

POPULATION ECOLOGY AND CONSERVATION OF TORTOISES: DEMOGRAPHIC ASPECTS OF REPRODUCTION IN *TESTUDO HERMANNI*

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ABSTRACT

Female *Testudo hermanni* at Alyki, northern Greece were sexually mature at a mean straight carapace length of 150mm, based on the frequency of finding eggs by X-ray or oxytocin injection during the nesting season. Oxytocin-induced egg laying behaviour is described. Egg width ($r = 0.49$), shape ($r = -0.32$) and weight ($r = 0.30$) were all significantly related to body size. Hatchling wet weight was strongly related to egg weight. Hatchlings of *T. hermanni* were lighter than those of *T. graeca* and *T. marginata* in terms of dry weight/egg weight. This pattern reflects the low energy content of *T. hermanni* eggs, and is possibly a consequence of the use of more productive habitats by this species. Clutch size and clutch mass were weakly related to body size; reproductive potential declined in the largest females. Simple and stratified calculations gave similar estimates of the total reproductive potential of adult females in two populations at Alyki. There were, in round numbers, 250 adult females on the main heath producing 1,700 eggs per year, and 65 adult females on the salt works heath producing 450 eggs per year. Nest predation was low at Alyki, judging from the rarity of destroyed natural nests. This was confirmed by burying chicken eggs to stimulate tortoise nests. About 90 per cent of nests probably remain undisturbed during the incubation period.

INTRODUCTION

Reproduction in chelonian species is known to vary between geographic regions (Tinkle, 1961), populations (Gibbons and Tinkle, 1969), individuals (Turner, Hayden, Burge and Roberson, 1986) and years (Schwarzkopf and Brooks, 1986). Information about reproduction used in a study of population dynamics should therefore be drawn from that population, and should cover as large a range of individuals and years as possible. We have previously (Hailey and Loumbourdis, 1988) compared reproduction of the three species of tortoise in Greece, including three separate populations of *T. hermanni*. The aim of the present study was to describe reproduction in more detail in one of these populations (Alyki in northern Greece), as part of a continuing study of tortoises at that site.

METHODS

FIELD OBSERVATIONS

The Alyki site has been described by Stubbs, Swingland, Hailey and Pulford (1985). Data from the whole site are pooled in this study. Females collected for X-ray examination or for oxytocin treatment were mostly from the salt works heath and the northern end of the main heath (sectors 1, 2, 3, 4, 7 and 10). These were the areas of highest population density, and had convenient enclosures where tortoises could be collected together.

General handling techniques have been described by Stubbs, Hailey, Pulford and Tyler (1984). Apart from urine, which is usually lost on handling, the body weight of a female tortoise may be increased by mature eggs, developing follicles and gut contents. The following measures are used here; *Field weight* is the gross weight of a tortoise as measured in the field. *Body weight* is the net weight of a female after laying a clutch of eggs, but including any developing follicles and gut contents. Note that a third measure (*cleaned soma weight*, excluding eggs, follicles and gut contents) is more appropriate for discussion of reproductive effort (Hailey and Loumbourdis, 1988).

OXYTOCIN-INDUCED EGG-LAYING

Eighty two individual females ranging from 139-198mm SCL were collected on eight days between 15 May and 1 June 1986. On each occasion the tortoises were found in the morning and the early evening, and held in a shaded empty drinking trough in sector 2, or in overturned skips in the salt water heath — the latter were transferred to disused rain collecting pans before oxytocin injection. A 1ml ampoule containing 5 IU oxytocin in saline (G. A. Pharmaceuticals) was diluted in a 5ml ampoule of 0.9 per cent sterile saline. Each tortoise was given an intramuscular injection of about 1.5 IU.kg⁻¹ between 18:00 and 19:00 local time. Tortoises laid their eggs on the floor of the arena, the first after about 20 minutes. Eggs were removed immediately, and labelled with pencil. The tortoises were released in the sector of capture in the

late evening, two hours after oxytocin treatment. Seven females were recaptured after 1-2 weeks and given a second oxytocin treatment.

Ninety seven females ranging from 113-207mm SCL were collected on nine days between 18 May and 1 June 1988. They were collected throughout the activity period, and held in a shaded trough or disused water tank before transport to Thessaloniki for X-ray analysis (below). Tortoises were kept for one to three nights in the arenas, which were liberally supplied with vegetation for shelter; those kept for a whole day or more were fed with tomatoes. Tortoises shown to have eggs by X-ray were placed in cleaned arenas and injected with undiluted oxytocin in saline, at a dose of about 3 IU.kg^{-1} , at either about 11:00 or 18:00 local time; the other tortoises were released immediately. The time of injection was noted for each tortoise, as were the times of each egg laid, all to the nearest minute. Tortoises were released 30 minutes after laying all the eggs identified by X-ray; rectal temperatures of some females were measured after egg-laying with a mercury thermometer.

X-RAY EXAMINATION

Tortoises were taken to Thessaloniki for X-ray examination, with the 70kv Atomscope portable X-ray generator used previously (Hailey and Loumbourdis, 1988), but with different film (loose Kodak X-Omat S 18 x 24cm films, cat. 501 6704). Machine power was reduced to 20mA, and exposure time increased to 5s. The generator was 70cm above the film, which was held in a standard X-ray-transparent metal cartridge. Two tortoises were placed side by side on each film.

The presence of two animals and the longer exposure time gave more chance of movement during the exposure, and so each tortoise was immobilised by wrapping plastic parcel tape around the carapace to cover the legs. One tortoise of each pair was identified by placing a key or paper clip next to it. Tortoises were kept in wooden cages in Thessaloniki for one to three days, before transport to Alyki for oxytocin treatment and/or release.

NEST PREDATION

An experiment on egg predation was carried out by burying chicken eggs (Swingland and Stubbs, 1985) in early June 1986. 100 eggs from a market (probably a few days old) were buried, 20 in each of five locations (Fig. 1);

A) Dry heath near the salt works, around a dump of rusted salt skips. This was the area most likely to house rats, which have been seen around the salt works.

B) Open dry heath where there were several burrows of the suslik ground squirrel (*Citellus citellus*), a possible excavator of tortoise nests.

C) Grassy heath around a small pool on the salt works heath.

D) Grassy heath around patches of brambles on the main heath, an area where opened tortoise nests were observed in autumn.

E) Dry heath on firm sandy soil on the main heath, in an area of regenerating hawthorn bushes.

These locations were chosen as having a range of habitats, and different potential nest predators. The 20 eggs at each location were buried individually, with

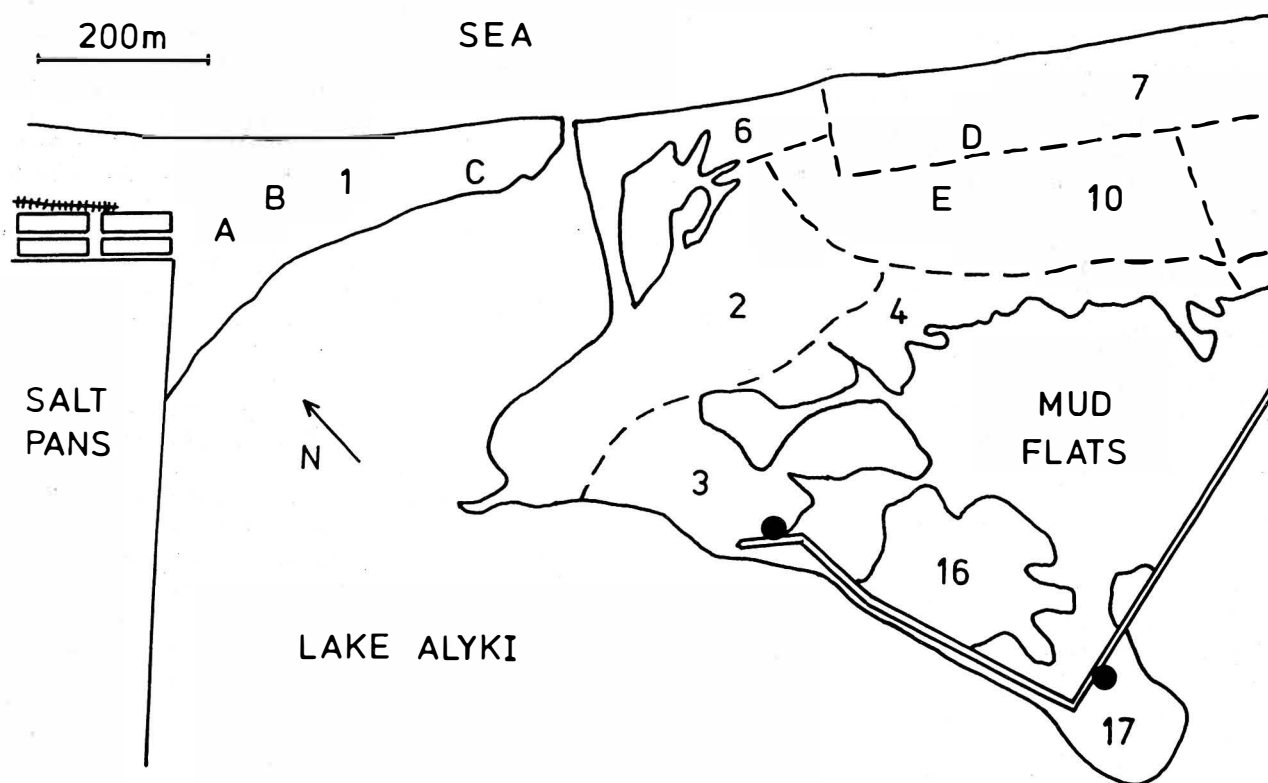


Fig. 1 The salt works heath (sector I) and the northern end of the main heath at Alyki, showing location of the five areas where hens eggs were buried in 1986 (A-E). The position of burrows in the banks of the new dyke is also shown (●).

about 5m between each egg. The egg was covered by 3-5cm of soil, and disguised with fragments of vegetation. The location of each 'nest' was marked by a 5cm square fragment of roof tile from the salt works. This was placed 50cm to the north of the 'nest', not directly above it, to prevent a predator finding the egg by the marker. The areas where eggs were buried were searched through the summer, sufficiently to note broken eggs on the surface but not to check each buried egg. This was initially done every 2-3 days, but it was soon apparent that egg removal was infrequent and the interval between searches was increased to 10-14 days. The experiment lasted until early September, when an attempt was made to retrieve each buried egg.

EGG MEASUREMENT AND INCUBATION

The length and width of eggs were measured with vernier callipers to 0.1mm. Both maximum and minimum widths were recorded. Eggs were weighed on an electronic balance the day after being laid.

196 eggs of *T. hermanni*, *T. graeca* and *T. marginata*, collected during the study of Hailey and Loumbourdis (1988), were incubated in 3cm deep trays of sand. Eggs were placed in these trays within 24h of being laid, and just covered with sand, which was moistened every week. The trays were placed in two constant temperature cabinets (31°C and 35°C) and in a shaded room fluctuating between 22°C and 27°C, for an experiment designed to test the relation between temperature, sex determination, incubation period, and hatchling size and energy reserves. However, few

hatchlings were obtained; these were weighed and measured within 12h of emergence, and then frozen. They were subsequently dissected to examine yolk remnants, then dried to constant weight at 70°C in an oven.

The 59 eggs collected during 1988 were incubated in two series, to determine whether low hatching success in 1985-86 was due to the incubation technique, or to non-viability of eggs laid after oxytocin injection. 32 eggs were incubated by Dr Theodora Sofianidou using a previously successful technique, in unheated boxes of soil. The other 27 eggs were left in a bare plastic box, also unheated.

Statistical analysis: values in the text are ± 1 S.D. Polynomial, exponential, and geometric regressions (IBM user group) were fitted to curved lines, and the model explaining the most variation (in terms of r^2) was used.

RESULTS

OXYTOCIN-INDUCED EGG LAYING

Details of the timing of oxytocin-induced egg laying were recorded for 25 females, and are given for comparison with natural nesting. Fig. 2 shows a raster plot of laying times for 24 females; the other had not started to lay after 100 minutes, and was given an additional injection. The solid line shows the cumulative proportion of females which had started laying. The first egg was laid after 13 minutes; the last first-egg after 68 minutes. Body temperatures

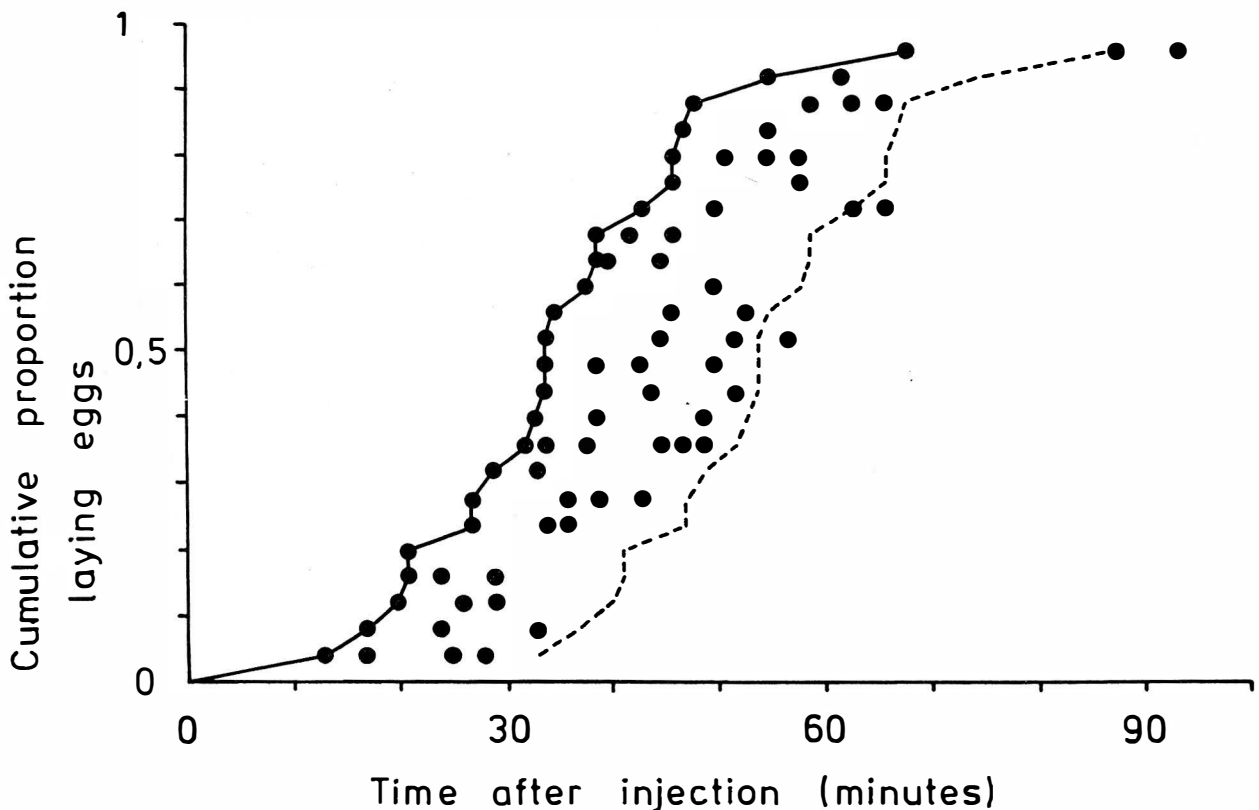


Fig. 2 The timing of egg laying after oxytocin injection. Each horizontal row shows eggs laid by one female, females ranked in order of laying. The solid line shows the cumulative proportion which had begun laying. The dashed line shows a period of 20 minutes after laying the first egg, during which most egg laying was completed.

measured immediately after laying ranged from 26.3–30.3°C. The mean time to the first egg was 35 ± 13 minutes ($n = 24$); the mean time for all eggs was 43 ± 15 minutes ($n = 75$) (Fig. 3). An individual female laid her eggs at intervals of 1–20 minutes, mean 6.2 ± 3.6 ($n = 51$; Fig. 3c), the most common interval being 3–5 minutes. The length of the laying period was much less variable than the latency before laying the first egg. Most eggs were produced within 20 minutes of the start of laying (Fig. 2).

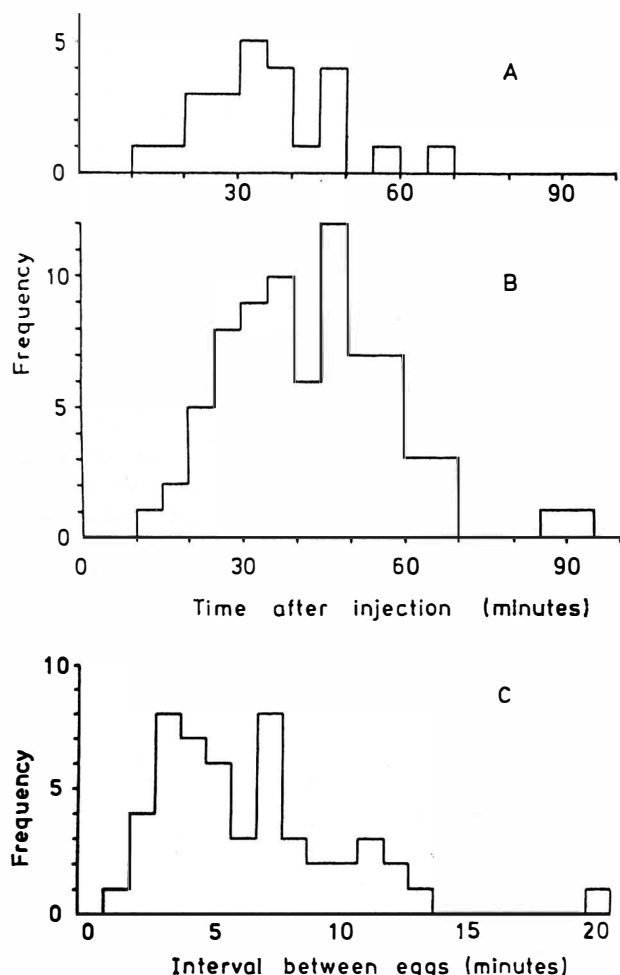


Fig. 3 Frequency histograms of the timing of egg laying after oxytocin injection. a) First eggs, b) all eggs. c) Interval histogram of the time between successive eggs.

Induced egg laying behaviour was stereotyped. The female walked rapidly around the arena in a normal gait after injection. The tail dropped after a few minutes, from the usual horizontal position to vertical, and was trailed along the ground while the female continued to investigate the arena. The female then assumed a posture with the head and forelegs withdrawn, and the front of the plastron resting on the ground. The hind legs were stretched out to raise the posterior of the body, and the tail was pointed horizontally backwards. The female moved away shortly after laying the egg. Females were kept for 30 minutes after laying the full complement of eggs observed by X-ray, but none laid any additional eggs.

SEXUAL MATURITY IN FEMALES

The size of females confirmed to be mature ranged from 142mm to 199mm SCL (Fig. 4a). However, only one (SCL 149mm) of the 13 females in the 140–149mm size class which were examined by X-ray or oxytocin had eggs, compared to 40–60 per cent of females larger than 150mm (Fig. 4b). Females of 150mm SCL or larger are therefore considered to be adults. In total, 146 females of this size were examined by X-ray or oxytocin injection in 1986 and 1988, and 57 of them (39.0 per cent) had calcified eggs. The proportions did not differ significantly between the two years; 28/78 in 1986 and 29/68 in 1988 ($X^2 = 0.44$, $P > 0.05$). In addition, 14 females injected with oxytocin in 1986 laid firm eggs with uncalcified shells (similar to the parchment shells of snake eggs), which would not be visible in X-ray photographs; these eggs were discarded.

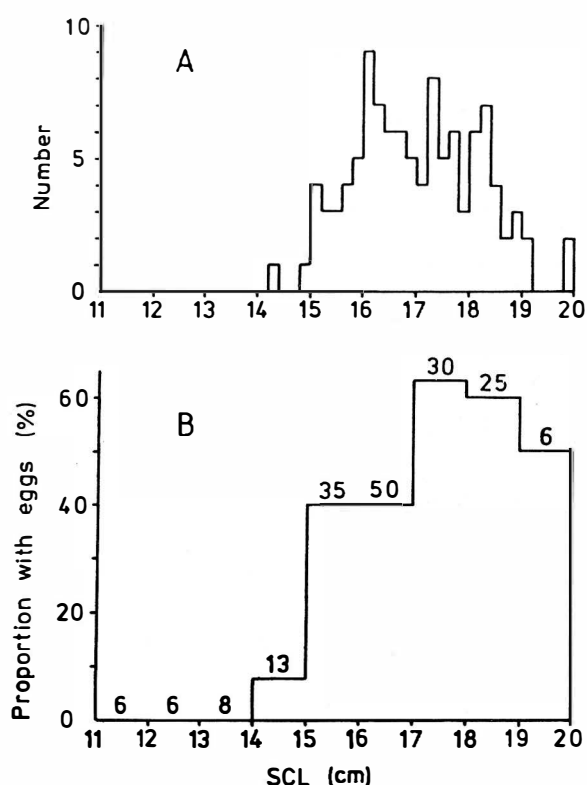


Fig. 4 The size at sexual maturity in females. a) Straight carapace length of all females from Alyki known to be mature, based on X-ray examination, oxytocin-induced egg laying or natural nesting. b) The proportion of females of different size which had eggs on X-ray examination or oxytocin injection. Sample size shown for each 1 cm class.

The field weight of females with eggs was compared to that of other females, in case the lightest females could be eliminated from future studies. The weight-length regression of females of 150mm or more handled in July (all years) was used as the standard:

$$2.785 \pm 0.144$$

$$\text{Weight (g)} = 0.354 \text{ SCL (cm)} \quad (1)$$

($n = 230$, $r = 0.93$). Results from July were used as this marks the low point of the female reproductive cycle, after eggs have been laid and while next years follicles are still small. Field weight, as a percentage of that

	1988	Total
SCL (mm)	171 ± 12 (30)	170 ± 12 (106)
Egg weight (g)	16.4 ± 3.1 (20)	15.5 ± 2.7 (66)
Egg length (mm)	37.2 ± 3.1 (20)	36.6 ± 2.7 (66)
Egg width (mm)	27.1 ± 2.0 (20)	27.0 ± 1.6 (66)
Clutch size	3.10 ± 1.03 (30)	3.59 ± 1.30 (59)
Clutch mass (g)	50.4 ± 17.3 (20)	54.5 ± 18.3 (41)
RCM (%)	5.52 ± 1.79 (20)	6.09 ± 2.00 (35)

TABLE 1: Egg and clutch size parameters for female *T. hermanni* from Alyki. Results for 1988, and pooled results 1983-88, shown as mean ± 1 S.D. (with number of tortoises).

expected from equation 1, was only slightly higher in females producing eggs compared to other females, in both years (with n):

	1986	1988
With eggs	104.2 ± 7.2 (42)	102.6 ± 6.7 (29)
Others	103.1 ± 7.5 (32)	102.0 ± 5.9 (39)

(Four injected females without eggs were not weighed in 1986.)

EGG SIZE AND CLUTCH SIZE

The results for 1988 are shown in Table 1, together with the pooled results for all years to show the mean reproductive pattern of Alyki females. The 1988 results are most similar to those from 1985-86 (Hailey and Loumbourdis, 1988); females laid more (clutch size 4-6) but smaller (mean egg size 12.9g) eggs in 1983 (Swingland and Stubbs, 1985). The average adult female at Alyki measured 170mm SCL, and laid a clutch of about 3.6 eggs, each weighing 15.5g. The measure of relative clutch mass in Table 1 is calculated as clutch weight/body weight, rather than field weight (as used in some studies).

Egg size and shape varied with body size in the Alyki population in 1985-86 (Hailey and Loumbourdis, 1988). The overall results including 1983 and 1988 are generally similar. The regression equations relating egg width (mm), egg shape (the ratio of length/mean width), and egg weight (g wet weight) to female SCL (cm) for the total sample (n = 66) are:

$$\text{Width} = 15.5 + 0.677 \text{ SCL} \quad (r = 0.486, P < 0.001) \quad (2)$$

$$\text{Shape} = 1.78 - 0.0252 \text{ SCL} \quad (r = -0.321, P < 0.01) \quad (3)$$

$$\text{Weight} = 3.24 + 0.728 \text{ SCL} \quad (r = 0.300, P < 0.05) \quad (4)$$

The correlation coefficients for width and for weight are similar to those reported previously, although the latter is now significant because of the increased sample size. The correlation between egg shape and

SCL is lower than the value ($r = -0.53$) found in 1985-86, because of additional unexplained variance from the particularly short eggs found in 1983.

The relation between weight, length and width of eggs from Alyki was investigated by multiple linear regression. The best fit was obtained using length (L), mean width (W)², and the difference between minimum and maximum width (dW):

$$\text{Weight} = 0.437L + 0.0200W^2 + 0.158dW - 14.7 \quad (5)$$

where dimensions are in mm (n = 59, $r^2 = 98.8$ per cent). However, the extra information provided by the difference between minimum and maximum widths did not produce a significantly better fit ($P = 0.081$); the other coefficients were significant at $P < 0.001$. The simpler equation:

$$\text{Weight} = 0.435L + 0.0203W^2 - 14.7 \quad (6)$$

($r^2 = 98.7$ per cent) may be used to predict egg weight from linear dimensions.

Clutch size (CS) was weakly related to female body size (Fig. 5). The regression equations for the 1988 results and for the overall sample were:

$$1988: \text{CS} = 0.208 \text{ SCL} - 0.5 \quad (n = 30, r = 0.240, P > 0.05) \quad (7)$$

$$\text{Total: CS} = 0.277 \text{ SCL} - 1.1 \quad (n = 59, r = 0.266, P = 0.05) \quad (8)$$

Body size therefore accounts for only about 7 per cent of the variation in clutch size. Clutch sizes ranged from 1 to 6, with a mode of three eggs (Fig. 5c). The clutches derived by oxytocin injection in 1986, and not confirmed by X-ray, were not included in this analysis as they may have been incomplete (Congdon and Gibbons, 1983). These clutches were smaller (mean 2.74 ± 0.88) than full clutches measured by X-ray or nesting, and were not related to body size:

$$\text{CS} = 0.029 \text{ SCL} + 2.3 \quad (n = 43, r = 0.04, P > 0.1) \quad (9)$$

This includes the clutch produced by one of the seven females given a second oxytocin treatment.

	22-27°C	31°C	35°C
<i>T. hermanni</i>	67, 67, 67, 72	56, 56	58, 58
<i>T. graeca</i>	—	56	66, 68
<i>T. marginata</i>	—	—	61, 61
Mean	68	56	62

TABLE 2: The time from laying to hatching at different incubation temperatures. Each value is the incubation period of one egg, in days.

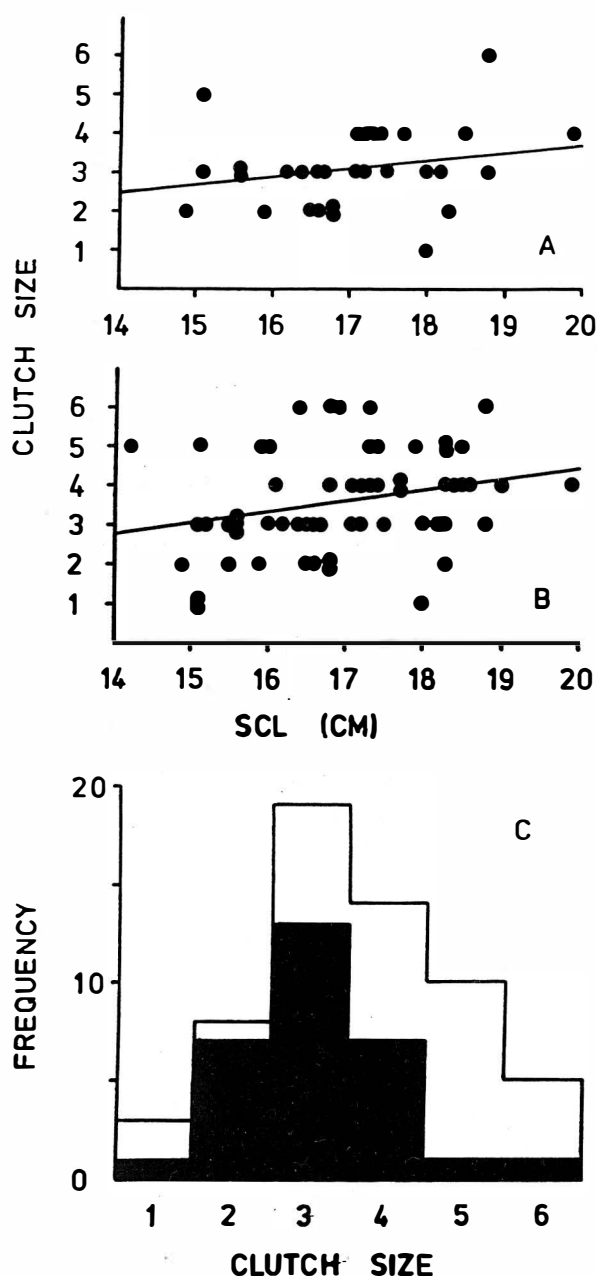


Fig. 5 a, b) The weak positive relationship between female size and clutch size (equations 7 and 8). a) X-ray examination in 1988. b) All complete clutches observed at Alyki. c) Frequency distribution of the sizes of complete clutches; data from 1988 are shaded.

Clutch mass was significantly related to female body size ($r = 0.326$, $n = 41$, $P < 0.05$), with a slightly higher correlation than for clutch size ($r = 0.266$) or egg weight ($r = 0.300$). A slightly improved fit ($r = 0.379$) was given by a polynomial regression rather than a linear model, which showed maximum clutch mass (CM) in intermediate sized females (Fig. 6):

$$CM = 7.9 SCL - 0.022 SCL^2 - 644 \quad (10)$$

where clutch mass is in g and SCL in mm.

INCUBATION

In total 196 eggs were incubated in 1985-86, including 30 *T. graeca* and 20 *T. marginata*. Only 13 of

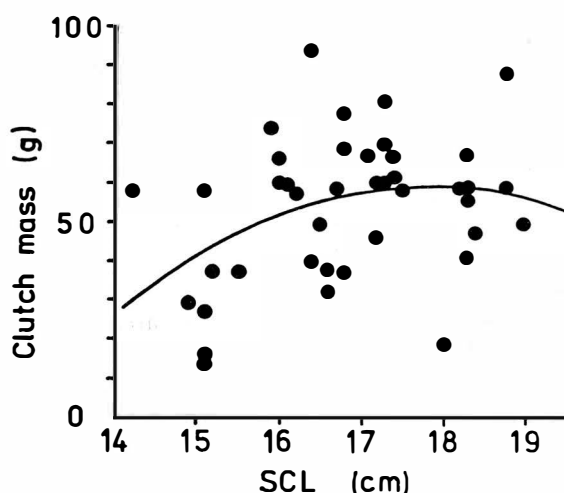


Fig. 6 Relation between clutch mass and female body size. The polynomial regression is given in the text (equation 10).

these hatched, after incubation periods from 56 to 72 days, 8-10 weeks (Table 2). Hatchling body weight (HW) and egg weight (EW) were strongly related, according to the equation:

$$HW = 0.574 EW + 0.79 \quad (11)$$

where HW and EW are wet weights in g ($n = 13$, $r = 0.87$, $P < 0.01$). The hatchling was on average 62.1 ± 5.6 per cent of egg weight. Hatchling size relative to the egg was similar in the three species for wet weight, but not for dry hatchling weight. The mean values, as percentages of egg weight, of the hatchlings incubated at 35°C ($n = 2$ for each species) were:

	Wet	Dry
<i>T. hermanni</i>	63.2	12.9
<i>T. graeca</i>	61.7	18.0
<i>T. marginata</i>	60.5	17.6

Wet weight for all temperatures was 62.2 ± 6.8 in *T. hermanni* and 61.9 ± 3.7 in the other species (t test, $P > 0.9$). The values for dry weight were 14.8 ± 2.3 in *T. hermanni* and 17.4 ± 1.1 in the other species ($t = 2.34$, 11 d.f., $P < 0.05$). Hatchling *T. graeca* retained large yolk sacs, which were partly external. Yolk remnants were smaller in *T. marginata*, and absent in *T. hermanni* incubated in these conditions. Mean hatchling SCL of the three species was:

<i>T. hermanni</i>	33.4mm
<i>T. graeca</i>	34.9mm
<i>T. marginata</i>	34.5mm

The *T. hermanni* eggs which hatched were from Litochoron and Deskati, and were slightly larger than eggs from Alyki.

Incubation was much more successful in 1988. The first group of eggs, laid from 19-28 May, were incubated in soil, and 30 out of 32 hatched before 29 July, after about 60 days incubation. The second group, laid from 31 May to 3 June, were incubated in a bare plastic dish, and 12 out of 27 hatched in late July and early August. Six of these eggs were laid on 31 May, and hatched from 25-29 July, after 55-59 days incubation. These six hatchlings weighed 9.6 ± 1.4 g,

61.3 $\pm$ 6.2 per cent of the wet weight of the eggs from which they hatched. Overall hatching success in 1988 was 71 per cent, compared to 6.6 per cent in 1985-86. Some of the 1988 hatchling *T. hermanni* from Alyki retained an external yolk sac. These hatchlings were all kept for growth and subsequent release, so the extent of yolk remnants and dry weight were not examined.

EGG PREDATION

The buried chickens eggs remained undisturbed in four of the five areas; only those in area B were disturbed. Five of the twenty eggs in this area were dug up, and were found intact on the surface. In the other areas, a few eggs were exposed or smashed within the hole, but the majority were recovered:

	A	B	C	D	E	Total
Removed from hole	1	5	0	1	0	7
Exposed in hole	0	0	2	1	0	3
Broken in hole	0	1	1	2	3	7
Recovered intact	19	14	17	14	16	80
Unable to locate	0	0	0	2	1	3

The eggs broken in the hole may have exploded after rotting, and so may not represent interference. The proportion remaining undisturbed through the incubation season was between 80 per cent (recovered intact) and 90 per cent (including eggs lost or broken within the hole).

Only one nesting female was observed at Alyki during the present study, and she did not complete her nest. The eleven nesting females found by Swingland and Stubbs (1985) were not grouped into any particular areas, and nests are thought to be widely dispersed over the heath. A few opened nests with fragments of eggshell were found, all of them in autumn rather than during the May-June nesting season. They are thus thought to result from successful hatching rather than nest predation.

Some large burrows, of fox or badger, were seen in the bank along the new dyke in sector 17 in 1984-86, and in 1988 a new burrow system was seen further along the dyke in sector 3 (Fig. 1). Many small holes, 10-15cm wide and deep, were found in grass within 50m of the burrows, but no fragments of eggshell were found in these holes. Tortoise nests which have been opened by predators at other sites in Greece and in France were always marked by eggshell. It is not known whether there was a localised nest predator in this area, or whether the animal was digging for roots or insects or, if a badger, making latrines (Bang and Dahlstrom, 1974).

Tortoises in the first few years of life were rarely seen at Alyki; animals with 0-3 growth rings made up only 2.6 per cent of all observations. Tortoises with 1-3 growth rings were seen throughout the year, with a similar seasonal pattern to that of larger animals (Fig. 7). The first hatchlings were seen in August, and remained active until November. They were relatively frequently seen in October and November, compared to larger animals, although this pattern is based on a small sample. Hatchlings with 0 growth rings were also seen in spring. They disappeared by June, presumably

as a result of growth of the first ring and incorporation into the 1 growth ring class.

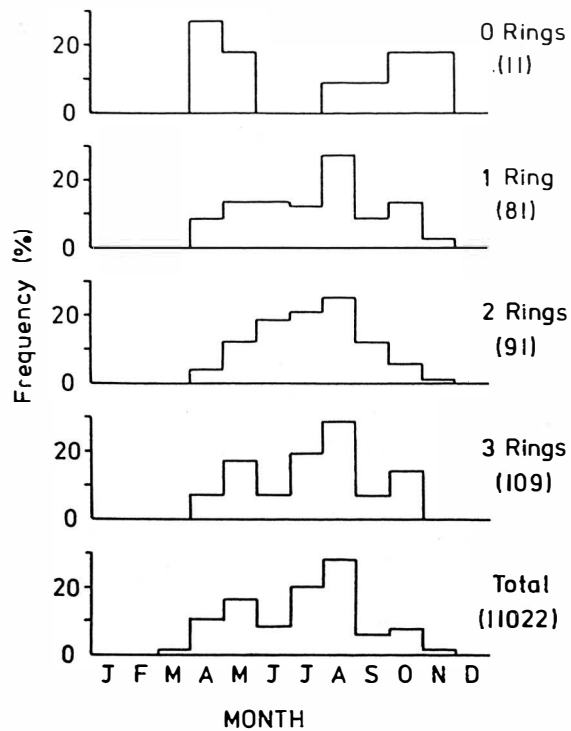


Fig. 7 Seasonal abundance of sightings of *T. hermanni* of different ages at Alyki. Each part shows the percentage of the total in each category (sample size at right) recorded in each month.

DISCUSSION

MATURITY AND EGG PRODUCTION

Female *T. hermanni* of 150mm are sexually mature at Alyki. This value represents the mid point of size at maturity, rather than the size of the smallest mature individual. Females between 100 and 149mm are termed subadults; they may be sexed by external characters (principally relative tail length; Stubbs *et al.*, 1984), but are not mature. Swingland and Stubbs (1985) noted that the smallest size of courting female *T. hermanni* at Alyki was 130mm. However, courtship of a female is not an indication of maturity, as male tortoises often court anything that moves; the smallest female seen being courted at Alyki was 108mm long. This is the reason for the large difference between 'mean adult size' (156mm) and mean size of nesting females (171mm) found by Swingland and Stubbs (1985).

The average reproductive parameters in Table 1 are provided for modelling purposes. The only parameters for which a large sample is not available are clutch frequency and reproductive potential. These are difficult to measure unless females can be restrained in enclosures or located by radio tracking throughout the whole nesting season. The alternative method based on dissection and counting eggs, follicles and corpora lutea (Gibbons, 1968) is only suitable for small samples of the protected *Testudo* species. Hailey and Loumbourdis (1988) used this method, which

suggested that reproductive potential (clutch size $\times$ clutch frequency) was greatest in intermediate sized *T. hermanni* from Alyki, and declined in large females.

Although additional measurements of clutch frequency were not made here, the proportion of females with eggs is an indication of *relative* clutch frequency. This was greater in females of 170–189 mm than those of 150–169 mm (Fig. 4b), with a decline in the largest size class (which however was based on a small sample). Clutch mass also showed a slight decrease in the largest females (Fig. 6). The lack of a clear relationship between size and reproductive parameters within this population is perhaps not surprising in view of the small size variation of adult female chelonians, compared to other groups of reptiles (Hailey and Davies, 1987). Body size explains (in terms of r^2) only about 5–15 per cent of the variation in reproductive parameters, and is likely to be of little importance in practical models of population dynamics.

Females with eggs weighed on average only 0.5–1.0 per cent more than females without eggs, so that field weight provided no indication of a female's reproductive status. It was expected that females with eggs would weigh about 6 per cent more, i.e. the mean relative clutch mass in the population (Table 1). It is probable that total body volume is constrained by the carapace; eggs are tightly packed within the body (see Fig. 6 of Hailey and Loumbourdis, 1988). This would lead to space for eggs being provided by reduced volume of some other part of the body, most probably by reduced gut contents.

INCUBATION

The low hatching success in 1985–86 was originally thought to result from non-viability of eggs laid after oxytocin treatment. For example, a maturational process within the oviduct may not have been completed, as suggested by Iverson's (1980) failure with oviducal eggs of the gopher tortoise. However the high success in 1988 indicates that the incubation conditions were at fault, possibly too high moisture level in the sand. The 71 per cent hatching rate in 1988 compares favourably with other studies of tortoises, such as 61 per cent in *Gopherus berlandieri* (Judd and McQueen, 1980).

When eggs of the three species were incubated together, hatchling *T. hermanni* had low dry weight compared to *T. marginata* and especially *T. graeca*, the eggs of which have a higher energy content (Hailey and Loumbourdis, 1988). The energy reserves of hatchlings are available to cover non-feeding periods, which may be due to hatchling behaviour or to fluctuating food supply. Reserves of hatchling sea turtles must supply them while digging out of a deep nest and then locating the nursery ground (Kraemer and Bennett, 1981). Many Chelydrid, Emydid and Kinosternid turtles delay emergence from the nest from autumn hatching until the following spring (Gibbons and Nelson, 1978). Hatchling *Geochelone gigantea* remain in their nests for a few weeks (Bourn, 1977), and *Testudo sulcata* are inactive and fast for 1–2 weeks after hatching (Cloudsley-Thompson, 1970). Hatchling *T. hermanni* were first found in the field in August, at about the

same time as eggs hatched in captivity. Prolonged delayed emergence from the nest therefore does not seem to occur at Alyki.

More work will be necessary to show whether the pattern of hatchling energy reserves in *Testudo* is truly species-specific, or whether this varies between populations as well. At present, it is suggested that the greater yolk remnants found in *T. graeca* and *T. marginata* are related to the occupation of less amenable habitats (differences in hatching times are another possibility — Willemsen, personal communication). In coastal areas in north-eastern Greece, *T. graeca* uses arid open heath habitat where *T. hermanni* occupies woodland or scrub (Wright, Steer and Hailey, 1988; Willemsen, 1988). *Testudo hermanni* becomes restricted to productive cultivated or riparian habitats inland at the extreme east of its range (Scholte, Helmer and Strijbosch, MS). The typical habitat of *T. marginata* is unproductive thorny scrub, while *T. hermanni* in southern Greece is largely restricted to more fertile cultivated areas (Willemsen, MS; Willemsen and Hailey, 1989).

Larger hatchlings emerged from larger eggs, with the hatchling being on average 62 per cent of egg weight. The loss of weight is not accounted for by the shell, which makes up about 13 per cent of egg weight in *Testudo* (Hailey and Loumbourdis, 1988), but rather reflects the metabolism of the embryo. This provides the simplest explanation of the higher relative clutch mass of oviparous compared to viviparous reptiles. Other factors being equal, RCM should be about 40 per cent lower in a species producing live young rather than eggs. There is therefore no need to invoke greater costs of reproduction in viviparous species, such as transporting the young or reduced food intake of the mother (for example, Seigel and Fitch, 1984).

No predation of natural nests was observed during the summer, and most buried chicken eggs remained undisturbed. Nesting success is usually about 20–30 per cent in apparently stable populations of tortoises and terrapins (Landers, Garner and McRae, 1980; Christens and Bider, 1987; Congdon, Breitenbach, van Loben Sels and Tinkle, 1987), but values up to about 70 per cent have been reported (Tinkle, Congdon and Rosen, 1981). The present study is directly comparable to that of Swingland and Stubbs (1985) on *T. hermanni* in France, where 71 per cent of chicken eggs suffered predation in the first two days after burial, and less than 10 per cent of natural nests were successful. Such differences in reproductive success must have important consequences for population dynamics; the French population was thought to be declining (Stubbs and Swingland, 1985), while the Alyki population is stable or increasing.

POPULATION REPRODUCTION

The reproductive potential of an animal population may be estimated using the size at maturity in females and the mean reproductive potential of adults, or the abundance of size or age classes and the reproductive potential of each class (for example, Table 9 of Turner and Berry, 1984). This section compares simple and stratified estimates at Alyki to assess the reliability of

<i>SCI</i>	<i>Reproductive potential (1)</i>	<i>Clutch size</i>	<i>Clutch frequency</i>	<i>Reproductive potential (2)</i>	<i>Females</i>	<i>Main Heath</i>		<i>Salt Works Heath</i>		
(mm)						<i>Eggs (1)</i>	<i>Eggs (2)</i>	<i>Females</i>	<i>Eggs (1)</i>	<i>Eggs (2)</i>
140s	-2.9	2.9	0.3	0.9	67	0	58	16	0	14
150s	4.6	3.2	1.6	5.0	88	402	440	21	96	105
160s	8.5	3.5	1.6	5.5	65	553	355	21	179	115
170s	8.9	3.7	2.5	9.2	58	517	532	15	134	138
180s	5.8	4.0	2.4	9.4	34	196	320	6	35	56
190s	-0.9	4.3	2.0	8.4	3	0	25	2	0	17
Total						1668	1730		444	445

TABLE 3: Estimates of the total reproductive capacity of *Testudo hermanni* on the main heath and on the salt works heath, by two methods (see text).

the simple method, and to provide gross measures of reproductive potential in the main heath (post-fire) and salt works heath populations.

A mark recapture study estimated the total number of females (of 100mm or larger) to be 478 in the main heath and 135 in the salt works heath (Table 1c and 1a of Stubbs *et al.*, 1985). This number was divided among 10mm size classes according to the relative frequencies shown in Fig. 3 of Stubbs *et al.*, (1985). Reproductive potential for the mid-point of each size class was calculated by two independent methods (see Table 3).

1) Using the polynomial regression of reproductive potential on carapace length given in Fig. 4 of Hailey and Loumbourdis (1988).

2) Clutch size was calculated from equation 8 above. Relative clutch frequency was taken from the data shown in Fig. 4b, with a maximum value of 1.0 for the 170s mm class. The average relative clutch frequency for the 146 females of 150mm or larger was then 0.767. Average clutch frequency for adult females at Alyki was 1.9 (Hailey and Loumbourdis, 1988). Relative clutch frequency was therefore converted to clutch frequency by multiplying by 2.48 (= 1.9/0.767). The product of clutch size and clutch frequency gave the reproductive potential of each size class.

248 of the 478 females estimated to live on the main heath were of 150mm or larger. The simple estimate of population reproduction is therefore (248 x 1.9 x 3.59) = 1,692 eggs per year. This value is similar to the two stratified estimates (1,668 and 1,730 eggs per year), and together give an estimate with a standard deviation only 1.8 per cent of the mean (1697 ± 31). 65 of the 135 females estimated to live on the salt works heath were of 150mm or larger, with a simple estimate of 443 eggs per year. The three estimates for the salt works are even closer (443, 444 and 445 eggs per year); surprisingly close in view of the crudity of the calculations. The main conclusion is that these methods are robust enough for use in models of the dynamics of the Alyki populations.

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