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CONTENTS

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THE MARSH FROG (*RANA ESCULENTA RIDIBUNDA* PALLAS) IN ENGLAND

By

J. I. Menzies

Introduction.

Since the early nineteenth century at least three species of anurans have been introduced at various times into this country. Only the edible frog (*Rana esculenta*) and the marsh frog (*Rana e. ridibunda*) seem to have become firmly established, existing without direct protection. The other two species are the midwife toad (*Alytes obstetricians*), and an Australian tree frog (*Hyla ewingi*) which have become established on a small scale in gardens in Bedford and Cornwall respectively (M. Smith, 1950, 1954; Larking, 1955).

The edible frog, which is apparently not indigenous to this country, at any rate in post-Pleistocene times, has been repeatedly introduced, the first recorded introduction being in 1837 (see M. Smith, 1954). It seems, however, to have died out after a few years in most places, and only survives in a few small colonies around London. None of these can compare with the huge colony of marsh frogs which now exists in south-east Kent and Sussex, occupying a territory of more than 100 square miles. Although small numbers of this frog have been liberated elsewhere at various times, it apparently survives only in this one huge colony, which forms the subject of the present study.

The origin of this colony is briefly described by M. Smith (1954), but the original account seems worth quoting. The frogs were liberated by E. P. Smith (Edward Percy, the playwright) who describes his experiment as follows (E. P. Smith, 1939).

"In the winter of 1934-5 I introduced twelve specimens of the European Edible Frog, *Rana esculenta* (the Hungarian variety), into a pond beside a running stream in my garden at Stone-in-Oxney, East Kent. The site of an old sea creek which, in Tudor times, formed a small landing stage; but now, since the sea has receded five miles or more, abuts upon a tract of land where the flats comprising the Walland, Romney and Denge Marshes usurp the place of the salt water. It is a maze of dykes, canals, meres and streams intersecting rich pastures."

Had Mr. Smith wished to establish the marsh frog as a permanent member of the British fauna he could hardly have chosen a better place. Probably nowhere in England is there such a large area of reclaimed land as that comprised by the Romney, Walland and Denge Marshes where nearly one hundred square miles of flat land jut out into the sea behind Dungeness.

Though the area is called a marsh it is not marshy at all (albeit entirely below sea level) being drained by a system of sewers and ditches running into the sea. Water level in the sewers is carefully controlled by a system of sluices and the seaward outfall of each is guarded by

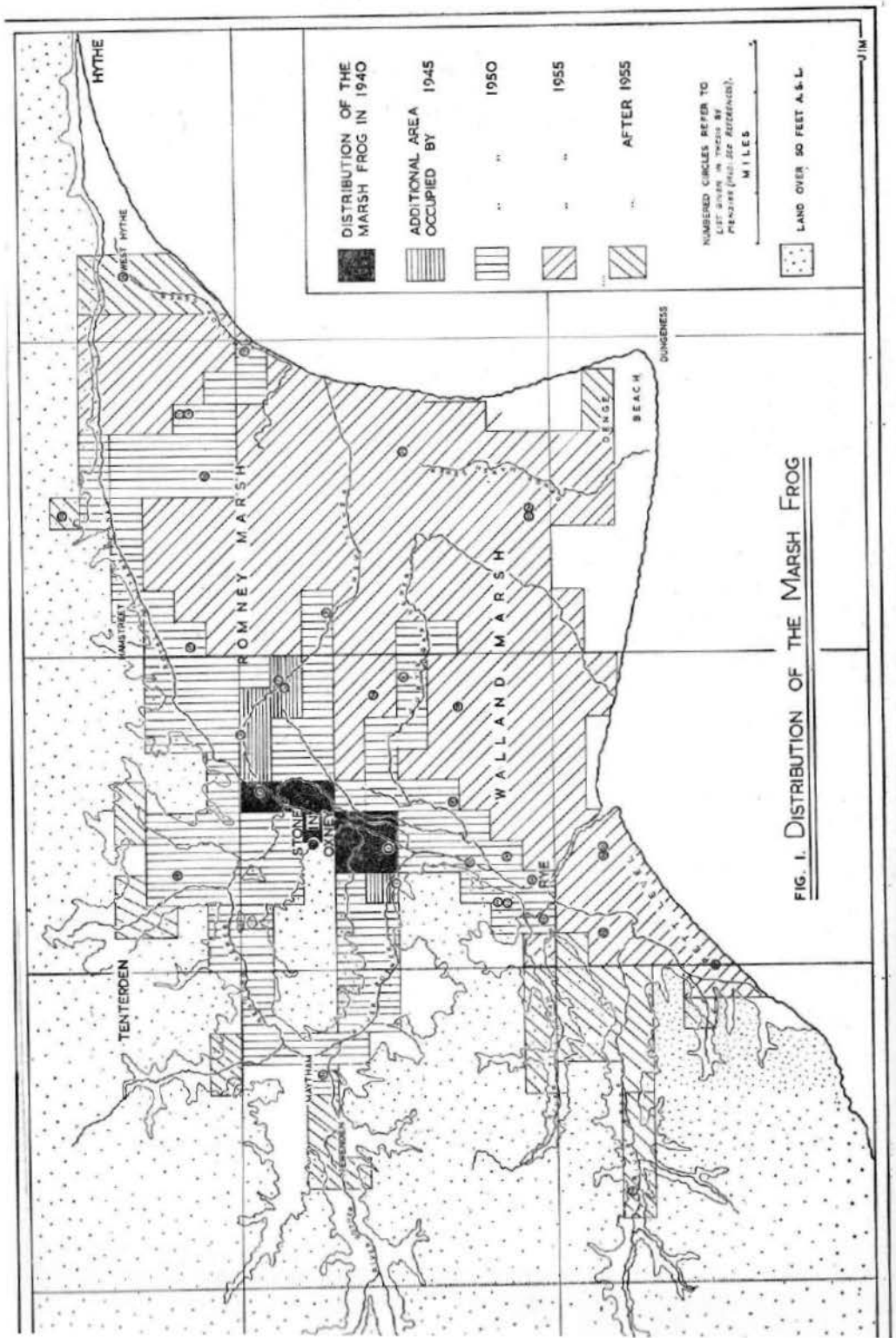

FIG. 1. DISTRIBUTION OF THE MARSH FROG

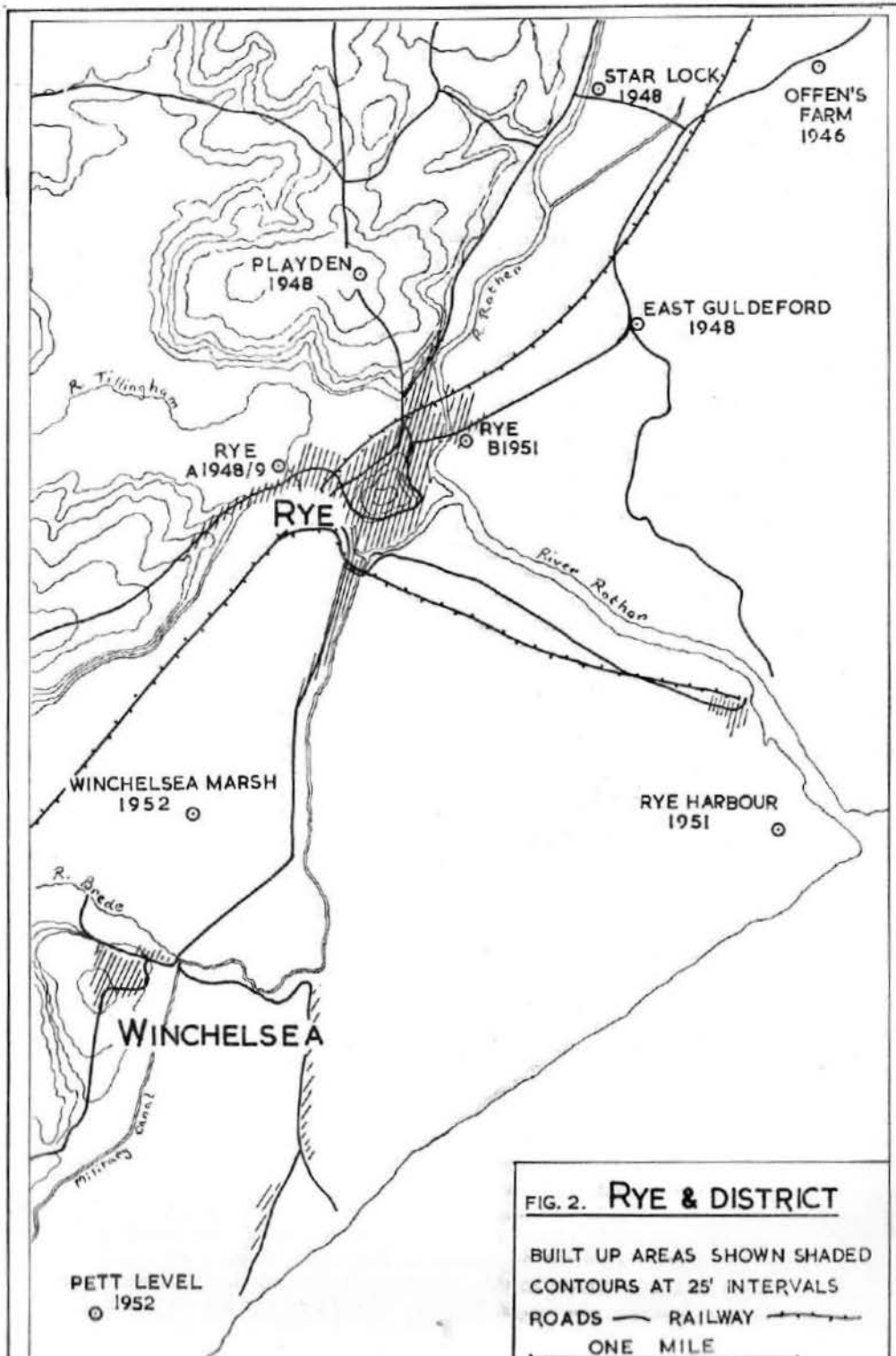

FIG. 2. RYE & DISTRICT

BUILT UP AREAS SHOWN SHADED
CONTOURS AT 25' INTERVALS
ROADS — RAILWAY
ONE MILE

tidal gates. These sewers often run between high banks and, in turn, receive water from high level ditches which drain the fields. Occasionally the tidal gates are opened on the flow and the sea allowed to enter. This scours out the channels and the salt water kills off the vegetation which would otherwise block the flow of water. It also has the effect of making the water in the sewers brackish, while that in the ditches remains fresh.

From Stone-in-Oxney the frogs have spread in all directions into Kent and Sussex. In 1948 a resident of Rye transferred a number from Walland Marsh to fields on the opposite of the River Rother, believing that he was making a new introduction. It is possible that the frogs had already reached that place by themselves. In 1949 eight were taken to Thorne, Yorkshire (Bunting, 1957) and in 1958 some were taken to Devon and others to Somerset. It is believed that these introductions have not survived. Today, the marsh frog continues to survive in Kent and Sussex and continues to extend its range.

Taxonomy.

According to Kauri (1959) the marsh frog can only be regarded as a sub-species of *Rana esculenta*, and I have followed his nomenclature. The relationship between the marsh and edible frogs has long been in doubt although Pallas (1771, p. 458), originally described the marsh frog as a distinct species. Boulenger (1885) came to the conclusion that it was best regarded as a sub-species and Mandeville & Spurway (1949) showed that interbreeding between the two forms can occur, though they did not succeeded in rearing the hybrid off-spring to maturity.

In England the two frogs are quite distinct in appearance. *R. esculenta esculenta* and *R. e. lessonae* (edible frogs) have been introduced from France and Italy and *R. e. ridibunda* from Hungary. The principal differences are in length of the legs and in the colouration. The marsh frog has longer legs and its basic colour is usually brownish, though the head and fore-body is suffused with green. It is rare for the marsh frog to have a dorsal stripe whereas the edible frog usually does have one. The basic colour of the edible frog is green though both types lose their green colour in captivity and become a dull brown. As the different types have not been introduced into the same localities no confusion, nor inter-breeding has been possible in England.

Distribution, Rate of Spread and Density of Population.

Up to the present no complete account of the distribution of the marsh frog in England has been published. Knight (1948) refers to the area involved as stretching from Hythe to Rye and from Tenterden to Hamstreet. In fact, the frogs have not yet reached Hythe or Tenterden and have gone beyond Rye. Smith (1954) gives a map of the area involved without actually indicating the boundaries but does not include the Rother, above Maytham, which they reached in 1949. Taylor (1948) gives no greater detail than vice-counties.

The area now occupied by the marsh frogs is shown in fig. 1, and extends from West Hythe to the south-west end of Pett Level, the valley of the Rother at least as far up as Newenden Bridge, the valley of the Brede as far as Brede Bridge, the lower valley of the Tillingham and all the land between the Royal Military Canal and the sea except those parts

of Denge Beach that have no suitable water. The total area is approximately one hundred and twenty-three square miles and representative parts of it are shown in plates 1 and 2.

The sparse published records of the marsh frog in England give some idea of its rate of spread from Stone. Additional information was obtained by distributing a circular amongst the inhabitants of the marshes asking for information about its spread. This information, which many persons so kindly sent me, is illustrated in fig. 1. A complete list of my informants and other details is given in a thesis (Menzies, 1960) available in the Senate House, University of London.

E. P. Smith (1939) states that the frogs can migrate overland as much as two miles and this is borne out by their presence in the long pond at Dungeness, one and a half miles from the nearest waterway. However, it cannot be proved that they were not carried there by human agency, or possibly the spawn was carried on a duck's or heron's feet. The main spread has probably been along the waterways that intersect the area. The smaller waterways carry the highest frog population, but these are not continuous. However, the larger sewers are, and by them the frogs could travel many miles without leaving the water at all. The frogs are powerful swimmers and when disturbed on the bank of a sewer frequently jump and swim right across before appearing on the surface. The early dates of appearance of the frogs in the main sewers at some distance from Stone seems to confirm this. For example, they first appeared at Baynham, on the White Kemp Sewer, and four and a half miles from the Military Canal, as early as 1946. This suggests a rate of spread of about half a mile per annum. A comparable rate of spread seems to have followed the course of the New Sewer from Appledore to Brenzett, a distance of four and a half miles in ten years. In both these cases the spread has been downstream but an even faster spread has been up the River Rother. Marsh frogs were first reported from the Rother in 1943 at Thornsdale, Iden. By 1949 they had reached Maytham, four and a half miles upstream. On the other hand, in places not situated on the main sewers, such as New Romney and Lydd, the frogs did not appear until the 1950's.

Up to 1943 the frogs had only colonised the land to the north of the Rother. In that year they crossed this river (Ticehurst, 1950, p. 198) and by 1946 had spread upstream as far as Maytham and down to Rye. Below Star Lock the Rother is tidal and has extensive muddy banks at low tide. However, this apparently proved no barrier and the frogs appeared at Rye Harbour (on the opposite bank) in 1951 and by 1952 had spread over Pett Level and Winchelsea Marsh (see fig. 2).

The number of frogs that any given waterway may support may be quite high but not every waterway in the same area always has the same number of frogs. For instance, one square kilometre near Appledore included 9.96 km. of waterways and 147 frogs were counted there but analysis of the count contains some interesting features. The area (fig. 3) is divided into two by the Appledore-Snargate road; on the north side of the road the land is entirely given to grazing and 141 frogs were counted in 4.76 km. of waterways. On the other side of the road the land is entirely arable and only 6 frogs were seen in 5.1 km. of water. More will be said about this later.

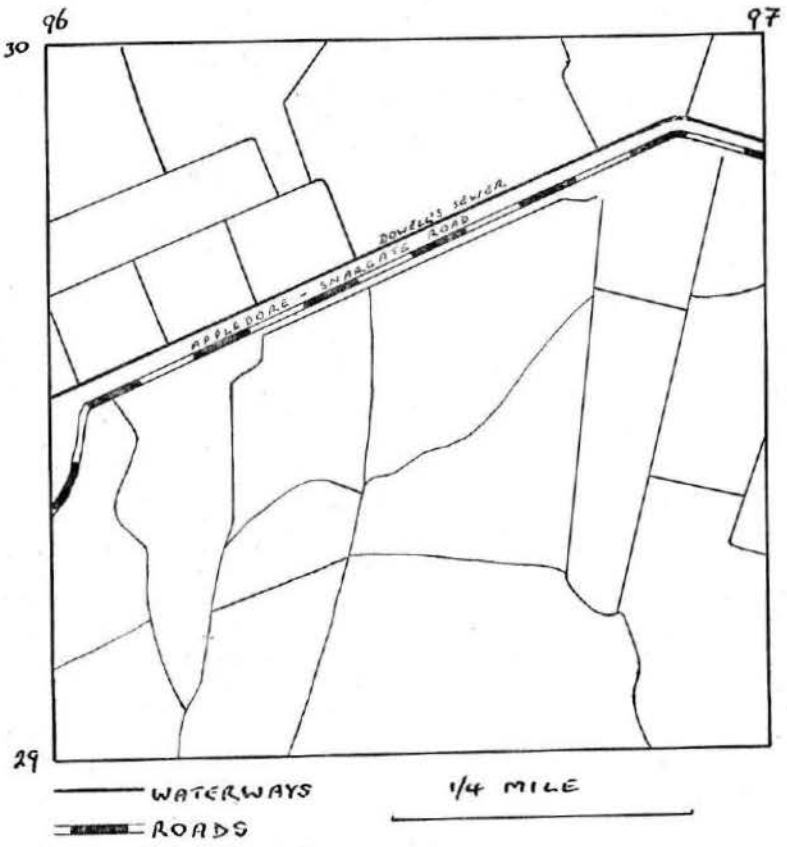

Fig. 3

It has already been mentioned that the waterways draining these marshes are of two types; sewers flowing to the sea and ditches in which the water is almost static. Where these ditches and sewers run through grazing land the bank vegetation is mostly grass that is kept short by the sheep. Where the ditch runs through arable land the bank vegetation is mostly rushes (*Phragmites*) which rapidly invade the water and ultimately block the canal. These conditions are apparently unsuitable for frogs, hence the low frog population of ditches through arable land. Small fields separated by ditches are advantageous in sheep farming as the ditches do away with the need for fences but the same ditches are disadvantageous in arable farming as they impede the movement of machinery. If these ditches are replaced by drains the frogs are deprived of their habitat and their continued existence on the Romney and Walland Marshes seems dependant on the continuance of sheep farming.

Reasons for the Success of the Marsh Frog in Kent and Sussex.

A. Habitat.

The marsh frog is largely aquatic and is seldom seen very far from water. On sunny days the frogs bask on the banks of the waterways or else lie floating on the water surface but on dull days they are hidden in the vegetation. The area occupied is largely reclaimed land and so presents a maze of ditches and sewers which has apparently proved a suitable habitat as the frogs can be found in nearly every ditch throughout the area.

The fact that some of the sewers contain brackish water seems to be no deterrent to their spread and frogs are to be seen in all types of ditches, whether brackish or fresh. Numerous authors (quoted by Menzies, 1960) have recorded amphibians living or breeding in brackish water in various parts of the world while Hardy (1943) and Mertens (1926) have recorded various European species including the marsh frog, doing the same nearer home. It is not surprising then that the marsh frog is to be found thriving in sewers that may have salinities approaching that of sea water.

B. Food

Juszyck (1950) examined the stomach contents of a number of *Rana esculenta* near Krakow and concluded that this species will eat practically anything. Stomach contents consisted of insects (mainly), other invertebrates, and fish, mice and smaller examples of their own species. M. Smith (1953) found that they feed on land as much as in water. Examination of seven stomachs during the present study seems to confirm this, as the following list shows.

Stomach 1. Bees, ants.
„ 2. Bees, snails, grasshoppers, wasp or syrphid, *Gerris*, duckweed.
„ 3. Spiders, beetles, earwigs, ants.
„ 4. Spiders, beetles.
„ 5. Beetles, bee.
„ 6. Beetles, spiders, *Nepa*.
„ 7. Snails, flies.

It is probable that the diet depends largely on the time of year and the type of ditch that the frogs happen to be living in at the time. By the summer the smaller ditches become so packed with vegetation that it would be difficult for the frogs to find food below the water surface but the larger sewers tend to remain relatively free during the year and so would provide easier feeding places for the frogs. In addition they contain a rather different fauna, largely prawns and mysids, due to their brackishness. Whether or not the frogs feed in the water, the sewers contain a large amount of suitable food. For instance, 96 cubic feet of water from Jury's Gap Sewer contained 6 prawns, 8 fish, 58 larvae of Odonata, 65 mysids, and *Gammarus* and corixids too numerous to count.

C. Reproduction and Growth.

Rana esculenta is more prolific than the common frog; three females that were examined contained 4,900, 5,500 and 7,880 eggs respectively, whereas the common frog may lay from one to two thousand (Boulenger, 1897-98; or from three to four thousand, Heron-Royer, 1885, 1887). It is possible that this greater prolificity enabled the marsh frog to fill a vacancy left by a declining common frog in that part of Britain.

It is almost certain that the marsh frog, in south-eastern England, becomes mature when two years old and commences to breed in the second summer after hatching. The rapid maturity of this frog is suggested by E. P. Smith (1939) whose introduction took place in the winter of 1934-5.

"In May, 1937, began what I might call the great year of *Rana esculenta*. There was an enormous amount of spawn and the minute frogs were to be seen everywhere. . . . They were appalingly vocal all through the summer.

It is scarcely possible that this enormous amount of spawn could have been produced by Mr. Smith's original twelve frogs. It must have been the beginning of a third generation. Spawn is laid in June and metamorphosis starts in August. In order to measure the natural growth of young frogs, collections were made in May, June, July, August and November, 1954-56. The frequency of the various sizes of young frogs are shown in fig. 4, and the growth rate is plotted graphically in fig. 5. Various authors (Angel, 1947; Savage, 1952; M. Smith, 1954), refer to the irregular growth rate of *Rana esculenta* and to the fact that many of the tadpoles have not metamorphosed by the onset of autumn. Though hibernating tadpoles have not been found by dredging of ditches in winter it is probable that they can do so and complete their development and metamorphose the following spring. Mrs. Green has kindly supplied me with the following data about the development of larvae from spawn produced by frogs with which I supplied her.

18 May 1955	Spawn laid.
2 July	1st metamorphosis.
18 Aug.	2nd metamorphosis.
2 April, 1956	penultimate metamorphosis.
1 June	1 tadpole remaining.

The lower curve in fig. 5, may suggest the growth rate of the natural spring metamorphs and is also derived from the table (fig. 4). Further evidence comes from Mr. Scott-Smith, a resident of Rye, who tells me that he has found large tadpoles as late as October and also in early spring.

Size	May	June	July	Aug.	Sept*	Nov.
58-60 mm			6	3		
55-57			5			
52-54			8	1		
49-51	1		2			
46-48	1	1	5			
43-45		4	4			
40-42	3	8	1			
37-39	2	8	1			
34-36	2	4				
31-33	5	1	1		40*	2
28-30	3	2				8
25-27	1			3		2
22-24				15		
19-21	1			7		
Totals	19	28	33	29	40*	12

Fig. 4. Size and frequency of monthly samples of young frogs.
* The figure for September is taken from M. Smith (1953).

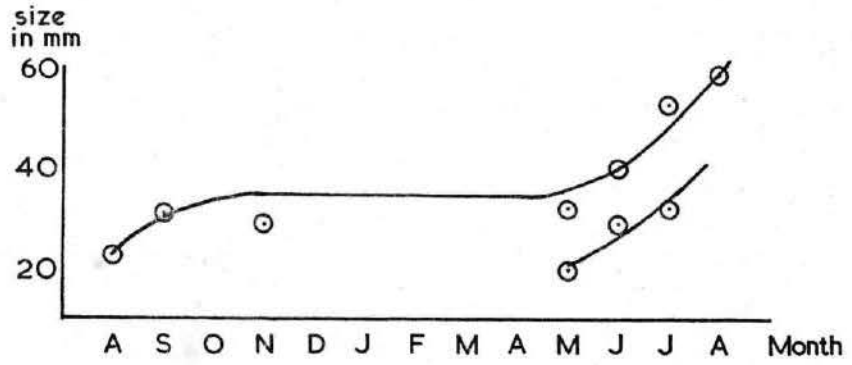

Fig. 5. Growth of the Marsh Frog
Upper curve - autumn metamorphs
Lower curve - spring metamorphs

D. Territoriality.

Aggressiveness of adult male marsh frogs towards other males of their own species may cause them to spread into new territories. *Rana temporaria* and *Bufo bufo* tend to congregate at spawning time, *R. esculenta* does not. Territory keeping has been noted in various amphibians (see Menzies, 1960), and observations in various parts of Romney and Walland Marshes

have shown that the marsh frog male tends to remain in the same place (up to fourteen days were recorded) and does not tolerate other males within his territory. The male frog has two calls, the first and loudest is uttered throughout the summer but falls off in intensity as the season progresses. This call starts rather slowly "kee-oink, kee-oink" but rapidly becomes faster and faster ending in a spluttering "kek, kek, kek". The second call of the male is a challenge or warning and is only uttered when one male enters the territory of another, it is a squeak sounding like "yip-yip" and can only be heard close by. The females' call is quite different, a very low-pitched "coo-uk" that cannot be heard by the human ear more than a few yards away. An observer who makes this call within the territory of a male frog during the breeding season will evoke an immediate response—the frog will swim towards him, whereas imitation of the warning call produces no such response.

Effect on the Native Amphibians.

That the common frog once existed on Romney and Walland Marshes is almost certain. It is equally certain that it is now extinct there and has become so after the introduction of the marsh frog. Boulenger (1908) records the common frog and the common toad from Kent without specifically mentioning the Marshes, while Butterfield (1905) records them as being common throughout Sussex. Mr. C. C. Hentschel tells me that frogs were common around Hythe in 1910, and Alderman C. T. Paine of Lydd says that they were common in that town when he was a child. Mr. M. W. F. Tweedie also reports plenty of frogs at Playden in 1929 but none of these residents has seen one in recent years. More direct evidence of the inability of marsh and common frogs to live together comes from Mr. Scott-Smith who introduced *ridibunda* to the Tillingham in 1948 or 1949 where *temporaria* was then common. *R. temporaria* has now disappeared from that area.

It is unlikely that there can be direct competition between the two species as, except during the breeding season, which is before *ridibunda* is active, *temporaria* does not remain in or near the water. *Ahrenfeldt* (1953) has seen the edible frog attacking and attempting to devour both common frogs and toads in captivity though his suggestion that this direct antagonism is responsible for the decline of the indigenous species is questionable. However, the tadpoles and metamorphosing froglets of *R. temporaria* and of *Bufo* would fall easy prey to the semi-aquatic adults of *R. ridibunda*. It is also likely that the territory-minded *ridibunda* males would not tolerate the presence of other frogs. Possibly *ridibunda* has filled a vacancy left by a declining *temporaria* population in the same way that the grey squirrel has replaced the red in Britain (Shorten, 1954).

Discussion.

This work has raised several interesting problems not the least of which is the disappearance of the common frog from the area now inhabited by the marsh frog. It cannot be proved that the disappearance of the one has anything to do with the introduction of the other; all that we know is that the common frog once existed on Romney and Walland Marshes

and that it no longer does so and that, in the meantime, the exotic species has established itself here. Only one author, E. P. Smith (1939) mentions the two species together, stating, that "the marsh frogs are still very numerous, far outnumbering the common *Rana temporaria*." But Smith lived at Stone which is just outside the Marsh and there are still plenty of frogs in the surrounding parts of Kent and Sussex.

Has the marsh frog really established itself as a member of the British fauna? At first sight the answer would appear to be "yes" as the species has been with us for twenty-five years and is still extending its range. On the other hand, Romney and Walland Marshes are a purely artificial creation of man and are maintained in the condition that they are now in because sheep farming is the principal occupation. Sheep-farming requires small fields surrounded by carefully maintained ditches and these provide the frogs with their food and habitat. If mechanical cultivation of crops continues to replace sheep-farming all but the larger sewers will be converted into closed drains and the frogs will lose their habitat. The numbers of marsh frogs that will be able to live under the present conditions will only be a fraction of the present population.

If the marsh frog is permanently established in this country will its numbers remain at the present high level? This question is prompted by a number of observers who believe that there has been a steady decline in recent years. Certainly the numbers in one district (around Appledore Station) seemed much higher about ten years ago and large quantities of frogs were carried away from there by naturalists and others. As no quantitative surveys have been made before, it is impossible to say whether there have been any profound changes in the numbers of frogs in any particular district. In years of bad weather the males are much less noisy than in hot summers and this may give the impression that there are fewer about but breeding seems unaffected by the weather. Most animal populations that have been studied quantitatively show periodic fluctuations in numbers, perhaps the early 1950's were boom years for the marsh frog and perhaps it has now settled down in more reasonable numbers. Only time will tell.

Acknowledgements.

In the first place I am indebted to Mr. C. C. Hentschel who has supervised this work from start to finish. I am also most grateful to the late Dr. Malcolm Smith who was always ready to discuss the problems and to help in the field, even when he was approaching eighty years of age. Mr. Kenneth Blackwell has been my companion during much of the field work; without his help many tasks would have taken more than twice as long.

This paper is based on a thesis approved for the degree of M.Sc., University of London, September, 1960.

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THE USE OF REPTILES IN EXPERIMENTAL EMBRYOLOGY.

By

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INTRODUCTION.

The science of experimental embryology is based mainly upon work with amphibian and chick embryos, though important observations have also been made on the embryos of fish, mammals and various groups of invertebrates. The reptiles have been almost completely neglected in such studies, probably because of the difficulty of obtaining adequate and reliable supplies of embryos. Reptiles seldom breed in captivity, at least in this country, and the eggs of oviparous species are difficult to find in the wild.

Recent observations suggest, however, that a new approach to the problem is offered by the use of cultured eggs removed from the oviducts of vivparous forms. A brief review of the scanty literature on the subject and an account of our own preliminary observations may therefore be of interest to other investigators.

PREVIOUS WORK.

Pasteels (1937), apparently the pioneer in the field, succeeded in marking the early embryos of chelonians with vital dyes in order to study the process of gastrulation. These experiments involved cutting a window in the eggshell, a technique often used in chick embryology. Nakao (1939) grafted parts of early lizard embryos on to the chorio-allantoic membrane of chick embryos and found that in some cases the various tissues continued to differentiate, and survived for periods of up to eight days. R. Bellairs (1951), also using techniques well established in chick embryology, removed blastoderms and somewhat older embryos from the eggs of English lizards and snakes, and was able to grow them in culture by the watchglass method for periods of up to 20 days.

Panigel (1956) made the important observation that embryos of the common lizard, *Lacerta* [=*Zootoca*] *vivipara* would develop in culture in Petri dishes after removal of the eggs from the mother. In some cases the embryos ultimately "hatched" from their membranes, though there was a considerable mortality just before the time of hatching. These experiments suggested that the embryos were not dependent on the mother for nourishment in the normal course of development, the supply of food in the yolk being sufficient for this purpose. Maderson and A. d'A. Bellairs (1962) found that it was possible to operate on these cultured embryos, removing a part of the tail. They also found that it was possible to culture embryos of the slow-worm (*Anguis fragilis*) by Panigel's Petri dish method.* Raynaud (1959a, b) described a more elaborate method of culturing the eggs of *Anguis* (see p. 59 here), and in a later note (1960) studied the effects of irradiation of the hypophysis in cultured embryos. Dufaure (1961) studied the influence of sex hormones on the development penes of common lizard embryos cultured in a medium of avian albumen, and Lutz and Dufaure (1960) cultured blastoderms and various embryonic organs of this species on artificial media.

Though of less relevance to our own work, the biochemical studies of Clark and his collaborators (1956, 1957) on the metabolism of various reptile embryos, and the observations of Gordon (1960) on the influence of moisture on the eggs and young of *Anolis* lizards may also be noted.

Criteria for assessing the growth stages of embryos are important in experimental work, especially in reptiles where the rate of developing varies with temperature and other conditions. Normal tables of development for reptiles have been prepared by the following authors: Peter (1904;

* Panigel also describes another technique in which lizard embryos are kept in tubes of oxygenated saline; this seems to be less satisfactory for our purposes.

Lacerta agilis), Pasteels† (1956-7; only the early stages of certain lizards and chelonians are described, but details of later stages, based on Pasteels' unpublished work are given by Milaire, 1957), Dufaure and Hubert (1961; *Lacerta vivipara*), and Zehr (1962; the garter snake, *Thamnophis sirtalis*).

OBSERVATIONS ON *Lacerta vivipara*.

During the summer of 1962, 155 fertile eggs of the common lizard were put up by us in Panigel dish culture. The great majority of the embryos were subjected to the operation of cutting off part of the tail, in order to investigate its regenerative potentialities during embryonic life. Some embryos developed gross anomalies of the hind part of the body and hind limbs which may have been due to the fact that after operation the embryos tended to prolapse through the rent in their membranes, the chorion and shell membrane. (Pl. 1F). It is hoped that this may be remedied in future by an improvement in technique (see p. 58) which unfortunately was introduced too late in the season to give conclusive results.

Preliminary analysis of our findings in those specimens where the condition of the tail could be readily interpreted essentially confirmed the earlier observations of Maderson and Bellairs (1962). No regeneration was observed in the embryos operated on at stages ranging from Dufaure-Hubert (subsequently called D.-H.) 29 (or Peter, figs. 21-22) to stage D.-H. 36 (Peter, fig. 34), although the tail stump healed over. Some evidence that the power of regeneration may develop shortly before the time of hatching was obtained. It is hoped to publish a full analysis of these findings elsewhere.

Maderson and Bellairs found that common lizard embryos could survive for up to 10 days at a temperature of 33-35°C., while others lived for quite long periods (in one case 29 days) at 4°C., during which development was almost at a standstill. Some of these refrigerated embryos continued to develop in apparently normal fashion when transferred to room temperature (21-24°C.). This finding seemed to have practical importance, suggesting that a pool of embryos might be stored in the refrigerator at a minimal rate of development, specimens being removed at convenient times for operation. The results of the comparatively few refrigeration experiments (on 35 embryos) performed this year suggest that the method, while probably useful up to a point, has limitations. The earlier embryos of D.-H. stage 32 and younger treated in this way showed after about a week at 4°C. a characteristic dilation of the peripheral chorio-allantoic blood-vessels (Pl. 1 H) which may have been due to stagnation of the blood resulting from slowing of the heart. Sometimes this would disappear when the embryos were transferred to room temperature, Pl.1,I); in other cases the embryos died. In older embryos these vascular changes after refrigeration were less marked and the blood vessels usually regained their normal appearance after some hours at room temperature.

Nearly all these later embryos lived for a fortnight at 4°C. and were then used for operation. Possibly refrigeration at a somewhat

† We are most grateful to Prof. Pasteels for sending us photographs illustrating his embryonic stages of the chamaeleon and the skink *Mabuya*.

higher temperature, say 8°C., would give better results for the storage of embryos.

The only five embryos kept at high temperatures (30-35°C.) this year survived for about a week. Further observations on the resistance of embryos to such conditions are also desirable in view of the possibility of grafting lizard embryo tissues to chick embryo hosts.

OBSERVATIONS ON *Anguis fragilis*.

89 fertile eggs of the slow-worm, taken from 11 females, were put up in Panigel dish culture. Although Maderson and Bellairs suggested on somewhat subjective grounds that temperatures of around 18°C. were most suitable for their maintenance, all the embryos used this year were kept at room temperature. No normal table of development for the slow-worm has been prepared, so that it was necessary to compare the embryos with those of *Lacerta* figured by Peter, and by Dufaure and Hubert. In view of the fact that the slow-worm differs markedly in body shape from lacertid lizards, especially in having no limb-buds (which are so useful as guides to the age of embryos) our staging of *Anguis* is inevitably subjective.

The fate of our slow-worm embryos is shown in the table below; the 23 embryos subjected to various operative procedures are not included in the figures.

SURVIVAL OF SLOW-WORM EGGS IN DISH CULTURE.

Group	A	B	C	D
Date when put up in culture	20 July	20 July	20 July-14 Aug.	1-20 Aug.
Total number of embryos	6	24	9	27
Stage at onset of culture	D.-H. 28; Peter fig. 20; chick, 3-3½ d	D.-H. 32; Peter fig. 27; chick, 5-5½ d	D.-H. 35-36; Peter figs. 33-34	D.-H. 39-40; Peter figs. 35-36; near hatching
No. fixed dead or dying	4	10	2	2
No. fixed alive before hatching	2	14	6	4
No. alive after 7 days in culture	3	20	6	24
,, 14 days	0	15	5	16
,, 21 days	0	8	2	1
,, 28 days	0	3	1	0
,, 35 days	0	2	0	0
Max. survival in culture and stage reached	10 d. D.-H. 30; Peter fig. 25	42 & 48 d. D.-H. 39; Peter fig. 35	34 d. hatching	27 d. hatching
No. died hatching	0	0	0	3 after 6-27 d. in culture
No. hatched alive	0	0	1	18

The embryos in group B, put up at D.-H. stage 32 (Pl. 1D) were of particular interest. The eight which lived for 21 days or more reached D.-H. stages of 36-37 (Peter, fig. 34), or in the case of the two which lived for over 40 days, D.-H. stage 39 (Peter, fig. 35)—so far as comparison with *Lacerta* could be made from features of head-shape, scale development and pigmentation. At least five of these embryos showed some external abnormality. Three were markedly oedematous (Pl. 1C), while the two which had lived for over 40 days were slightly abnormal in head or body shape. These two embryos also seemed considerably smaller than embryos showing similar morphological features (i.e. of D.-H. stage 39) which had recently been removed from the mother or only kept for a short time in culture (Pl. 1A, B). No abnormalities were noticed in embryos of the other groups.

Our figures for the survival of the embryos are influenced unfavourably by the fact that over one-third of them were fixed when still alive and apparently healthy, in order to obtain a series for descriptive work. Had they been allowed to remain in culture until they died or hatched, like the rest, the number surviving for longer periods would very probably have been greater. Nevertheless, the results show that over half of the group of embryos put up in culture during the middle period of development (as at D.-H. stage 32) are likely to survive for a fortnight or more, and a third of them may live for over 21 days. After such long periods, however, they often, if not always, become abnormal in some way. Only those put up at late stages (D.-H. 36 or later) seem to have a good chance of hatching.

Experimental Technique for Dish Cultures.

The procedure followed differs slightly from that of Panigel (1956). Pregnant lizards or slow-worms are killed by ether or chloroform which appears to have no effect on the viability of the embryos. The body of the mother is slit open and the thin-walled oviducts with the eggs inside are removed and placed in a large Petri dish containing 0.6% sodium chloride. The oviduct is then pulled away with fine forceps and the eggs released. These are then transferred individually by means of a wide pipette to small Petri dishes, each containing a circle of gauze moistened with 0.6% sodium chloride. Slightly deeper dishes are necessary for the slow-worm eggs, which are bigger than those of the common lizard. All glassware, instruments, gauze and saline are sterilised. Although not used by us, the addition of antibiotics to the saline might be of value in preventing infection. Fungus infections are prone to develop after long periods in culture, but do not necessarily cause the death of the embryos, at least for a considerable time.

The small Petri dishes containing the eggs are then placed in a large covered glass or plastic receptacle containing an open dish of water to prevent evaporation. Each egg is arranged in its dish with the embryo uppermost and the yolk downwards, so that the progress of development can be inspected daily under a dissecting microscope; the lid of the dish can be briefly removed for this purpose. It is usually possible to tell if an embryo is alive by observing the flow of blood through the chorio-allantoic vessels; in the later stages, movements of the embryo can readily be seen.

At least one embryo from each litter should be fixed after removal from the mother to give an indication of the stage reached at the onset of culture: with rare exceptions, all the embryos in one female are at about the same stage of development.

Operations are performed by cutting through the embryonic coverings with fine scissors; although the embryos seem remarkably resistant to haemorrhage, the larger chorio-allantoic blood-vessels should be avoided if possible. The tail is then seized with watchmaker's forceps and cut off proximal to the forceps grip with scissors; limb-buds can also be removed in this way. To prevent prolapse, small pieces of cotton handkerchief sterilised by boiling are placed over the rent in the membranes, to which they soon become adherent (Pl. 1G).

Operations should be delayed until the embryos have been in culture for at least 24 hours. This apparently allows the thin embryonic coverings to harden slightly, so that the eggs do not collapse after incision. The frequent failure of common lizard embryos to hatch in Panigel culture may perhaps be partly due to difficulty in breaking through the hardened membranes. Embryos which hatch overnight are liable to drown in the humid dishes, and this cause of death might be reduced by draining off excess moisture shortly before hatching is expected.

Discussion.

The slow-worm seems to be more highly adapted to viviparity than the common lizard which has been reported to lay eggs under exceptional circumstances in the wild (see Panigel, 1956, for discussion). The eggs of the former species are larger than those of the latter and have thinner coverings so that the embryos remain clearly visible throughout development. Although no well developed placentation has been described in the slow-worm, it seems likely that physiological exchange, perhaps including the transfer of food substances, between mother and embryo, are important. This is indeed, suggested by the abnormalities shown in a large group (B) of our cultured embryos. It is also supported by the fact that Raynaud's culture method, in which a much greater attempt is made to simulate the normal conditions of development, apparently gives better results.

Raynaud (1959 a, b) placed his slow-worm eggs in a series of specially blown glass chambers to support their thin coverings, and perfused them with a special solution containing chick albumen and other substances. He succeeded in raising embryos from "leurs premiers stades" for up to 26 days, while embryos put up around the middle of development survived until they were about ready to hatch. His account suggests that comparatively normal growth was obtained. It is not clear whether ordinary surgical operations could be performed on embryos cultured in this way. Perhaps the much simpler Panigel dish technique may still be useful in certain types of experiment on slow-worm embryos, where only short survival times are required, or where only the later stages are being studied.

Generally speaking, culture methods of various kinds seem likely to be most valuable in the study of problems relating to the later phases of embryonic life. Operations involving the removal and perhaps the grafting of certain organs are feasible. Other methods for producing lesions such as irradiation (which was used by Raynaud, 1960) may yield further

interesting results. The invention of improved culture methods which are not too elaborate is clearly desirable, and a search should be made for other species of viviparous reptiles with embryos amenable to such techniques.

One serious criticism may be levelled at any results based on experiments with cultured embryos. How far can these embryos be regarded as normal, and to what extent do any disturbances of growth to which they may be subject invalidate the experimental data? The answer will clearly depend to a considerable extent on the species of reptile and the method of culture employed. A careful study of the weights and measurements of cultured embryos, microscopic examination of their tissues and a comparison with conditions in normal controls may throw some light on this important problem.

A practical drawback to the use of culture methods is the difficulty of collecting sufficient numbers of pregnant females in the breeding season, which only occurs once a year in temperate climates. Since, however, pregnant common lizards usually contain some five to seven fertile eggs and slow-worms six to twelve or more, it is not necessary to sacrifice very large numbers of them in order to obtain embryos in significant quantities. The danger of exterminating natural populations of these common reptiles does not, therefore, seem very serious, especially if care is taken to collect them from several different localities.

SUMMARY.

The literature on the experimental embryology of reptiles is briefly reviewed. Some experiments on embryos of *Lacerta vivipara* kept in Panigel dish culture, involving tail removal and refrigeration, are described in outline. Results are given for the survival of 66 embryos of *Anguis fragilis* kept in dish culture. A third of the embryos put up around the middle of development survived for long periods (21 to 48 days) but most of them became abnormal in shape or size. Only embryos cultured very late in development were likely to hatch. Our experimental technique is described and problems involved in the use of culture methods are discussed.

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LIVING WITH REPTILES: by KATHLEEN PICKARD-SMITH, Nelson, Edinburgh. Price 18s.

This is an easy-to-read "chatty" type of book, which might encourage those who have never kept reptiles to do so. It is a great pity that more care has not been taken to verify the correct scientific name of the species mentioned.

The description of a vivarium for keeping *Bombina* does not mention the fact that they need water in which to soak. This species is not happy without water, as it spends most of the time sitting in shallow water. The yellow-bellied toad is not *Bombina salsa* but *B. variegata*; also the edible frog is not *Rana edulis* but *R. esculenta*. The *Handbook of Turtles* was written by Dr. A. Carr; Ivor and Audrey Nöel-Hume wrote *Tortoises, Terrapins and Turtles*, published by F, Muller.

It is often written that the painted terrapin has been naturalised in England, but no colony has yet been found; also there is no sexual dimorphism with regard to shell colour in this group. The colour differences are sub-specific and not sex differences. The photographs in this book are excellent. The author has a great fondness for her animals, and this is shown throughout the book. MONICA GREEN.

LEECHES ATTACKING COMMON NEWT

By R. A. LITTON

In the spring and summer of 1961, I visited a pond in this district. The pond is of concrete construction with vertical sides, and has a layer of mud and debris with a little water weed.

This pond abounded in specimens of the common newt (*Triturus vulgaris*) and, out of 20 specimens captured, 18 were infested with leeches. One, which died soon after capture, had no less than 12 leeches upon various parts of its body. As far as could be seen, the leeches merely remained on the newts long enough to appease their hunger, whereupon they dropped off.

From my own observations and those of other naturalists in and around Bradford, this pond would appear to be the only one in which this happening occurs, but perhaps this is only a rare occurrence in and around this area. No other relatively large animal life was found in the pond, so that the leeches may be forced to prey mainly upon the newts.

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THEY SHALL TAKE UP SERPENTS : by Weston La Barre. University of Minnesota Press, Minneapolis. 1962.

A study of the psychology of snake-handling cults in the U.S.A. and elsewhere.

THE GIANT SNAKES : by Clifford H. Pope. Routledge & Kegan Paul Ltd., London, 1962. 289 pp., 25 photographs. Price 50s.

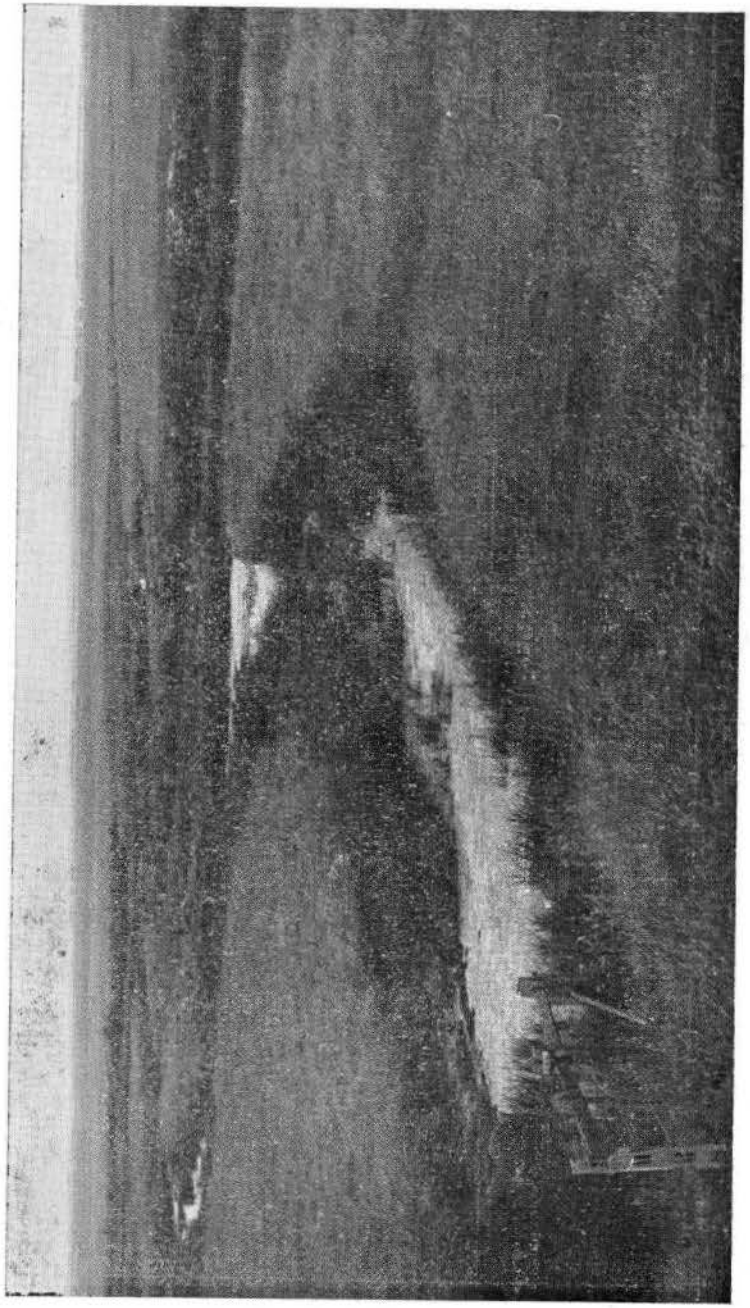
I have heard this author respectfuliy referred to both as "the grand old man of herpetology" and "the ambassador of reptiles". He deserves both titles. All his books can be read with pleasure by both the herpetologist and the layman alike, and it is such a pity that reptiles can't read.

What makes this book different from the author's previous works of a general nature is that it covers a more limited field, dealing as it does with only six species. These are, however, no mean members of the world's herpetofauna, but the six snakes which fully justify the term "giant"—the Anaconda, the Common Boa, and the four largest pythons (African, Indian, Reticulate and Amethystine). Apart from a certain amount of background information applicable to snakes generally, the whole book is devoted to facts and figures directly concerning these six snakes.

The basic method of presentation is to consider in turn various facets of snake life—habitat, senses, feeding, reproduction, growth, parasites and many others—and give in each case a general survey followed by a "breakdown" for each of the six species. Extra chapters under the heading "Relations to Man" follow the same principle as far as it is convenient to do so. This is a very suitable method of compiling a book dealing with such a limited number of subjects, although its extension to the "Special References" might be objected to on the grounds of unnecessary complication and length (the 24 pages they occupy could probably be reduced to about six if all repetition of titles were omitted).

Within the scope of what it has set out to do, this is a most interesting and informative book. It can be very well recommended both to the layman who likes to read something better than fiction and to the expert as a book not only worth reading once, but of permanent value as a work of reference.

J. W. Steward.



Walland Marsh : Jury's Gap Sewer

Menzies Pl. 1

Menzies Pl. 2 Wittersham Levels and Walland Marsh, from Stone Cliff, looking towards Thornsdale.