male C.taeniolatus and their variation between years, and (ii) by determining other important reproductive parameters such as size at sexual maturity and the relationships between clutch size and weight, and lizard size.

6.2 METHODS

To determine the annual reproductive cycle 10 to 20 lizards were collected approximately monthly using the schedule detailed in section 2.2. Standard body measurements were taken (Section 2.2) and gross and histological examination of the reproductive organs were made. Fat-free dry weight (FFDW) (Section 7.2.2) was used throughout this study as an unbiased measure of body weight.

6.2.1 Histological techniques

Histological examination was carried out on the ovaries and testes from lizards collected in 1979. Gonads were fixed in Bouin's fixative, dehydrated through xylene to paraffin using standard techniques, sectioned (7µm) and stained with Masson's Trichrome (Humanson 1972). Two to three slides were made of each gonad, with the exception of one ovary which was serially sectioned to determine the number of germinal beds, a characteristic which is species specific (Jones et al. 1982).

Sectioned gonads were examined under high power (400x). In the ovary, maximum follicle diameter (mm) and in the testes, the diameter of 10 seminiferous tubules (μm) and heights of associated epithelia (μm) were measured, using an ocular micrometer. Observations were made on cellular differences throughout the year for ovaries and testes.

Sexual maturity was determined by histological examination of gonads; the smallest lizards with active gonads in the spring were deemed to be at the minimum size for sexual maturity.

6.2.2 Gross analysis of gonads

Gross morphological changes in gonads were estimated by measuring the following parameters:

FEMALE	MALE
ovary length (mm)	testes length (mm)
ovary width (mm)	testes width (mm)
ovary wet weight (g)	testes wet weight (g)
number of developing follicles in left and right ovaries	
number of left and right oviducal eggs	
wet weight (g) of oviducal eggs	
number of corpora lutea and number of corpora atretica (estimated from gross morphology and checked with histology)	

Gonad weight, thought to be one of the more accurate measures of seasonal development, was used as it allowed ready comparisons with other studies. In the rare instances when measurements were missed or could not be taken, the following relationships, calculated from complete sets of data allowed reliable estimates to be made.

Ovary wt = 56.97^{***}.(follicle diameter)$^{2.354***}$, R^2 = 0.79, P < 0.001, n = 70

Ovary wt = 18.59^{***}.(ovary width)^2.(ovary length) + 0.55^{ns}, R^2 = 0.93, P < 0.0001, n = 105.

Testes wt = 22.01^{***}.(testes width)^2.(testes length) + 1.05^{**} , R^2 = 0.65, P < 0.0001, n = 65.

6.3 RESULTS

6.3.1 Sexual maturity and dimorphism

C.taeniolatus hatched at 33.0 (SD = 1.7) mm and reached sexual maturity at 43.0 mm for males and 52.0 mm for females. At this stage both males and females were most likely to be in their third summer season or two years old, although some males could be in their second summer of their first year (Section 5.4). At no stage in their life cycle could males and females be distinguished using external morphology alone, although females grew larger than males. Figure 23 details the snout-vent length (SVL) to weight (Wt) relationship for males and females, which through tests of homogeneity, after log-log transformations, were shown to be significantly different at the 0.2% level (slopes: $F_{1,328}$ = 13.5306, elevations: $F_{1,328}$ = 9.4012), indicating that females, once past sexual maturity, grow larger and heavier than males. The resultant lines were;

Female : Wt = $e^{-9.97***}$.$SVL^{2.75***}$, R^2 = 0.79, P < 0.0001, n = 196,

Male : $Wt = e^{-11.89***} . SVL^{3.25***}$, $R^2 = 0.91$, $P < 0.0001$, $n = 136$.

6.3.2 Gonad weight and lizard size

The ratio of gonadal weight to body weight is often used as a parameter of seasonal development (Goldberg 1975, Sherbrook 1975, Shine 1977b, Shine 1977c, Duvall et al. 1982 review others), so that any possible relationship between these two variables can be taken into account. However, such indices do not correct for body size unless (i) the relationship between gonad weight and body weight is linear, (ii) the coefficient of variation of gonadal weight is constant over the entire body weight range and most importantly (iii) that the relationship intercepts the y-axis at zero (de Vlaming et al. 1982, Weil 1962, Tanner 1949). If these conditions are not fulfilled then gonosomatic indices, or any other similar ratios, may not be valid and either the raw gonad weight or an analysis of covariance procedure with body weight as the covariate should be considered.

Therefore to determine the validity of using gonosomatic indices in C.taeniolatus, testes and ovary weights were regressed against FFDW, an estimate of body weight (Section 7.2.2). As the variation within each month was large and the sample sizes often small only lizards captured in October and June were examined. These months represented times when ovary and testes weights were at a maximum and a minimum respectively. Accordingly, it was found that while ovary weight was linearly related to FFDW ($P < 0.01$) in both months, it did not pass through the origin, and that testes weight was not significantly related to body weight. However, even though no gonosomatic indices were required, they were still included here as gonad weight/FFDW, so that comparisons could be made with previous work. Further, such measures are not the only indication of seasonal activity in gonads, as histological examination

of the gonads was also used to confirm any seasonal changes.

6.3.3 Male reproduction

6.3.3.1 Morphology and histology of the reproductive tract

The testes of C.taeniolatus are paired white ellipsoid organs and lie dorsally about 2/3 of the way down the abdominal cavity, the right testes lying anterior to the left. The epididymis and vas deferens lie posterior to the testes, the epididymus capping the posterior portion of each testis. The testes is suspended from the body wall by a mesorchium and is covered with a transparent tunica albuginea; only in very developed testes can the seminiferous tubules be seen through the membrane.

Figure 24 summarises the histological cycle in lizard testes. The cycle can be divided into 4 stages.

Stage 1 : Inactive - from March to May seminiferous tubules have a mean diameter of 110 µm and have no spermatozoa present in the lumen of the tubule. Most cells in the epithelium of the tubule are in the spermatogonial stage, with a few spermatocytes present. The epididymus is undeveloped with no sperm in the lumen.

Stage 2 : Onset of activity - from June to September seminiferous tubule diameter and epithelial height gradually increase. Small amounts of spermatozoa are present in the lumen of the seminiferous tubules. By September there are many primary spermatocytes in the epithelium and a large number of spermatids at the final stages of division are evident in some tubules. Very small amounts of sperm are evident in the epididymus.

Stage 3 : Maximum activity - between September and November the seminiferous tubules and epithelium reach maximum dimensions. Very large amounts of spermatozoa choke the lumen of all seminiferous tubules. Many primary spermatocytes are present as are layers of spermatids in the final stages of division. The epididymus is now approximately twice the size of those in stage 1, and has large amounts of sperm in the lumen.

Stage 4 : Regressing - from December to March seminiferous tubule and epithelium dimensions are greatly reduced; tubules have collapsed. Only spermatogonia are present in the epithelium, no spermatozoa are present in the lumen of the seminiferous tubules. The epididymus has returned to stage 1 size, and has only small amounts of sperm in the lumen.

6.3.3.2 Seasonal cycle in testis weight

The seasonal cycle in testis weight and testis weight index for 1979-82 are shown in figure 24. The gross changes concur with the histological changes detailed in figure 25, with the exception that cellular preparations for spermatogenesis commence during the winter (June) while weight changes are not detected until spring - the peak time for spermatogenesis. Figure 24 shows that there is some variation in the timing of peak spermatogenesis, October being the principal time in the spring of both 1979 and 1981, while in 1980 it ocurred in September. Note here that the two measures of testis activity (testis weight and testis weight index) show similar trends at all times except during October and November of 1980, where testis weight index indicates a constant level of testis activity over spring, while testis weight indicates a declining level. Note also that as testis weight is not related to body weight, it provides an unbiased estimate of reproductive

activity.

All adult males examined during the spring seasons of 1980 to 1982 had enlarged and active testes.

6.3.4 Female reproduction

6.3.4.1 Morphology and histology of the reproductive tract

The ovaries of C.taeniolatus are fusiform and lie dorsally about 2/3 of the way down the abdominal cavity, the right ovary lying anterior to the left. The paired oviducts lie laterally to each ovary. The ovary is suspended from the body wall by a mesovarium, and is covered by a transparent epithelium, through which can be seen the germinal bed, developing follicles, corpora lutea and corpora atretica.

Figure 26 summarises the histological cycle in the ovary of C.taeniolatus. The cycle can be broken up into four stages.

Stage 1 : Inactive - from March to May each ovary contain 8 to 19 white unyolked follicles. The majority of these are less than 1 mm diameter, although 1 to 4 in each ovary are slightly larger (1 to 2 mm). In these larger follicles the granulosa, the layer of cells between the theca and vitelline membranes, is distinct and made up of a number of cell types. Pyriform cells, typical of Squamata (Guraya 1978) are obvious. In some instances corpora lutea and corpora atretica were seen.

Stage 2 : Onset of cellular activity - from June to September there is little change in the gross appearance of the ovary and follicles. In the slightly enlarged follicles described in stage 1, the granulosa is less distinct and pyriform cells are now more squamous in shape.

Stage 3 : Vitellogenesis and ovulation - from September to November 1 to 4 follicles per ovary become greatly enlarged (maximum 8.7 mm) and yellow coloured. Vitellogenesis begins in September and continues through October or November when eggs are ovulated. In the developing follicle the granulosa changes gradually from the form described in stage 2 to being virtually invisible between the theca and the vitelline membranes. Yolk granules and fat vacuoles are very distinct within the follicle.

Stage 4 : Inactive, regressing ovary - from November to March follicles are as described in stage 1. Corpora lutea are present and although they equal the number of oviducal eggs, they are not necessarily equal in number in the left ovary and oviduct - crossing over can take place during ovulation (Cuellar 1970). Corpora atretica are sometimes also seen. Eggs are present in the oviduct between November and December when oviposition takes place, after which the oviduct remains distended indefinitely.

6.3.4.2 Annual ovarian cycle

The seasonal cycle in ovary weight index for 1979-82 is shown in figure 27. The gross changes in the ovary concur with the histological changes detailed in figure 26, with the exception that histological changes in the ovary commence during winter (June) while weight changes are not detected until vitellogenesis commences in September - October. Figure 27 and table 10 show that there is some variation in the timing of commencement of vitellogenesis; however, this is slight, with the majority of females ovulating between October and November and oviposition taking place between November and December. No further follicles have ever been observed developing after this time so a second clutch is unlikely. Females of reproductive age probably reproduce

every year, as the percentages of females known to be in reproductive condition (with enlarged follicles, oviducal eggs or corpora lutea) in the three years were 100, 80 and 85.7% respectively. Corpora atretica were found more commonly in 1981 and 1982 than in 1979, and was probably a result of the harsh conditions imposed by the drought in eastern Australia which commenced in the winter of 1979. Note that the figure detailed in table 10 represents percentages of lizards with atretic follicles within a month, in all cases there was only one atretic follicle per lizard in all months.

Table 10 : Percentage of C.taeniolatus with developing follicles, oviducal eggs and corpora lutea and corpora atretica from 1979 to 1982.

	Sept	Oct	Nov	Dec
1979-80				
Developing follicles	11.1	71.4	57.1	0
Oviducal eggs	0	0	42.9	25.0
Corpora lutea	0	0	42.9	100.0
Corpora atretica	0	0	0	0
Percentage in reprod. condition	11.1	71.4	100.0	100.0
1980-81				
Developing follicles	30.0	76.5	40.0	0
Oviducal eggs	0	11.8	30.0	0
Corpora lutea	0	11.8	30.0	80.0
Corpora atretica	0	0	7.1	20.0
Percentage in reprod. condition	30.0	88.3	70.0	80.0
1981-82				
Developing follicles	0	64.3	14.3	-
Oviducal eggs	0	0	71.4	-
Corpora lutea	0	0	71.4	-
Corpora atretica	0	0	33.0	-
Percentage in reprod. condition	0	64.3	85.7	-

6.3.5 Clutch size and weight

Female lizards lay on average 3.7 eggs (SD = 1.17, range = 1-7) with a mean total wet clutch weight of 0.87 g (SD = 0.194, range = 0.55-1.17), which was not significantly different for clutches of different size ($F_{3,16} = 0.064$).

Clutch size, determined from the number of oviducal eggs or yolked follicles, was significantly related to the size of the lizard, which is measured in two ways - snout-vent length (mm, SVL) and FFDW (g).

Clutch size = $-4.40^{***} + 0.12^{***}.SVL$, $R^2 = 0.54$, $P < 0.0001$, $n = 42$, Fig. 28.

Clutch size = $-10.48^{**} + 37.55^{**}.(FFDW) - 32.07^{**}.(FFDW)^2 + 9.04^{**}.(FFDW)^3$, $R^2 = 0.55$, $P < 0.01$, $n = 42$, Fig. 28.

The large variation in clutch size within each body size class is unlikely to be a result of the variation between sampling years, as a comparison of snout-vent length-clutch size relationships for 1979, 1980 and 1981 revealed no significant differences among the lines (homogeneity of lines; slope - $F_{2,39} = 1.346$, $P > 0.5$; elevation - $F_{2,39} = 0.0732$, NS).

Wet clutch weight, measured as the weight of oviducal eggs (eggs were at early, but similar stages of development (stages 2-8; Defaure and Hubert 1961 cited in Porter 1972)), was also significantly related to body size.

Clutch weight = $-0.17^{ns} + 0.016^{*}.SVL$, $R^2 = 0.21$, $P < 0.05$, $n = 20$, Fig. 29.

Clutch weight = $0.348^{n.s.} + 0.652^{*}.(FFDW)$, $R^2 = 0.23$, $P < 0.05$, $n = 20$, Fig. 29.

Note that all above relationships were first checked to see if polynomial regressions had significant quadratic or cubic components.

Due to the considerable variation in clutch size and weight present in all of the above relationships, the primary result from this section is that the maximum clutch size (or weight) a lizard can produce is a function of body size (snout-vent length or body weight).

6.3.6 Nesting and incubation

Eggs, at stage 30 (Defaure and Hubert 1961), were laid around December approximately one month after ovulation and fertilization. Although clutches are usually deposited singly in burrow up to 30 cm deep, in one instance two clutches were found in the same burrow system. Adult female lizards were occasionally found in the same burrow system as the eggs, but no direct evidence of egg guarding was found.

The first hatchlings appear in mid January, approximately one month ofter oviposition. R.Shine (pers.comm) has found in the laboratory that the incubation time for C.taeniolatus eggs at 30 C was 40 days. Experiments designed to duplicate these results for lizards from the New England region were unsuccessful because of problems with bacterial and fungal infections.

Figure 23 : Relationship between snout-vent length and body weight for female (circles) and male (crosses) Ctenotus taeniolatus. Lines represent the regression lines detailed in section 6.3.1. Intact lines represent females and dashed lines represent males.

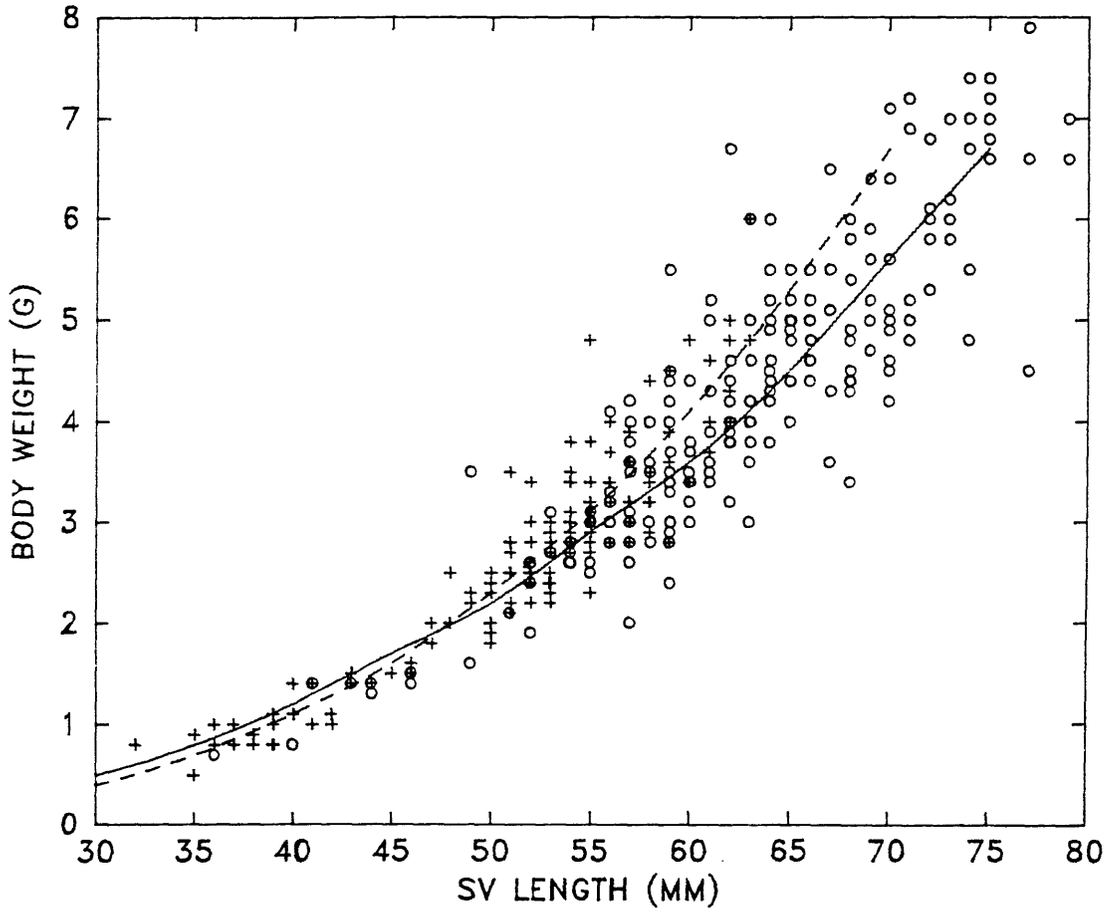

BODY WEIGHT (G)
SV LENGTH (MM)
0
1
2
3
4
5
6
7
8
30
35
40
45
50
55
60
65
70
75
80

Figure 24 : Reproductive cycle of male Ctenotus taeniolatus captured in 1979-80, 1980-81, 1981-82. Circles represent means, thin lines SD of testes weight/FFDW and thick lines SD of testes weight. Months are represented by their first initial.

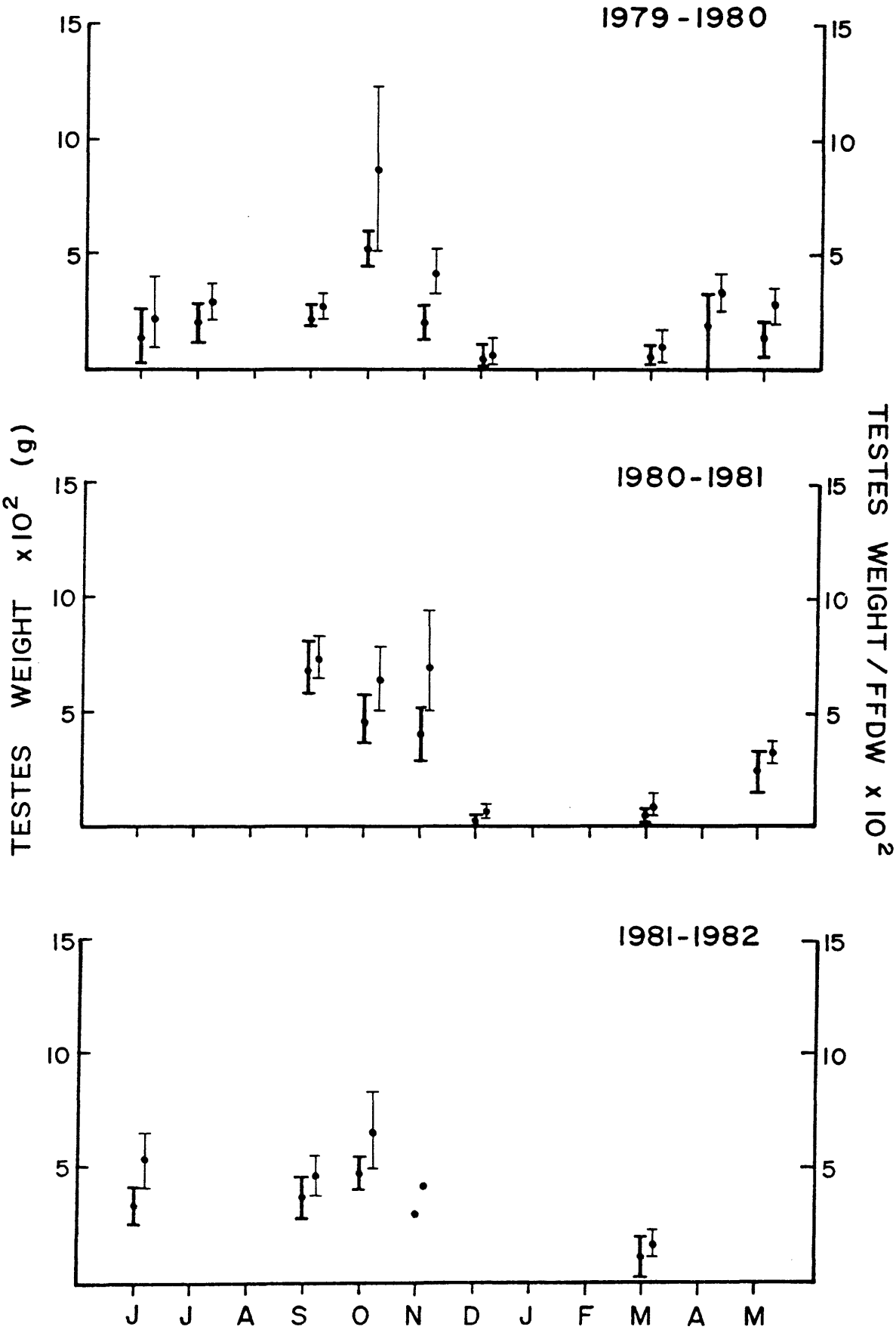
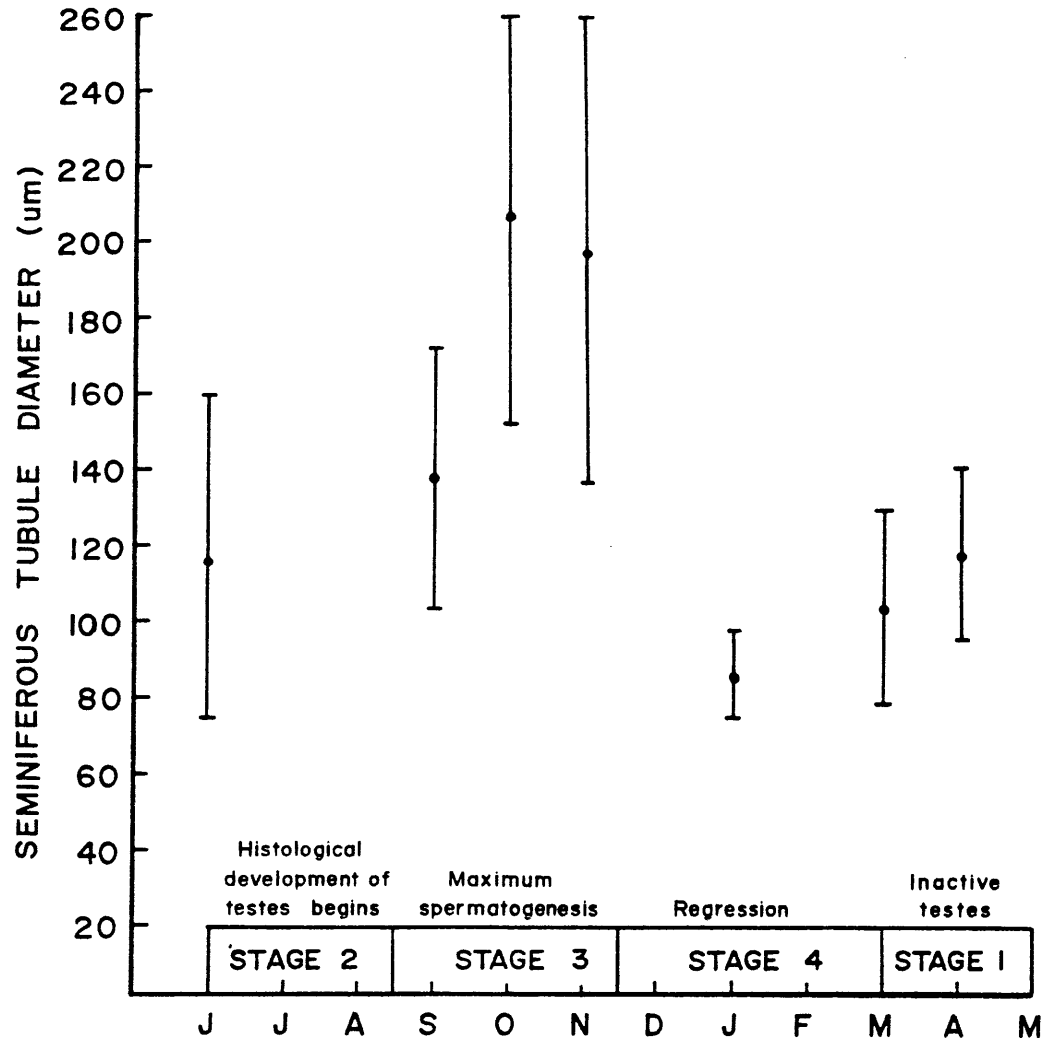

1979-1980
1980-1981
1981-1982
15
10
5
TESTES WEIGHT x 10² (g)
TESTES WEIGHT / FFDW x 10²
J J A S O N D J F M A M

Figure 25 : Annual testicular cycles of Ctenotus taeniolatus. Means are represented by circles, SD by vertical lines and months by their first initial.


SEMINIFEROUS TUBULE DIAMETER (um)
260
240
220
200
180
160
140
120
100
80
60
40
20
Histological development of testes begins
Maximum spermatogenesis
Regression
Inactive testes
STAGE 2
STAGE 3
STAGE 4
STAGE 1
J J A S O N D J F M A M

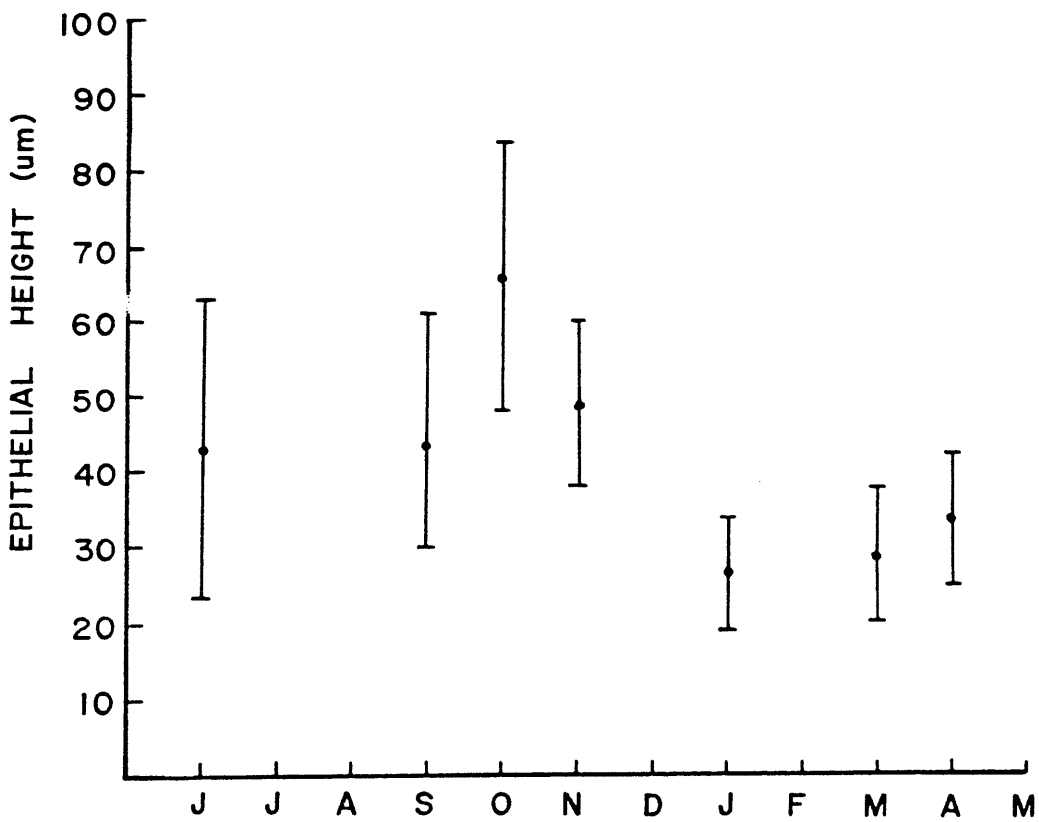

EPITHELIAL HEIGHT (um)
100
90
80
70
60
50
40
30
20
10
J J A S O N D J F M A M

Figure 26 : Ovarian cycle of Ctenotus taeniolatus. Means are represented by circles, SD by vertical lines and months by their first initial.

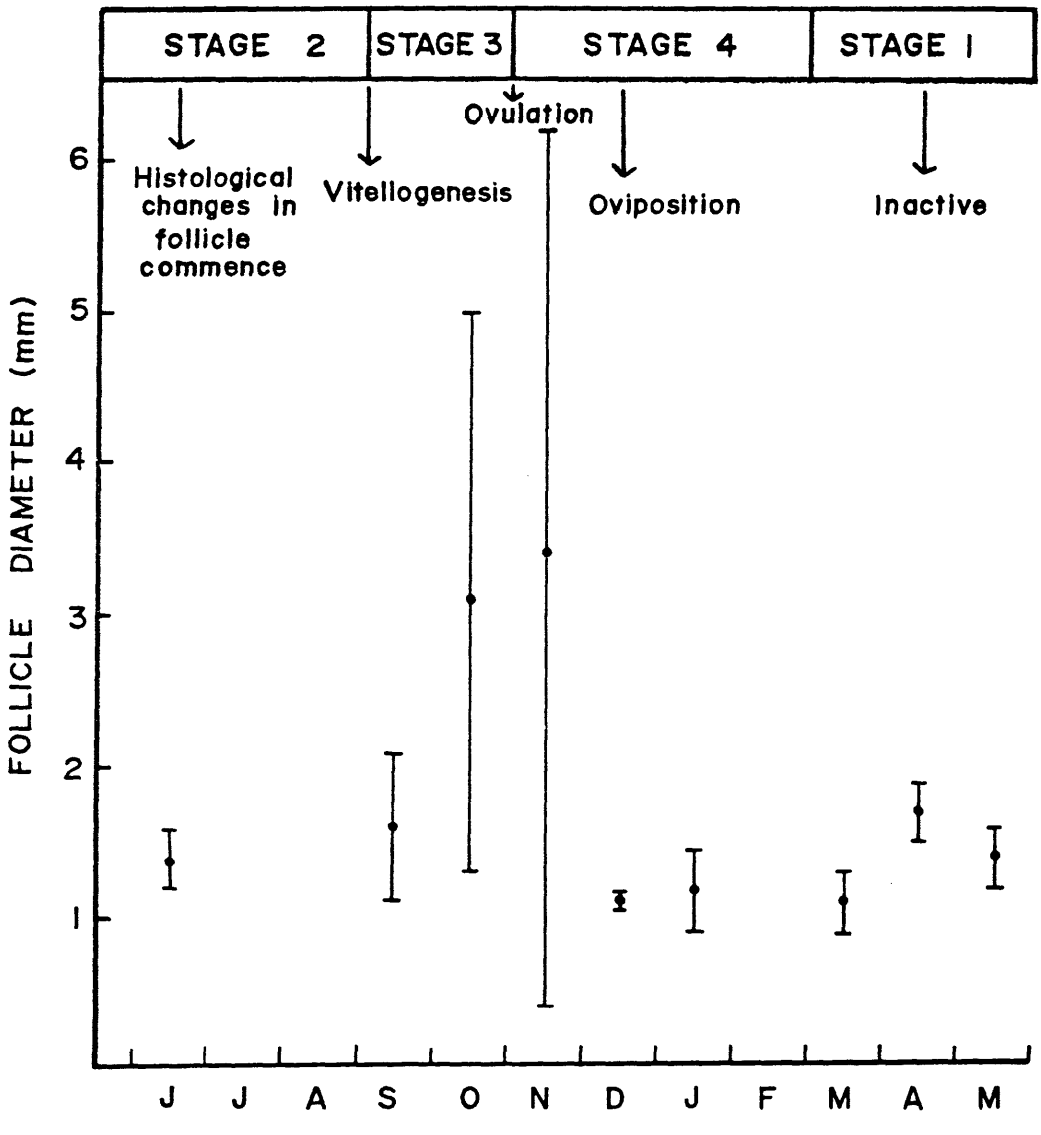

STAGE 2
STAGE 3
STAGE 4
STAGE 1
Ovulation
Histological changes in follicle commence
Vitellogenesis
Oviposition
Inactive
FOLLICLE DIAMETER (mm)
6
5
4
3
2
1
J
J
A
S
O
N
D
J
F
M
A
M

Figure 27 : Reproductive cycle of female Ctenotus taeniolatus in 1979-80, 1980-81, 1981-82. Circles represent means, thick lines represent SD of Ovary weight/FFDW and thin lines represent oviductal weight/FFDW. Months are represented by their first initial.

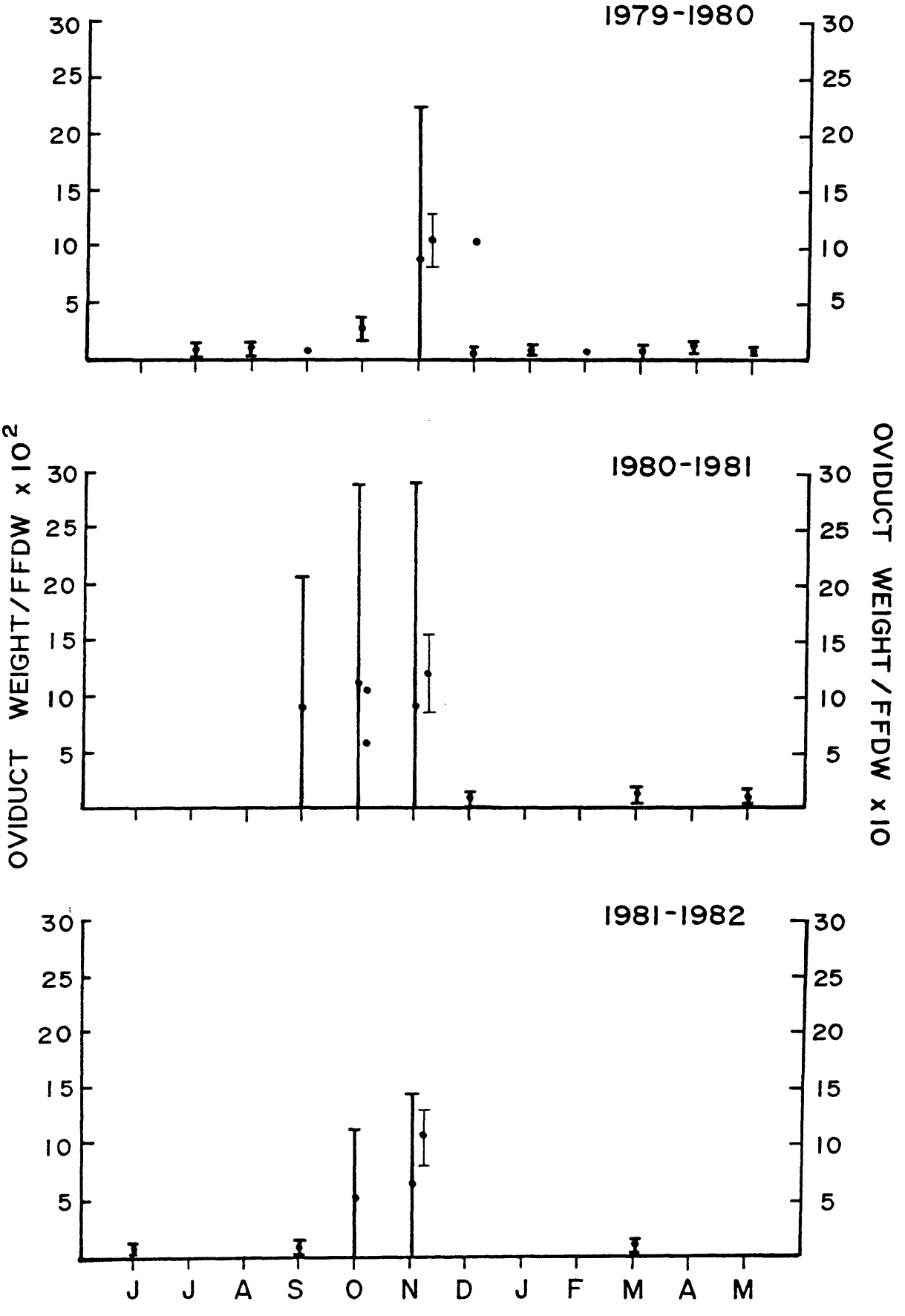
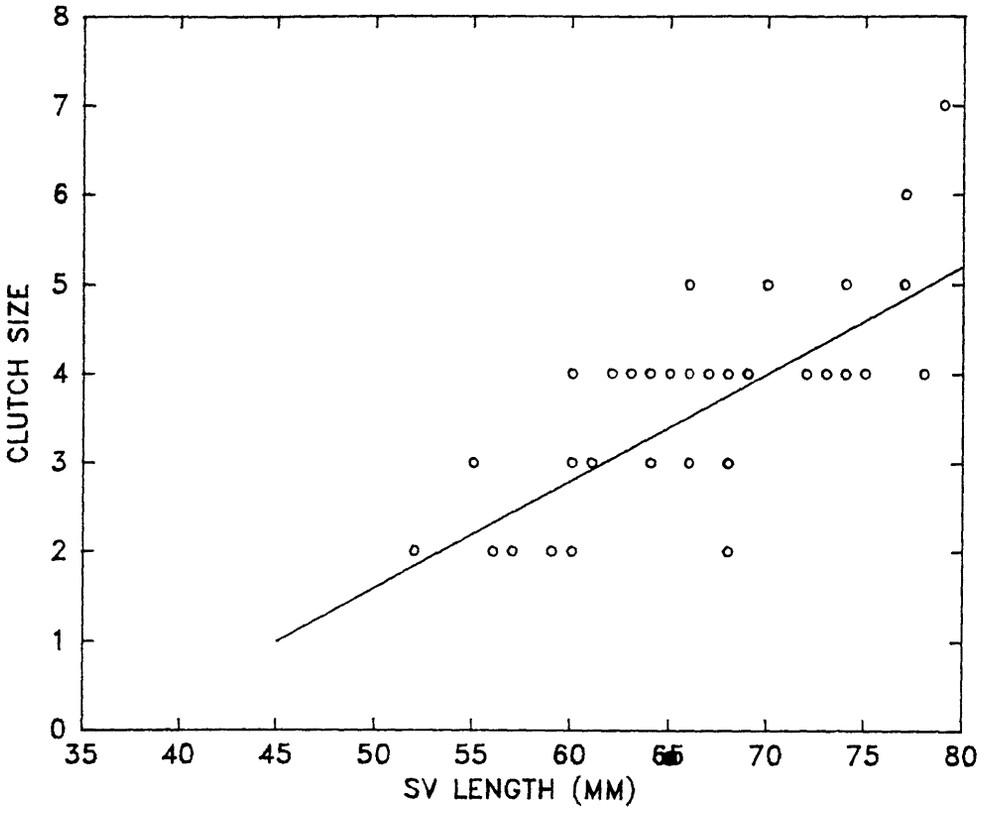

1979-1980
1980-1981
1981-1982
OVIDUCT WEIGHT/FFDW x 10²
OVIDUCT WEIGHT/FFDW x 10
30
25
20
15
10
5
J
J
A
S
O
N
D
J
F
M
A
M

Figure 28 : Relationship between clutch size and snout-vent length and clutch size and FFDW of Ctenotus taeniolatus. Lines represent regression lines detailed in section 6.3.5.


CLUTCH SIZE
0
1
2
3
4
5
6
7
8
35
40
45
50
55
60
70
75
80
SV LENGTH (MM)

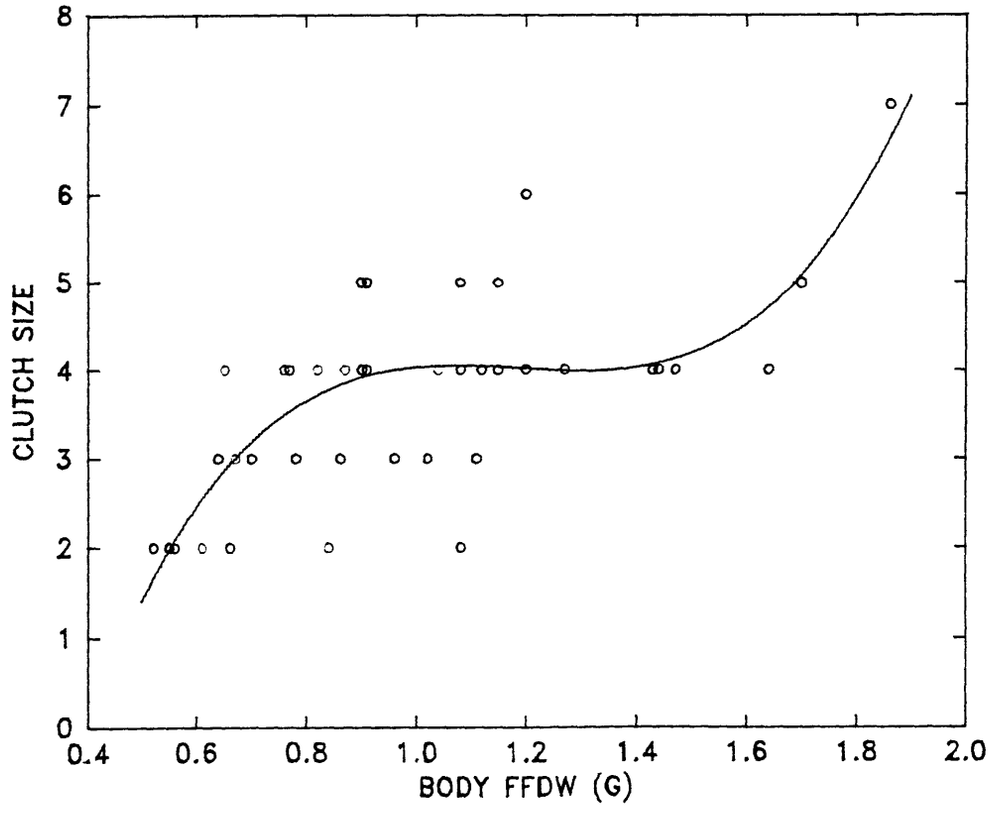
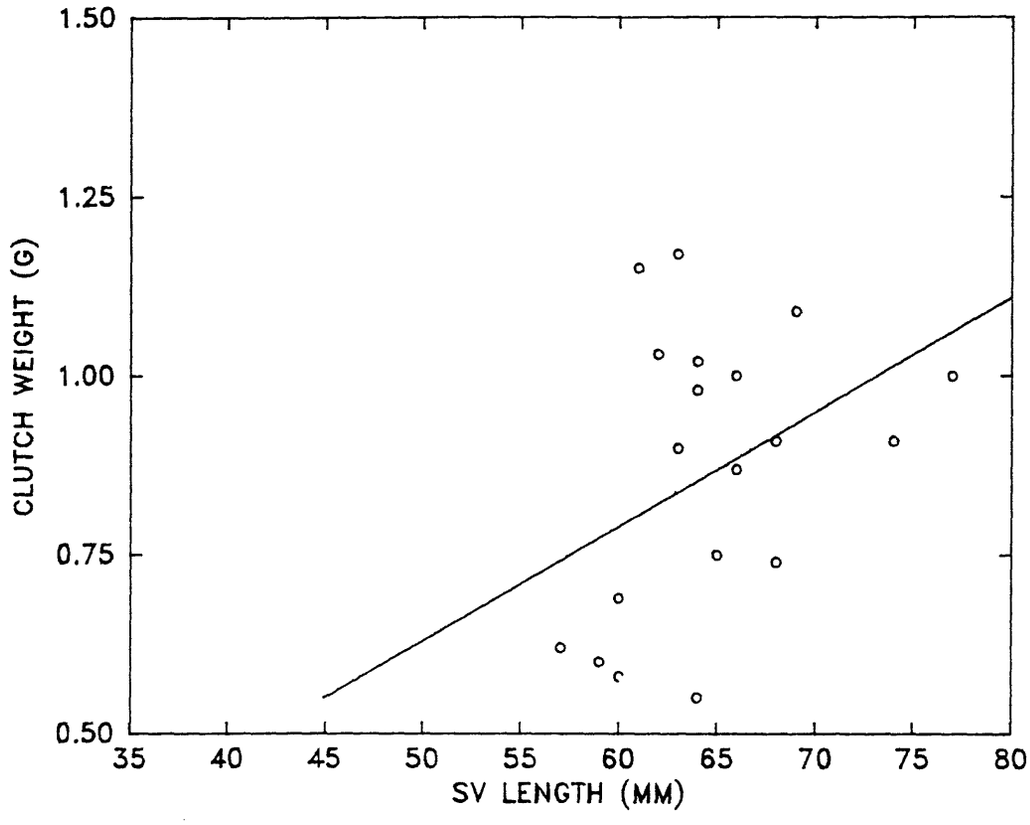
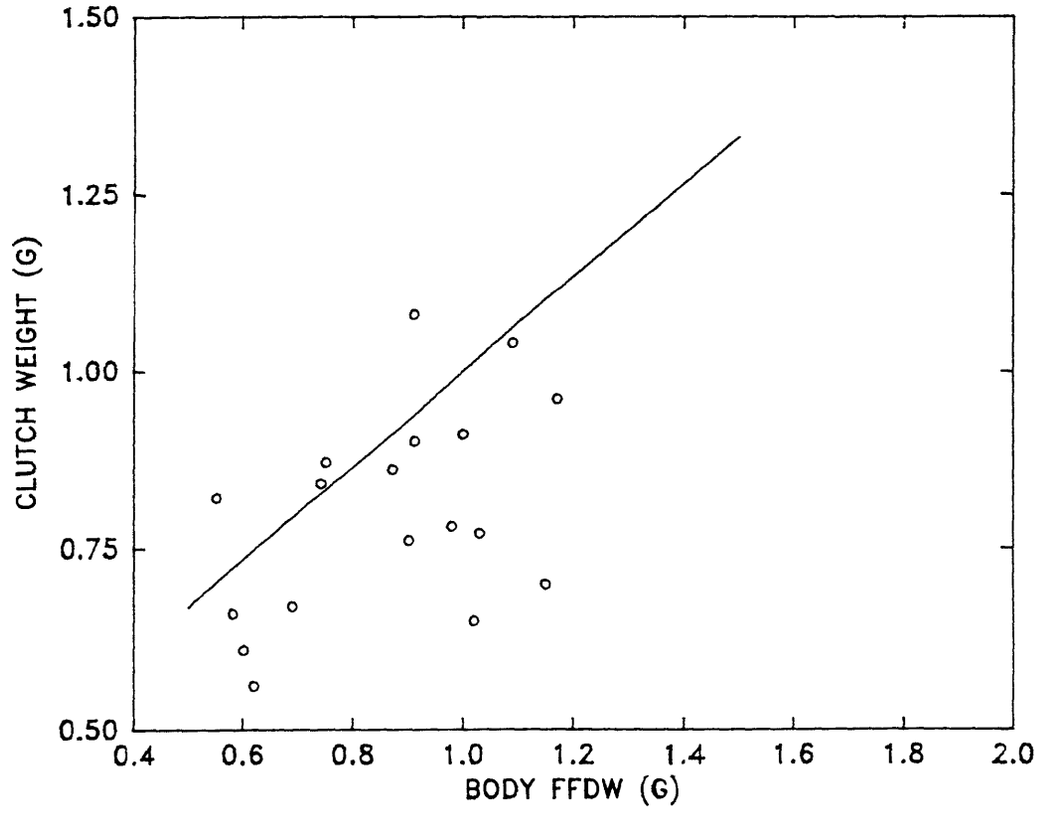

CLUTCH SIZE
0
1
2
3
4
5
6
7
8
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
BODY FFDW (G)

Figure 29 : Relationship between wet clutch weight and snout-vent length and wet clutch weight and FFDW of Ctenotus taeniolatus. Lines represent regression lines detailed in section 6.3.5.


1.50
1.25
1.00
0.75
0.50
CLUTCH WEIGHT (G)
35
40
45
50
55
60
65
70
75
80
SV LENGTH (MM)


1.50
1.25
1.00
0.75
0.50
CLUTCH WEIGHT (G)
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
BODY FFDW (G)

6.4 DISCUSSION

The study of reproductive cycles and associated phenomena is one of the most popular fields of herpetological research. Fitch (1970) summarised the available literature on reproductive cylces in lizards and snakes up to 1970 and Duvall et al. (1982) gave a further, but briefer, survey of more recent research. No attempt is made here to catalogue all known reptilian reproductive cycles, as a more relevant approach to understanding reptilian reproduction is their categorisation, a procedure followed by a number of researchers (Table 11). Each of these researchers has her/his own criteria for classification depending on the questions asked and on their research animals. These classifications have been based on ovarian cycles, testicular cycles or 'evolutionary stategies'. Perhaps the type of classification most useful in a discussion of skink reproductive patterns is one first presented by Pengilley (1972), who categorised some Australian skink reproductive cyles on the basis of a combination of male and female cycles. Note that skinks are primarily autochronic in their patterns of ovulation, so the classification of Smith et al. (1972) (see table 11 for definitions) is not particularly useful in expanding our understanding of skink reproduction. The other types of classification presented in table 11 can then be pooled to produce an integrated classification that embraces all knowledge of Australian skinks to date.

Skink reproductive patterns can thus be divided into seven types, which typically describe the reproductive patterns of temperate (highland, in particular) skinks - a direct reflection of the distribution of herpetologists rather than the distribution of the herpetofauna.

Type I : Spring spermatogenesis and mating, spring ovulation

Type II: Autumn spermatogenesis and mating, spring ovulation

Type III : Winter to spring spermatogenesis, spring mating and ovulation

Type IV : Spring and autumn spermatogenesis and mating, early spring and summer ovulations.

Type V : Spring and autumn spermatogenesis and mating, spring ovulation

Type VI : Spermatogenesis and mating all year, ovaries active all year with a peak in spring.

Type VII : Spermatogenesis and mating all year, ovaries active all year.

Table 12 presents a summary of our present knowledge of Australian skink reproduction. This table lists the reproductive cycles of 18 of the approximately 250 known species of skinks in Australia.

These cycles can in some instances be further subdivided as some lizards show development in ovaries and testes over winter. Cycles I and II have examples of lizards that show increases in ovary weight or follicle diameter over the autumn and winter periods and others that do not. C.taeniolatus, in particular, shows no gross changes in ovary weight or follicle diameter prior to spring, although cellular changes commence in winter, while vitellogenesis proper, with its large energy demand, does not commence until after emergence in spring. Aldridge (1979) described these two distinct phases as primary vitellogenesis and secondary vitellogenesis in the snakes Arizona elegans and Crotolus viridus. Dessauer and Fox (1959) also observed these

differences in Thamnophis sauritus. Similarly, changes in weight of testes alone are not a good indicator of testicular activity; for example in Sphenomorphus tympanum testis weight approaches high levels in autumn, but is not a sign of the commencement of mating, for although a few sperm are present, the majority of cells are still at the spermatocyte stage (Pengilley 1972). In addition, C.taeniolatus also begins to produce sperm slowly in early winter, but the majority of cells are still primarily at early stages over the winter period. The final stages of sperm production, when the majority of cells are producing sperm and the seminiferous tubules are packed with sperm, occurs in spring. The above demonstrates the importance of examining the histological condition of the gonads as well as observing gross characteristics.

The variety of reproductive cycles is somewhat surprising in what are mainly temperate skinks. And further, there appears to be few discernible patterns to relate the reproductive cycle to the mode of reproduction (viviparity or oviparity), climate or habitat, except in the obvious case of the tropical lizards, Carlia fusca and C.rhomboidalis (Wilhoft 1963b). Robertson (1981) proposed that the low preferred body temperature and thigmothermic habits of Anotis maccoyi allow it to feed and hence develop eggs overwinter, compared with Hemiergis decresiensis which having a higher preferred body temperature did not. This pattern extends to Lampropholis guichenoti and L.delicata (Pengilley 1972, Joss and Minard in press) which are known to be active, to develop eggs and to have motile sperm at times during winter, even on the New England Plateau (Heatwole 1976, personal observations). C.taeniolatus on the other hand is inactive over winter with no sign of vitellogenic activity, although cellular preparation for vitellogenesis has begun. Further, Robertson (1981) supports the proposition of Smyth

and Smith (1968) that post-ovulatory mating allows earlier spring ovulation which then allows the young to be born earlier giving them a better chance to feed and grow before winter. One could further postulate that a combination of follicular development and sperm storage over winter would be the ideal way to produce young earlier or to produce two clutches in a good season. Most lizards appear not to use this alternative, Hemiergis decresiensis uses only post-ovulatory mating and sperm storage while C.taeniolatus, probably as a result of its high activity temperatures (personal observations), practises neither of these alternatives and leaves all of its reproductive development until after emergence.

However, one species of lizard does use this strategy. L.guichenoti, a lizard with a wide distribution through a range of climates and habitats, has a type II cycle with follicular development and sperm storage over winter in the southern highlands (Pengilley 1972) with some variation in the commencement time of vitellogenesis (Heatwole 1976). While in the warmer coastal environment of Sydney it has a type IV reproductive pattern in a wet year and a type V one in a dry year. That is, under warmer wetter conditions L.guichenoti is capable of developing follicles over winter, producing sperm twice a year and producing two clutches over the summer season. Similarly, L.delicata has two spermatogenic periods in a year, but only has one clutch, although follicles did begin to develop after the first ovulation, indicating that under better conditions another clutch could be possible (Joss and Minard in press). Joss and Minard (in press) are more conservative in their discussion of L.delicata saying that there is no good evidence to suggest a 2nd clutch but rather that the spermatogenesis in spring is necessary to fertilize the females in their first reproductive season. Little is known about the intraspecific

diversity of lizard reproductive cycles over the Australian continent but one might expect to find that only lizards with type II reproduction would be able to produce multiple clutches under the right conditions. C.taeniolatus does not fall into that category.

C.taeniolatus, then, classically fits into the type I reproductive cycle, with pre-ovulatory mating in spring. It is oviparous and reproduces every year in the New England region. There was little variation in the timing of reproduction in the three years of the study, although these years all occurred during a severe drought. As with other Australian skinks (Pengilley 1972) clutch size and weight increase with body size. Although clutch size was very variable, the nature of its relationship with body size did not change appreciably between years. Also, even though this study was done in a severe drought, few atretic follicles were observed. Many factors are known to affect variation in clutch size (review by Ballinger 1978) and it is obvious that more information is necessary to determine why such large variations occur. As it stands now the relationship between clutch size (or weight) and body size really serves to predict the upper limit in the number and weight of eggs that a lizard of a certain size can produce.

Table 11 : Classification of reptilian reproductive cycles.

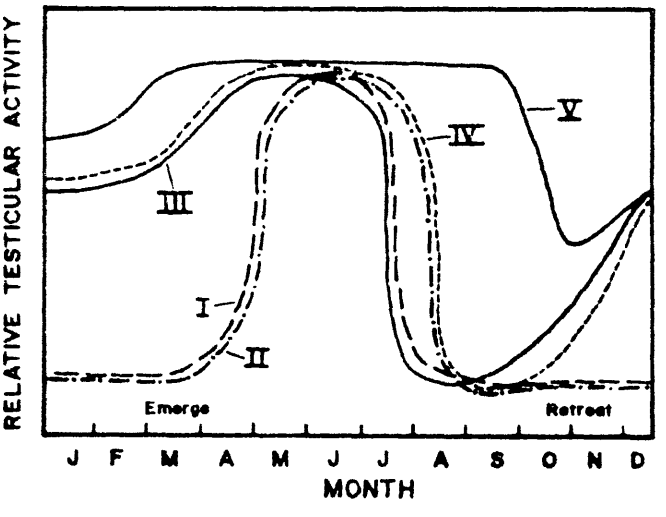
Basis of cycle	Categories	Source
Continuity of cycle	1. Continuous, without variation in reproductive activity 2. Continuous, with variation in reproductive activity 3. Non-continuous	Sherbrooke 1975
Evoluntionary stategies	1. Early maturing/multiple brooded/small clutch size 2. Late maturing/single brooded/large clutch size	Tinkle, Wilbur and Tilley 1970
Intra- and inter-ovarian patterns of ovulation	1. Allochronic (mono and poly) - ovulation proceeds alternatively from paired ovaries throughout breeding season 2. Autochronic (mono and poly) - ovulation occurs simultaneously from both ovaries 3. Monochronic - only a single ovary functional	Smith, Sinelik, Fawcett and Jones 1972
Ovarian cycles	1. Secondary vitellogenesis restricted to spring 2. Secondary vitellogenesis begins in autumn	St.Girons 1966; Aldridge 1979
Testicular cycles of snakes	1. Aestival (summer) and post-nuptial : spermatogenesis occurs in warm season and spermatozoa stored over winter in males and females 2. Mixed : spermatogenesis begins in late spring, completed one year later (a) 2 periods of spermiogenesis and mating (spring and autumn) (b) 1 period of spermiogenesis and mating (spring) 3. Prenuptial : spermatogenesis is completed towards end of mating season 4. Continuous reproductive activity	St.Girons 1982
Testicular cycles of lizards		Licht, Hoyer and van Oordt 1969; Licht and Gorman 1970

Table 12 : Summary of reproduction and reproductive cycles in Australian skinks. Reproductive cycles I - V are detailed in section 6.4. A refers to lizards that show no development of ovaries or testes overwinter and B refers to lizards that do show such development. (O = oviparous, V = viviparous).

Species	Reproductive Cycle		Reproductive Mode	Method	Habitat	Clutch size	Source
Anotis maccoyi	I	B	O	gonad dimensions	temperate (highlands)	2-7	Robertson 1976, 1981
				gonad dimensions histology	temperate (highlands)	1-4	Pengilley 1972
Carlia fusca	I	-	O	gonad dimensions histology	tropical	2	Wilhoft and Reiter 1965
C.rhomboidalis	VI	-	O	gonad dimensions histology	tropical	2	Wilhoft 1963a,1963b
Ctenotus robustus	I	-	O	gonad dimensions	temperate (highlands)	4-7	Way 1979
C.taeniolatus	I	A	O	gonad dimensions histology	temperate (highlands)	2-7	this study
Egernia cunninghami	I	-	V	gonad dimensions histology	temperate (highlands)	3-8	Barwick 1965
Hemiergis decresiensis	II	A	V	gonad dimensions	temperate	2-4	Robertson 1976,1981
H.peroni	II	-	V	gonad dimensions	temperate	1-6	Smyth 1968
Lampropholis delicata	V	B	O	gonad dimensions histology	temperate (coastal)	-	Joss and Minard in press
L.guichenoti	II	B	O	gonad dimensions histology	temperate (highlands)	1-5	Pengilley 1972
	IV,V	B	O	gonad dimensions histology	temperate (coastal)	-	Joss and Minard in press
Leiolopisma entrecasteauxii	II	B	O	gonad dimensions histology	temperate (highlands)	1-6	Pengilley 1972
L.x rawlinsoni	II	B	O	gonad dimensions histology	temperate (highlands)	2-3	Pengilley 1972
L.trilineata	III	-	O	gonad dimensions histology	temperate (highlands)	2-10	Pengilley 1972
Menetia greyi	I	-	O	gonad dimensions	temperate	1-3	Smyth and Smith 1974
Morethia boulengeri	I	-	O	gonad dimensions	temperate	2-5	Smyth and Smith 1974
Pseudomoia spenceri	II	-	V	gonad dimensions histology	temperate (highlands)	1-2	Pengilley 1972
Sphenomorphus quoyi	III	-	V	gonad dimensions	temperate (highlands)	2-7	Veron 1969b
S.tympanum	III	-	V	gonad dimensions histology	temperate (highlands)	2-5	Pengilley 1972

7.0 ENERGY STORAGE

7.1 INTRODUCTION

The study of energy storage and utilisation in relation to certain environmental parameters can provide valuable information for understanding reptilian life histories. Within an animal, energy is usually stored in two ways, either by the storage of lipids or the storage of glycogen, with lipid storage being the more efficient in terms of energy production (Derickson 1976). Structural lipids which represent about 1% of the total body lipids (Giese 1966, 1967) are mainly composed of lipids other than triglycerides and are thus not regarded as storage lipids. Studies on the patterns of energy storage within and between years although common in the literature from the northern hemisphere, are rare in the literature on Australian reptiles (Barwick and Bryant 1966, Cogger 1978, Pengilley 1972, Robertson 1976, 1981, Smyth and Smith 1974). Consequently the aims of this chapter are to describe, in a quantitative way, the energy cycles for male, female and juvenile C.taeniolatus within a year and to examine the variation in these among years. The energy sources examined were carcass and tail lipids, liver as a whole and components of the liver, glycogen and lipid.

7.2 METHODS

7.2.1 Lipid analysis - histological

To determine the position of lipid deposits in the tail two histological techniques were employed. Firstly, tails were fixed in AFA (acetic acid, formalin, alcohol), decalcified, dehydrated through xylene

to paraffin, sectioned using standard techniques (7um) and stained with Masson's Trichrome (Humanson 1972). Secondly, as lipid stored in fat cells is dissolved during fixing and dehydration, to confirm that the evacuated cells contained lipid, segments of the tail were frozen, sectioned by hand and stained with Sudan Black, a stain specific for lipids.

7.2.2 Lipid analysis - gross

To determine annual lipid cycles, 10 to 20 lizards were collected approximately once per month using the schedule detailed in section 2.2. Standard body measurements were taken on lizards prior to dissection (Section 2.2), which involved removal of the internal organs and separation of the lizard into carcass and tail (beginning at the vent). The carcass and tail were then dried to constant weight using freeze-dry lyophilisation.

Lipids were extracted using a Soxhlet apparatus, which consists of an extractor enclosing a fat-free cellulose extraction thimble, a condenser and a flask with a solvent, following the procedure of Sawicka-Kapusta (1975). The thimbles selected for lipid extraction from lizards were of two sizes: 22.5 mm diameter for carcasses of adults and 10 mm diameter for carcasses of juveniles and all tails. Giese (1966, 1967), Morris and Gulkin (1976) and Simpson (1982) indicated that Soxhlet extraction using mild heat with a non-polar solvent extracts only stored lipids which occur as free globules in cells, while polar solvents are required to extract structural and more tightly bound lipids. The aim of this study was to follow changes in loosely held stored lipids within a lizard and consequently a non-polar solvent, petroleum ether (BP 40-60 C) was used.

After extraction, lipid content of carcass and tail (g) was determined, and was subsequently used to calculate fat-free dry weight (FFDW);

FFDW = (dry weight of carcass - lipid weight in carcass) + (dry weight of tail - lipid weight in tail)

FFDW of a whole lizard is related to wet weight (W_w) by the equation,

$W_w = 0.533^{***} + 4.17^{***}.FFDW$, $R^2 = 0.6673$, $P < 0.00001$, $n = 208$.

7.2.3 Liver analysis

To determine the annual cycle of liver weight, 10 to 20 lizards were collected approximately once per month using the schedule detailed in section 2.2. Standard body measurements were taken and livers were removed, blotted dry and weighed (0.005 g) within 24 hours of capture.

Annual glycogen and lipid content of livers was determined in 1981 from lizards captured in March, May, September, October and December which corresponded to pre-winter, emergence and reproductive periods. Twelve to 20 lizards were collected at each time, pithed and dissected immediately. Once removed, livers were quickly blotted dry and weighed, with approximately 100 mg of tissue being analysed immediately for glycogen and the rest being frozen for lipid analysis.

Glycogen was estimated with anthrone reagent using the method of Seifter et al. (1950) modified by the addition of a drop of 10% (w/v) sodium sulphate solution to the sodium hydroxide digest before precipitating the glycogen with ethanol (Patterson et al. 1978). By this method glycogen was first determined as mg of glucose per liver and then converted to mg of glycogen per liver using the conversion factor

of Morris (1948). Total lipid in the liver was determined using the diphasic extraction (chloroform and methanol) method of Bligh and Dyer (1959) modified for small samples by Thomson (1981). As usual for lipid extraction, liver samples were first dried to constant weight using freeze-dry lyophilisation. Lipid weights are presented as grams of lipid per whole liver.

7.2.4 Statistical analysis

Seasonal and annual trends in all body components were first investigated for periodic effects by fitting a Fourier series of the form

$$y = a_0 + a_1\cos u + a_2\cos 2u + a_3\cos 3u + b_1\sin u + b_2\sin 2u + b_3\sin 3u$$

where y = weight of lipid/FFDW, u = 2π (month)/12 and a_i, b_i are constants.

This function would seem to be the most appropriate as it is a combination of sine and cosine functions and is capable of allowing for periodic variations in the dependent variable. The Fourier series was fitted, using stepwise multiple regression procedures (Bar3; Burr 1975), for all body components which had been monitored approximately monthly over a year, and tested for the existence of significant trends over these periods. In cases where data were available for 3 years, the Fourier series was also fitted as confirmation of the above.

In all, this series was fitted to 18 subsets of data - to carcass and tail indices, and liver weight indices for males and females collected in 1979-80 (1 year) and 1979-82 (3 years), to carcass and tail lipid indices for juveniles collected in 1979-80 (1 year) and to gylcogen and liver lipid indices for males and females collected in 1980-81 (1 year). These subdivisions were necessary because in 1979-80

lizards were sampled monthly, while between 1980 and 1982 lizards were collected only at times deemed to be crucial on the basis of the 1979-80 data, i.e. reproductive, pre-winter and post-winter periods. Juveniles were collected only during 1979-80, and glycogen and liver lipid estimations were made in 1980-81 only.

Further analysis of the differences among males and females, and years are described in the relevant results sections. In all cases an analysis of variance was used whereby the year was in general divided into 4 crucial times - emergence (September), reproduction (October, November) and pre-winter (March). As the annual cycle was determined after fitting Fourier series, these analyses were not designed to compare between months. All relevant analyses were completed using the NEVA computer package for analysis of variance of complete factorial experiments (Burr 1981).

7.3 RESULTS

7.3.1 Storage sites of lipids

C.taeniolatus stores lipids in the tail, general carcass and liver. In original tails, lipid is stored in 4 circum-skeletal blocks associated with each fracture plane down the length of the tail (Fig. 30), while in regenerated tails, the lipid forms a continuous mass around the cartilaginous rod that replaces the vertebrae.

No distinct lipid depots were seen in the carcass of C.taeniolatus but lipids were probably stored in small amounts subcutaneously.

7.3.2 Lipid stores and lizard size

Both tail and carcass lipid weights increased directly with weight of lizard, measured as FFDW, and passed through the origin, confirming the validity of the lipid index expression (Section 6.3.2). Table 13 shows the relationships between lipid weight and FFDW for females and males from June and October, as determined by linear regression procedures. As a consequence, lipid weight was corrected for body weight in all ensuing analyses, and expressed as a lipid index (lipid weight(g) / FFDW(g)).

7.3.3 Lipid stores and seasonality

7.3.3.1 Tail lipid

Figures 31, 32 and 35 show the changes in tail lipid index in females and males from 1979 to 1982, and for juveniles in 1979-80 respectively. Fourier series of the form described in section 7.2.4, accounted for a significant proportion of the variation in the monthly tail lipid indices for females, males and juveniles collected in 1979-80 (Table 14, Fig. 36). In all cases there was indicated a yearly periodicity, which peaked in the pre-winter period (March), decreased throughout winter and early spring (reproductive period) to a minimum around November and increased to the maximum described previously. Similar Fourier series were also suitable models (Table 14) for the 3 years of tail lipid indices for females and males, confirming the continuity of the annual cycles over the duration of the study.

7.3.3.2 Carcass lipid

Figures 33, 34 and 35 show the changes in carcass lipid index in females and males from 1979 to 1982, and for juveniles in 1979-80 respectively. Fourier series of the form described in section 7.2.4 accounted for a significant proportion of the variation in the monthly carcass lipid indices for females and juveniles collected in 1979-80, but was not a suitable model for the male carcass lipid indices from the same period (Table 15). Figure 37 shows the Fourier series that fit the female and juvenile cycles. Carcass lipid indices of females followed a similar pattern to that of associated tail lipid indices, with a peak from March throughout winter, decreasing to a minimum in spring (November - December). A similar Fourier series was a suitable model for the 3 years of carcass lipid indices for females (Table 15), confirming the continuity of this annual cycle over the duration of the study.

The juvenile cycle was described by a Fourier series with a primary periodic cycle of 4 months with peaks in early winter (May - June) and Spring (November) and a trough in early spring (September). This pattern was different from the expected one and may be related to the very large variation present in all juvenile lipid indices.

7.3.3.3 Multiple comparisons

(a) A comparison of carcass and tail lipid indices between females and males, within and between years was carried out using split-plot analysis of variance with sex (S) at 2 levels (male, female), year (Y) at 3 levels (1979-80, 1980-81, 1981-82) and month at (M) 4 levels (September, October, November, March), as the main plot factors. Lipid (L), the sub-plot factor, had 2 levels (tail, carcass) which were split within each lizard. Five lizards, randomly selected in order to balance

the design, were compared from each cell.

Table 16 shows the results of the analysis, the most important of which are the significant interactions between year, month and lipid (YxMxL), and sex and lipid (SxL).

Further analysis of the YxMxL interaction using Newman-Keuls' multiple comparisons at the 5% level, with appropriate errors (Steel and Torrie, 1960) revealed (Fig. 38):

1. Tail lipid was significantly greater than carcass lipid in all months, except November. This was consistent over all years.

2. There were significant differences in tail lipid between months, the basic pattern following that determined after fitting Fourier series in section 7.3.3.1. The three years differed in their pattern of variation; in 1979-80 only September and October were not significantly different, in 1980-81 September and March were not significantly different and in 1981-82 November and October, and September and March were not significantly different.

Further analysis of the SxL interaction (as above) revealed that female tail lipid was significantly greater than male tail lipid (Fig. 39).

(b) A comparison of carcass and tail lipid indices between females, males and juveniles, within a year (1979-80) was carried out using a split-plot analysis of variance with sex (S) at 3 levels (female, male, juvenile) and month (M) at 4 levels (September, October, November, March), as the main plot factors. Lipid (L), the sub-plot factor, had 2 levels which were split within each lizard. The rest of the design was as described in (a) above.

Table 17 shows the results of the analysis, the most important of which is the significant interaction between month, sex and lipid (MxSxL). Further analysis using the methods described in (a) revealed (Fig. 40):

1. Males, females and juveniles had significantly different patterns of lipid distribution in tail and carcass throughout the year. Tail lipid in females was significantly greater than carcass lipid in October and March only, while tail lipid in males was significantly greater than carcass lipid in March only.

2. There were significant differences in tail lipid of males, females and juveniles between months. In September and October, female tail lipid was greater than male and juvenile tail lipid, but the latter two were not significantly different from each other, while in March, female tail lipid was significantly greater than that of males which was greater than juvenile tail lipid.

Table 13 : Relationship between lipid weight in tail and carcass (y) and body weight, FFDW (x), for male and female Ctenotus taeniolatus captured in June and October.

		Male	Female
October	Body	$y = 0.014^{ns} + 0.037^{*}x$ $R^2 = 0.17$, NS, $n = 27$	$y = 0.019^{ns} + 0.044^{***}x$ $R^2 = 0.31$, $P < 0.001$ $n = 50$
	Tail	$y = -0.02^{ns} + 0.107^{***}x$ $R^2 = 0.49$, $P < 0.001$, $n = 27$	$y = -0.027^{ns} + 0.203^{***}x$ $R^2 = 0.44$, $P < 0.001$, $n = 50$
June	Body	$y = 0.011^{ns} + 0.059^{**}x$ $R^2 = 0.28$, $P < 0.001$, $n = 26$	$y = -0.01^{ns} + 0.187^{***}x$ $R^2 = 0.362$, $P < 0.001$. $n = 48$
	Tail	$y = 0.006^{ns} + 0.083^{*}x$ $R^2 = 0.28$, NS, $n = 26$	$y = -0.058^{ns} + 0.349^{***}x$ $R^2 = 0.81$, $P < 0.001$, $n = 48$

Table 14 : Fourier series describing female, male and juvenile tail lipid indices (y) at different times of the year (u = 2Π(month)/12).

	1 year	3 years
Female	$y = 0.257^{***} - 0.09^{***}\cos u + 0.064^{**}\sin u - 0.049^{**}\cos 2u$ $R^2 = 0.524$, $P < 0.01$, $n = 60$	$y = 0.229^{***} - 0.062^{***}\cos 2u + 0.050^{***}\sin 2u + 0.027^{*}\sin u$ $R^2 = 0.408$, $P < 0.01$, $n = 172$
Male	$y = 0.197^{***} - 0.04^{**}\cos u + 0.078^{***}\sin u + 0.047^{**}\sin 2u + 0.032^{*}\sin 3u$ $R^2 = 0.785$, $P < 0.01$, $n = 41$	$y = 0.145^{***} + 0.046^{***}\cos 2u - 0.050^{***}\cos u + 0.050^{***}\cos 3u$ $R^2 = 0.645$, $P < 0.001$, $n = 106$
Juvenile	$y = 0.077^{***} - 0.039^{***}\cos u$ $R^2 = 0.20$, $P < 0.001$, $n = 54$	-

Table 15 : Fourier series describing female, male and juvenile carcass lipid indices (y) at different times of the year (u = 2Π(month)/12).

	1 year	3 years
Female	$y = 0.103^{***} - 0.02^{**}\cos u$ $R^2 = 0.138$, $P < 0.01$, $n = 60$	$y = 0.087^{***} + 0.020^{***}\sin 2u$ $R^2 = 0.064$, $P < 0.001$, $n = 172$
Male	NS	
Juvenile	$0.093^{***} + 0.034^{**}\cos 2u$ $R^2 = 0.69$, $P < 0.01$, $n = 54$	

Table 16 : Split-plot analysis of variance on the effects of sex (S), month (M) and year (Y) on tail and carcass lipid indices of Ctenotus taeniolatus.

Source	DF	MS	Probability
Sex (S)	1	0.1785	< 0.001
Month (M)	3	0.1605	< 0.001
Year (Y)	2	0.0134	< 0.05
Month x sex	3	0.0194	< 0.001
Year x sex	2	0.0030	NS
Month x year	6	0.0075	< 0.05
M x Y x S	6	0.0086	< 0.05
Main plot error	96	0.0029	
Lipid (L)	1	0.4844	< 0.001
Sex x lipid	1	0.1320	< 0.001
Month x lipid	3	0.1063	< 0.001
Year x lipid	2	0.0015	NS
M x S x L	3	0.0035	NS
Y x S x L	2	0.0036	NS
M x Y x L	6	0.0071	< 0.05
M x Y x S x L	6	0.0023	NS
Sub-plot error	96	0.0026	
Total	239		

Table 17 : Split-plot analysis of variance on the effects of sex and month on tail and carcass lipid indices during 1979-80, in Ctenotus taeniolatus.

Source	DF	MS	Probability
Sex (S)	2	0.1124	< 0.001
Month (M)	3	0.0542	< 0.001
Month x sex	6	0.0171	< 0.001
Main plot error	48	0.0029	
Lipid (L)	1	0.0753	< 0.001
Sex x lipid	2	0.0595	< 0.001
Month x lipid	3	0.0388	< 0.001
M x S x L	6	0.0083	< 0.001
Sub-plot error	48	0.0025	
Total	119		

7.3.4 Liver weight and components

7.3.4.1 Liver weight

Liver weight (g) increased directly with the body weight of the lizard, measured as FFDW (g), and passed through the origin, confirming the validity of the liver expression (Section 6.3.2). Table 18 shows the relationship between liver weight and FFDW for males and females from October and June determined by linear regression procedures. As a consequence, it was necessary to correct liver weight for body weight in all ensuing analyses, with liver weight being expressed as a liver index (liver weight(g)/FFDW(g)).

Figures 41 and 42 show the changes in the liver index in females and males from 1979 to 1982. A significant proportion of the variation in the monthly lipid indices for females collected in 1979-80 (Table 19) was accounted for by a Fourier series of the form described in section 7.2.4. The significant periodic effect resulted because liver weight index (i) peaked in the pre-winter period (March), (ii) decreased through early winter, (iii) increased again around emergence time in early spring, (iv) followed by a slight decrease during the reproductive period (October - December) and (v) increased to the maximum described previously (Fig. 43). Fourier series also significantly described (Table 19) the 3 years of liver indices for females and confirmed the continuity of the annual cycle over the duration of the study.

Liver indices for males did not change significantly ($P > 0.05$) throughout the year.

A comparison of liver indices between females and males, within and between years was carried out using a 3-way analysis of variance with sex (S) at 2 levels (male, female), year (Y) at 3 levels (1979-80,

1980-81, 1981-82) and month (M) at 4 levels (September, October, November, March). Five lizards, randomly selected to balance the design, were compared within each cell. A logarithmic transformation was applied in order to stabilise the variance. The rationale behind the selection of September, October, November and March as representative times of the year is presented in section 7.2.4.

Table 20 shows the results of the above analysis, the most important of which was the significant interaction between month, year and sex (MxYxS). Further analysis of this using Newman-Keuls' multiple comparisons at the 5% level revealed the following results (Fig. 44):

1. Female liver indices were significantly larger than male liver indices in all months over all years, except in 1979-80 when September and November were not significantly different.

2. Female liver indices were significantly different between years, within each month. These differences showed no consistent pattern. In September, liver indices for 1979-80 were significantly less than those for 1980-81, which were less than those for 1981-82; in October, liver indices for 1979-80 were significantly different from those for 1981-82, while in November, liver indices for 1981-81 were significantly greater than those for the other two years which were not significantly different.

Table 18 : Relationship between liver weight (y) and body weight, FFDW (x), for male and female Ctenotus taeniolatus captured in October and June.

	June	October
Male	$y = 0.009^{ns} + 0.079^{*}x$ $R^2 = 0.30$, $P < 0.05$, $n = 11$	$y = 0.024^{ns} + 0.079^{***}x$ $R^2 = 0.59$, $P < 0.01$, $n = 20$
Female	$y = 0.042^{ns} + 0.054^{*}x$ $R^2 = 0.25$, $P < 0.05$, $n = 14$	$y = -0.049^{ns} + 0.254^{***}x$ $R^2 = 0.47$, $P < 0.00001$, $n = 34$

Table 19 : Fourier series describing female liver indices (y) of Ctenotus taeniolatus at different times of the year ($u = 2\pi(month)/12$).

1 year

$y = 0.132^{***} - 0.026^{*}\cos 2u + 0.029^{**}\cos u + 0.030^{*}\sin u$
$R^2 = 0.23$, $n = 55$, $P < 0.05$

3 years

$y = 0.146^{*} - 0.027^{***}\cos 2u - 0.023^{ns}\sin 2u + 0.037^{***}\sin u + 0.035^{**}\cos u - 0.029^{*}\cos 3u$
$R^2 = 0.23$, $n = 138$, $P < 0.05$

NB : male liver indices did not change significantly over the year.

Table 20 : Three-way analysis of variance on the effects of sex (S), year (Y) and month (M) on liver indices of Ctenotus taeniolatus.

Source	DF	MS	Probability
Sex (S)	1	2.555	< 0.0001
Year (Y)	2	0.519	< 0.0001
Month (M)	3	1.078	< 0.0001
YxS	2	0.040	NS
MxS	3	0.031	NS
MxY	6	0.224	< 0.0001
MxYxS	6	0.091	< 0.05
Error	96	0.040	
Total	119		

7.3.4.2 Glycogen

Glycogen weight (mg) increased directly with the body weight of the lizard and passed through the origin in males only, confirming the validity of the use of the glycogen index expression in this group (Section 6.3.2). Table 21 shows the relationship between glycogen weight (mg) and FFDW (g) for males and females from October determined by linear regression procedures. As a consequence, it is necessary to correct glycogen weight for body weight in males only. However, for the sake of uniformity the female levels were also corrected so that in both sexes glycogen weight was expressed as an index (glycogen weight/FFDW).

Figure 45 shows the changes in glycogen index in females and males from 1980-81, which were significantly described (Table 22) by Fourier series of the form described in section 7.2.4. These Fourier series were similar for females and males in that the female cycle peaked in October (male - December) and reached a minimum around February (male - April).

A comparison of glycogen indices between females and males within each month was completed using a two-way analysis of variance with sex (S) at 2 levels (female, male) and month at 5 levels (September, October, December, March, May). In order to balance the design, six lizards were selected randomly from the total number of lizards within each cell, and compared. One missing value was estimated using the technique described by Burr (1981). A logarthmic transformation was applied to stabilise the variances. Table 23 shows the results of this analysis, the most important of which was the significant ($P < 0.05$) interaction between month and sex (MxS). Further analysis of this interaction using Newman-Keuls' multiple comparisons at the 5% level revealed that there were no significant differences in glycogen levels

between females and males in any month, except May when male levels were greater than those of females. This is most likely due to the lack of synchronisation of the glycogen cycles at this time.

Finally a visual comparison of figure 43 with figure 45 indicated that changes in liver weight were not a reflection of changes in glycogen content alone. This was confirmed when glycogen index was regressed against liver index for each month, and the variation about the resultant lines compared using the variance ratio test. In both males and females there was a significant ($P < 0.0005$) heterogeneity in the variation about the line (Fig. 46).

Table 21 : Relationship between glycogen weight (y) and body weight, FFDW (x), for male and female Ctenotus taeniolatus captured in October.

Male

$y = -0.524^{ns} + 1.318^{*}x$
$R^2 = 0.72$, $P < 0.05$, $n = 6$

Female

$y = 0.344^{ns} + 0.518^{**}x$
$R^2 = 0.05$, NS, $n = 12$

Table 22 : Fourier series describing male and female glycogen indices (y) at different times of the year ($u = 2\pi(month)/12$).

Female

$y = 0.424^{***} - 0.454^{***}\sin 2u + 0.204^{*}\cos u + 0.147^{*}\cos 2u$
$R^2 = 0.37$, $P < 0.05$, $n = 50$

Male

$y = 0.652^{***} + 0.347^{***}\cos 2u + 0.418^{***}\cos u$
$R^2 = 0.73$, $P < 0.0001$, $n = 31$

Table 23 : Two-way analysis of variance on the effect of sex (s) and month (M) on glycogen indices of Ctenotus taeniolatus (1 missing value).

Source	DF	MS	Probability
Sex (S)	1	0.0041	NS
Month (M)	4	2.3297	< 0.0001
MxS	4	0.5793	< 0.05
Error	49	0.1848	
Total	58		

7.3.4.3 Liver lipid

As many of the livers analysed weighed less than 100 mg the number of lipid estimations was small and does not correspond to the number of glycogen estimations. Further, as a consequence there were too few points for a realistic examination of the relationship between liver lipid weight and body weight, so a linear relationship was assumed and concentrations were calculated as a liver lipid index (liver lipid(mg)/FFDW(g)).

Figure 47 shows the changes in liver lipid index in females and males throughout the year. A Fourier series of the form $y = 0.036^{***} - 0.020^{*}\cos 3u$ (where y is liver lipid index and u is 2π(month)/12), described significantly ($R^2 = 0.23$, $P < 0.05$, $n = 19$) female liver lipid indices throughout the year. Figure 47 shows this function, which peaked in March and September, and had a minimum in December. Male liver lipid indices did not change significantly throughout the year.

Figure 30 : Transverse sections of tail of Ctenotus taeniolatus (x 150). Figure in top left is adjacent to the body, while lower right is closest to the tail tip. The large clear areas surrounding the vertebrae are lipid deposits.

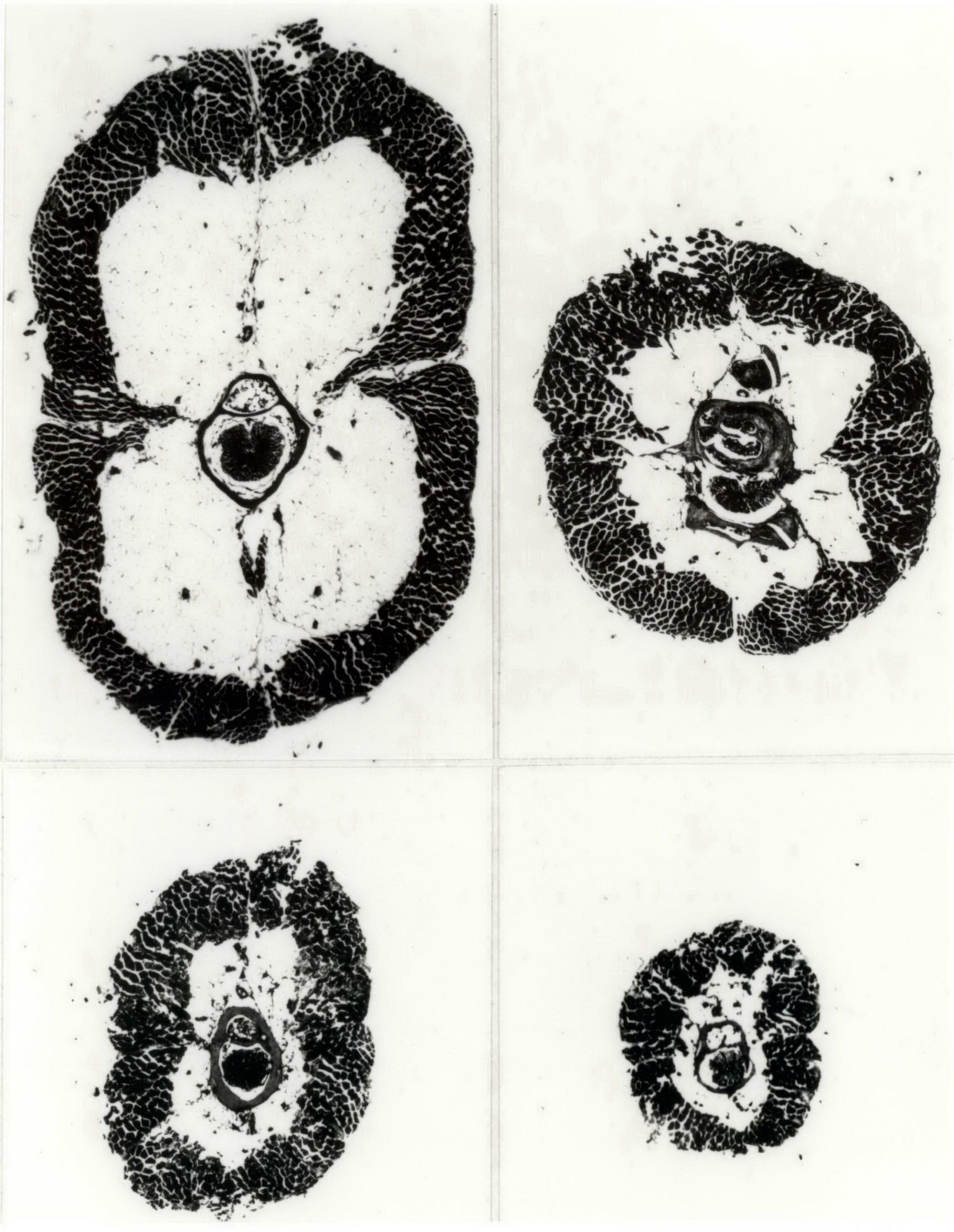


Figure 31 : Tail lipid index of female Ctenotus taeniolatus throughout the year for 1979-80, 1980-81 and 1981-82. Means are represented by circles, SD by vertical lines and months by their first initial.

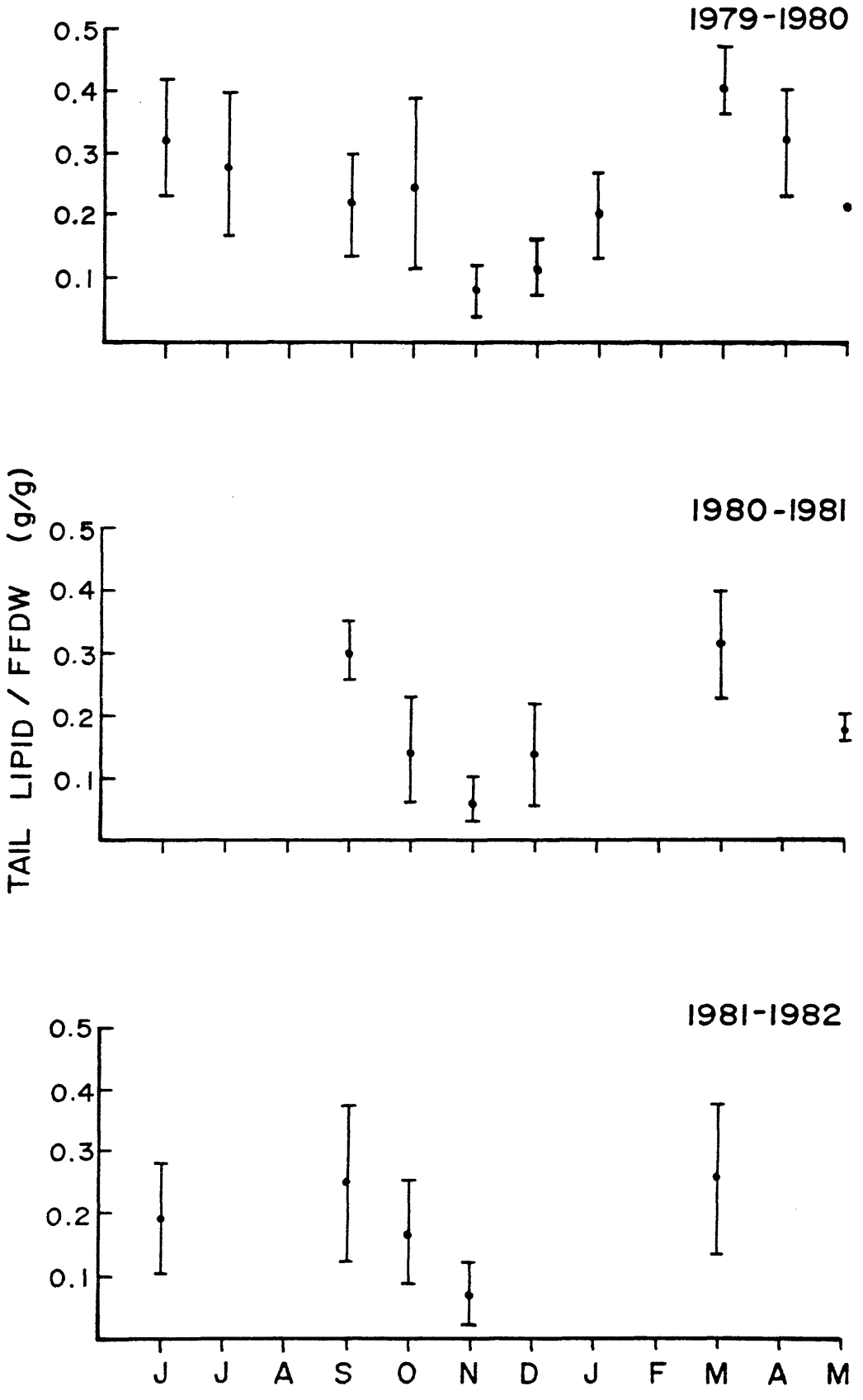

1979-1980
1980-1981
1981-1982
TAIL LIPID / FFDW (g/g)
0.5
0.4
0.3
0.2
0.1
J J A S O N D J F M A M

Figure 32 : Tail lipid index of male Ctenotus taeniolatus throughout the year for 1979-80, 1980-81 and 1981-82. Means are represented by circles, SD by vertical lines and months by their first initial.

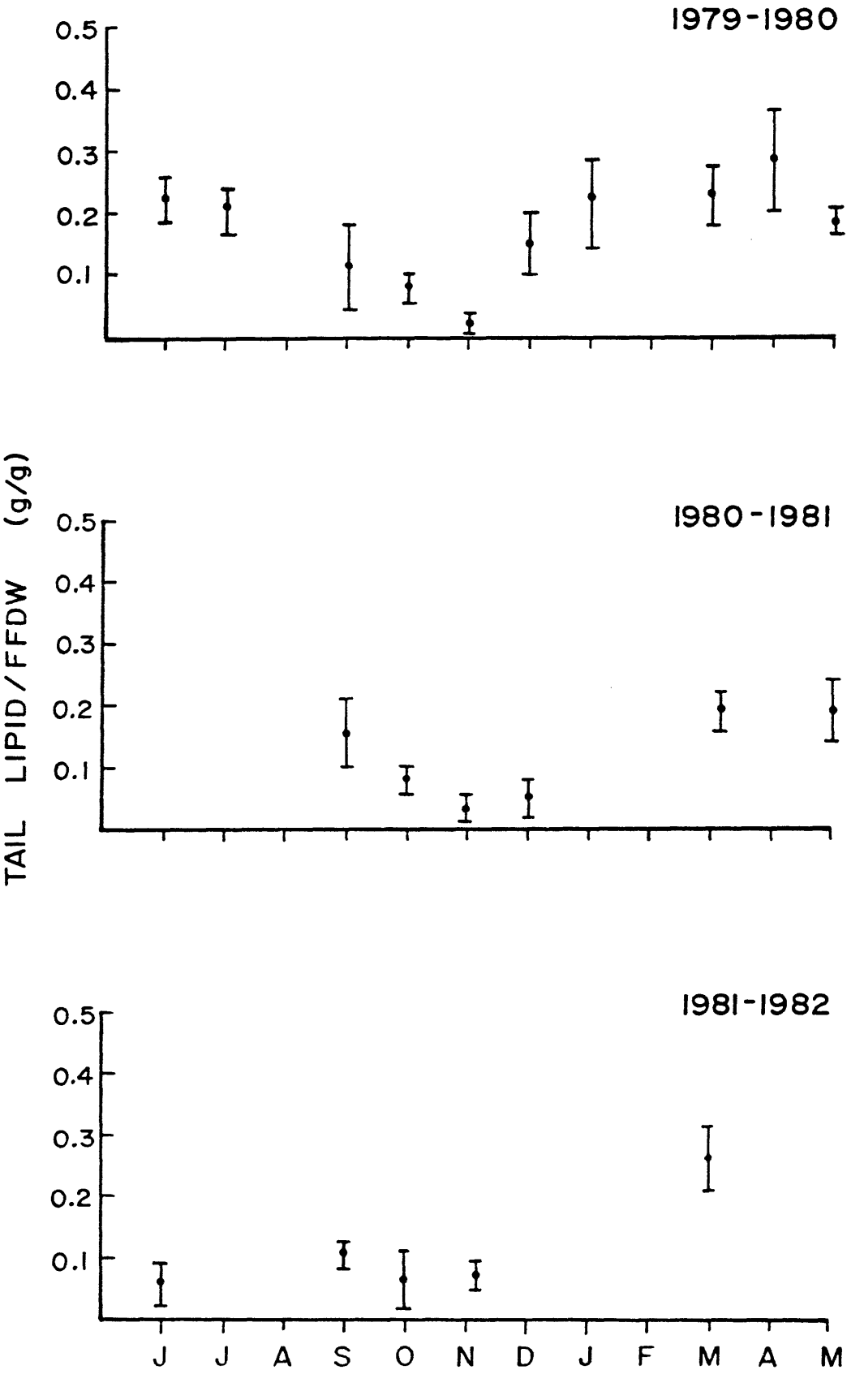

1979-1980
1980-1981
1981-1982
TAIL LIPID/FFDW (g/g)
0.5
0.4
0.3
0.2
0.1
J J A S O N D J F M A M

Figure 33 : Carcass lipid index of female Ctenotus taeniolatus throughout the year for 1979-80, 1980-81 and 1981-82. Means are represented by circles, SD by vertical lines and months by their first initial.

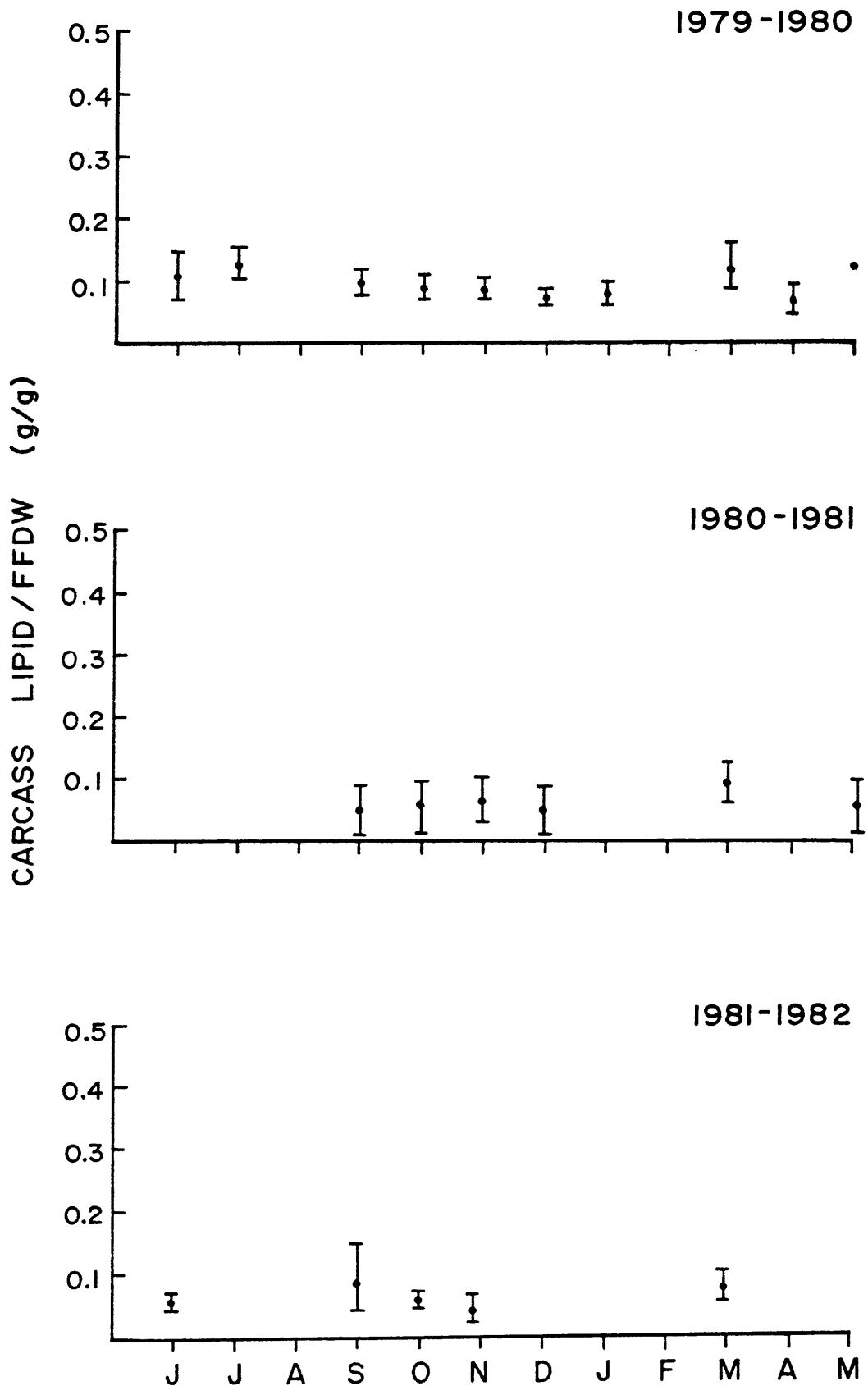

1979-1980
1980-1981
1981-1982
CARCASS LIPID / FFDW (g/g)
0.5
0.4
0.3
0.2
0.1
J
J
A
S
O
N
D
J
F
M
A
M

Figure 34 : Carcass lipid index of male Ctenotus taeniolatus throughout the year for 1979-80, 1980-81 and 1981-82. Means are represented by circles, SD by vertical lines and months by their first initial.

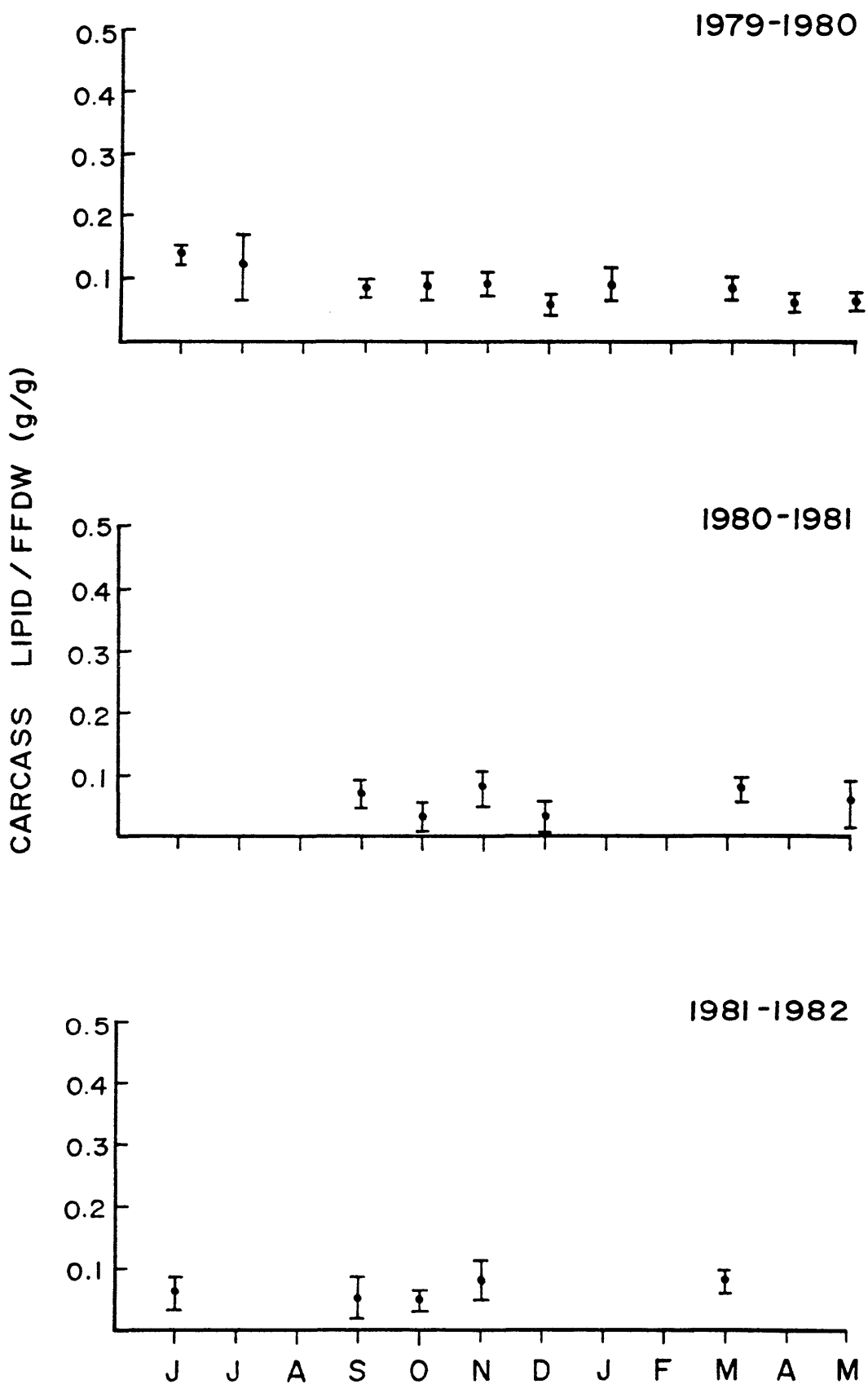
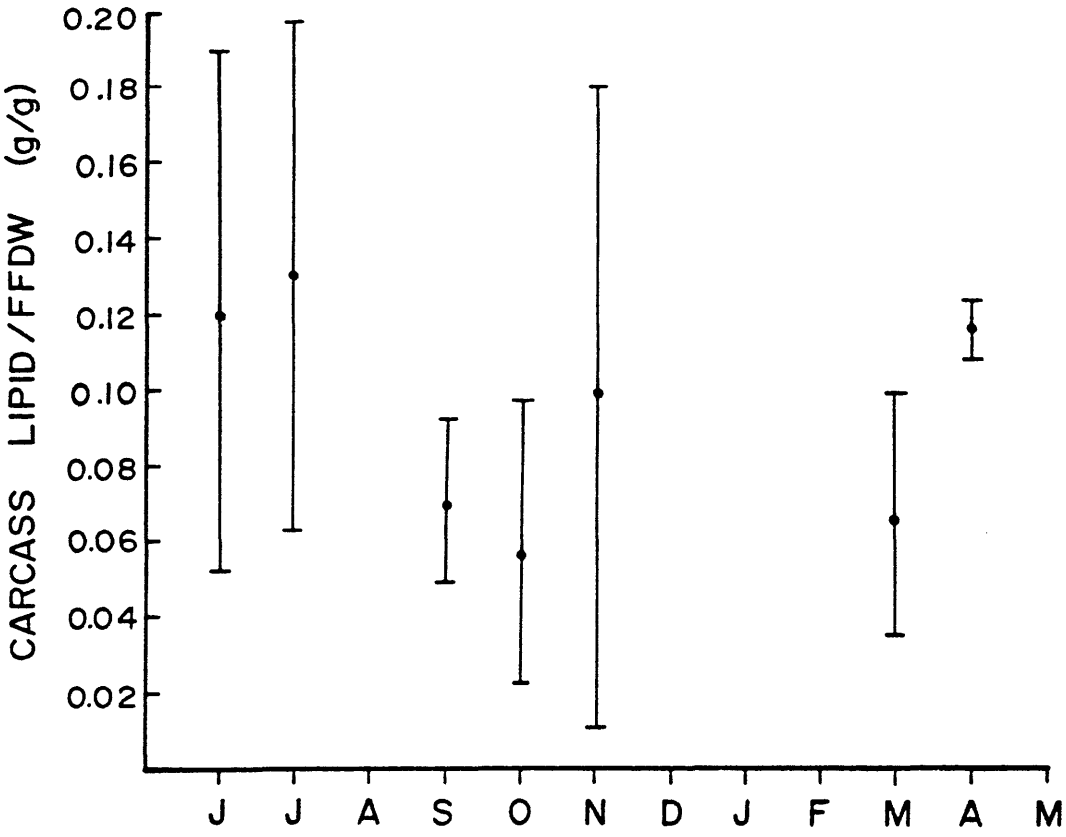

1979-1980
1980-1981
1981-1982
CARCASS LIPID / FFDW (g/g)
0.5
0.4
0.3
0.2
0.1
J J A S O N D J F M A M

Figure 35 : Tail and carcass lipid index of juvenile Ctenotus taeniolatus throughout the year, 1979-80. Means are represented by circles, SD by vertical lines and months by their first initial.


CARCASS LIPID/FFDW (g/g)
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
J J A S O N D J F M A M

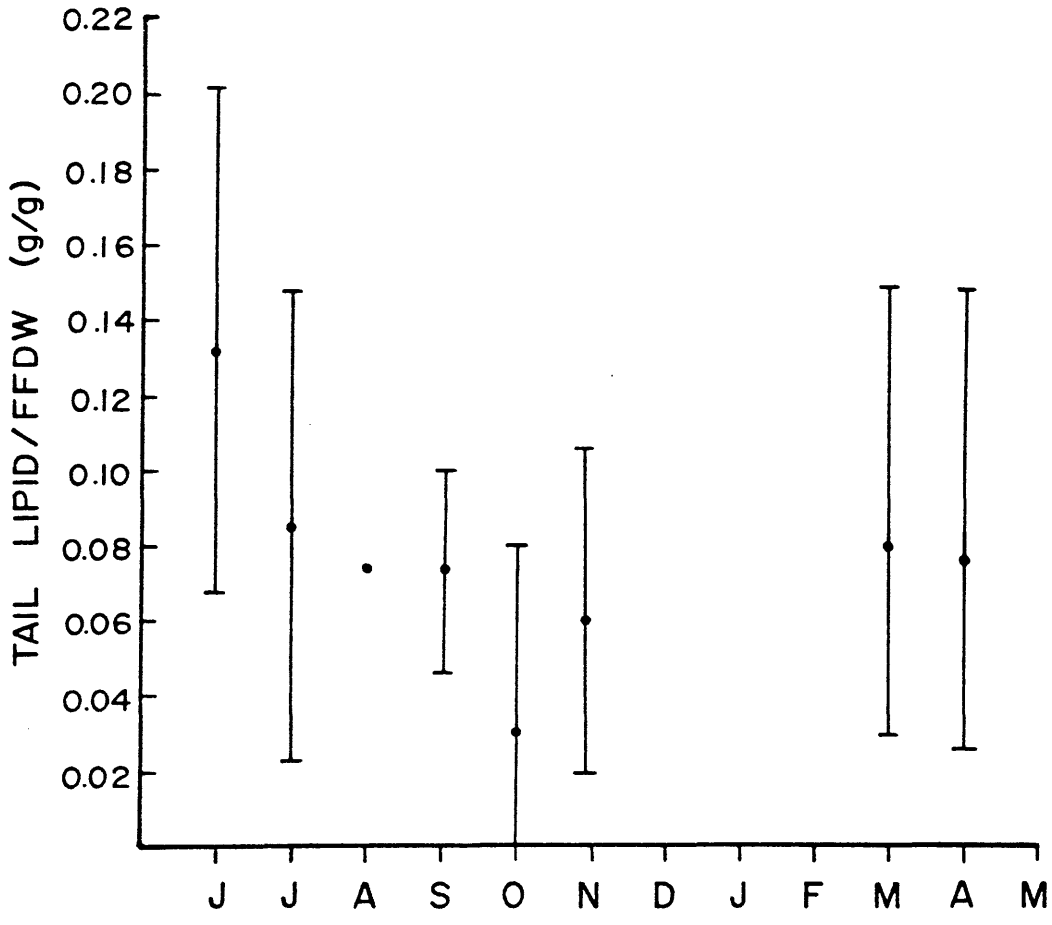
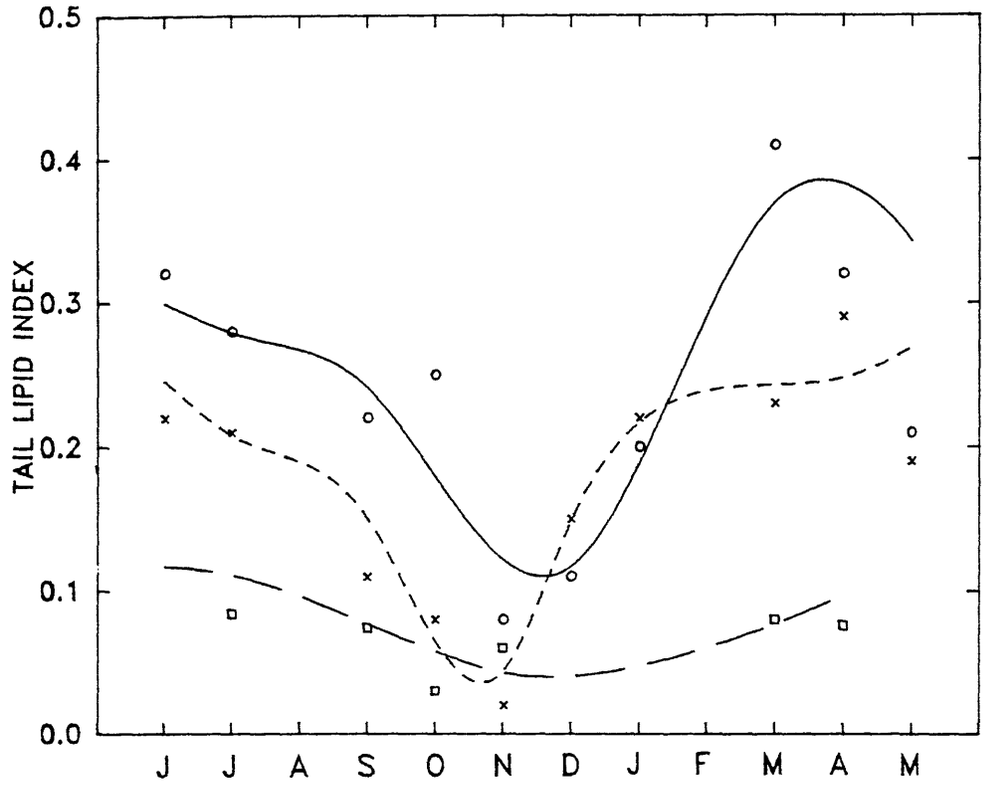

TAIL LIPID/FFDW (g/g)
0.22
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
J J A S O N D J F M A M

Figure 36 : Fourier series describing female (_____), male (-----) and juvenile (— — —) tail lipid indices of Ctenotus taeniolatus throughout the year, 1979-80. Means are presented for comparison: female (circles), male (crosses) and juvenile (square). Months are represented by their first initial.

Figure 37 : Fourier series describing female (_____) and juvenile (— — —) carcass lipid indices of Ctenotus taeniolatus throughout the year, 1979-80. Means are presented for comparison: female (stars) and juvenile (circle). Months are represented by their first initial.


TAIL LIPID INDEX
0.5
0.4
0.3
0.2
0.1
0.0
J J A S O N D J F M A M

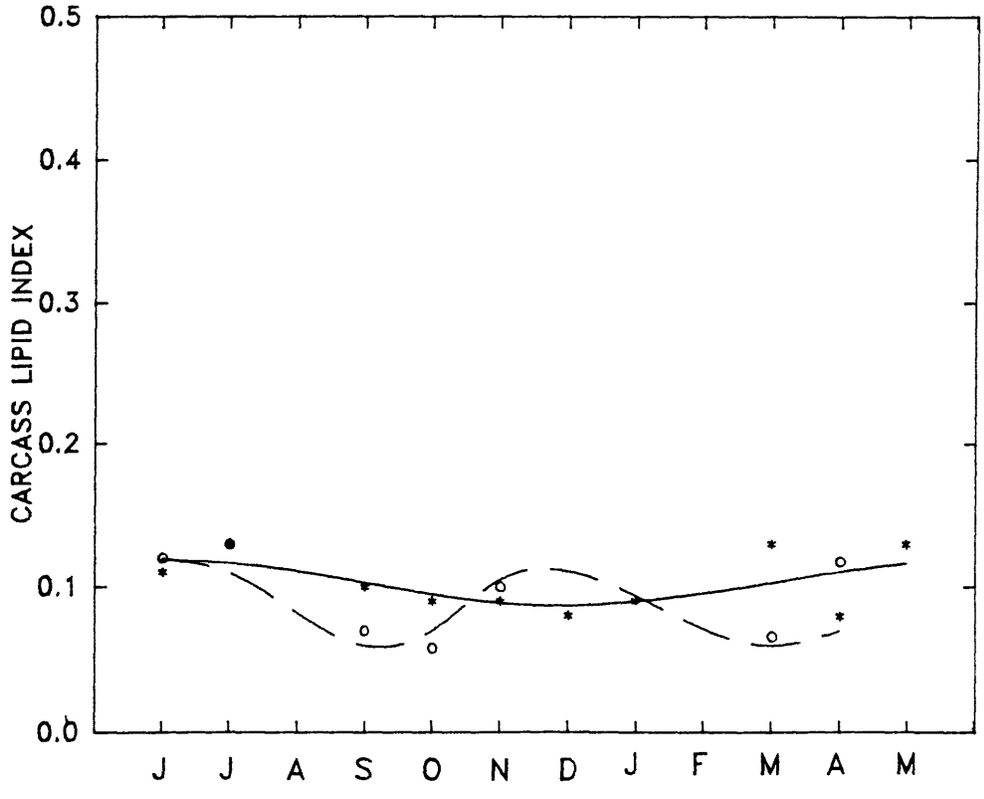

CARCASS LIPID INDEX
0.5
0.4
0.3
0.2
0.1
0.0
J J A S O N D J F M A M

Figure 38 : Graphical representation of the interaction between month, lipid and year (Table 15). Months are represented by their first initial.

(a) Comparison between year and lipid, for each month (tail lipid = circle, carcass lipid = cross).

(b) Comparison between month and lipid, for each year (tail lipid = circle, carcass lipid = cross).

(c) Comparison between year and month, for tail and carcass lipid (Y1 (1979-80) = circle, Y2 (1980-81) = cross and Y3 (1981-82) = open circle).

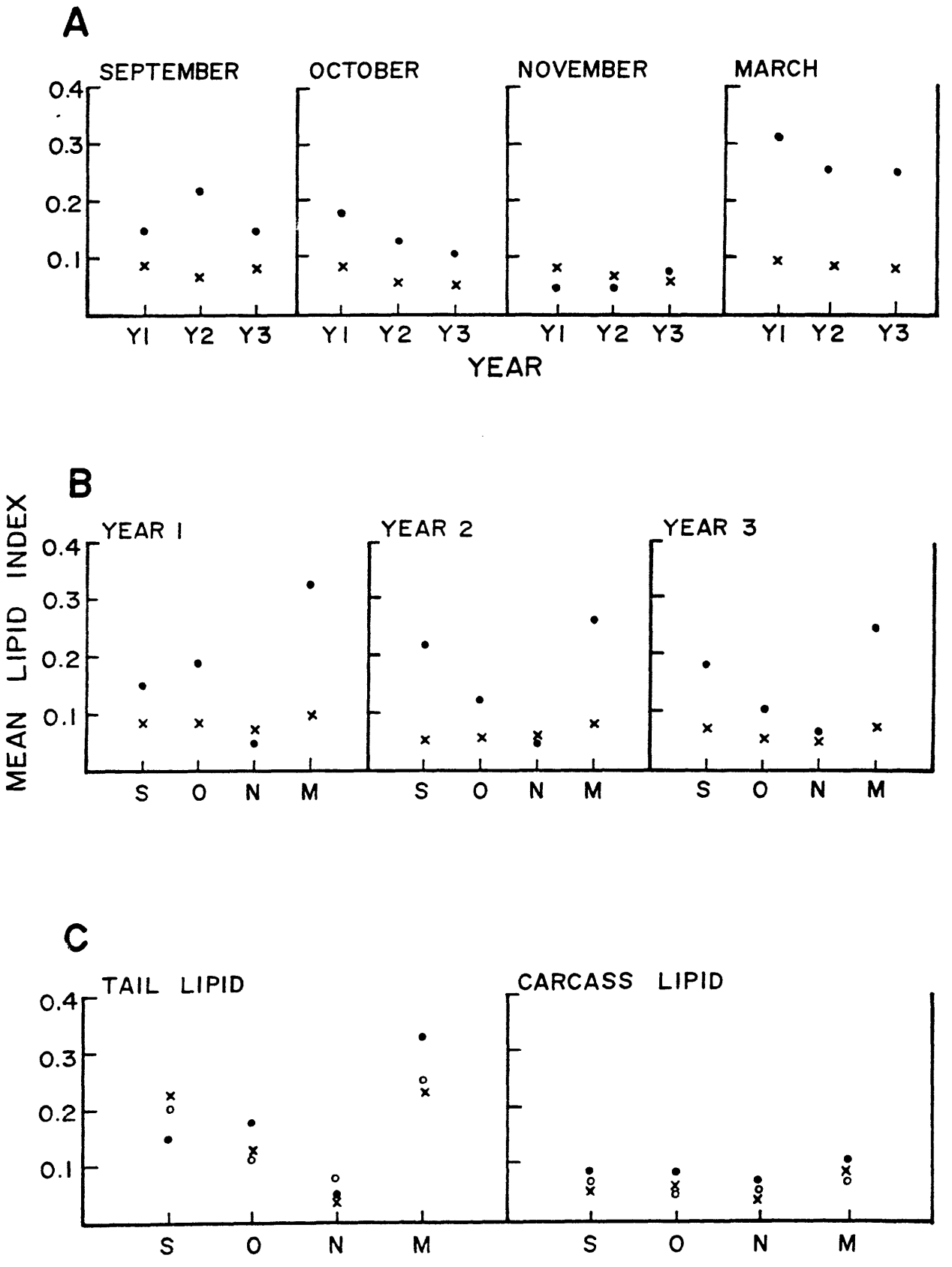

A
SEPTEMBER
OCTOBER
NOVEMBER
MARCH
0.4
0.3
0.2
0.1
Y1 Y2 Y3 Y1 Y2 Y3 Y1 Y2 Y3 Y1 Y2 Y3
YEAR
B
MEAN LIPID INDEX
YEAR 1
YEAR 2
YEAR 3
0.4
0.3
0.2
0.1
S O N M S O N M S O N M
C
TAIL LIPID
CARCASS LIPID
0.4
0.3
0.2
0.1
S O N M S O N M

Figure 39 : Graphical representation of interaction between sex and lipid (Table 15). Black histograms represent mean tail lipid index, while clear histograms represent mean carcass lipid index.


MEAN LIPID INDEX
0.3
0.2
0.1
MALE
FEMALE

Figure 40 : Graphical representation of the interaction between month, sex and lipid (Table 16). Months and sexes are represented by their first initial.

(a) Comparison between sex and lipid, for each month (tail lipid = circle, carcass lipid = cross).

(b) Comparison between month and lipid, for each sex (tail lipid = circle, carcass lipid = cross).

(c) Comparison between month and sex, for tail and carcass lipid (female = circle, male = cross, juvenile = open circle).

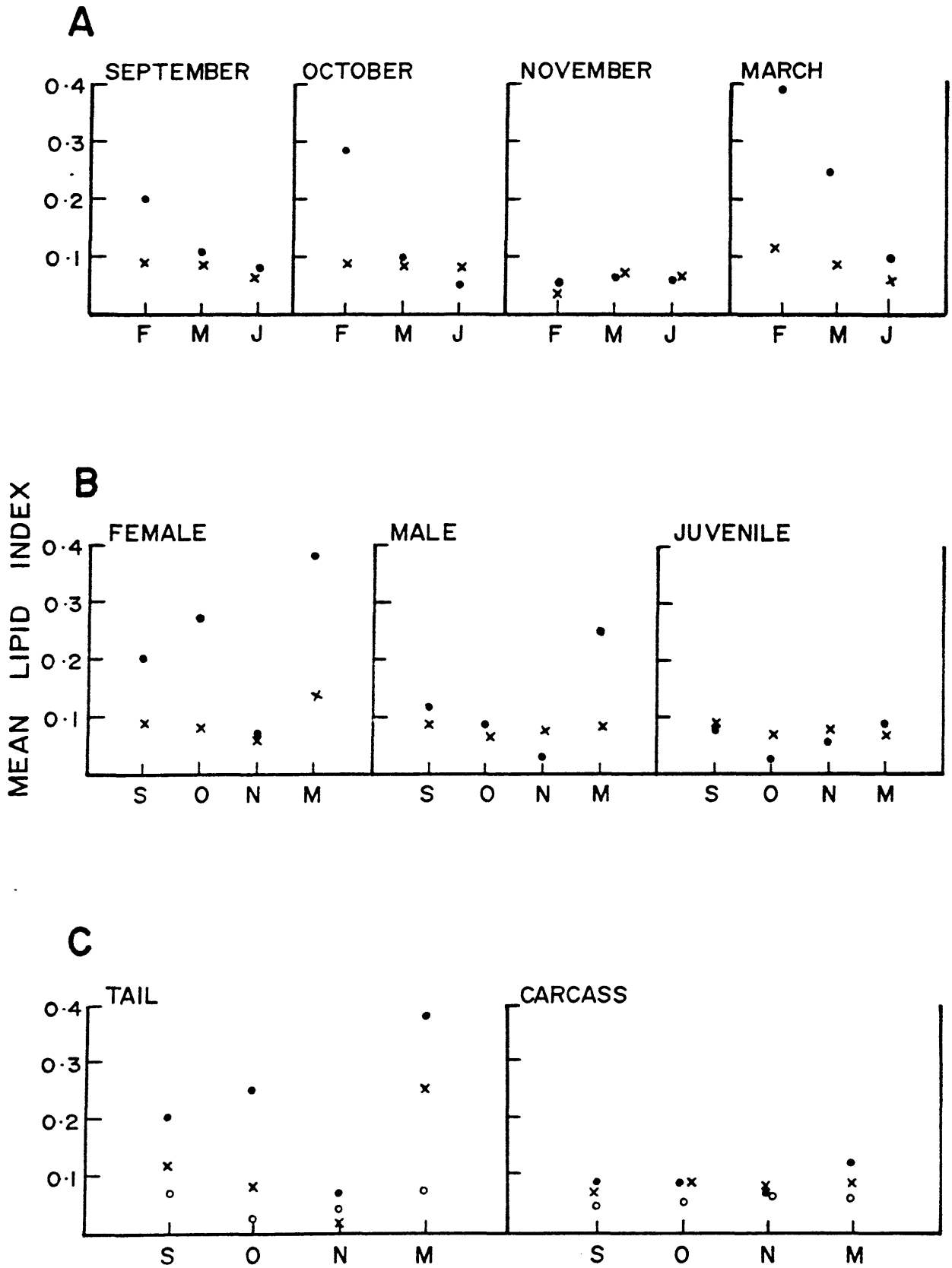

A
SEPTEMBER
OCTOBER
NOVEMBER
MARCH
0·4
0·3
0·2
0·1
F M J F M J F M J F M J
B
MEAN LIPID INDEX
FEMALE
MALE
JUVENILE
0·4
0·3
0·2
0·1
S O N M S O N M S O N M
C
TAIL
CARCASS
0·4
0·3
0·2
0·1
S O N M S O N M

Figure 41 : Liver weight index of female Ctenotus taeniolatus throughout the year for 1979-80, 1980-81, and 1981-82. Means are represented by circle, SD by vertical lines and months by their first initial.

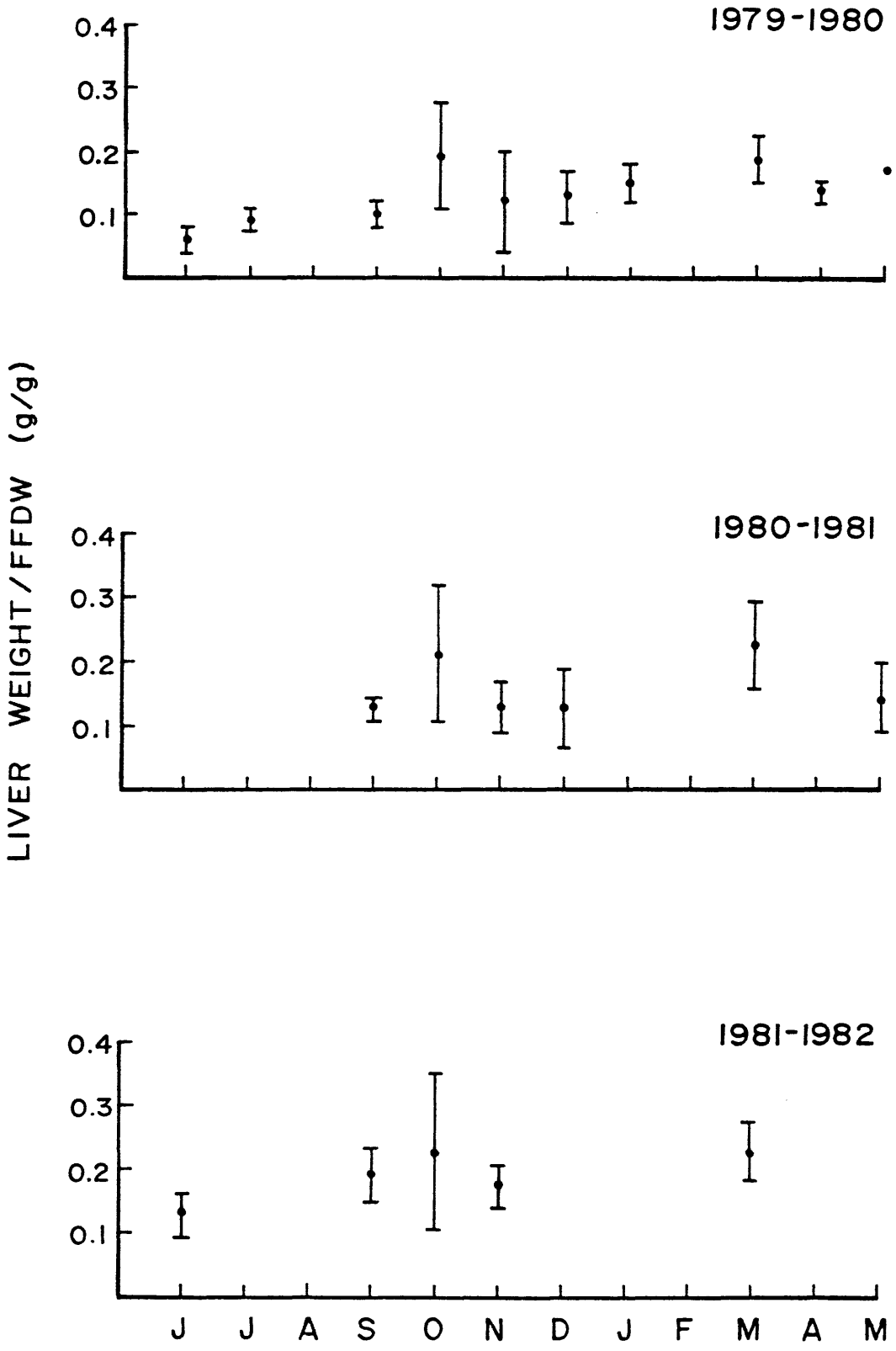

1979-1980
1980-1981
1981-1982
LIVER WEIGHT/FFDW (g/g)
0.4
0.3
0.2
0.1
J J A S O N D J F M A M

Figure 42 : Liver weight index of male Ctenotus taeniolatus throughout the year for 1979-80, 1980-81, and 1981-82. Means are represented by circles, SD by vertical lines and months by their first initial.

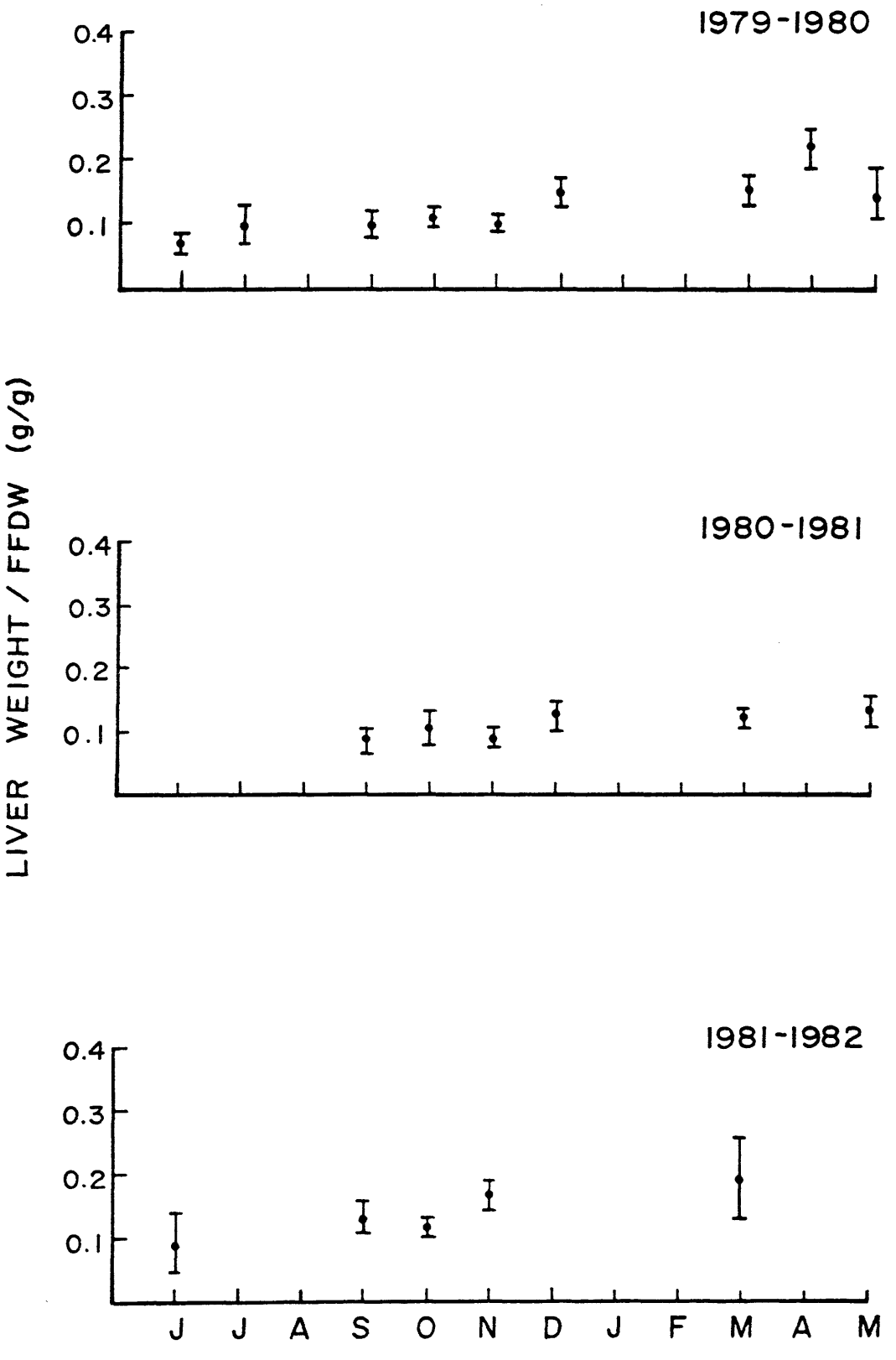

1979-1980
1980-1981
1981-1982
LIVER WEIGHT / FFDW (g/g)
0.4
0.3
0.2
0.1
J J A S O N D J F M A M

Figure 43 : Fourier series describing the liver weight indices for female Ctenotus taeniolatus throughout the year, 1979-80. Means are presented for comparison.

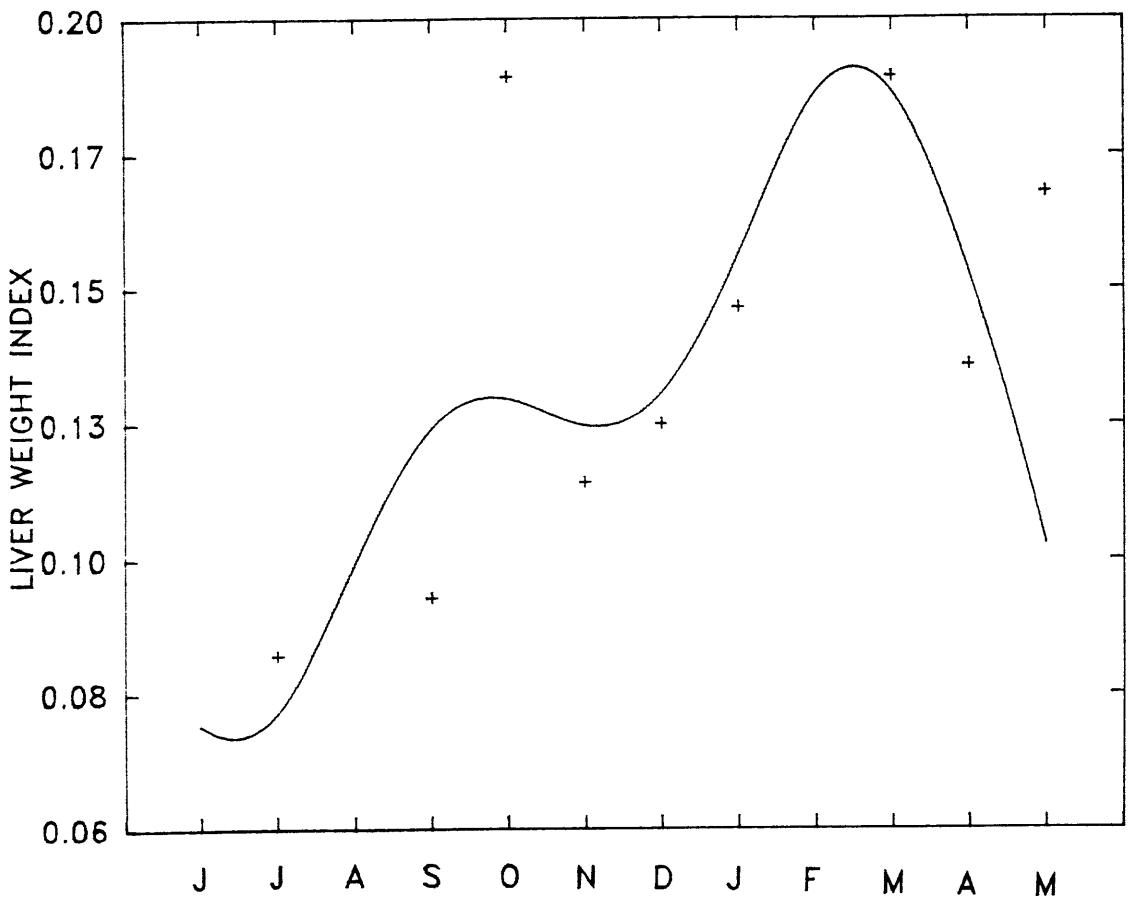

LIVER WEIGHT INDEX
0.20
0.17
0.15
0.13
0.10
0.08
0.06
J
J
A
S
O
N
D
J
F
M
A
M

Figure 44 : Graphical representation of the interaction between month (M), year (Y) and sex (S) (Table 20). Months are represented by their first initial.

(a) Comparison between sex and year for each month (female = circle, male = cross).

(b) Comparison between sex and month for each year (female = circle, male = cross).

(c) Comparison between year and month for each sex (Y1 (1979-80) = circle, Y2 (1980-81) = cross, Y3 (1981-82) = open circle).

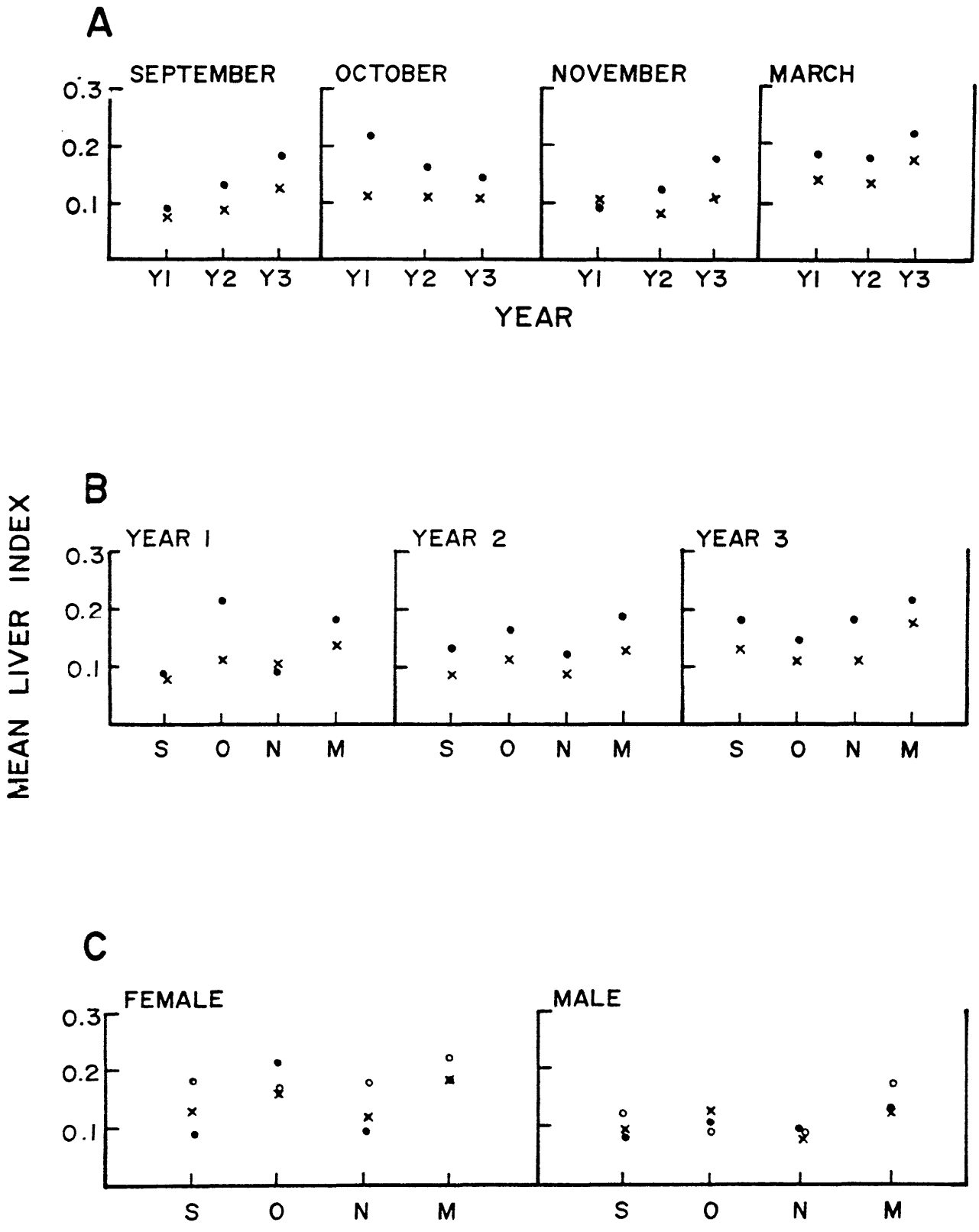

A
SEPTEMBER
OCTOBER
NOVEMBER
MARCH
0.3
0.2
0.1
Y1 Y2 Y3 Y1 Y2 Y3 Y1 Y2 Y3 Y1 Y2 Y3
YEAR
B
YEAR 1
YEAR 2
YEAR 3
0.3
0.2
0.1
S O N M S O N M S O N M
MEAN LIVER INDEX
C
FEMALE
MALE
0.3
0.2
0.1
S O N M S O N M

Figure 45 : Fourier series describing female (_____) and male (-----) glycogen indices of Ctenotus taeniolatus throughout the year, 1980-81. Raw glycogen indices are included for females (star) and males (circle).

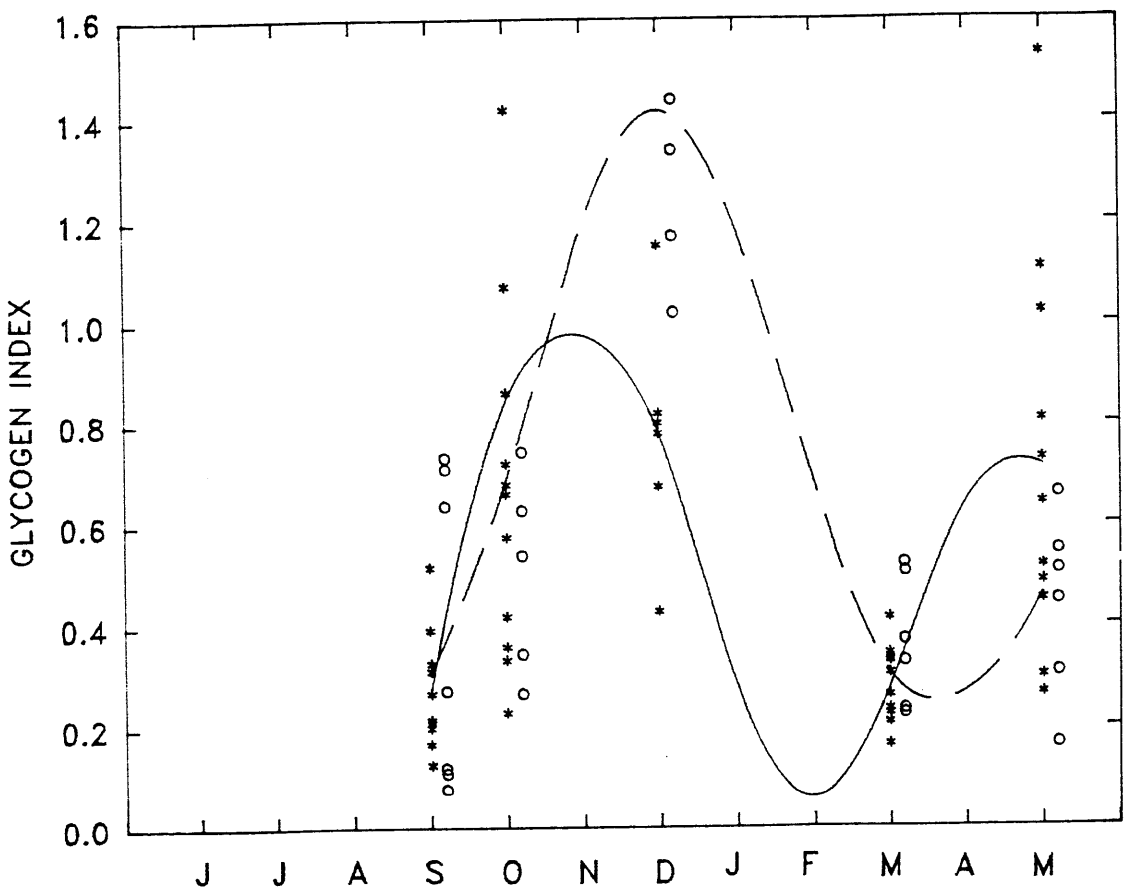
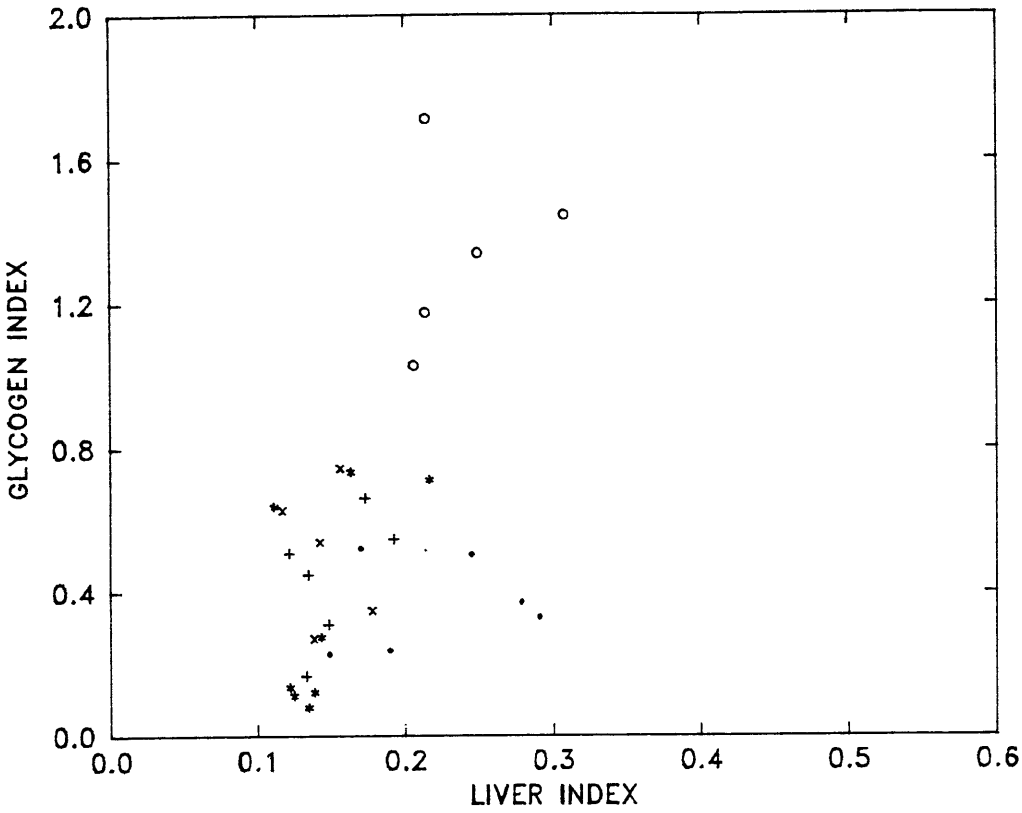

GLYCOGEN INDEX
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
J
J
A
S
O
N
D
J
F
M
A
M

Figure 46 : Relationship between glycogen index and liver weight index for male (upper) and female (lower) Ctenotus taeniolatus. Months of capture are March (circle), May (+), September (*), October (x) and December (open circle).


2.0
1.6
1.2
0.8
0.4
0.0
GLYCOGEN INDEX
0.0
0.1
0.2
0.3
0.4
0.5
0.6
LIVER INDEX

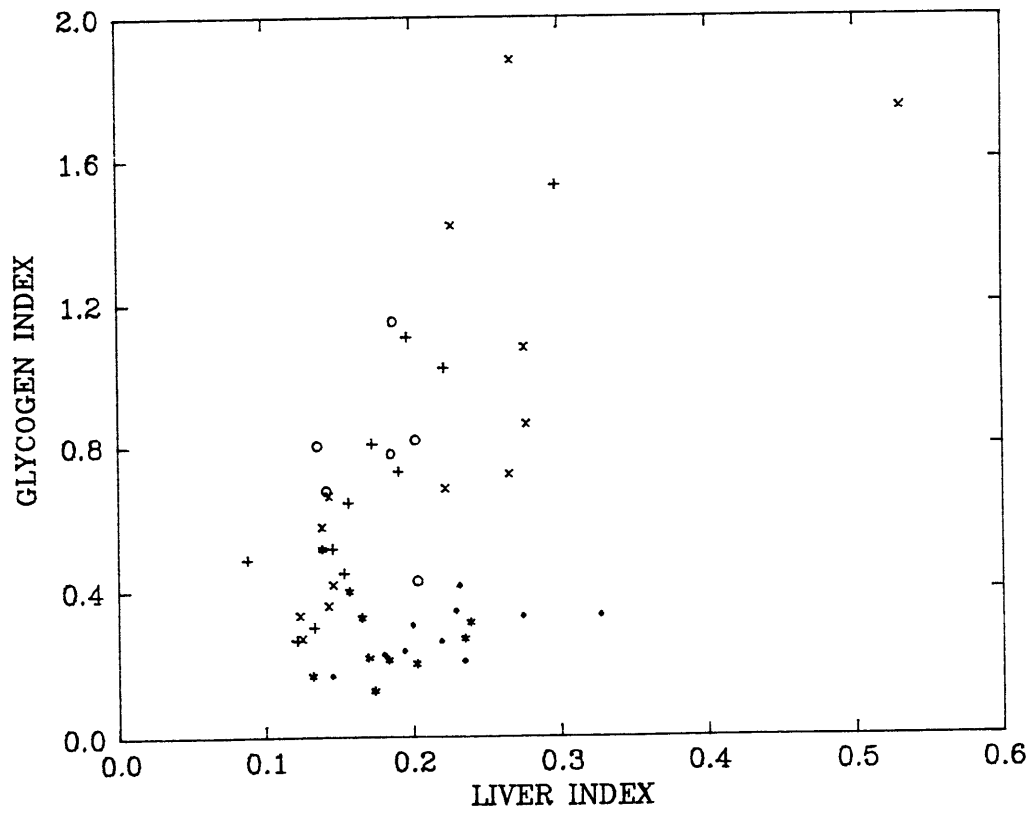
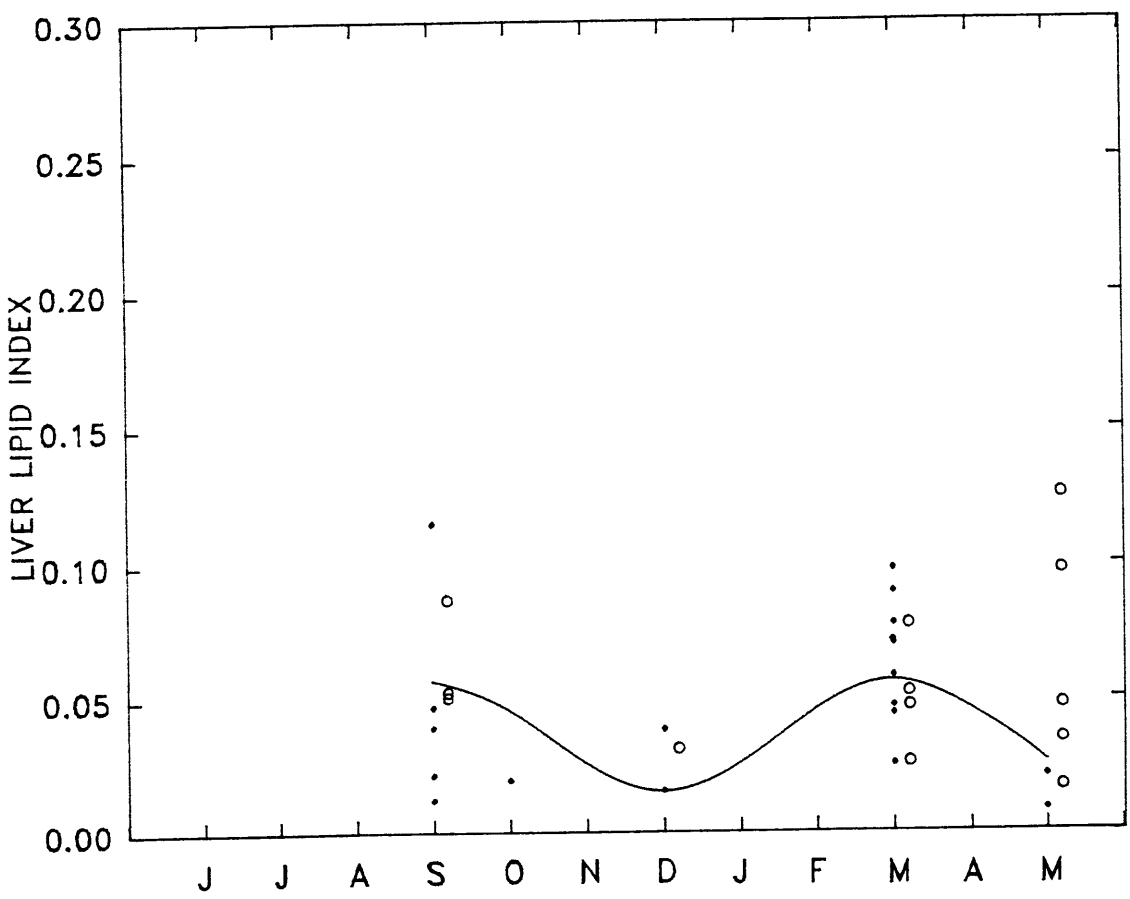

2.0
1.6
1.2
0.8
0.4
0.0
GLYCOGEN INDEX
0.0
0.1
0.2
0.3
0.4
0.5
0.6
LIVER INDEX

Figure 47 : Fourier series describing female liver lipid indices of Ctenotus taeniolatus throughout the year, 1980-81. Raw liver lipid indices are included for females (circle) and males (open circle).


0.30
0.25
0.20
0.15
0.10
0.05
0.00
LIVER LIPID INDEX
J
J
A
S
O
N
D
J
F
M
A
M

7.4 DISCUSSION

As with reproductive cycles, the study of lipids and associated body components has become one of the most popular fields of herpetological research and a requirement for many autecological studies. As Derickson (1976), Gregory (1982) and Pond (1978) present reviews of this for reptiles (in general and during hibernation) and wild vertebrates respectively, no attempt is made here to catalogue all known knowledge of reptilian lipids, their storage and utilisation.

Derickson (1976) identifies four basic patterns of lipid storage and utilisation. These patterns include no lipid cycling, cycling associated only with winter dormancy, cycling associated only with reproduction, and cycling associated with both winter dormancy and reproduction. The work of Pengilley (1972), Robertson (1981), Smyth (1974) and Cogger (1978) show that many Australian lizards conform to these patterns. The only exception is found in the temperate skink, Egernia cunninghami, in which a wide variety of tests failed to reveal any consistent pattern of seasonal change, either in intact fat bodies, in lipid content or in dry weight of fat extracted tissue (Barwick and Bryant 1966). Although Derickson's patterns were all determined after examination of lizards with abdominal fat bodies, they are equally relevant to lizards that do not possess these organs , but which store lipids in the tail or general carcass. Avery (1973) showed that in Lacerta vivipara, although fat bodies and tail lipid stores are quite distinct, there are no major differences in their composition - 95.5% of lipid fractionated into neutral components and 4% into polar components, with the most abundant fatty acid being oleic acid. The results of MacAvoy (1976) for the skink, Leiolopisma zelandica and the gecko, Hoplodactylus pacificus, agree with those of Avery (1973). She found also that carcass lipid was predominantly phospholipid in the skink,

with decreasing predominance in the gecko. She suggested consequently that carcass lipid could not be readily mobilised to meet metabolic demands. The neutral lipids in the carcass were again composed mainly of oleic acid (MacAvoy 1976, Brian et al. 1972).

Lizards without abdominal fat bodies studied to date do fit into the patterns described by Derickson (1976) : two tropical skinks from New Guinea, Lobulia morokana and L.stanleyana, show no cycling in total lipids (Allison 1979); two temperate skinks from southeastern Australia, Hemiergis decresiensis and H.peronii, have cycles in tail lipids associated with both winter dormancy and reproduction (Robertson 1980, Smyth 1974). In the present study adult Ctenotus taeniolatus have lipid cycles similar to those of Hemiergis; that is, in both sexes tail lipids peak prior to winter dormancy, decrease over winter, continue to decrease during vitellogenesis and spermatogenesis in spring to a minumum at the beginning of summer, after which lipids again increase. Females have significantly greater amounts of tail lipid than males, which in most cases are also greater than those of juveniles. Although there is a gradation in size from juveniles to males to females, this gradation can not be assumed to account for the similar trend in tail lipid levels, as lipid levels were all corrected for body size. Lipid levels and body size are isometrically related in C.taeniolatus, and not allometrically related as found in Lacerta vivipara by Avery (1974).

Carcass lipid appears to be less labile than tail lipid which is significantly greater in quantity in all months except November,the end of the reproductive period. Carcass lipid in females does show an annual cycle similar to tail lipid but with a reduced amplitude, whereas males show no significant change throughout the year. Overall, because, there were no significant differences in carcass lipid in females, males and juveniles, it can be concluded that carcass lipid is not, under

normal conditions, used for either overwintering or reproduction.

One interesting and somewhat confusing result is that the tail lipid cycle for juvenile C.taeniolatus, although based on fewer measurements than those of the adults, produced a significant annual cycle similar to the adult cycles, although with a reduced amplitude. That is, the tail lipids continued to decrease throughout spring in juveniles even though they were, of course, not reproducing. Carcass lipids also show a significant, and also confusing, annual cycle. Avery (1974) found similar results for juvenile Lacerta vivipara. However, his suggestion that these changes may be due to qualitative changes in the diet, as invertebrates of high-lipid content (Orthoptera, Lepidoptera) form an increasing proportion of the diet as the year progresses, does not hold for Ctenotus as these high lipid invertebrates collectively are distributed evenly throughout the season (Section 4.3.2).

The fact that juvenile lipids decrease during the spring does not detract from the significance of the decrease in tail lipids of females over this period. Hahn and Tinkle (1965) and Smith (1968) in experiments removing fat bodies from pre-vitellogenic lizards determined a firm relationship between fat stores and ovarian development, as did Smyth (1974) in experiments removing the tails from the skink, Hemiergis peronii, a lizard in which the tail is the only usable fat store.

Environmental conditions characteristically affect life history phenomena. In particular food availability, usually directly related to precipitation levels (Dunham 1978, French 1971, Janzen and Schoener 1968), is known to affect growth (Dunham 1978, Ballinger and Congdon 1980), reproductive effort (Sherbrooke 1975, Goldberg 1975) and lipid levels (Derickson 1976, Duvall et al. 1982). In the present study,

tail and carcass lipid levels in C.taeniolatus were monitored for 3 years, during which time there were no significant changes in either carcass or tail lipid between years. The variation in weather conditions between years was minimal (Section 2.1), because the region was under the influence of a severe drought.

The liver is the most important organ with respect to distribution and maintenance of fuel levels. As a consequence many studies have examined seasonal changes in liver weights and components with the hope of understanding the nature of the metabolic processes occurring throughout the season. Many researchers in the past have considered the liver to be a storage organ (Haggag et al. 1966). However, this could be an overestimation of its worth. Livers do store glycogen and lipid. However, the amounts of these are very small; less than 1% of the body weight (Fig. 49) or 2-3% of the energy store in Lacerta vivipara (Patterson et al. 1978). Hence energy storage in the liver could not sustain a stressed animal for a long period of time, even though it may be important as a source of glucosyl residues (Patterson et al. 1978). Rather, liver components and their respective seasonal changes should be viewed as indicators of the nature of the metabolism occurring at any point in time. Thus high levels of glycogen indicate feeding while low levels indicate starvation in lizards (Gist 1972, Vladescu et al. 1970). Increases in liver lipid can represent either an increase of lipid from dietary sources or from mobilisation from other depots to the liver, while decreases in liver lipid can represent either metabolism of that lipid or mobilisation to depots (MacAvoy 1976). Gist (1972) found that starvation of Anolis carolinensis decreased liver lipids which did not then increase after refeeding for 10 days, while Gillett and da Cruz (1981) in a similar experiment with Ameiva ameiva found that refeeding for 21 days after a period of starvation increased

liver lipid levels.

Although few researchers have examined the biochemistry of these metabolic processes in reptiles, except for Coulson and Hernandez (1964, 1983), many have surveyed changes in liver weight, glycogen and liver lipid over a season. Gregory (1982) reviews many of these; other papers not included in the review are by MacAvoy (1976), McPherson and Marion (1982), Reddy et al. (1972), Khalil and Yanni (1961) and Gillett and da Cruz (1981). Further, many researchers have routinely included seasonal changes in liver weight while studying the ecology of reptiles, but their results are not catalogued here.

In the present study liver weight, glycogen and liver lipid of C.taeniolatus were all measured throughout the year. Only in females was liver weight found to change significantly throughout the year, with a peak in autumn decreasing to a minimum during winter, increasing again in spring, with a slight drop during the reproductive period, followed by an increase to the maximum described previously. Female livers in most cases accounted for a greater proportion of the body weight than did those of males, but exhibited much greater variation between years. Male liver weights were not significantly different within or between years. Overall, female livers showed greater fluctuation than did those of males.

In the literature detailed above many different patterns of liver weight have been recorded; however, the value of examining liver weight is questionable because seasonal changes in liver weight can reflect changes in many parameters including water, protein, glycogen and lipid content (Dessauer 1955). Although Dessauer (1953) found a positive correlation between liver weight and glycogen weight, this does not apparently hold for all reptiles as MacAvoy (1976), Barwick and Bryant

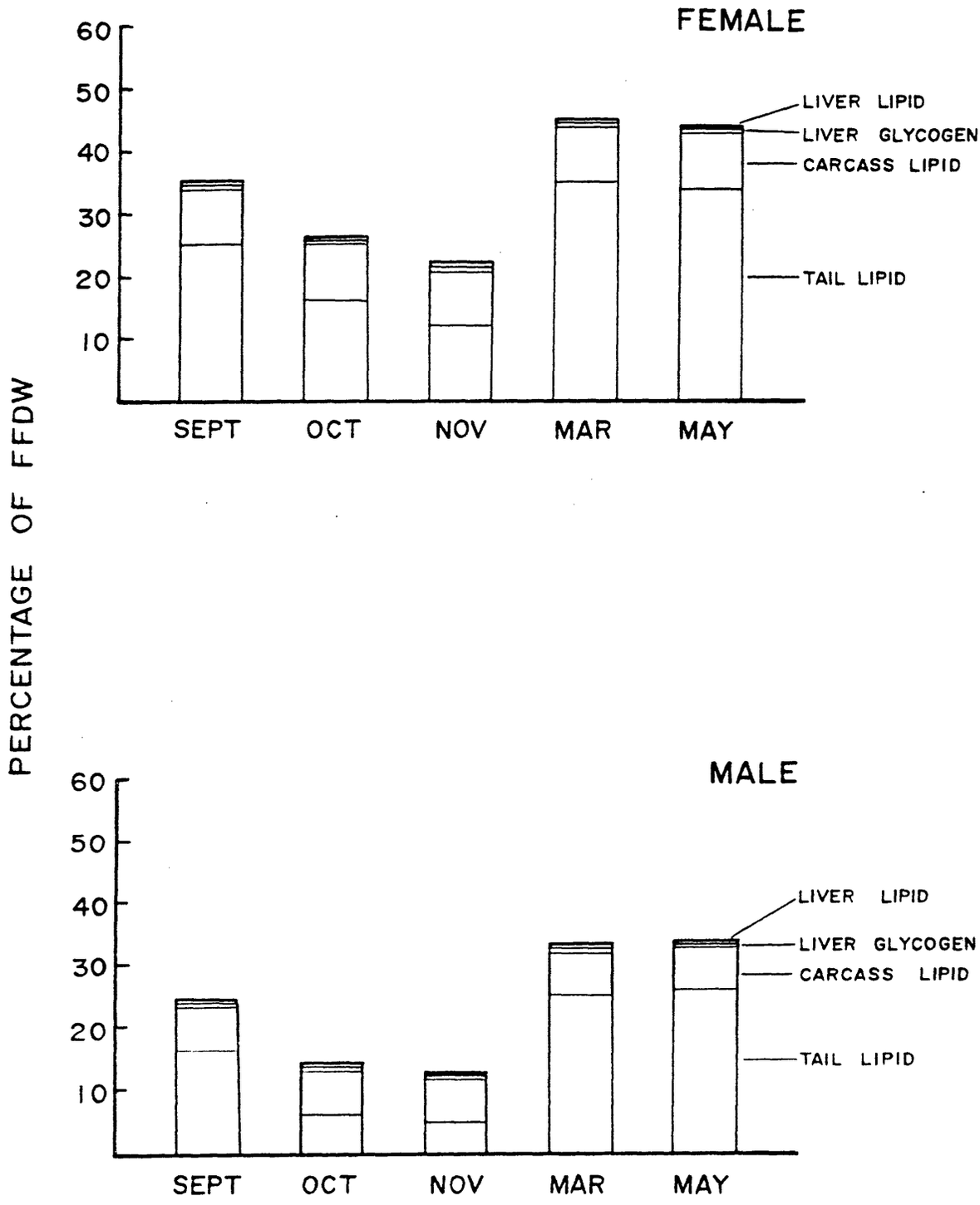
(1966) and this study all found that glycogen levels, although having a significant seasonal trend, do not correspond to the trend in liver weight. In C.taeniolatus glycogen levels in males and females were not significantly different, except in late autumn when female glycogen was slower to begin increasing, and followed patterns similar to those described by MacAvoy (1976) and Barwick and Bryant (1966): glycogen levels were maximum in summer, decreased in late summer, increased in autumn after which they decreased to a minimum over winter. Liver lipid in females followed a different pattern with peaks after emergence and pre-winter, and troughs after reproduction and during winter. Liver lipid in males showed no significant seasonal trend. In general liver lipids seem to follow the liver weight cycle, although the data are inconclusive.

So in C.taeniolatus information on liver lipid and glycogen serve as useful tools in determining the nutritional state of a lizard. They can be further used to confirm the relationships found between lipid cycles and seasonality and those found for feeding in section 4.0 (Table 24).

Table 24 : Summary of tail lipid, liver lipid and glycogen cycles in Ctenotus taeniolatus.

	REQUIREMENTS EXCEED INTAKE	
Emergence (September)	Tail lipid low Glycogen low Liver lipid high	Feeding commences; requirements exceed intake; mobilising depot lipids
	REQUIREMENTS EXCEED INTAKE	
During reproduction (October)	Tail lipid decreasing Glycogen high Liver lipid decreasing	Feeding; requirements exceed intake; mobilising depot lipids
	INTAKE EXCEEDS REQUIREMENTS	
End reproduction (Nov-Dec)	Tail lipid minimum Glycogen high Liver lipid low	Feeding; requirements exceed intake; mobilising depot lipids
	REQUIREMENTS EXCEED INTAKE	
Autumn (March)	Tail lipid maximum Glycogen low Liver lipid high	Feeding; but sporadic
	REQUIREMENTS EXCEED INTAKE	
Pre-winter (May)	Tail lipid decreasing Glycogen high Liver lipid low	Feeding; but sporadic; mobilisation of lipids commencing

Figure 48 : Percentage distribution of body components of female and male Ctenotus taeniolatus throughout a year.


FEMALE
MALE
PERCENTAGE OF FFDW
60
50
40
30
20
10
SEPT
OCT
NOV
MAR
MAY
LIVER LIPID
LIVER GLYCOGEN
CARCASS LIPID
TAIL LIPID