Studies on the biology of the common frog rana temporaria temporaria (linnaeus) with particular reference to altitude

Robert C. Beattie

STUDIES ON THE BIOLOGY OF THE COMMON FROG *Rana temporaria temporaria* (LINNAEUS) WITH PARTICULAR REFERENCE TO ALTITUDE

by

ROBERT C. BEATTIE, B.Sc. (Dunelm)

..... being a thesis presented in candidature for the degree of Doctor of Philosophy in the University of Durham, 1977.

ACKNOWLEDGMENTS

I should like to express my sincere gratitude to the following people:

My Supervisor, Dr J. C. Coulson, for his constructive criticism throughout the study, and for his thorough reading of the manuscript.

Professor D. Barker for providing excellent research facilities in the Zoology Department, Durham University.

Dr A. H. Seheult and Mr S. K. Cook (Department of Mathematics, Durham University) for their expert computer analysis of the data in section 9.2.5.

Mr M. Rawes and his staff at the Nature Conservancy Council Moor House Field Station for providing meteorological data.

Mr J. Richardson for his assistance in locating breeding ponds and obtaining the right of access over certain key study areas.

Miss E. A. Beattie for checking the manuscript, Mr E. Henderson for photographing the figures, and Mrs R. L. Reed for typing the thesis.

Finally, my special thanks are due to my wife Margaret for her continuous encouragement and support throughout my studies.

The work was carried out whilst in receipt of a grant from the Science Research Council.

ABSTRACT

Aspects of the breeding biology of the Common Frog, *Rana temporaria temporaria* L., were studied in relation to altitude. Work centred on 55 breeding ponds in northern England, ranging in altitude from 86 to 838m. A chemical temperature integration technique was used to measure pond and soil temperatures at a series of sites. The temperature fell by approximately 0.4°C for every 100m increase in altitude. Between 1974 and 1977 spawning was always later at higher altitudes. By delaying spawning, highland embryos were less likely to encounter lethally low temperatures. Highland female frogs were about five per cent smaller in snout-vent length than lowland females and produced 707 eggs on average, less than half the mean number of 1,586 eggs produced by lowland frogs. These differences were thought to be due to the short growing season and possibly to the lack of food at higher altitudes. At 6°C the eggs from highland females developed four per cent faster than those from lowland females. Highland eggs had a lower lethal limit for normal development of 2.8°C; one Celsius degree below the limit for lowland eggs. The mucopolysaccharide capsules covering the eggs of Common Frogs act as insulators, keeping the embryos warmer on average than the surrounding water. This is thought to be of importance as Common Frogs breed early in the year when pond temperatures are often close to the lower lethal limit. The volume of the capsules and their insulating efficiency varied in different pond waters. The concentration of the ions in the pond water used as the culture medium was found to be the major factor influencing capsular swelling, but the valency of the ions, the temperature and pH of the water were also important. These findings were discussed in terms of their adaptive significance.

CONTENTS

CHAPTER 1

Introduction

Physiological variation within a population can arise through genotypic differences produced by gene mutation, chromosome aberration and the recombination of alleles during meiosis. Most organisms can also modify the full expression of a single genotype during the course of development, particularly in response to stressful environmental conditions. Differences produced in this way are phenotypic and, therefore, non-inheritable.

Natural selection will favour those individuals which are equipped with useful genotypic and phenotypic characteristics, and select against individuals with disadvantageous features. Neutral features may also be incorporated into the gene pool, however, especially in a population experiencing few selective pressures. For example, in a population of Tree Frogs from Santo Domingo there are two forms, one having a rhomboidal pupil and the other an oval pupil. Both forms are nocturnal and inhabit the same valley so there would seem to be little reason for the difference (Noble, 1954). It is unwise to assume, therefore, that all the variation within a population is the result of natural selection.

If a particular characteristic is shown to have a selective advantage, it may be said to be adaptive. Adaptation was defined by Prosser (1964) as "any property of an organism which favours survival in a specific environment, particularly a stressful one". An adaptive characteristic permits maintenance of physiological activity and survival when the environment alters with respect to one or more parameters. In this study the term 'adaptive' was restricted to characteristics which complied with Prosser's definition.

It was noted by Darwin (1859) that wide-ranging species usually exhibit more variation than forms having a smaller range. The Common Frog, *Rana temporaria temporaria*, is a northern species with a range extending over most of Europe and palearctic Asia. In parts of northern Scandinavia and Lapland it is found beyond the Arctic Circle, while in southern Europe it becomes an inhabitant of the colder mountainous regions such as the Pyrenees and Italian Alps. As one might expect in such a wide-ranging species there is considerable variation, but only two forms appear to be entitled to varietal distinction. *Rana temporaria parvipalmata* is found in North-West Spain and can be constantly distinguished from *R. t. temporaria* by a shorter web and somewhat more slender form (Boulenger, 1897). *Rana temporaria chensinensis* is the far eastern form, found north of the Yang-Tse-Kiang River in central China. This is distinguished from European *R. t. temporaria* principally by its smaller size (Liu, 1950).

R. t. temporaria is the only subspecies found in Great Britain and Ireland. It occurs in all the counties of England, Wales and Scotland, and breeds at sea level as well as at altitudes above 900m in the Scottish Highlands. In Ireland it is more common on the west than the east coast, and flourishes in the mountainous areas.

In the present study research centred on *R. t. temporaria* from northern England. A climatic gradient exists in this area between the comparatively equable lowland regions and the harsher high moorland areas of the northern Pennines, which are invariably colder (Manley, 1936) and subject to more violent climatic fluctuations (Cragg, 1961). Any rigorous habitat will tend to foster more adaptations than a less selective one, so one might expect to find adaptive differences between highland and lowland populations.

Many poikilothermic animals which inhabit a range of altitudes have specialized adaptations allowing the completion of the life-cycle under different temperature regimes. This was found to be the case in two cranefly species *Tipula subnodicornis* and *Molophilus ater* studied in the northern Pennines by Coulson *et al.* (1976). They were shown to have two adaptations which facilitated the completion of the annual life-cycle at different temperatures. Firstly *M. ater* had an apparently temperature-independent growth rate and, secondly, there was a period of reduced development triggered by short autumn daylength. As only fully grown larvae respond to this change in daylength it allowed larvae developing at lower temperatures to catch up with those that were fully grown. In some species such as the Psyllid, *Strophingia ericae*, the generation time is extended at higher altitudes (Hodkinson, 1973), and in other species such as the Simuliid, *Prosimulium hirtipes*, long egg diapause and rapid larval development facilitates the completion of larval development at low temperatures (Davies and Smith, 1958). Common Frogs in northern England have a wide altitudinal range, and the larvae are subject to different developmental temperatures. In certain cases the larval period in highland areas is extended to two years. Gadow (1901) observed overwintering tadpoles in some of the mountain tarns of North Wales but this is uncommon even in highland *R. temporaria*. It may be that overwintering is associated with iodine deficiency rather than low water temperature (Lynn and Brambel, 1935).

There has been little work done on altitudinal populations of *R. temporaria*. Kozlowska (1971) compared lowland and highland Common Frogs from Poland with respect to female body length, mean number and size of eggs and larval development rate. A similar study was carried out by Aebli (1966) on Common Frogs from the Canton of Glarus, Switzerland. Mullally and Cunningham (1956) studied the ecological relations of the Yellow-legged Frog,

Rana mucosa, in the Sierra Nevada Mountains of California. Ruibal (1955 and 1957) and Volpe (1957) studied altitudinal populations of the Leopard Frog, *Rana pipiens*, and Pettus and Angleton (1967) compared the reproductive biology of highland and lowland Chorus Frogs, *Pseudacris triseriata*, in northern Colorado. The results of these studies are discussed in subsequent chapters.

In the present study several parameters were compared in lowland and highland Common Frogs. Research centred on morphological features and physiological processes which were thought to be of importance in the survival of adult and larval frogs at low temperatures.

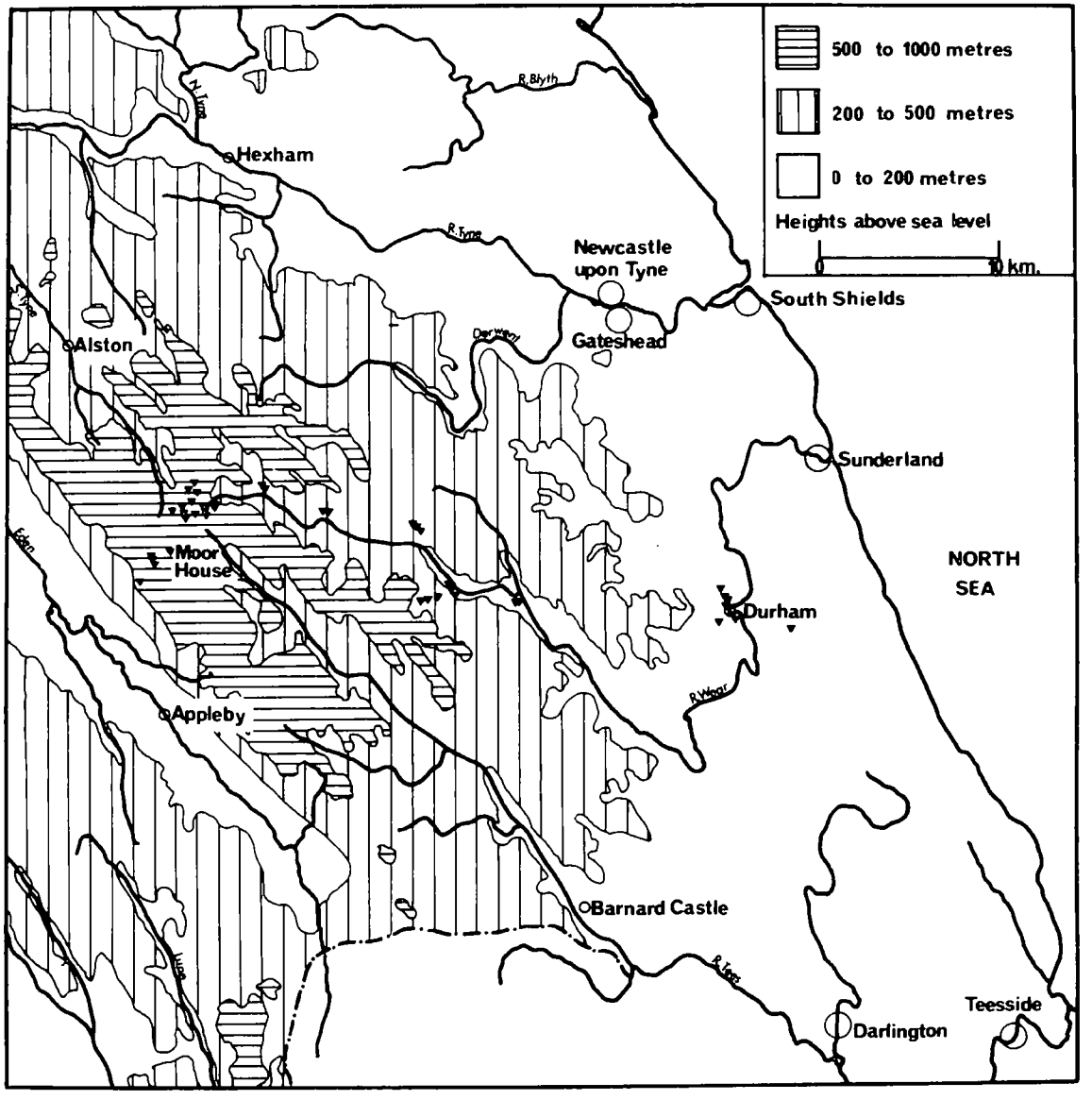
CHAPTER 2

The Study Area

Work centred on 55 breeding ponds in northern England, ranging in altitude from 46 to 838m (Table 1). All the ponds were within a 20km range of latitude, mostly in the Wear Valley to the west of Durham City (Fig. 1). In general the ponds lay on areas of carboniferous limestone, millstone grit and coal measures (Taylor *et al*. 1971). The amount and

Table 1. The location and altitude of the 55 breeding ponds used in this study

Pond No.	Location (Nat. Grid ref.)	Altitude (m)	Pond No.	Location (Nat. Grid ref.)	Altitude (m)
1	NZ 277438	46	28	NY 856410	427
2	NZ 291451	61	29	NY 781435	442
3	NZ 288456	61	30	NY 781434	457
4	NZ 274405	61	31	NY 780433	472
5	NZ 254497	76	32	NY 786434	488
6	NZ 224421	91	33	NY 786434	488
7	NZ 084366	122	34	NY 786434	488
8	NZ 079368	137	35	NY 757422	488
9	NZ 343385	137	36	NY 701297	488
10	NZ 026363	183	37	NY 788437	511
11	NZ 026363	183	38	NY 788437	511
12	NZ 026363	183	39	NY 788437	511
13	NZ 026363	183	40	NY 811433	518
14	NZ 026363	183	41	NY 806451	556
15	NZ 026363	183	42	NY 761328	556
16	NZ 026363	183	43	NY 797447	564
17	NZ 026363	183	44	NY 758327	572
18	NZ 004349	244	45	NY 795445	602
19	NY 992349	305	46	NY 794445	602
20	NY 989348	335	47	NY 794445	602
21	NY 991347	335	48	NY 794445	602
22	NY 977413	335	49	NY 795446	602
23	NY 977413	335	50	NY 800433	610
24	NY 977413	335	51	NY 799433	617
25	NY 913388	373	52	NY 799433	617
26	NY 913388	373	53	NY 717314	747
27	NY 857409	411	54	NY 711318	808
			55	NY 710321	838


500 to 1000 metres
200 to 500 metres
0 to 200 metres
Heights above sea level
0
10 km.
R. Blyth
R. Tyne
Hexham
Newcastle upon Tyne
South Shields
Gateshead
Alston
Sunderland
NORTH SEA
Moor House
Durham
Appleby
Barnard Castle
Teesside
Darlington

composition of the vegetation surrounding the ponds varied, but there was a trend for ponds at altitudes above 400m to be in moorland areas with a substratum of peat, and surrounded by plants such as *Festuca*, *Carex*, *Calluna* and *Sphagnum* spp.

2.1 Variation in Pond Water Chemistry with Altitude

The water chemistry of ponds varied, but it was found that as the altitude of a pond increased, the pH and conductivity of the water in them tended to decrease (Table 2). The conductivity of the pond water at

Table 2. The pH and conductivity of water from ponds of different altitude

(Measurements were taken on 1 April 1976)

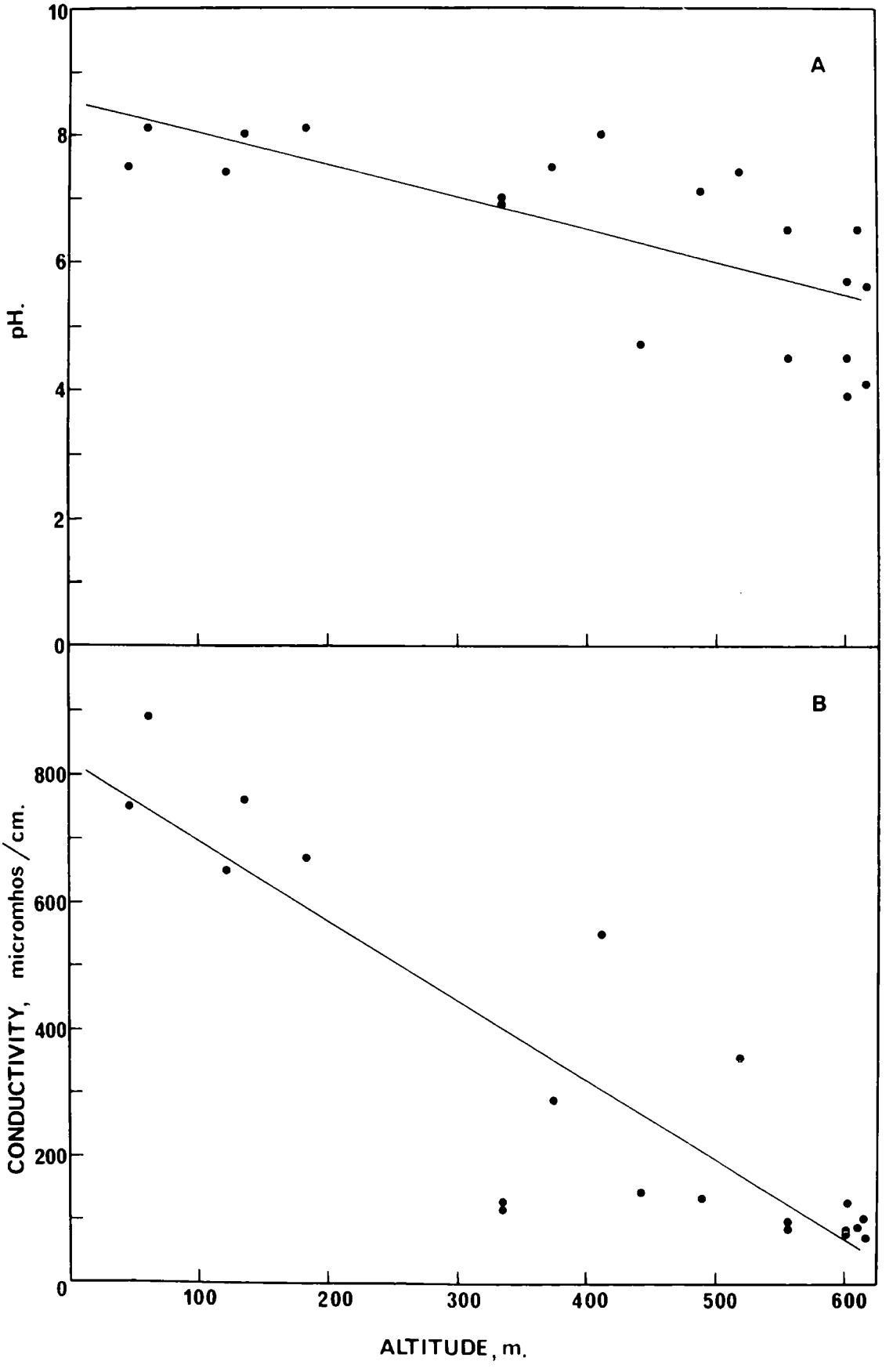
Pond No.	Pond altitude (m)	Conductivity (micromhos/cm at 15°C)	pH
2	61	890	8.1
9	137	760	8.0
1	46	750	7.5
10	183	670	8.1
7	122	650	7.4
27	411	550	8.0
40	518	355	7.4
25	373	290	7.5
29	442	145	4.7
32	488	135	7.1
22	335	130	7.0
45	602	130	3.9
20	335	115	6.9
51	617	105	4.1
41	556	100	4.5
50	610	90	6.5
46	602	87	4.5
47	602	80	5.7
42	556	77	6.5
52	617	75	5.6

$15^{o}C$ measured in micromhos/cm ($\underline{y}$) was related to the altitude of the pond in metres ($\underline{x}$) by the following relationship: $y = -1.26(\pm 0.15)x + 824.8$.

The coefficient of correlation of regression, $r = -0.89$, was significantly different from zero, $t = 8.21$, d.f. = 18, $P < 0.001$ (Fig. 2B). Conductivity is a measure of the water's ability to convey an electric current and is related to the concentration of the ions in the water. As pond water conductivity decreased with altitude, highland pond water must have a comparatively low ionic concentration.

Inorganic ions mainly enter a pond by the weathering of surrounding rocks combined with the leaching of local soils. Input also occurs through aerial precipitations including dust, wind transported minerals, soil particles and ions in aerosol form derived from the sea. The relative importance of these sources of ions varies with hydrology, geology and factors affecting the aerial transport of ions, such as proximity to the sea (Moore and Bellamy, 1974). The low level of ions in highland ponds probably results from the high percentage input of rainwater, which contains few inorganic ions (Crisp, 1966; Gore, 1968), compared with the water seeping into a pond. Furthermore, the seepage water entering a highland pond usually percolates through peat which releases fewer inorganic ions than carboniferous limestone, which is the main substratum of lowland ponds.

The pH of the pond water ($\underline{y}$) was related to the altitude of the pond in metres ($\underline{x}$) by the following relationship: $y = -0.005(\pm 0.001)x + 8.54$. The coefficient of correlation of regression, $r = -0.72$, was significantly different from zero, $t = 4.45$, d.f. = 18, $P < 0.001$ (Fig. 2A). The acidic conditions prevailing in highland ponds are probably the result of organic acids leaching into the ponds from the surrounding peat and the selective take up of cations by *Sphagnum* (Clymo, 1964). Highland ponds tend to have a poor water flow and few bicarbonate ions, the only relatively strong base found in natural waters (Clarke, 1930), so hydrogen ions are neither neutralized or flushed away. They tend to accumulate, weakly conjugated


A
B
pH.
CONDUCTIVITY, micromhos / cm.
ALTITUDE, m.
0
2
4
6
8
10
200
400
600
800
100
200
300
400
500
600

with whatever anions are being supplied to the system. In lowland ponds, hydrogen ions combine with bicarbonate ions to form carbon dioxide and water and/or will be flushed out of the system.

CHAPTER 3

The Measurement of Soil and Pond Temperatures

3.1 Introduction

The climate of the Moor House Nature Reserve has been described by Manley (1936) as sub-arctic, having many features comparable to those at sea-level in southern Iceland. Manley compared air temperatures recorded in standard Stevenson Screens at Moor House (altitude 561m) with average temperatures from four northern lowland stations at Newton Rigg (altitude 170m), Appleby (altitude 134m), Houghall (altitude 49m) and Durham (altitude 102m). On average, Moor House was found to be about 3.1°C colder than the lowland sites. The mean difference varied over the temperature range, the mean maximum at Moor House being about 3.9°C lower, and the mean minimum about 1.8°C lower. The typical pattern can be seen in the data taken from the 1975 Meteorological Station records for Durham and Moor House (Table 3). As expected from Manley's work, Moor House was colder than Durham throughout the year (Fig. 3), the greatest difference in temperature occurring during August.

Using a sucrose inversion technique, Coulson *et al.* (1976) measured soil temperatures (1cm deep) at a series of sites on the Moor House Nature Reserve, ranging in altitude from 370 to 820m. There was a progressive decrease in temperature with increase in altitude (1°C per 207m), temperature differences between sites being present throughout the year. The highest temperature difference between sites occurred in autumn and the lowest in winter and spring.

In the present study two techniques were employed to measure winter soil and pond temperatures prior to spawning, and pond temperatures during embryonic development. These techniques are described overleaf.

Table 3. Mean daily air temperatures for each month in 1975 from the Durham and Moor House Meteorological Station records

	½ (Max + Min)		Mean Daily Maximum		Mean Daily Minimum	
Month	Durham (102m)	Moor House (558m)	Durham	Moor House	Durham	Moor House
Jan	5.6	1.9	8.8	4.0	2.4	-0.3
Feb	3.5	1.5	6.3	3.5	0.7	-0.4
Mar	4.1	1.3	7.6	4.1	0.6	-1.6
Apr	7.6	3.5	11.5	8.9	3.7	-1.9
May	7.9	6.5	12.2	10.8	3.7	2.2
Jun	13.1	8.5	18.6	12.8	7.6	4.2
Jul	15.9	10.3	20.1	13.4	11.7	7.1
Aug	17.6	10.5	22.8	13.8	12.3	7.2
Sep	11.9	7.5	16.0	10.0	7.8	4.9
Oct	8.8	3.3	12.5	5.7	5.1	1.0
Nov	5.4	2.7	8.7	4.9	2.1	0.6
Dec	5.4	3.3	8.4	5.7	2.4	1.0
Mean	8.9	5.1	12.8	8.1	5.0	2.0

The difference in mean daily air temperature $\pm$ 1S.E. between Durham and Moor House, by the method of paired comparisons:

½ (Max + Min) = 3.8 ± 0.49, $t = 7.82$, d.f. = 11, $P < 0.001$

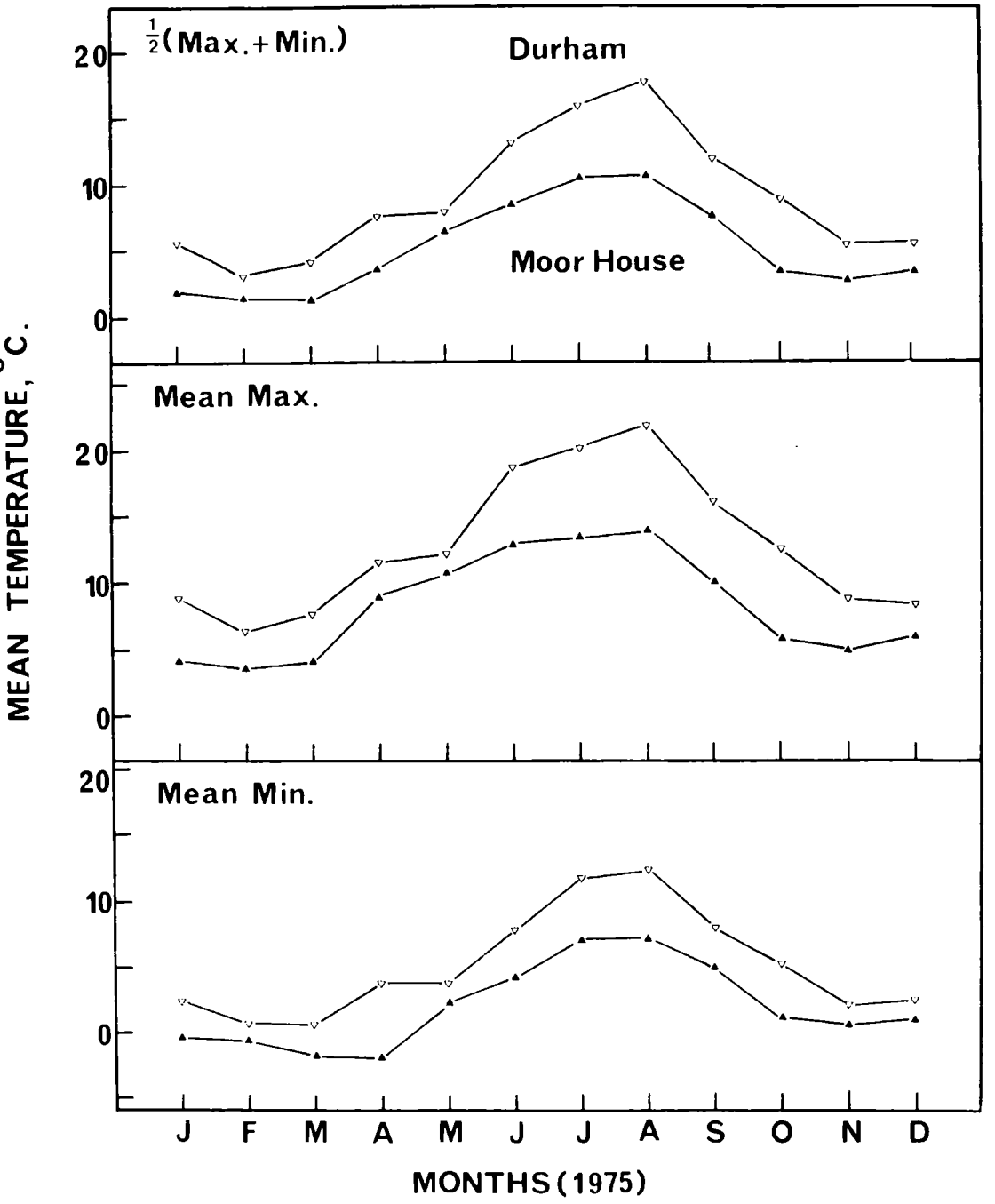
Mean Max = 4.7 ± 0.65, $t = 7.17$, d.f. = 11, $P < 0.001$

Mean Min = 3.0 ± 0.45, $t = 6.69$, d.f. = 11, $P < 0.001$

3.2 Methods

3.2.1 Cambridge Thermograph Recorders

Two Cambridge thermographs were used during the present study. One was positioned near pond (4) in the Durham area at an altitude of 61m,


½(Max.+Min.)
Durham
Moor House
Mean Max.
Mean Min.
20
10
0
MEAN TEMPERATURE, °C.
J F M A M J J A S O N D
MONTHS (1975)

and the other near pond (42) on the Moor House Nature Reserve at an altitude of 556m. From 1 January to the time of spawning the thermographs were used to measure soil temperatures at a depth of 5cm. As soon as spawning occurred, the sensing bulb of the recorder was transferred to the pond and positioned in the middle of a spawn mass at a depth of 10cm, giving information on the temperature experienced by the eggs during development. The two recorders were frequently checked for accuracy using a mercury in glass thermometer placed adjacent to the sensing bulb of the recorder.

3.2.2 Chemical Temperature Integration

Integrated temperature records were obtained using a sucrose inversion technique introduced by Pallmann *et al.*(1940). This uses the temperature-dependent hydrolysis of sucrose as the sensing reaction:

sucrose + water → fructose + glucose

The rate of this irreversible reaction is proportional to temperature and hydrogen ion concentration. As all the sugars are optically active in solution, their effective concentration at any time during the reaction can be determined polarimetrically. Thus if the pH is constant and the amount of inversion is known, the integrated temperature experienced by the solution can be calculated.

Lee (1969) investigated the accuracy of this method and concluded that the probable overall precision was $\pm$ 0.1°C. The main inaccuracy of this method is the exponential relationship between sucrose hydrolysis and the temperature of the solution. This means that the chemically derived 'exponential' mean temperature (T_e) consistently overestimates the arithmetic mean temperature (T_a). Coulson *et al.* (1976) showed that the relationship between T_e and T_a over the temperature range 0 to 16°C could, nevertheless,

be adequately described by the straight line equation, $y = +0.87(\pm 0.03)x - 0.04$, where ($\underline{y}$) is the arithmetic mean and ($\underline{x}$) is the exponential mean. Pallmann and Frei (1943) found that the temperature range experienced by the inversion solution was most important, and where this was less than $5^{\circ}C$ the values of T_e and T_a were virtually the same. In the present study 'sucrose' tubes were used to measure winter soil and pond temperatures at a series of sites, together with two Cambridge thermographs. The maximum daily temperature range recorded by the thermographs was $3.6^{\circ}C$, so exponential and arithmetic mean temperatures were assumed to be equal.

3.2.2.1 Practical details

The 'Slow Inversion Solution' described by Berthet (1960) was used. The maximum change in the optical rotation of this sucrose solution is about 60°, but in practice it is desirable to utilize only half of the total range (Lee, 1969) so that maximum rotation does not occur in the field. When all the sucrose has hydrolysed to fructose and glucose, the solution ceases to reflect changes in environmental temperature. The relationship between the time for half inversion, temperature and pH was described graphically by Berthet (1960). This figure was used to select the pH of each batch of solution so that approximately half of the sucrose would convert to fructose and glucose during the time in the field.

Approximately 15ml of the inversion solution was placed inside each of a series of 55mm x 25mm specimen tubes with polythene stoppers. These tubes were then immediately frozen at around $-25^{\circ}C$, in which state inversion was negligible. They were transported to and from the field in vacuum flasks containing dry ice.

Three of the tubes were used to determine the initial rotation of the sucrose solution before inversion (a_o). A tube was placed in each of three constant temperature rooms at 5°, 10° and $20^{\circ}C$ to permit the

calculation of the constant C_1. The rotation angle of the solution was measured using a polarimeter manufactured by Bellingham and Stanley. Care was taken to warm up each tube to room temperature before taking a reading, because Lawton (1969) found that thawed but cold solutions showed a rotation which was as much as one degree smaller than their value after reaching room temperature. For each tube two readings were taken and the mean calculated. A specimen calculation is shown in the appendix.

3.3 Results

3.3.1 Soil and pond temperatures prior to spawning

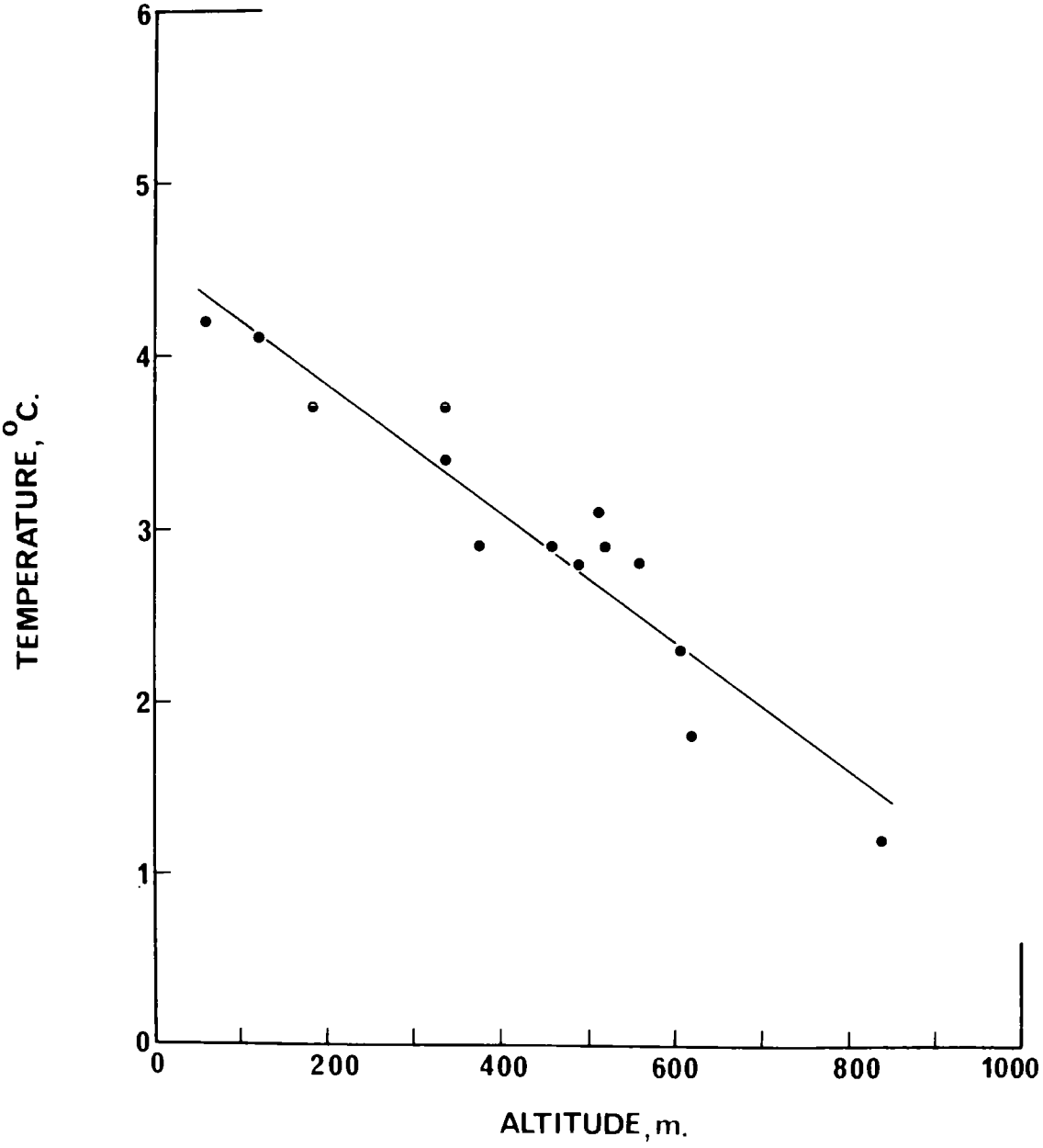
Mean soil temperatures at a depth of 5cm and pond temperatures at a depth of 10cm were measured at 14 different altitudes, using a sucrose inversion technique (Tables 4 and 5). The sucrose tubes were placed in the field on 9 November 1974 and removed on 1 March 1975. Soil temperature in degrees Celsius ($\underline{y}$) was found to be related to the altitude of the pond in metres ($\underline{x}$) by the following relationship: $y = -0.0037(\pm 0.0003)x + 4.57$. The coefficient of correlation of regression, $r = -0.95$, was significantly different from zero, $t = 10.34$, d.f. = 12, $P < 0.001$ (Fig. 4). Pond temperature in degrees Celsius ($\underline{y}$) was related to the altitude of the pond in metres ($\underline{x}$) by the following relationship: $y = -0.0037(\pm 0.0004)x + 5.21$. The coefficient of correlation of regression, $r = -0.94$, was significantly different from zero, $t = 9.24$, d.f. = 12, $P < 0.001$ (Fig. 5). As one might expect, mean soil and pond temperatures were closely correlated with altitude. The temperature fell in both cases by $1^{o}C$ for every 270m increase in altitude.

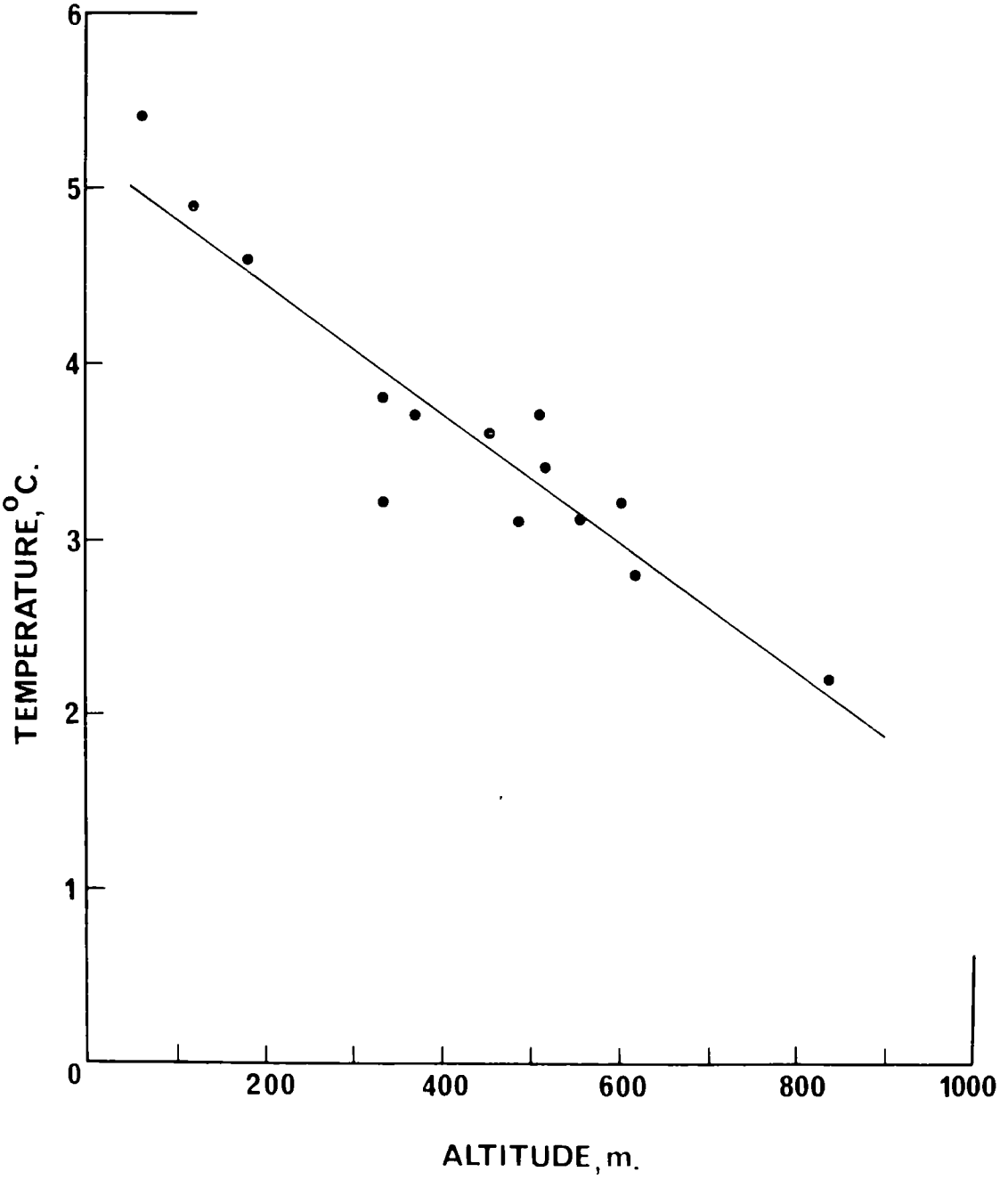
Table 4. Mean soil temperatures measured at 14 different altitudes at a depth of 5cm, using a sucrose inversion technique. The tubes were placed in the field on 9 November 1974 and removed on 1 March 1975

Altitude (m)	Soil temperature (°C)
61	4.2
122	4.1
183	3.7
335	3.4
335	3.7
373	2.9
457	2.9
488	2.8
511	3.1
518	2.9
556	2.8
602	2.3
617	1.8
838	1.2

Table 5. Mean pond temperatures measured at 14 different altitudes at a depth of 10cm, using a sucrose inversion technique. The tubes were placed in the field on 9 November 1974 and removed on 1 March 1975

Pond no.	Pond altitude (m)	Pond temperature (°C)
2	61	5.4
7	122	4.9
11	183	4.6
20	335	3.2
24	335	3.8
26	373	3.7
30	457	3.6
32	488	3.1
37	511	3.7
40	518	3.4
42	556	3.1
46	602	3.2
52	617	2.8
55	838	2.2


TEMPERATURE, °C.
6
5
4
3
2
1
0
0
200
400
600
800
1000
ALTITUDE, m.

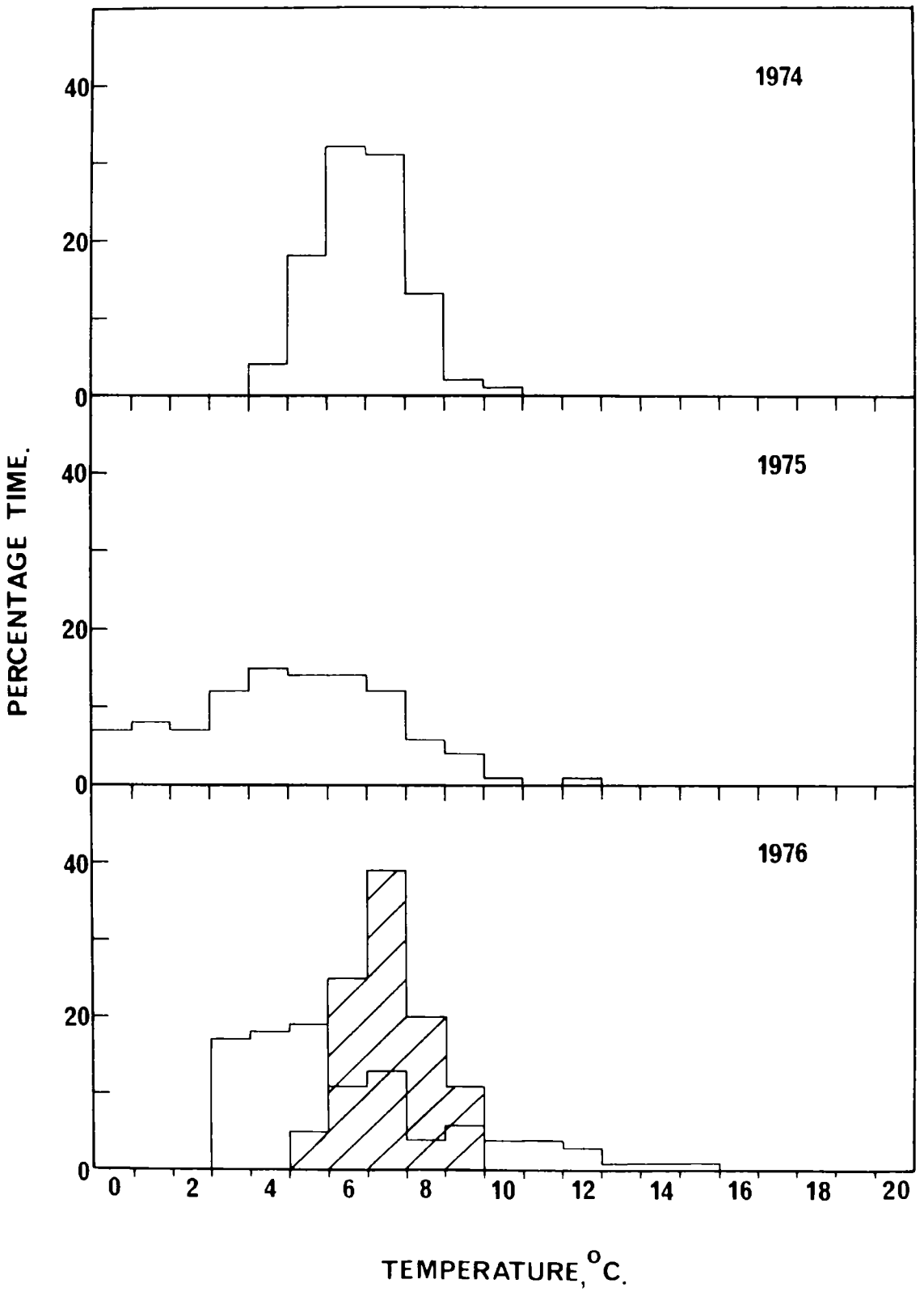

6
5
4
3
2
1
0
TEMPERATURE, °C.
200
400
600
800
1000
ALTITUDE, m.

3.3.2 Pond temperatures during development

In 1976 two thermographs were used to measure the temperature in lowland pond (2) which was in the Durham area, and highland pond (42) at Moor House. The sensing bulb of the recorder was positioned in the middle of a spawn mass, at a depth of 10cm. Spawning occurred in the Durham pond on 29 March, and in the Moor House pond on 6 April. The mean temperature of the eggs in the Moor House pond between spawning and hatching was 6.0°C, 1.1°C colder than the eggs in the Durham pond. Table 6 gives the percentage time that the eggs in the two ponds were exposed to a particular temperature. The eggs in the Durham pond were at or below 5°C

Table 6. The percentage time between spawning and hatching, eggs in lowland pond (4) and highland pond (42), were exposed to a particular temperature. Percentages were calculated from hourly temperature values taken from Cambridge Thermograph recordings

Temperature (°C)	The percentage time at a particular temperature			
	1976		1975	1974
	Pond (4)	Pond (42)	Pond (42)	Pond (42)
0	0	0	7	0
1	0	0	8	0
2	0	0	7	0
3	0	17	12	0
4	0	18	15	4
5	5	19	14	18
6	25	11	14	32
7	39	13	12	31
8	20	4	6	13
9	11	6	4	2
10	0	4	1	1
11	0	4	0	0
12	0	3	1	0
13	0	1	0	0
14	0	1	0	0
15	0	1	0	0


1974
1975
1976
40
20
0
PERCENTAGE TIME.
0
2
4
6
8
10
12
14
16
18
20
TEMPERATURE, °C.

for five per cent of the time between spawning and hatching, the minimum temperature being 4.5°C (Fig. 6). In the Moor House pond the temperature of the eggs was at or below 5°C for 54 per cent of the time, the minimum temperature being 2.9°C.

The low temperatures recorded in the Moor House pond during 1976 were by no means unusual. From Table 6 it would appear that the eggs in this pond were at or below 5°C for 22 per cent of the time in 1974, and 63 per cent of the time in 1975.

The maximum egg mass temperature recorded in these two ponds during the three year period was 15°C. High temperatures are seldom recorded in northern England during early spring. During the period 1966 to 1976 the maximum air temperature recorded at Durham during March (the month during which most lowland embryos develop) and at Moor House during April (the month during which most highland embryos develop) was 17.2°C in both cases (Table 7).

3.4 Discussion

Highland frogs spawn later in the year, which tends to compensate for the lower temperatures at higher altitudes. As a result there is probably little difference in the mean developmental temperatures experienced by the eggs in different ponds. Thermograph and Meteorological Station data suggest, however, that eggs in highland ponds are particularly subject to low temperatures.

Several authors have estimated the minimum temperature for the normal development of *R. temporaria* eggs (Table 18), which appears to be between 4° and 5°C in most cases. Douglas (1948) reported that English embryos could develop for some time at 3.3°C, and were able to tolerate at least 12 hours at 0°C. During 1975 the eggs in Moor House pond (42) were subjected to temperatures below 3.3°C for a continuous period of 103 hours,

Table 7. Mean maximum and minimum daily air temperatures recorded during March at a lowland site (Durham Meteorological Station, altitude 102m), and during April at a highland site (Moor House Meteorological Station, altitude 561m), over a 10 year period. Absolute maximum temperatures are also shown.

Year	½(Max + Min) (°C)		Mean Max (°C)		Mean Min (°C)		Absolute Max (°C)	
	Durham (March)	M. House (April)	Durham	M.House	Durham	M.House	Durham	M.House
1975	4.1	3.8	7.6	7.1	0.6	0.5	10.7	16.0
1974	4.6	3.5	7.7	8.9	1.6	-1.9	13.2	14.8
1973	6.5	2.0	10.7	5.4	2.3	-1.4	17.2*	12.9
1972	5.3	3.8	8.8	7.2	1.9	0.4	15.5	10.6
1971	5.0	3.5	8.2	7.3	1.8	-0.2	11.4	14.7
1970	3.2	1.3	7.4	3.9	-0.9	-1.2	12.8	10.0
1969	2.1	2.2	4.9	5.9	-0.6	-1.5	11.7	14.4
1968	6.2	3.7	9.9	7.9	2.2	-0.6	17.2*	13.9
1967	6.5	3.5	9.6	6.9	3.4	0.1	14.4	17.2*
1966	6.2	1.5	10.2	3.6	2.1	-0.7	13.9	-
Mean	5.0	2.9	8.5	6.4	1.4	-0.7		

The difference in mean daily air temperatures $\pm$ 1 S.E., between Durham and Moor House, by the method of paired comparisons:

½(Max + Min) = 2.1 $\pm$ 0.51, t = 4.11, d.f. = 9, $P < 0.01$

Mean Max = 2.1 $\pm$ 0.80, t = 2.61, d.f. = 9, $P < 0.05$

Mean Min = 2.1 $\pm$ 0.42, t = 4.96, d.f. = 9, $P < 0.001$

*The maximum air temperature recorded during March at Durham and April at Moor House was 17.2°C.

and temperatures below 0.5°C for 36 hours. These data recorded at Moor House during April 1975 were not atypical, in fact the April of 1975 was one of the warmest recorded at Moor House in a decade (Table 7). It was not uncommon for the eggs on top of a spawn mass to be frozen and when these eggs were returned to the laboratory and cultured at 10°C they failed to develop.

The maximum temperature for the normal development of English *R. temporaria* embryos was estimated by Douglas (1948) to be between 24° and 25°C. The March and April max. air temperatures at Durham and Moor House respectively have never exceeded 17.2°C over the last ten years (Table 7), so the likelihood of pond temperatures in northern England exceeding the maximum developmental temperature seems remote.

Low environmental temperature seems to be a selective force acting on the eggs of northern frogs, particularly those in highland ponds. The possibility that Common Frogs have adapted in response to this pressure was investigated and the results discussed in the following chapters.

CHAPTER 4

Emergence from Hibernation and Spawning

4.1 Introduction

Considerable evidence suggests that temperature plays a dominant role in the regulation of Anuran reproductive cycles. The breeding season is often delayed in populations from more northern latitudes or higher altitudes. *Rana sylvatica*, for example, breeds in January and February in the southern states of America (Martof and Humphries, 1959), but spawning is delayed until late April and May in Alaska, and June in some parts of Canada (Herreid and Kinney, 1967). Breeding in *R. temporaria* can take place from January in Brittany (Juszczyk and Zamachowski, 1965) until the end of May in Finland (Koskela, 1973), and as late as July in the Alps at an altitude of 2,231m (Terentiev, 1950). At high elevations in the Sierra-Nevada of central California (2,200 to 2,612m), the breeding season of *Hyla regilla* extends into the middle of July (Livezey, 1953), while the breeding season at lower elevations normally ends in mid-May.

The external trigger for breeding activity in the Amphibia may be a complex interaction involving a number of factors, but temperature and rainfall appear to be of particular importance (Gallien, 1959). Balinsky (1969), for example, found a highly significant correlation between spawning and rainfall in most of the twelve species of frogs and toads studied near Johannesburg. The emergence of the Australian Burrowing Frog, *Limnodynastes dorsalis* depends on both soil moisture and temperature (Martin, 1969). It was discovered that as long as the soil moisture reached some critical but undetermined level, the exact time of emergence depended on the soil temperature exceeding 12.5^{o}C. It was shown by Heusser (1968) that rainfall, temperature and twilight acted complementary to internal factors in

initiating migration in the Common Toad, *Bufo bufo*. The emergence of the Cricket Frog, *Acris crepitans*, and the Green Frog, *Rana clamitans*, is associated with the attainment of a minimum air temperature of 10°C (Brenner, 1969), and spawning in *Rana aurora* occurs when the water temperature rises above about 7°C (Licht, 1969). Temperature also appears to be involved in the onset of breeding in the Australian Burrowing Frog, *Pseudophryne corroboree* (Pengilley, 1973).

The present study was particularly concerned with emergence from hibernation and spawning in *R. temporaria*. The gonads of the Common Frog develop during hibernation and are capable of producing gametes very early in the spring, if the right stimulus is provided. It has been firmly established that the condition of the gonads in Amphibia is controlled by gonadotrophins, secreted by the anterior lobe of the pituitary (van Oordt and van Oordt, 1955; Smith, 1955). Thus *R. temporaria* can be induced to breed earlier than normal by the administration of anterior pituitary substances or gonadotrophic hormones. In a series of experiments van Oordt (1956) showed that gonadotrophic hormone secretion was determined by environmental temperature, the secretory activity being low when the temperature was low, and *vice versa*.

There is some evidence to suggest that the stimulus causing frogs to emerge from hibernation is different from the stimulus that initiates breeding. In some years, for example, Common Frogs breed as soon as they arrive at the pond, but in other years frogs are found in the ponds several weeks before they breed (Savage, 1961). In England there is often a lengthy delay between emergence and spawning in the Edible Frog, *Rana esculenta*, and the Marsh Frog, *Rana ridibunda* (Smith, 1969).

The timing of emergence in the Common Frog was thought by Juszczyk and Zamachowski (1965) to depend on a temperature-independent 'physiological clock'. *R. temporaria* from Poland were kept in aquaria containing tap water,

from 1 November to the middle of March. The temperature of the water was maintained between 0° and 1°C by placing the aquaria in a refrigerator, the interior of which was completely dark. During this period the frogs remained motionless and gave the impression of being dead. In the middle of March their behaviour suddenly changed, they became active and reacted violently to weak stimuli such as light or noise. Some pairs went into amplexus even though the temperature remained between 0° and 1°C. Heusser and Ott (1968) found that migration in the Common Toad, *Bufo bufo*, was under the control of an intrinsic temperature-independent rhythm, which acted in conjunction with extrinsic factors, temperature and rainfall. The set time for migration was specifically determined by belonging to a certain population. When two different toad populations were hibernating in the same area, those toads belonging to the cooler pond migrated later than the population belonging to the warmer pond.

Low temperatures can delay ovulation and spermatogenesis in the Common Frog, presumably by retarding the secretion of gonadotrophins from the pituitary gland. Juszczyk and Zamachowski (1965) observed that female frogs kept at temperatures between 1° and 3°C during hibernation ovulated at the end of March and the beginning of April, the same time as females in the wild. Frogs kept at temperatures between 0° and 1°C, however, did not ovulate until the middle of April when the water temperature rose from 1° to 2°C. Experimental males which had been kept at temperatures below 5°C during hibernation were found to have no spermatozoa in the seminal vesicles or bladder when they were examined during the breeding season. As soon as these males were exposed for even a few hours to a water temperature of approximately 10°C, they accumulated large quantities of mature spermatozoa.

Spawning in *R. temporaria* can be triggered by a rise in temperature. In the experiments conducted by Juszczyk and Zamachowski (1965), pairing and calling in the males occurred when the water temperature rose to 7°C, and

spawning occurred when the temperature of the water reached 11° to 15°C. Similarly, Smith (1969) observed that frogs caught in Cornwall and brought back to London laboratories in January would spawn almost at once in a warm tank, while others in an adjacent tank under conditions identical save for colder water would not. Allison (1956) has shown, however, that *R. temporaria* will still spawn even when kept at a consistently low temperature. A group of Common Frogs from England were kept in a refrigerator at 3.3°C from late September onwards. Spawning took place from the middle of May until early July in spite of the low water temperature. This suggests that spawning can be delayed by low temperatures, but not prevented.

Savage (1961) found that the date of spawning in *R. temporaria* was significantly correlated with temperature and rainfall several months before spawning. He concluded that spawning was controlled by specific olfactory stimuli provided by certain chemical products of the algal flora in the ponds. In experiments with *Xenopus laevis* (Savage, 1965, 1971) it was established that a water soluble substance associated with the algae, probably an algal metabolite, triggered spawning. There is no direct evidence, however, to suggest that algal metabolites trigger spawning in Common Frogs under natural conditions, and Common Frogs kept in the laboratory will spawn in tap water with little or no variation in algal metabolites (Allison, 1956).

Research in the present study centred on the effect of temperature on the emergence from hibernation and spawning of Common Frogs from northern England.

4.2 Methods

Ponds in the Wear Valley, ranging in altitude from 46 to 838m, were visited regularly during the early spring of 1974, 1975, 1976 and 1977, a record being taken of the first appearance of spawn in each pond. Pond and soil temperatures were measured by the methods outlined in Chapter 3.

4.3 Results

In the four years of study, spawning was always later at higher altitudes. The difference between years was examined statistically in Table 8. In general, there was little annual variation in the date of spawning, only in 1974 was the date of spawning significantly different from other years. This consistency occurred in spite of fairly large annual differences in temperature, several months before spawning (Table 9).

Each pond appears to have its own characteristic spawning period. Spawning in pond (7), for example, was always much later than would have been predicted from the altitude of the pond (Fig. 7), or the temperature of its environs (Fig. 8). Similarly, spawning in pond (15) was always earlier than expected.

It is the lower winter temperatures at higher altitudes, presumably, that cause the delay in spawning (Table 10). The date of spawning in days after 1 January 1975 ($\underline{y}$) was related to the soil temperature in degrees Celsius, between 9 November 1974 and 1 March 1975 ($\underline{x}$) by the following relationship: $y = -17.6(\pm 3.25)x + 141.7$. The coefficient of correlation of regression, $r = -0.82$, was significantly different from zero, $t = 5.40$, d.f. = 14, $P < 0.001$ (Fig. 8).

Temperature obviously has an effect, but it is difficult to imagine how it could act as an accurate trigger for synchronous emergence and spawning. Frogs hibernate in a multitude of different hibernaculae all at different temperatures. To give some indication of the variation in temperature a population of hibernating frogs might experience, 'sucrose' tubes were buried in the soil at a depth of 5cm, and left there from 1 November 1974 to 15 April 1975. They were arranged in four lines radiating north, south, east and west from a pond on the Moor House Nature Reserve. There were four tubes in each line, 50m apart. A difference of 145 day-

Table 8. The date of spawning in 14 different ponds ranging in altitude from 61 to 838m, over a four year period. The spawn date is expressed as the number of days from the first of January

Pond No.	Pond altitude (m)	Spawn Date			
		1974	1975	1976	1977
2	61	64	62	63	63
7	122	86	82	87	72
15	183	59	62	63	63
18	244	72	70	79	72
20	335	78	77	93	72
22	335	72	85	79	79
25	373	74	85	87	79
27	411	78	66	87	79
32	488	89	97	97	83
37	511	89	97	97	87
40	518	86	107	104	107
46	602	92	107	100	107
52	617	89	107	104	107
55	838	101	114	110	114

The mean difference $\pm$ 1 S.E. between the spawn dates in four successive years, and the values of 't' computed by the method of paired comparisons are shown below (each value of 't' has 13 degrees of freedom):

	1974	1977	1975	1976
1974	-	3.93 ± 2.68, t = 1.47, P > 0.05 insignificant	6.36 ± 2.56, t = 2.49, P < 0.05 significant	8.64 ± 1.42, t = 6.08, P < 0.001 significant
1977	-	-	2.43 ± 1.79, t = 1.35, P > 0.05 insignificant	4.71 ± 2.24, t = 2.12, P > 0.05 insignificant
1975	-	-	-	2.29 ± 2.17, t = 1.06, P > 0.05 insignificant
1976	-	-	-	-

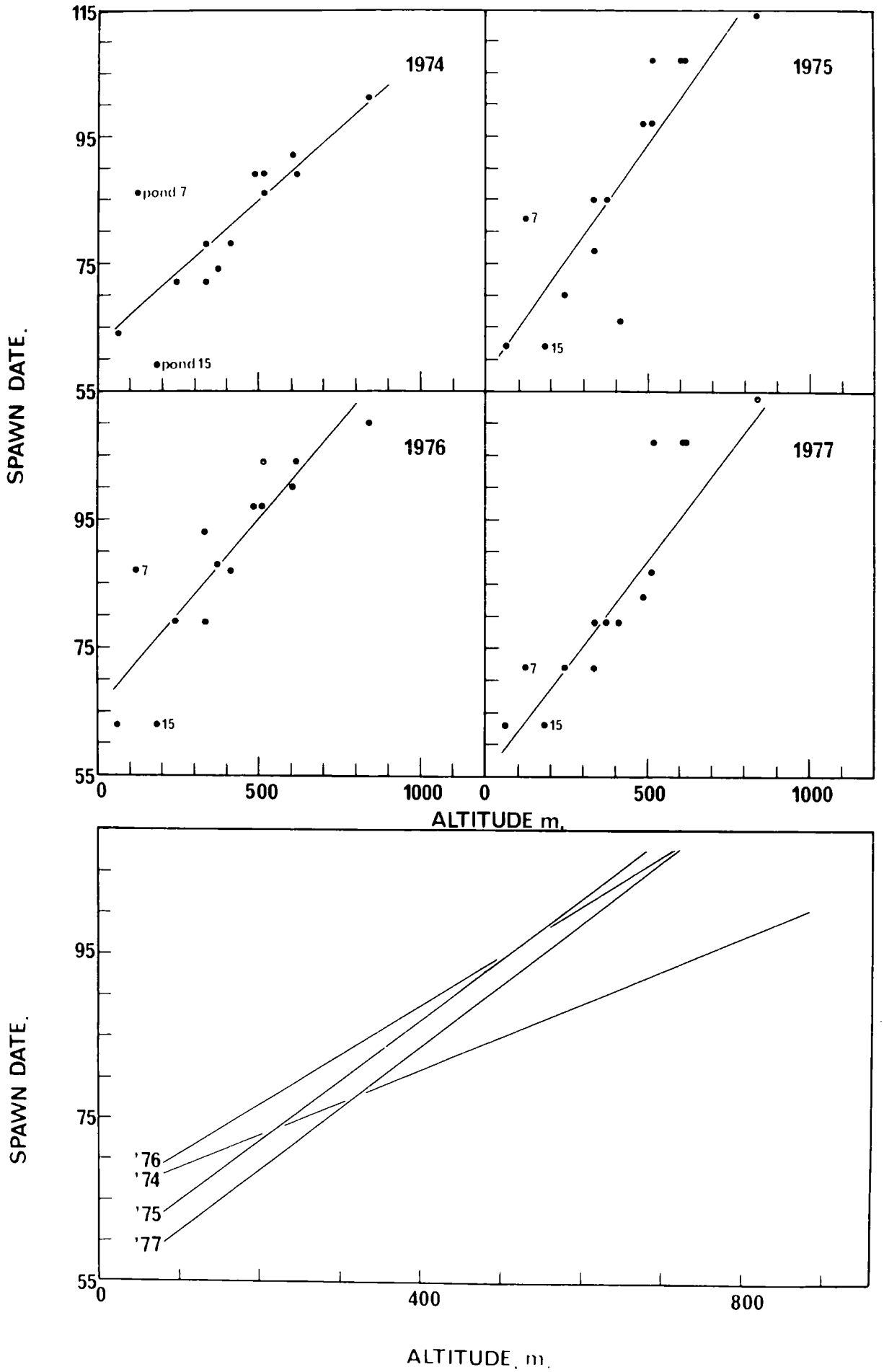
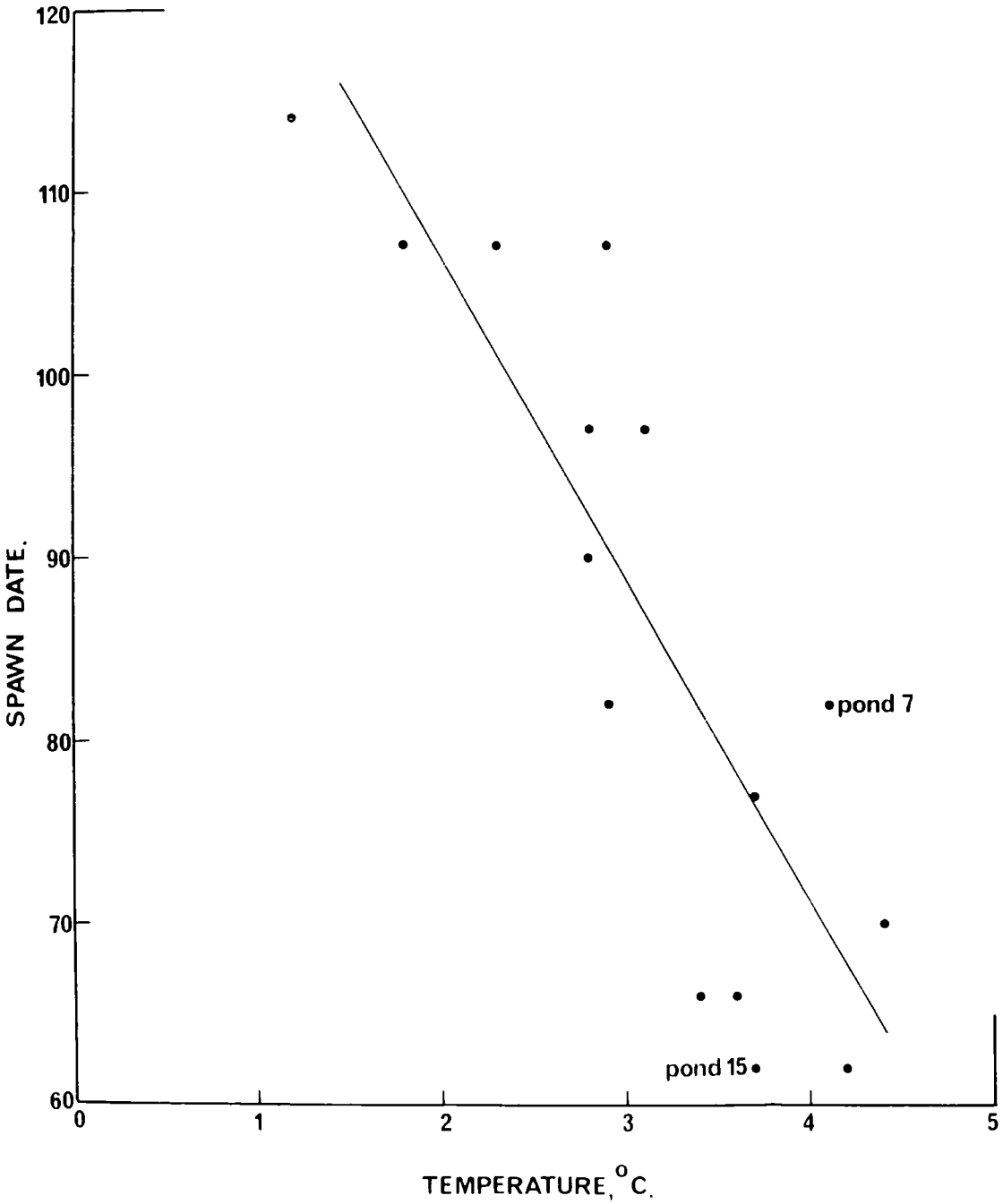

SPAWN DATE.
115
95
75
55
1974
1975
1976
1977
•pond 7
•pond 15
•7
•15
0
500
1000
ALTITUDE m.
SPAWN DATE.
95
75
55
'76
'74
'75
'77
0
400
800
ALTITUDE, m.

Table 9. Mean daily air temperature recorded in four successive years from the beginning of January to the end of February at the Durham Meteorological Station (altitude 192m) and from the beginning of January to the end of March at the Moor House Meteorological Station (altitude 558m)

	Temperature (°C)			
	1974	1975	1976	1977
Moor House	1.6	1.4	0.7	0.1
Durham	4.7	4.6	4.4	2.7
Mean	3.2	3.0	2.6	1.4

Table 10. The date of spawning in 16 different ponds ranging in altitude from 61 to 838m in relation to mean soil temperatures measured between 9 November 1974 and 1 March 1975. Temperatures 3m away from each of the 16 ponds were measured using 'sucrose' tubes buried in the soil at a depth of 5cm

Pond no.	Pond altitude (m)	Integrated soil temperature (°C)	Spawn date (Days after 1 Jan 1975)
15	183	3.7	62
2	61	4.2	62
24	335	3.4	66
27	411	3.6	66
18	244	4.4	70
20	335	3.7	77
7	122	4.1	82
26	373	2.9	82
42	556	2.8	90
32	488	2.8	97
37	511	3.1	97
30	457	2.9	107
40	518	2.9	107
46	602	2.3	107
52	617	1.8	107
55	838	1.2	114


120
110
100
90
80
70
60
SPAWN DATE.
0
1
2
3
4
5
TEMPERATURE, °C.
pond 7
pond 15

degrees was found between two tubes only 150m apart (Table 11), which suggests that a temperature-dependent 'clock' running for the full period of hibernation would not be accurate enough to give the synchronised emergence and spawning observed in nature. A temperature-dependent 'clock', however, may be sufficiently accurate over a shorter time period.

Table 11. Mean soil temperatures measured with 'sucrose' tubes between 10 November 1974 and 15 April 1975 (156 days), expressed as the number of day-degrees. The tubes were 50m apart

					North					
					435					
					441*					
					370					
					353					
West	423	307	365	329	329	357	356	395	312	East
					317					
					296*					
					339					
					353					
					South					

*Maximum difference = 145 day-degrees

Prior to spawning, maximum and minimum temperatures were measured in six different ponds (Table 12). Spawning tended to occur when the maximum pond temperature was greater than 7°C, but eggs were laid in a pond in which the maximum temperature one week before spawning was 3.1°C.

Table 12. Maximum pond temperatures (depth 10cm) measured at intervals from 14 February 1975 until the date of spawning

Date of each thermometer reading, expressed as the number of days from 1 January

Pond No.	51	59	60	62	66	67	70	72	77	85	90	97	101
2	5.2	-	8.5	8.6*									
11	8.4	-	8.5	8.7*									
20	3.4	-	5.4	-	-	6.1	4.8	3.1	3.1*				
33	4.6	5.6	-	-	5.3	-	4.9	-	5.0	5.7	-	5.6	9.5*
42	-	-	-	-	-	-	-	-	-	7.1	7.0*		
47	5.8	7.3	-	-	8.0	-	5.1	-	5.0	8.0	-	7.0	7.5*

* = Date of spawning in each pond

4.4 Discussion

In nearly every case spawning followed immediately after emergence from hibernation and migration, which suggests that when frogs reach the pond they are physiologically ripe, and that any conditions necessary in the pond for spawning already prevail. Juszczyk and Zamachowski (1965) found that breeding in Common Frogs from Poland began when the water temperature reached 7°C, and spawning commenced between 11° and 15°C, whereas spawning in English Common Frogs will take place at 3.3°C (Allison, 1956). In the present study, spawning was found to occur in the field when maximum pond temperatures were above 7°C, but eggs were laid in one pond where the maximum temperature one week before spawning was 3.1°C. The minimum temperature required for spawning is unknown and may well vary in different populations.

There was a positive correlation between the date of spawning and pond altitude in each of the four years of study. The date of spawning in individual ponds, however, was fairly consistent from year to year, and appeared to be little affected by the temperature several months before spawning. Variation in the date of spawning between ponds is a reflection of the different emergence times of various breeding populations. It is not known which factor or group of factors trigger emergence in nature. The laboratory experiments of Juszczyk and Zamachowski (1965) suggest that awakening from hibernation in *R. temporaria* occurs independently of environmental stimuli, and appears to depend on an intrinsic temperature-independent rhythm. An effective test of this hypothesis would be to transfer highland frogs to a lowland location and *vice versa*, and observe the effect on the date of spawning. A series of breeding experiments between highland and lowland populations would establish the degree to which the timing of the rhythm was under genetic control.

CHAPTER 5

The Rate of Development of *Rana temporaria temporaria* Embryos from different Latitudes and Altitudes in relation to temperature

5.1 Introduction

A large literature exists on the physiological differences in animal and plant populations from different climatic areas. For reviews of the literature see Prosser (1955), Bullock (1955), Fry (1958), Vernberg (1962) and Precht *et al.* (1973). Several animal studies have shown that variation in the rate of embryonic development can occur in populations breeding at different environmental temperatures. Runnström (1936) and Fox (1938) produced evidence of physiological races with respect to the rate of embryonic development, in tunicate and echinoderm embryos from different climatic areas.

Some amphibian species have adapted their embryonic development rate to a climatic gradient. Moore (1943, 1949a) and Volpe (1954) studied populations of *Rana pipiens* from different latitudes. Embryos developing in cooler northern waters were found to develop faster at low temperatures than embryos from a southern population, which usually develop in warmer waters. The latter were found to develop relatively faster than the former at higher temperatures. Ruibal (1955) found variation in the rate of embryonic development with temperature in *R. pipiens* embryos from different altitudes in Mexico. The embryos from the highland location (altitude 3,000m) developed slower at all the temperatures studied than embryos from the lowland location (altitude 350m). Highland embryos were 33 per cent slower at 27.8^{o}C and 18 per cent slower at 12.6^{o}C. The temperature in the highland region was about 13^{o}C lower throughout the year, so one might have expected highland embryos to have developed faster than lowland embryos at low

temperatures in the same way as northern *R. pipiens* embryos develop faster than southern *R. pipiens* embryos at low temperatures. Ruibal found that in the highland regions of Mexico only a few species occur in the same breeding ponds as *R. pipiens* in contrast to lowland regions where many species were found together. He suggested that a faster rate of development with respect to temperature had evolved in lowland embryos because it shortened the period of time that the embryos were exposed to intense interspecific (and possibly intraspecific) competition in the breeding ponds. Presumably this species has become widely distributed, partly because it has been able to adapt the rate of embryonic development to different environmental conditions. Other amphibian species appear to be less adaptable. Moore (1942) and Volpe (1957a) found no significant difference in the rate of embryonic development with temperature in geographically separated populations of *Rana catesbeiana* or *Bufo valliceps*. The inability of these two species to adapt their embryonic development rate presumably accounts to some extent for their comparatively restricted distribution. Pettus and Angleton (1967) found no significant difference in the rate of embryonic development over the temperature range 16° to 32°C in two populations of the Chorus Frog, *Pseudacris triseriata*, from altitudes of 1,524m and 2,835m in northern Colorado. They used hatching as the experimental end-point, however, which cannot be regarded as a definite developmental stage because the stage at which hatching takes place differs with temperature (Grainger, 1959).

R. temporaria has a wide distribution, being found over the greater part of Europe and palearctic Asia, so one might expect to find adaptive variation in the rate of embryonic development in populations breeding at different temperatures. The rate of development of *R. temporaria* embryos from northern England was measured at various constant temperatures and the results were compared with similar studies on *R. temporaria* from Europe and Japan. In northern England *R. temporaria* breed in ponds in the comparatively

equable lowland regions as well as in the colder high moorland ponds of the northern Pennines. The rate of development with temperature was measured using embryos from both areas to see if adaptation to local climatic conditions had occurred.

5.2 Methods

Adult *R. temporaria* from northern England were caught in the breeding ponds in early spring, just prior to spawning. They were transported to the laboratory and stored in a cold room at 1°C until required. Fertile eggs were obtained by stripping the eggs from gravid females into a sperm suspension, which was produced by crushing the testes of a male frog in 10 to 15ml of pond water. For details, see Rugh (1934). The eggs were cultured in perspex containers, divided into 20 compartments, 6 x 10 x 12cm, which kept batches of eggs separated but allowed the free circulation of water. The tap water in the containers was continuously aerated and stirred, and had been allowed to stand in sunlight for several days to remove the chlorine. Constant temperatures were maintained in the containers with a variation of less than 0.1°C by placing them in constant temperature rooms about 1°C below the temperature required, and raising and controlling the temperature by means of a 500 watt immersion heater controlled by a 'Jumo' electrical contact thermometer (Gallenkamp Ltd) and a Type F102-4 hot wire vacuum switch relay (Sunvic Controls Ltd). This method allowed batches of eggs from different females to be kept at the same temperature and oxygen concentration.

The starting-point of the experiments was when the furrow of the first division of the eggs became visible (stage 3, Pollister and Moore, 1937). The end-point of the experiments was when 50 per cent of the embryos had circulation through the gills (stage 20, Pollister and Moore, 1937), the same end-point as that used by Douglas (1948), Moore (1949a),

Volpe (1953, 1954, 1955, 1957a and 1957b), Ruibal (1955, 1962), Kobayashi (1962), Zweifel (1968) and Kozlowska (1971).

Table 13 is an example of results taken to calculate the end-point of an experiment. The embryos were examined microscopically at intervals near the end-point, and the percentage of embryos with corpuscles circulating through the gills was noted. The time at which 50 per cent of the embryos had gill circulation was taken from a curve similar to that drawn in Fig. 9.

Table 13. The percentage of embryos with gill circulation at different time intervals; the sample was based on 20 eggs, kept at 16°C. All the eggs were from the same female

Time (hours)	% of embryos with gill circulation
101.8	0
102.8	5
103.3	10
103.8	15
104.8	40
105.3	55
105.8	65
106.3	70
107.3	80
107.8	85

5.3 Results

(a) A comparison of the rate-temperature curves of *R. temporaria* embryos from northern England, France/Belgium and Japan.

The time taken by 50 per cent of the frog embryos from northern England to develop from stage 3 to stage 20 at 6°, 8°, 10°, 15°, 16° and 20°C was recorded in Table 14. These data were used to produce the rate-temperature curve in Fig. 10, which could be compared with those produced

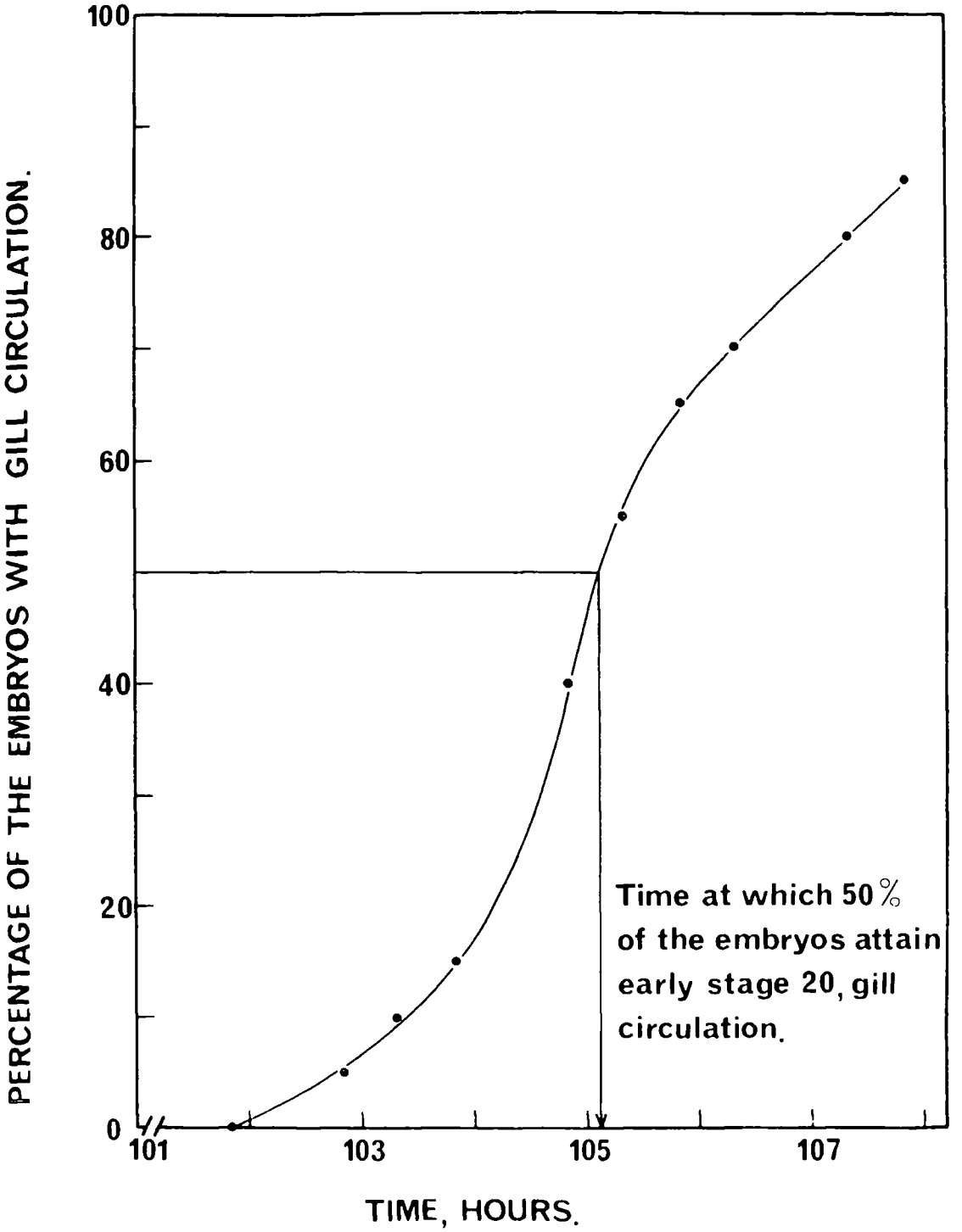
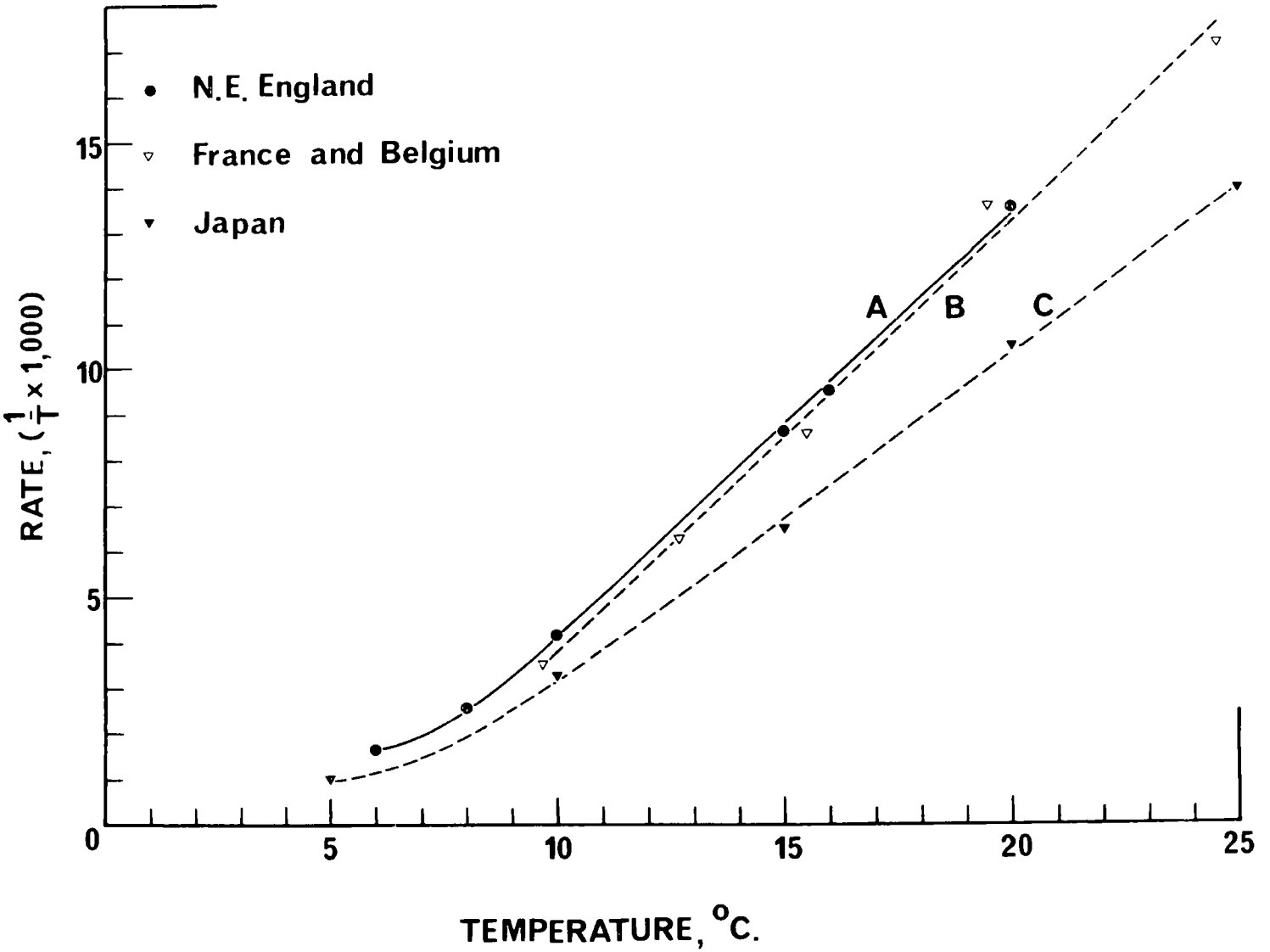

100
80
60
40
20
0
101
103
105
107
PERCENTAGE OF THE EMBRYOS WITH GILL CIRCULATION.
TIME, HOURS.
Time at which 50% of the embryos attain early stage 20, gill circulation.

Table 14. The mean time ± 1 S.E. taken by the eggs from several frogs to develop from early stage 3 (appearance of the furrow of the first division) to stage 20 (initiation of gill circulation), at various constant temperatures. Six batches of eggs, with 20 eggs in a batch, were taken from each one of a total of five frogs. One batch of eggs from each female was kept at 6°, 8°, 10°, 15°, 16° and 20°C. Two batches of eggs were taken from each one of a further 25 frogs, and one batch from each frog was cultured at 6° and 16°C.

	Temperature					
	6°C	8°C	10°C	15°C	16°C	20°C
Mean time ± 1 S.E. to stage 20 (hours)	593.1±2.99	390.6±1.80	241.7±1.27	116.6±0.63	105.7±0.18	74.1±0.90
Rate ($\frac{1}{T}$ x 1,000)	1.69	2.56	4.14	8.58	9.46	13.49
No. of females used in each case	29	5	5	5	30	5


N.E. England
France and Belgium
Japan
A
B
C
RATE, ($\frac{1}{T}$ x 1,000)
0
5
10
15
5
10
15
20
25
TEMPERATURE, °C.

for *R. temporaria* by Moore (1951) and Kobayashi (1962). Moore obtained his specimens from the Vosges district of eastern France and Louvaine in Belgium. Kobayashi caught his frogs in the suburbs of Sapporo, Hokkaido Island, Japan.

The relationship between embryonic development rate and temperature was curvilinear, but over the temperature range 8° to $25^{\circ}C$ the relationship could be adequately described by a straight line:

For English embryos, $y = 0.91(\pm 0.02)x - 4.87$ (results from the present study)

$(r = +0.999, t = 38.71, d.f. = 3, P < 0.001)$

For French/Belgian embryos, $y = 0.94(\pm 0.05)x - 5.57$ (calculated from Moore, 1951)

$(r = +0.996, t = 18.83, d.f. = 3, P < 0.001)$

For Japanese embryos, $y = 0.72(\pm 0.02)x - 4.04$ (calculated from Kobayashi, 1962)

$(r = +0.999, t = 37.79, d.f. = 2, P < 0.001)$

where ($\underline{y}$) is the rate of development and ($\underline{x}$) is the developmental temperature in degrees Celsius. All of the regression coefficients were significantly different from zero. The slopes of these curves were compared statistically in Table 15. The slope of the English rate-temperature curve was not significantly different from the slope of the French/Belgian curve ($t = 0.56$, d.f. = 4, $P < 0.05$). The slopes of these two curves, however, were both significantly larger than the slope of the curve for Japanese embryos ($t = 6.71$, d.f. = 5, $P < 0.002$)($t = 4.08$, d.f. = 4, $P < 0.02$).

Fig. 11 shows the percentage difference in the rate of development between the three populations, over the temperature range 8° to $24^{\circ}C$. The French/Belgian embryos developed at the same rate as English embryos, at $24^{\circ}C$, but as the temperature decreased, the French/Belgian embryos developed progressively slower, until at $8^{\circ}C$ they developed 21 per cent slower than

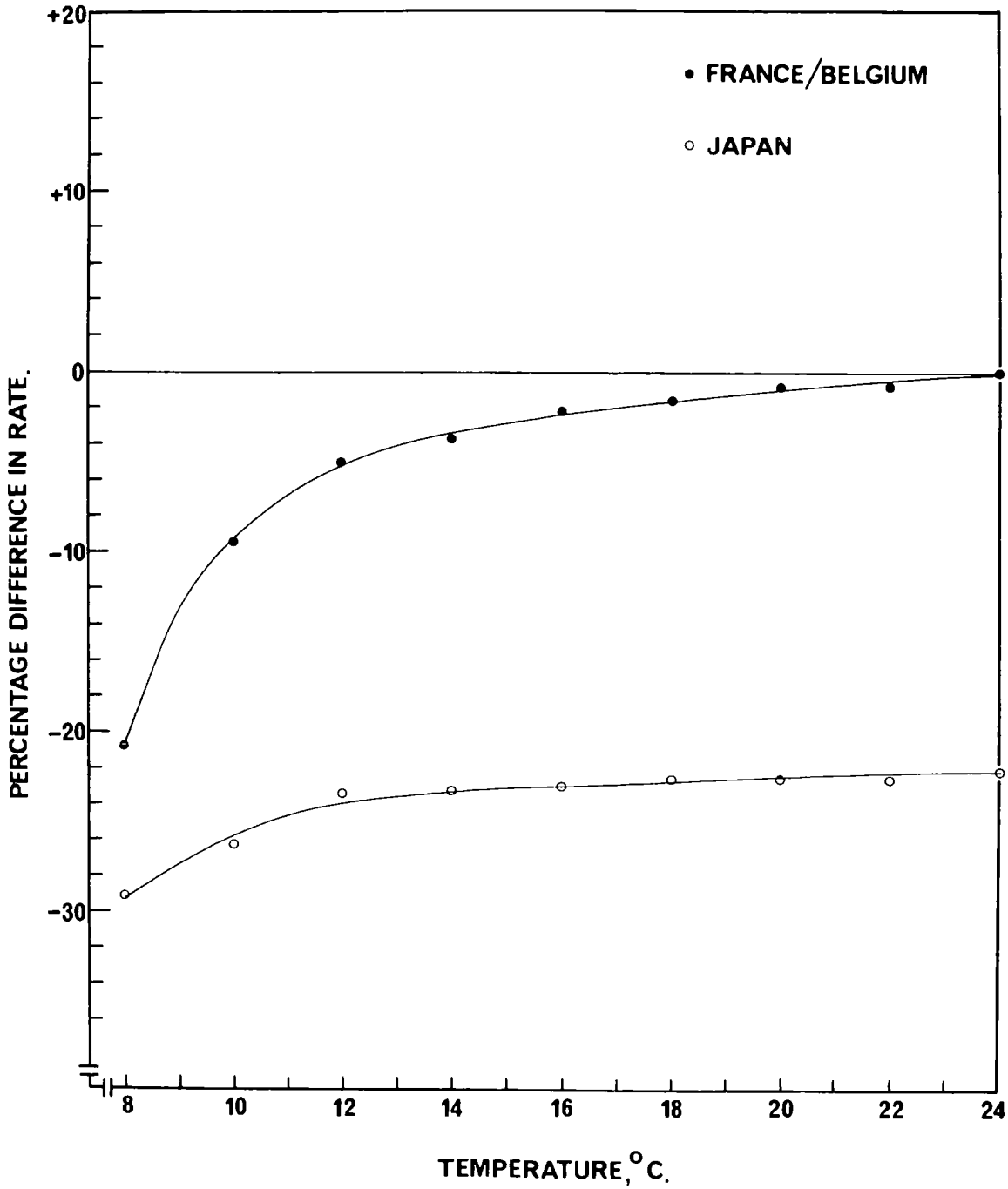

+20
+10
0
-10
-20
-30
• FRANCE/BELGIUM
○ JAPAN
PERCENTAGE DIFFERENCE IN RATE.
8
10
12
14
16
18
20
22
24
TEMPERATURE, °C.

Table 15. The mean difference in the slopes of the rate-temperature curves, ± 1 S.E., between embryos from England, France/Belgium and Japan

	England	France/Belgium	Japan
England		0.03 ± 0.05, t = 0.56, d.f. = 4, P > 0.05. insignificant	0.19 ± 0.03, t = 6.71, d.f. = 5 P < 0.002. significant
France/Belgium			0.22 ± 0.05, t = 4.08, d.f. = 4, P < 0.02. significant
Japan			

English embryos. Between 24° and 12°C, Japanese embryos developed about 23 per cent slower than English embryos. Below 12°C the Japanese embryos developed even slower and, for example, at 8°C, they were 29 per cent slower than English embryos.

(b) The rate of development of lowland and highland *R. temporaria* embryos from northern England, at 6° and 16°C.

The time taken by lowland and highland embryos to develop from stage 3 to stage 20 at 6° and 16°C is shown in Table 16. The rate of development of lowland embryos was significantly different from highland embryos at 6° and 16°C, although the differences were small. Compared

Table 16. The mean time ± 1 S.E. taken by the eggs from frogs caught in lowland and highland ponds to develop from early stage three (appearance of the furrow of the first division) to stage 20 (initiation of gill circulation). Two batches of eggs, with 20 eggs in each batch, were taken from each one of a total of 30 frogs, and one batch from each frog was cultured at 6° and 16°C. The mean altitude ± 1 S.E. of the ponds in metres was: lowland ponds, 187.0 ± 12.3; highland ponds, 621.2 ± 20.6

	Altitude of the pond in which the frogs were caught (m)	The mean time taken to develop from stage 3 to stage 20 at 16°C (hours)	The mean time taken to develop from stage 3 to stage 20 at 6°C (hours)
Eggs from lowland frogs	91	105.45	598.73
	91	105.67	610.33
	183	106.60	600.92
	183	105.85	611.25
	183	105.75	613.50
	183	103.52	617.08
	183	105.25	623.83
	183	106.00	618.08
	183	104.58	610.17
	183	105.83	621.58
	183	103.92	587.92
	244	106.08	600.42
	244	104.35	592.42
	244	104.98	590.58
	244	104.58	592.75
Mean ± 1 S.E.	(187.0 ± 12.3)	(105.23 ± 0.23)	(605.97 ± 3.07)
Eggs from highland frogs	556	106.42	571.50
	556	105.00	577.75
	556	105.08	583.20
	556	105.17	580.08
	602	106.40	-
	602	106.70	577.33
	602	107.27	579.58
	602	107.00	577.67
	602	107.08	580.85
	617	104.75	578.00
	617	106.83	581.92
	617	106.25	578.83
	617	105.68	575.08
	808	107.27	584.65
	808	106.62	584.57
Mean ± 1 S.E.	(621.2 ± 20.6)	(106.23 ± 0.23)	(579.36 ± 0.97)

The difference in mean time ± 1 S.E. taken by lowland and highland eggs to develop from stage 3 to stage 20 at 16°C is 1.0 ± 0.32, $t = 3.15$, d.f. = 28, $P < 0.01$.

The difference in mean time ± 1 S.E. taken by lowland and highland eggs to develop from stage 3 to stage 20 at 6°C is 26.6 ± 3.22, $t = 8.26$, d.f. = 17, $P < 0.001$.

to highland embryos, the embryos from lowland regions developed about one per cent faster at 16°C, but about four per cent slower at 6°C.

5.4 Discussion

Variation does occur in the rate of development of *R. temporaria* embryos from different latitudes, particularly at low temperatures. At 8°C the embryos from northern England (latitude 55°) developed 21 per cent faster than the embryos from France and Belgium (latitude 45° to 52°) and 29 per cent faster than the embryos from Japan (latitude 41°). The embryos from northern regions, therefore, developed faster at low temperatures than the embryos from southern regions, a result similar to that found in *R. pipiens* embryos by Moore (1943) and Volpe (1954). Increasing latitude usually results in a lower environmental temperature and a reduction in the growing season. This, presumably, has selected for a rapid rate of development in northern embryos at low temperatures.

The taxonomic position of *R. t. temporaria* from Hokkaido Island (Japan) is somewhat controversial. Although *R. t. temporaria* from Japan bear a striking resemblance to *R. t. temporaria* from Europe, except for the smaller body size (Kawamura, 1962), differences exist in the karyotype (Kobayashi, 1962b) and the chromosome number.

R. t. temporaria from Europe have 26 chromosomes in the spermatagonia (Witschi, 1922a, 1922b and 1924; Prokofieva, 1935, and Wickbom, 1945), whereas *R. t. temporaria* from Hokkaido Island (Japan) and Sakhalin Island (U.S.S.R.) have a diploid number of 24 (Witschi *et al.* 1958 and Kobayashi, 1962b). Japanese *temporaria* are also completely isolated from European *temporaria* by hybrid inviability (Kawamura and Kobayashi, 1960, and Kawamura and Nishioka, 1962). *R. t. temporaria* from Japan are very similar to *R. t. chensinensis* in many respects (Kawamura, 1962), and Kawamura suggested that *R. t. temporaria* from Hokkaido and Chinese *R. t. chensinensis* should be

provisionally classified together as a distinct species and given the name *Rana chensinensis* DAVID. The substantial difference in the larval development rates of *R. t. temporaria* from Japan and Europe tends to substantiate Kawamura's proposed taxonomic change.

A small difference was found in the rate of development with temperature of *R. temporaria* embryos from different altitudes in northern England. Highland embryos developed about four per cent faster at 6°C, but about one per cent slower at 16°C than lowland embryos. The variation in the rate of embryonic development in local altitudinal populations of *R. temporaria* is fairly insignificant compared to the variation found in latitudinal populations from Europe and Japan. Aebli (1966) studied the rate of development of *R. temporaria* embryos from different altitudes in the Canton of Glarus, Switzerland. He found that the duration of development from the egg to metamorphosis at room temperature (18°C ± 3°) varied from two months (in samples from 2,000m) to five months (in samples from 400 to 600m). Such a large genetic difference seems unlikely when most Amphibia appear to have a rather low genetic adaptibility (Bachmann, 1969). Polish *R. temporaria* embryos were studied by Kozlowska (1971) from altitudes of 200, 700 and 1,000m. No significant difference in the embryonic development rate was found at 13°C, but as this was the only experimental temperature used, it is possible that there could have been significant differences at other temperatures. Guyétant (1969) observed that highland *R. temporaria* embryos from an altitude of 880m in South-East France developed faster at all the temperatures studied when compared with lowland embryos from altitudes of 230 and 550m.

There are several environmental factors which may have selected for a rapid development rate at low temperatures in highland embryos. In northern England spawning is later at higher altitudes (Fig. 7) and the ponds are colder during the period of embryonic development (Fig. 6).

Both these factors will delay metamorphosis, giving young highland frogs little time to feed and accumulate energy reserves for their first winter. A rapid rate of development will tend to compensate for this to some extent. Spawning ponds in highland areas were often shallow and tended to dry up, exposing the eggs to more extreme temperatures and desiccation. A rapid rate of development will reduce the time that frail immobile embryos are exposed to these conditions.

Volpe (1957a) thought that the rapid development rate in *Bufo valliceps* embryos was correlated with the limited time period available for larval growth in temporary pools. Similarly, the rapid rate of development in the embryos of *Rana nigromaculata* comn. race was thought by Moriya (1960) to result in part from breeding in ponds which were liable to dry up. Kobayashi (1962) suggested that the limited water in the highland breeding ponds of *Rana temporaria ornativentris* had selected for the rapid rate of development in this species.

CHAPTER 6

The lower limiting temperature for the normal development of *Rana temporaria temporaria* embryos from lowland and highland areas of northern England

6.1 Introduction

It was observed by Moore (1949b) that the temperature tolerance of Ranid embryos from North America was closely related to the temperature of the breeding habitat. Volpe (1953) found a similar relationship between the geographical distribution and embryonic temperature tolerance of four toad species from North America in the genus *Bufo*. The lower limiting temperature for the normal development of *B. americanus* embryos was 10°C, several degrees lower than *B. terrestris*, *B. fowleri* and *B. woodhousie* which had lower limiting temperatures of 15.3, 15.0 and 15.3°C respectively. This correlates well with the fact that *B. americanus* breeds in lower water temperatures than any of the other species, has the most extensive northern distribution and is the characteristic species of higher altitudes. Kobayashi (1962) observed a similar situation among two species and one subspecies of Japanese Brown Frog. Specimens of *Rana japonica* were caught in the suburbs of Hiroshima where the average annual temperature is 14.7°C, specimens of *Rana temporaria ornativentris* were collected from mountainous districts of the Hiroshima Prefecture where the average annual temperature is 10.3°C, and specimens of *Rana temporaria temporaria* were caught in the suburbs of Sapporo where the average annual temperature is 7.6°C. At 5°C, the percentage of *R. japonica*, *R.t. ornativentris* and *R.t. temporaria* embryos that developed normally was 47, 86 and 93 per cent respectively. The lower limiting temperature for the normal development of these frogs, therefore, appears to be closely related to the temperature of the breeding habitat.

Intraspecific adaptation of the lower limiting temperature to the climate of the breeding habitat has also been observed. Moore (1949a) found that northern populations of *R. pipiens* from Quebec, Vermont, Wisconsin and New Jersey were able to develop normally at temperatures about 5°C lower than southern populations from Florida, Texas and Mexico.

In the present study the lower limiting temperatures for the normal development of lowland and highland *R. temporaria* embryos from northern England were compared.

6.2 Methods

Batches of 20 eggs obtained from four frogs caught in lowland ponds and five frogs caught in highland ponds were cultured in perspex containers, as described in Chapter 5, at 1°, 3°, 4° and 5°C. The percentage of the embryos which developed normally to stage 20 (initiation of gill circulation) at each temperature was noted.

6.3 Results

The percentage of normal embryos reaching stage 20 was plotted against developmental temperature. From these curves the temperatures for 50 per cent normal development were obtained (Table 17). The lower limiting temperature for the normal development of lowland and highland embryos was found to be 3.8° and 2.8°C respectively. The difference between the two samples $\pm$ 1 S.E., 1.0°C $\pm$ 0.41, was significant, $t = 2.42$, $d.f. = 7$, $P < 0.05$.

6.4 Discussion

Several authors have studied the development of *R. temporaria* embryos at low temperatures (Table 18). The results of these eight authors suggest that the lower limiting temperature for 50 per cent normal embryonic

Table 17. The mean temperature ± 1 S.E. at which 50 per cent of the eggs from frogs caught in lowland ponds (mean altitude ± 1 S.E. = 152.3m ± 37.5) and highland ponds (mean altitude ± 1 S.E. = 649.2m ± 39.8) developed normally to stage 20 (the initiation of gill circulation). Samples of 20 eggs were taken from four lowland frogs and five highland frogs

	Eggs from frogs caught in lowland ponds	Eggs from frogs caught in highland ponds
Temperature at	4.1	2.4
which 50 per	4.4	3.0
cent of the eggs	3.5	3.8
developed normally	3.1	2.4
to stage 20 (°C)		2.3
Mean temperature ± 1 S.E.	3.8 ± 0.29	2.8 ± 0.28

The difference in mean temperature ± 1 S.E. at which 50 per cent of the eggs from frogs caught in lowland and highland ponds developed normally to stage 20 is 1.0 ± 0.41, $t = 2.42$, d.f. = 7, $P < 0.05$.

development can vary from about 3.3° to > 8°C in this species. The observed variation may result from adaptation to different environmental temperatures, but legitimate comparisons cannot be made due to the heterogeneous methods used by the different authors. In the present study the lower limiting temperature was found to be 3.8°C for lowland embryos and 2.8°C for highland embryos. The value for highland embryos is 0.5°C lower than any of the estimates for *R. temporaria* quoted in Table 18. Compared with most of the embryos previously investigated, the highland embryos used in the present study were from an area of high altitude and latitude, which might account for this low value. Adaptation to the colder

Table 18.

Author	Date of Publication	Origin of the living material	Author's comments	Estimated lower limiting temperature
Mikulski	1938	Poland	Seven per cent of the embryos survived at $5^{\circ}C$, 19 per cent at $8^{\circ}C$.	$> 8^{\circ}C$
Douglas	1948	Lea Valley in southern England	The embryos would develop at $3.3 \pm 2.2^{\circ}C$ and could tolerate at least 12 hours at $0^{\circ}C$	$3.3^{\circ}C$
Moore	1951	Vosges district of eastern France and Louvaine in Belgium	Development was not always normal at $4.1^{\circ}C$ so the lower limiting temperature was set at $5^{\circ}C$.	$5^{\circ}C$
Balcells	1956	Maranges and Vidrá in northern Spain	-	$4^{\circ}C$
Grainger	1959	-	Two per cent of the embryos survived as far as the appearance of the neural fold at $7^{\circ}C$	$> 7^{\circ}C$
Kobayashi	1962	Suburbs of Sapporo, Hokkaido Island, Japan	Ninety three per cent of the embryos developed normally at $5^{\circ}C$	$< 5^{\circ}C$
Angelier and Angelier	1968	From high altitude ponds in the Central Pyrenees	Constant temperatures below $7^{\circ}C$ could not be achieved, but the lower limiting temperature was estimated to be $4^{\circ}C$	$4^{\circ}C$
Guyétant	1969	Verrière-du-Grois-Bois in south-eastern France	80.1 per cent of the embryos died at $6^{\circ}C$	$> 6^{\circ}C$

breeding ponds at higher altitudes may have produced the difference in the lower limiting temperature found between lowland and highland embryos.

CHAPTER 7

The difference in the body length and the number of eggs produced by lowland and highland female frogs

7.1 Introduction

Many poikilotherms including several Amphibian species have been shown to obey Bergmann's rule and are, therefore, larger at lower temperatures (Ray, 1960). There is a positive correlation, for example, between latitude and body size in the Leopard Frog, *Rana pipiens* (Moore, 1949a). Similarly, highland specimens of the Chorus Frog, *Pseudacris triseriata*, were found to be larger than lowland specimens (Pettus and Angleton, 1967). There is also a trend for Amphibia inhabiting colder climates to produce fewer but larger eggs (Baxter, 1952 and Pettus and Angleton, 1967). The present research was conducted to discover if altitudinal populations of *R. temporaria* from northern England follow these zoo-geographical rules.

7.2 Methods

The body length of female frogs caught in the breeding ponds during early spring was measured from the tip of the snout to the vent, using a steel rule graduated in millimetres. Care was taken on each occasion to fully straighten the frog.

The number of eggs produced by lowland and highland frogs was found by estimating the number of eggs in clumps of spawn deposited in ponds of varying altitude. Female Common Frogs lay their full complement of eggs very rapidly in one large clump (Savage, 1961), so each clump of spawn in a pond represents the total number of eggs laid by one female. When spawn is freshly laid there is no difficulty in distinguishing one clump of spawn from another. A gravimetric method was used to estimate

the number of eggs in a clump. Clumps of eggs were removed from the ponds using a net, the excess water being allowed to drain off. The whole clump was then weighed (using a Torbal torsion balance accurate to 0.1g) and a sample of approximately 100 eggs cut from the clump. The sample was then weighed and the number of eggs in the sample counted exactly. The estimated number of eggs in a clump was calculated from:

$$\frac{\text{Weight of the whole clump} \times \text{Number of eggs in the sample}}{\text{Weight of the sample}}$$

7.3 Results

The snout-vent length of 11 lowland and 23 highland female frogs was measured. To compensate for the small sample of lowland frogs, snout-vent measurements of 12 lowland female frogs caught in the same area by Falconer (1971) were also used (Table 19). Lowland frogs were found to be approximately five per cent longer than highland frogs, the difference between the two samples $\pm$ 1 S.E. = 3.8mm $\pm$ 1.87, being significant, $t = 2.03$, $d.f. = 38$, $P < 0.05$.

The number of eggs in 48 clumps laid in six lowland ponds and in 50 clumps laid in five highland ponds was estimated (Table 20). Highland females produced an average of 707 eggs, less than half the mean number of 1,586 eggs produced by lowland females. The difference between the two samples $\pm$ 1 S.E. = 879 $\pm$ 83.38, being significant, $t = 10.54$, $d.f. = 96$, $P < 0.001$.

7.4 Discussion

Lowland female frogs were about five per cent longer than highland frogs, the reverse of that expected from zoo-geographical rules. Whether this difference in size is a reflection of the different age structure of the two populations, or perhaps the result of the shorter growing season at higher altitudes, is unknown.

Table 19. The body length from snout to vent of mature female frogs from lowland and highland ponds

Lowland Frogs			Highland Frogs	
Altitude of the pond in which the frog was caught (m)	Body length (mm)		Altitude of the pond in which the frog was caught (m)	Body length (mm)
183	74		556	80
183	80		556	70
183	73		556	77
244	80		556	65
335	73		556	73
335	84		556	72
335	78		556	74.5
373	84		556	70
373	84		556	67.5
411	87		556	69.5
427	90		556	72.5
76	69)		556	77
76	79)		602	73
76	67)		602	78
76	77)		602	75
76	66)	From	602	84
76	64)	Falconer	617	71
76	82)	(1971)	617	69
76	77)		617	64
76	65)		617	68
76	69)		617	71
76	76)		808	66
76	67)		808	71
Mean $\pm$ 1 S.E. (186.7 $\pm$ 27.9)	(75.9 $\pm$ 1.56)		(599.2 $\pm$ 14.8)	(72.1 $\pm$ 1.02)

The mean difference $\pm$ 1 S.E. between the body length of female lowland and highland frogs is 3.8 $\pm$ 1.87, $t = 2.03$, d.f. = 38, $P < 0.05$

Highland frogs on average produce 707 eggs, less than half the mean number of 1,586 eggs produced by lowland frogs. This may partly result from the fact that highland females are slightly smaller than lowland females, and there is a tendency for smaller females to produce fewer eggs. Pettus and Angleton (1967), for example, showed that egg number in the Chorus Frog, *Pseudacris triseriata*, was positively correlated with female body size. Terentjev (1960) found a similar relationship in several Ranid

Table 20. The number of eggs in clumps of spawn laid by lowland and highland frogs

	Pond altitude (m)	No. of clumps counted	Mean no. of eggs per clump ± 1 S.E.
(Lowland ponds)	46	8	2,315 ± 131.6
	183	15	1,554 ± 65.5
	183	8	1,615 ± 192.3
	183	8	1,166 ± 147.1
	183	4	1,369 ± 280.2
	183	5	1,314 ± 183.2
Mean egg number ± 1 S.E. (Calculated from the raw data)		(48)	(1,586 ± 75.9)
(Highland ponds)	556	10	621 ± 77.9
	602	10	878 ± 54.6
	617	10	766 ± 53.7
	747	10	515 ± 82.4
	838	10	755 ± 69.3
Mean egg number ± 1 S.E. (Calculated from the raw data)		(50)	(707 ± 34.5)

The mean difference ± 1 S.E. in the number of eggs in clumps of spawn laid by lowland and highland frogs is 879 ± 83.38, $t = 10.54$, d.f. = 96, $P < 0.001$

species, and Kozlowska (1971) observed that small female *R. temporaria* laid fewer eggs. The difference in egg number is perhaps also due to the fact that highland frogs have a comparatively short period of activity during the summer in which to feed and produce the energy supplies for growth and reproduction, as well as for enduring the long winter. There is also some evidence to suggest that frogs from high moorland areas of northern England are short of food at certain times of the year (Houston, 1973).

CHAPTER 8

An investigation of the jelly capsule surrounding the frog's egg, particularly its insulating properties in different pond waters

8.1 Introduction

The egg capsule surrounding the vitellus is composed of a mucoid material secreted by the oviduct, often in complex layers. These mucoid layers are composed of acid mucopolysaccharides which on hydrolysis yield hexoses, hexosamines and amino acids (Bolognani *et al.* 1966; Folkes *et al.* 1950; Giacosa, 1882; Hiyama, 1949a, b and c; Kusa and Ozu, 1961; Lee, 1967; Masamune and Yosizawa, 1953; Masamune *et al.* 1951; Minganti, 1955; Minganti and D'Anna, 1957 and 1958; Monroy, 1965 and Shulz and Becker, 1935). The number of mucoid layers in the jelly capsule varies in different species (Salthe, 1963) and histochemical studies of oviducts and egg jelly has shown that the chemical constitution of these layers can vary (Freeman, 1968; Ghiara, 1960; Humphries, 1966; Humphries and Hughes, 1959; Kambara, 1956a and b, 1957; Kelly, 1954; Salthe, 1963; Shaver, 1966 and Steinke and Benson, 1970). The English *R. temporaria* egg capsules examined in the present study had two mucoid layers, compared to four layers in the capsules of Japanese *R. temporaria* examined by Katagiri (1961). This difference does not necessarily have a functional significance. Salthe (1963) studied the egg capsules of 72 Amphibian species and found no obvious correlation between capsule structure and environmental conditions. Some species, however, have adapted the overall shape of the egg clump. Moore (1940) found that frogs such as *Rana sylvatica* and *Rana pipiens* which breed early in the spring when the water is cold have a submerged compact jelly mass, which presumably presents the least surface area for cooling, whereas summer breeding frogs such as *Rana clamitans* and *Rana catesbeiana* deposit their eggs in a surface film.

The jelly capsule is reported to have several functions. It protects the vitellus from injury, against ingestion by larger organisms and from fungal and other infections (Gabayeva, 1962). It is generally accepted that the jelly is necessary for successful fertilization of amphibian eggs but its rôle is uncertain (Kambara, 1953; Katagiri, 1966; Barbieri and Villeco, 1966). The suggestion has also been made that the jelly capsule acts as a lens, focusing light on the eggs, thereby increasing the supply of heat. Bragg (1964) cites a case of the jelly of *R. pipiens* eggs acting as a lens. Cornman and Grier (1941), however, measured the refractive indices of the egg jelly of *Ambystoma maculatum* and *R. sylvatica* and found no significant refraction at the water-jelly surface, or the surface between the capsules and the outer layer of jelly. It was also pointed out by Rugh (1951) that as the jelly is largely water which is a non-conductor of heat rays, the jelly cannot concentrate the heat rays of the sun. There is good evidence to suggest, however, that the jelly capsule acts as an insulator and heat accumulator. Savage (1950) experimented with the eggs of *R. temporaria* in the laboratory and discovered that a mass of frogs' eggs exposed to intermittent radiation was on average warmer than a similar mass of water. From measurements in the field he found that, in general, the eggs in a pond were warmer than the surrounding water by an average of 0.63^{o}C. The eggs of *R. sylvatica* observed by Hassinger (1970) averaged 1.60^{o}C higher than the surrounding water, and those of *R. pipiens* averaged 0.63^{o}C. The higher temperature within the egg mass was presumably due to the absorption of solar radiation by the darkly pigmented vitelli, and the insulation of the vitelli by their jelly capsules.

During visits to ponds in the Wear Valley it was noticed that the egg capsules in some ponds were much larger than egg capsules in others. This observation was thought to be of importance as the size of the jelly

capsule could affect its heat retaining characteristics and thus influence the survival chances of the frog embryo. This hypothesis was tested by conducting the following experiment.

8.2 Methods

The eggs from the two ovisacs of a single female were removed and one clump of eggs was placed in a litre of water from pond (46) and the other in a litre of water from pond (2). It was known that the water from pond (46) allowed the rapid swelling of egg jelly while that from pond (2) tended to inhibit swelling. A thermometer was placed inside each of the egg clumps, and one at the same level in the water just outside the clumps. The four thermometers had been calibrated, and over the temperature range 0° to 12°C there was no difference in their readings. They were all accurate to 0.05°C. The egg clumps were allowed to swell for 24 hours, then the temperature inside and outside of the clumps was measured at intervals over a period of 12 hours. The experiment was conducted in a constant temperature room set at 1°C. The eggs and water were heated by a batch of light bulbs for six hours, the light bulbs were then switched off, and the eggs and water were allowed to cool for six hours.

8.3 Results

The results shown in Table 21 were plotted in Fig. 12. From Fig. 12 the spawn and water temperature at 20 minute intervals was taken and the differences were examined statistically by the method of paired comparisons (Table 22). It is apparent from these results that over the 12 hour period both batches of eggs were significantly warmer than the surrounding water, and the eggs with large jelly capsules in water from pond (46) were significantly warmer than the eggs with small jelly capsules in water from pond (2). During the heating phase the eggs with large

Table 21. The difference in temperature between two egg masses and the water surrounding them, with heating and cooling

	Time (Hours)	Temperature of the water from pond 46 (°C)	Temperature of egg mass (A) (°C)	Temperature of the water from pond 2 (°C)	Temperature of egg mass (B) (°C)
Heating	0.00	1.55	1.70	1.80	1.85
	0.42	2.10	1.95	2.23	2.23
	1.00	3.55	3.13	3.60	3.50
	1.83	4.15	4.00	4.00	4.23
	2.67	4.70	5.00	4.68	5.08
	3.33	5.13	5.55	5.10	5.55
	4.00	5.50	6.03	5.53	6.00
	4.75	5.85	6.38	5.85	6.30
	5.50	6.15	6.70	6.15	6.63
	6.00	6.38	6.90	6.40	6.83
Cooling	6.50	5.63	6.48	5.73	6.18
	7.00	5.00	5.88	5.03	5.50
	7.50	4.35	5.28	4.53	4.95
	8.50	3.40	4.10	3.70	4.03
	9.25	3.00	3.48	3.23	3.50
	10.00	2.63	2.93	2.88	3.00
	11.00	2.25	2.50	2.48	2.58
	12.00	2.00	2.18	2.20	2.28

(Eggs 20 hours old)

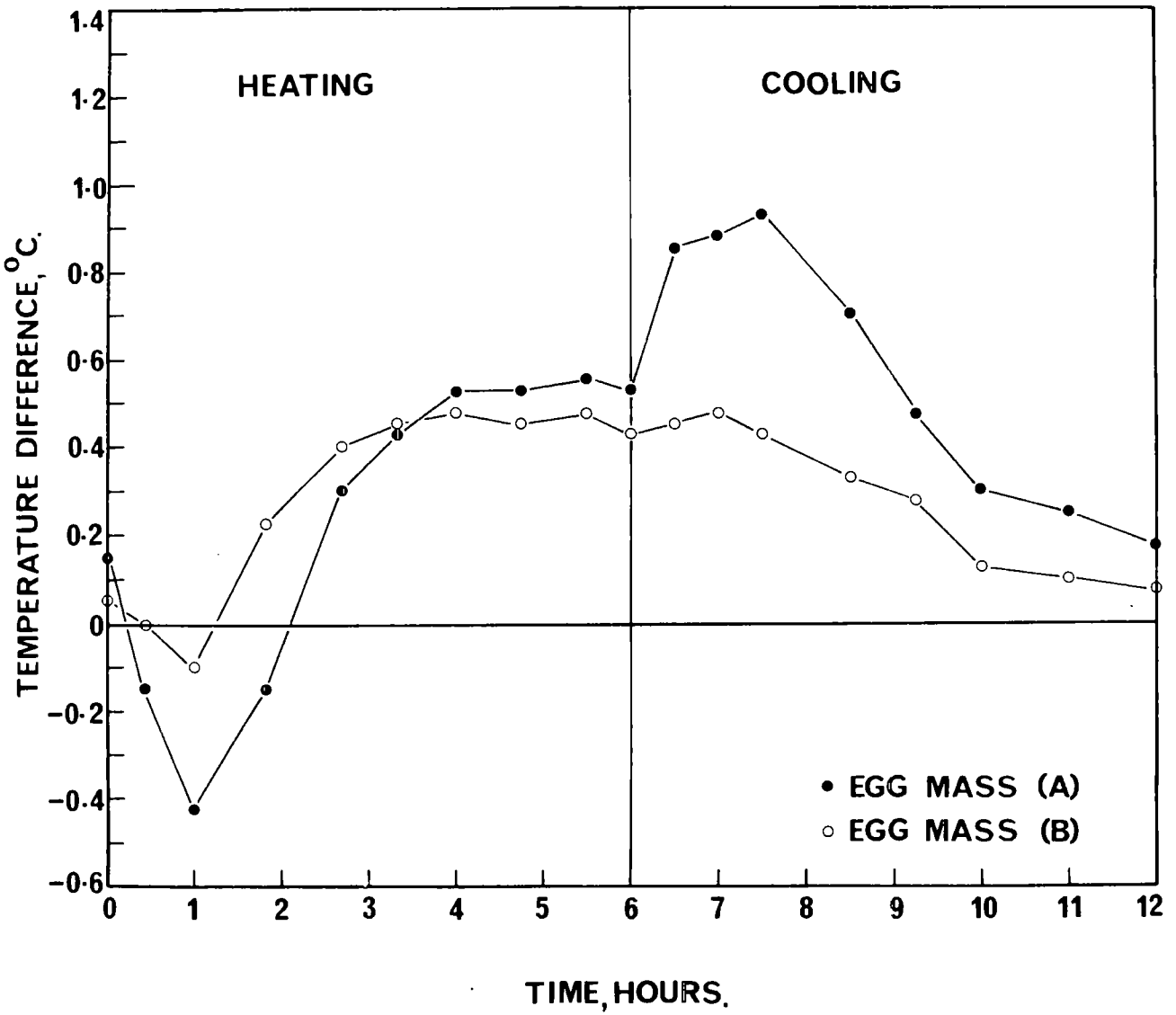

HEATING
COOLING
TEMPERATURE DIFFERENCE, °C.
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
−0·2
−0·4
−0·6
0
1
2
3
4
5
6
7
8
9
10
11
12
TIME, HOURS.
• EGG MASS (A)
○ EGG MASS (B)

Table 22. The difference in temperature between two clumps of spawn and their surrounding water at 20 minute intervals (taken from Fig. 12) and examined by the method of paired comparisons

	Difference in temperature between:								
	egg clump (A) and pond water (46)			egg clump (b) and pond water (2)			egg clump (A) and egg clump (B)		
	difference during heating	difference during cooling	overall difference	difference during heating	difference during cooling	overall difference	difference during heating	difference during cooling	overall difference
$\bar{X}$	0.22	0.53	0.38	0.31	0.26	0.28	-0.09	0.27	0.09
1 S.E.	0.08	0.07	0.06	0.05	0.04	0.03	0.04	0.03	0.04
't'	2.71	8.17	6.56	6.62	7.45	10.08	2.25	9.05	2.38
	(all with 35 degrees of freedom)								
P	< 0.02	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.05	< 0.001	< 0.05

capsules were slightly cooler on average than the eggs with small capsules, but they had reached a higher temperature at the end of the six hour heating period. Eggs with large capsules were much more effective in conserving heat during the cooling phase.

The experiment was repeated with the same eggs after they had been swelling for 120 hours. The results were similar to those described previously except that the insulating effect was enhanced, as one might have expected with larger capsules.

At the end of these experiments the two clumps of eggs were weighed, and the number of eggs in each clump counted (Table 23). There was a similar number of eggs in both clumps, yet after 132 hours the mean wet weight of eggs in water from pond (46) was 0.83g, over two and a half times heavier than the mean weight of 0.31g for eggs in water from pond (2).

Table 23. The weight of two clumps of eggs after 132 hours in water from pond (2) and pond (46)

	eggs in water from pond (2)	eggs in water from pond (46)
Egg number	648	615
Clump wet weight (g)	200.8	509.7
Mean egg weight (g)	0.31	0.83

8.4 Discussion

From these experiments it would appear that the mucopolysaccharide egg capsule does act as an insulator, keeping the vitelli warmer on average than the surrounding water. It is also apparent that the pond water, by limiting the swelling of the insulating jelly capsule, can influence the temperature of the embryo. The difference in temperature between the two

clumps of spawn in different pond waters may not seem very large but, as Savage (1950) pointed out, small differences in temperature are very important to animals breeding early in the spring when temperatures are often only slightly above the lower lethal limit. Furthermore, the experiment was performed on two relatively small egg clumps, whereas in practice clumps of spawn are laid together in a pond, which will have a cumulative effect on temperature differences.

The large difference in mean egg wet weight observed in this experiment gives some indication of the effect that different pond waters can have on egg swelling. The swelling process was investigated further, and the results reported in the following chapter.

CHAPTER 9

Factors affecting the swelling of the jelly capsule surrounding the frog's egg

It has long been known that the egg jelly of frog's spawn swells in water, but little work has been done on the factors influencing swelling. Krogh *et al.* (1938) found that the swelling of *R. temporaria* egg jelly was large in distilled water and appeared to be specifically inhibited by calcium. Later work by Kobayashi (1954) on the swelling of *Bufo vulgaris formosus* eggs showed that swelling was greater at higher temperatures, and that the optimum pH for swelling was between 6.4 and 6.8. He also noted that at low concentrations anions inhibited swelling in accordance with the order of the lyotropic series, while at high concentrations the order was reversed. The lyotropic or Hofmeister series lists ions according to their effect on the swelling of macromolecular gels (Kruyt, 1949). Lee (1964) studied some of the factors affecting the swelling of *R. pipiens* egg jelly and found that solutions of low pH and high ionic concentration inhibited swelling.

The swelling of the gelatinous capsules surrounding the eggs of *R. temporaria* could play an important part in temperature regulation, and the physical factors involved were investigated.

9.1 The methodology for swelling experiments

9.1.1 The method for measuring the whole egg volume

Infertile eggs, obtained by gently squeezing the abdomen of a gravid female, were transferred by means of a fine brush to clear plastic jars. The eggs naturally adhered to the bottom of the jars and remained in the same position even after the culture medium was added. The bottoms of the jars were marked so that each egg could be individually recognized.

The maximum and minimum diameters of the whole egg, including the gelatinous capsule, were measured using a graticule in a binocular microscope. The radius, obtained from the equation:

$$\frac{\text{Maximum diameter + minimum diameter}}{4}$$

was used to calculate the whole egg volume by the following formula, since the eggs were approximately spherical:

$$\text{volume} = \frac{4}{3}\pi r^3$$

where r = radius.

Changes in the whole egg volume in different culture media are almost entirely the result of changes in the gelatinous egg capsule. The volume of the perivitelline chamber of fertile eggs does increase during development (Salthe, 1965), but this has no significant effect on the whole egg volume.

The whole egg volume was affected by exposure to the air. When the volume of 16 infertile eggs was measured at different time intervals in air at 15°C, with a 50% RH, it was found that compared to the initial volume the eggs contracted by eight per cent after five minutes, and 52 per cent after 100 minutes (Table 24). The saturation deficit of the air will presumably affect the degree of contraction. Lee (1964) discovered that the percentage water loss from the eggs of *R. pipiens* was correlated with the amount of water vapour in the atmosphere.

Before the culture medium was added to the eggs they were often exposed to the atmosphere for several minutes. To test whether this brief exposure affected the subsequent swelling of the gelatinous egg capsule, the following experiment was performed. A batch of 16 eggs (A) was exposed to the atmosphere for 100 minutes before having 300ml of two millimolar $CaCl_2$ solution added. A control batch of 16 eggs (B) had the

Table 24. The mean volume at various intervals of eggs exposed to the air; the sample was based on 16 eggs

The length of time eggs were exposed to the air (mins)	Mean egg volume $\pm$ 1 S.E. (mm^3)
0	9.0 $\pm$ 0.17
5	8.3 $\pm$ 0.14
10	7.7 $\pm$ 0.15
20	6.4 $\pm$ 0.15
40	5.0 $\pm$ 0.09
60	4.4 $\pm$ 0.08
80	4.3 $\pm$ 0.08
100	4.3 $\pm$ 0.07

same volume of $CaCl_2$ solution added immediately after they were extracted from the female. There was only a five per cent difference between the mean whole egg volume of batch (A) and batch (B) after 24 hours at 15°C. This difference was not significant ($t = 0.99$, d.f. = 30, $P < 0.05$) (Table 25).

The jelly of the frog's egg can, therefore, be dehydrated without affecting the normal swelling pattern of jelly. Banta and Gortner (1914) showed that *Ambystoma* egg jelly could be dehydrated and rehydrated with no outward change in appearance. Lee (1964) demonstrated this reversibility in the jelly from *R. pipiens* eggs, which is a typical property of hydrophilic gels.

Table 25. The mean volume $\pm$ 1 S.E. of two batches of eggs (16 eggs per batch) after 24 hours in 300ml of two millimolar $CaCl_2$. Batch (A) was exposed to the air for 100 minutes before the $CaCl_2$ solution was added. The $CaCl_2$ solution was added to batch (B) immediately

			Batch (A)	Batch (B)
Mean egg volume $\pm$ 1 S.E. (mm^3) measured:	(i)	straight from the female	9.0 $\pm$ 0.17	8.5 $\pm$ 0.39
	(ii)	after being exposed to the air for 100 minutes	4.3 $\pm$ 0.07	-
	(iii)	after 24 hours in 2 millimolar $CaCl_2$	265.8 $\pm$ 6.45	254.0 $\pm$ 9.89

The difference in mean egg volume $\pm$ 1 S.E. between batch (A) and batch (B) after 24 hours in two millimolar $CaCl_2$ is 11.8 $\pm$ 11.8, t = 0.99, d.f. = 30, P > 0.05.

9.1.2 A comparison between the swelling of the gelatinous capsules of fertile and infertile eggs

There are certain disadvantages associated with the use of fertile eggs in swelling experiments. Before a test solution can be added eggs must be placed in a sperm-pond water solution, which will itself influence the swelling of the gelatinous egg capsule. Furthermore, not all of the eggs will be fertilized, which means that more than the required number of eggs must be measured initially until fertile eggs can be distinguished from infertile ones. These problems do not arise when infertile eggs are used. It was not known, however, whether the gelatinous capsules of infertile eggs had the same swelling pattern as fertile eggs. Krogh *et al.* (1938) thought that the mucus surrounding the frog's egg

behaved in the same way in solution, whether fertilization took place or not. No evidence was produced to justify this statement however. Accordingly, the following experiment was performed to discover if the gelatinous capsules of infertile eggs behaved in the same way as those from fertile ones. A batch of 64 eggs was taken from the same female and was divided equally between four plastic jars, A, B, C and D. 100ml of sperm-pond water solution was added to jars A and B, and 100ml of the same pond water, but without the sperm, was added to jars C and D. After five minutes the fluid was emptied from all four jars and 300ml of tap water was added to each. The volume of all 64 eggs was measured until fertile eggs could be distinguished by the appearance of the furrow of the first division. Ten out of the 32 eggs in jars A and B were fertilized. The volume of these 10 eggs was compared at intervals with 10 selected infertile eggs from jars C and D, each one of which had the same volume after half an hour as one of the fertile eggs. The eggs were kept in a constant temperature room set at $15^{o}C$ and the whole egg volume was measured at intervals over a 146 hour period. The maximum percentage difference between the mean egg volume of fertile and infertile eggs was nine per cent. Overall, there was no significant difference between fertile and infertile eggs over the 146 hour period ($t = 1.22$, d.f. = 9, $P > 0.05$) (Table 26). The gelatinous capsules of fertile and infertile eggs had a very similar swelling pattern (Fig. 13). The mean egg volume in cubic millimetres ($\underline{y}$) was related to the square root of time in hours ($\underline{x}$) by the following linear relationships:

(1) Mean egg volume of infertile eggs, $y = 56.8(\pm 1.93)x + 11.4$

$(r = +0.995)$

(2) Mean egg volume of fertile eggs, $y = 53.6(\pm 1.31)x + 27.6$

$(r = +0.998)$

The mean difference between the slopes of the two regression lines is 3.2 ± 2.33, which is not significant. Therefore it is justifiable to use infertile eggs in subsequent swelling experiments.

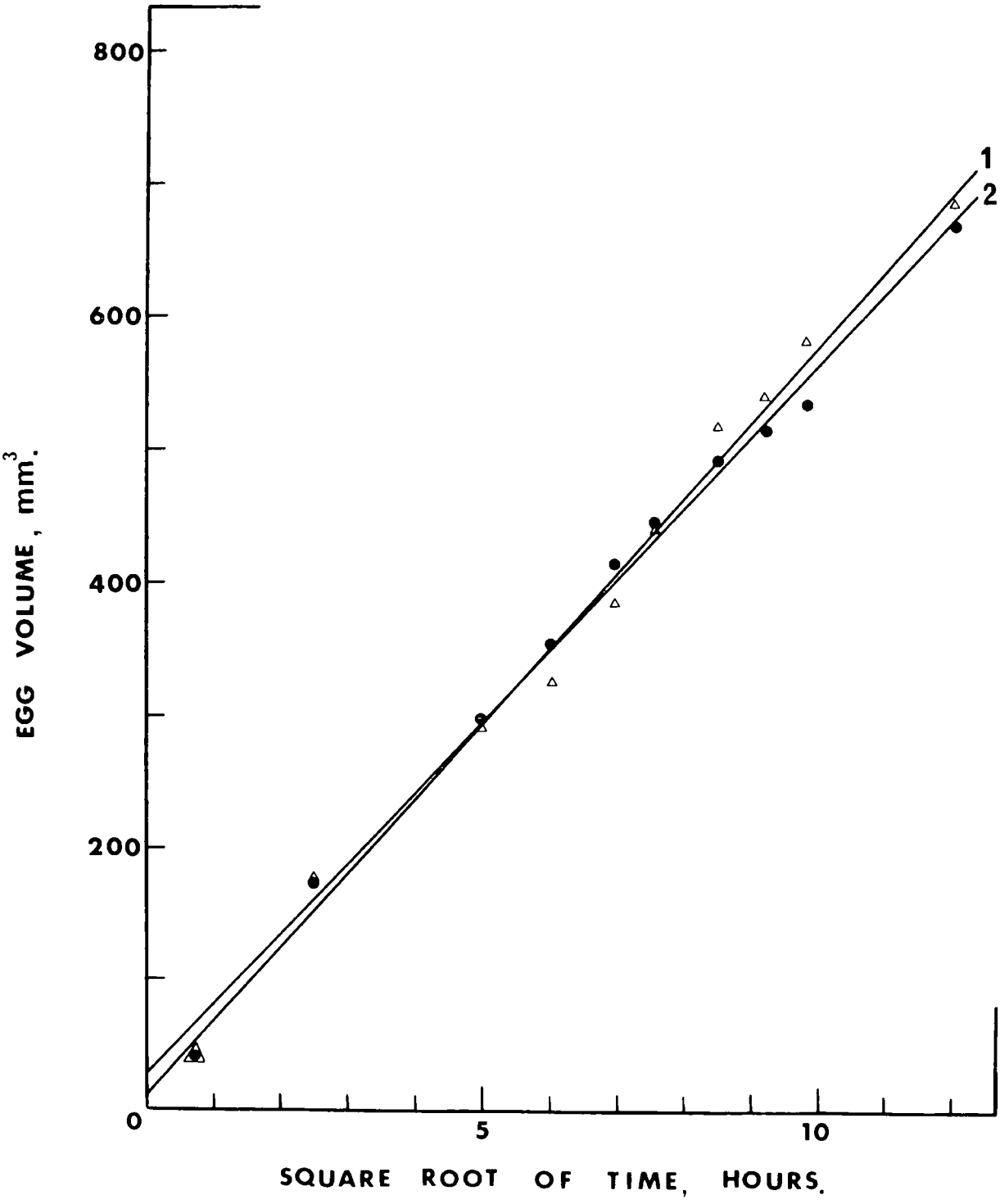
Table 26. The mean volume at different intervals ± 1 S.E. of fertile and infertile eggs kept under the same conditions; each sample was based on 10 eggs

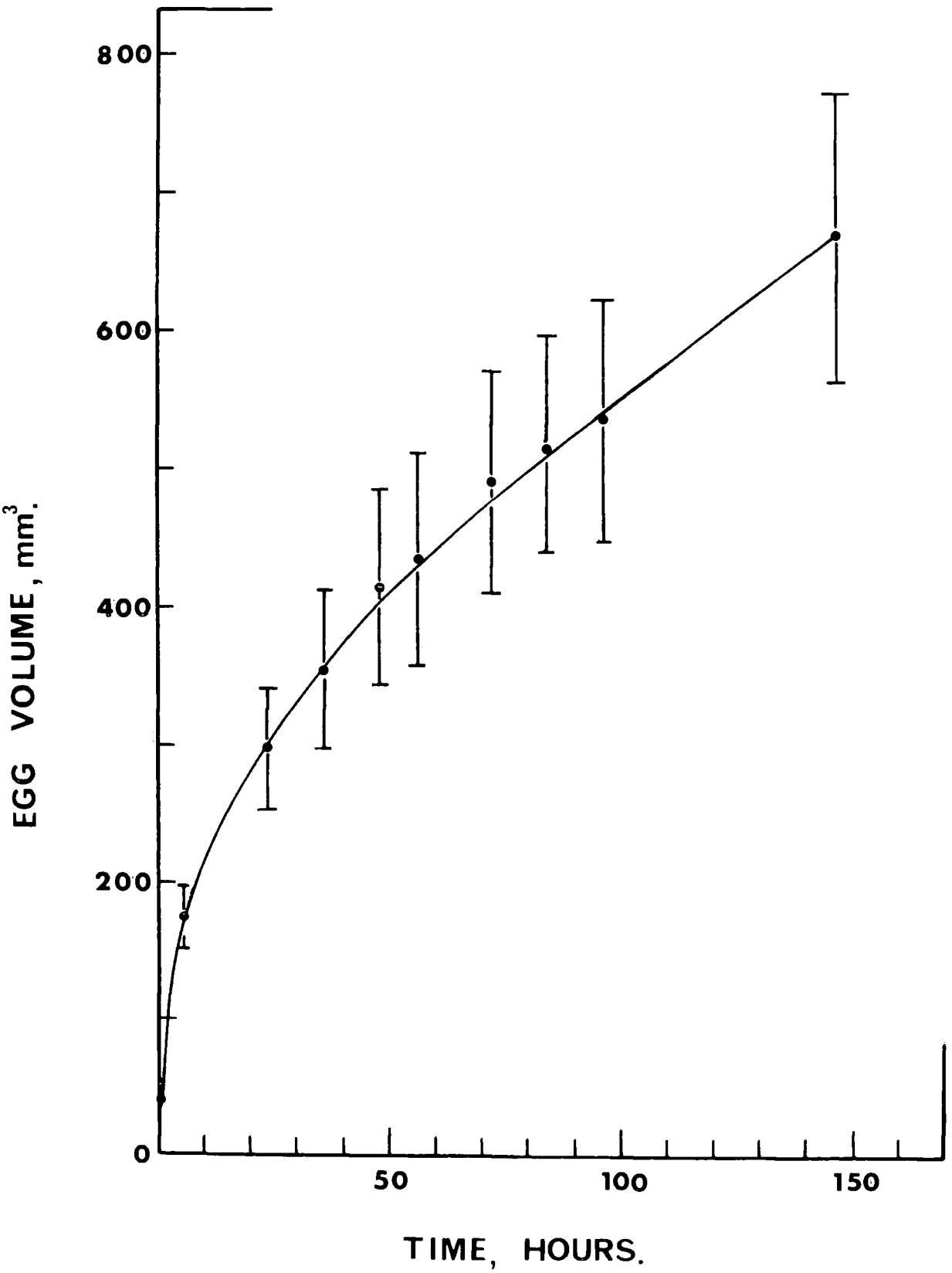
Time (Hours)	Mean egg volume of fertile eggs ± 1 S.E. (mm^3)	Mean egg volume of infertile eggs ± 1 S.E. (mm^3)
0.5	41.7 ± 1.97	41.7 ± 1.97
6.3	174.3 ± 11.78	178.3 ± 6.59
24.0	297.5 ± 21.53	295.4 ± 22.96
36.3	355.1 ± 28.40	324.1 ± 29.93
48.3	415.0 ± 35.06	385.1 ± 35.31
56.5	434.7 ± 38.09	430.8 ± 36.38
72.0	490.2 ± 39.72	518.9 ± 38.32
84.0	514.0 ± 40.37	539.9 ± 36.64
96.0	535.1 ± 43.56	582.0 ± 36.59
146.0	669.9 ± 53.58	685.6 ± 37.30

The difference in mean egg volume ± 1 S.E. between fertile and infertile eggs is 5.4 ± 4.46, $t = 1.22$, d.f. = 9, $P > 0.05$ (by the method of paired comparisons).

9.1.3 The duration of swelling experiments

The swelling of the gelatinous egg capsule was rapid at first, then started to moderate after about 24 hours (Fig. 14). The egg capsule continued to swell slowly until the whole system was completely liquified. In the later stages of swelling the egg capsule started to disintegrate and the volume became difficult to measure. Most swelling experiments, therefore, were terminated after 24 hours. There was a good correlation


800
600
400
200
0
5
10
1
2
EGG VOLUME, mm3.
SQUARE ROOT OF TIME, HOURS.

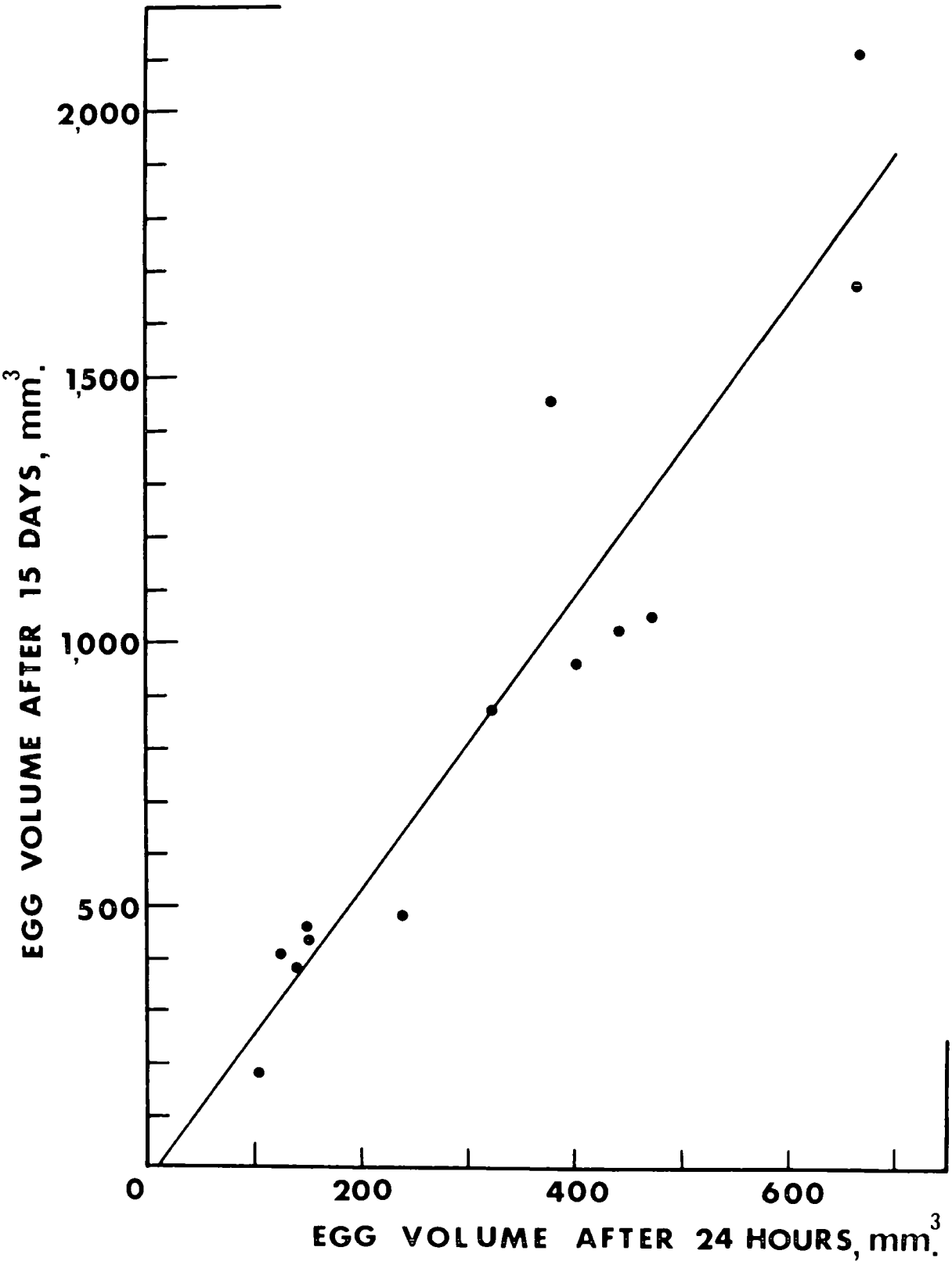

800
600
400
200
0
EGG VOLUME, mm3.
50
100
150
TIME, HOURS.

between the egg volume measured after 24 hours and the egg volume measured at other times. For example, the egg volume of 13 batches of eggs (eight eggs in a batch) was measured after 24 hours and 15 days in different pond waters (Table 27). The egg volume after 15 days in cubic millimetres (y) was related to the egg volume after 24 hours in cubic millimetres (x) by the following relationship:

Egg volume after 15 days, $y = 2.8(\pm 0.3)x - 19.8$. The coefficient of correlation of regression, $r = +0.94$, was significantly different from zero, $t = 9.48$, d.f. = 11, $P < 0.001$ (Fig. 15).

Table 27. The volume of 13 batches of eggs (eight eggs per batch) after 24 hours and 15 days in different pond waters. All the eggs were from the same female.

No. of the pond from which the water was taken	Egg volume after 24 hours (mm^3)	Egg volume after 15 days (mm^3)
1	140	389
2	126	413
8	150	465
20	441	1,028
22	377	1,455
25	151	440
27	105	188
32	324	877
37	401	961
41	663	1,676
45	239	485
46	471	1,052
51	666	2,117


2,000
1,500
1,000
500
0
200
400
600
EGG VOLUME AFTER 15 DAYS, mm.3
EGG VOLUME AFTER 24 HOURS, mm.3

9.1.4 Estimation of the sample size

Two hundred and sixteen eggs were taken from the same female and their volume was measured after 24 hours in two millimolar $CaCl_2$ (Table 28). These values were used to calculate the sample size required for a 10 per cent error of the mean, which in the present case was found to be 14. In swelling experiments, therefore, samples of 14 eggs or more were used.

Table 28. The volume of 216 eggs from the same female after 24 hours in 2mM $CaCl_2$, used to calculate the sample size for a 10 per cent error of the mean

Egg volume after 24 hours (mm^3)	Frequency	Egg volume after 24 hours (mm^3)	Frequency
493	1	268	9
463	1	258	12
449	4	249	7
435	3	239	7
421	3	230	5
408	10	221	7
395	3	212	8
382	6	204	2
369	2	195	2
357	5	187	2
345	6	180	1
333	11	165	2
322	16	158	2
310	16	151	1
299	18	144	1
289	29	113	1 (216)
278	13		

The mean egg volume $\pm$ 1 S.E. is 297.9 $\pm$ 4.52.

The number of samples required (N) is given by: $N = \{\frac{ts}{\bar{x}D}\}^2$

where s = standard deviation, $\bar{x}$ = mean, D = the required level of accuracy expressed as a decimal, and t is a quantity which depends on the number of samples and is obtained from the tables.

$$N = \{\frac{1.658. \quad 66.36}{0.1. \quad 297.9}\}^2 = 14$$

9.2 The major factors affecting the swelling of the gelatinous egg capsule

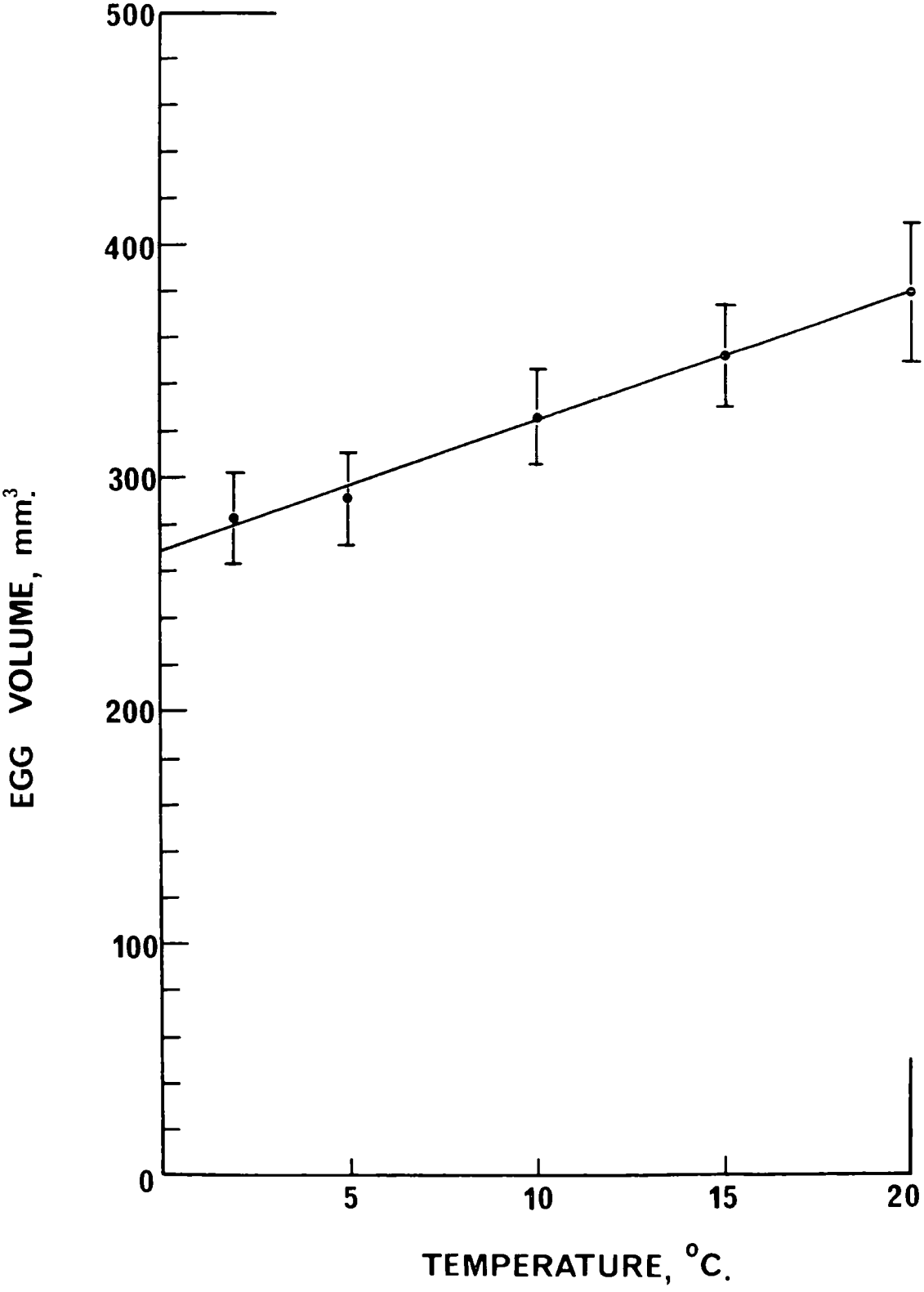
9.2.1 The effect of temperature on the swelling of the egg capsule

Batches of 16 eggs were placed in five plastic jars and 300ml of two millimolar $CaCl_2$ was added to each one. A jar of eggs was cultured at each of the following temperatures: 2°, 5°, 10°, 15° and $20^{\circ}C$, and the volume of the eggs was measured after 24 hours (Table 29). These results were plotted in Fig. 16.

Table 29. The mean volume $\pm$ 1 S.E. of five batches of eggs (16 eggs in each batch) after 24 hours in two millimolar $CaCl_2$, kept at different temperatures

	Temperature				
	$2^{\circ}C$	$5^{\circ}C$	$10^{\circ}C$	$15^{\circ}C$	$20^{\circ}C$
Mean egg volume $\pm$ 1 S.E. after 24 hours (mm^3)	282.7 $\pm$9.70	291.1 $\pm$9.70	326.0 $\pm$9.99	352.6 $\pm$10.98	380.0 $\pm$14.90

The mean egg volume after 24 hours in cubic millimetres (y) was related to the temperature in degrees Celsius (x) by the following relationship: Mean egg volume, $y = 5.6(\pm 0.26)x + 268.3$. The coefficient of correlation of regression, $r = +0.997$, was significantly different from zero, $t = 21.94$, d.f. = 3, $P < 0.001$. Between 2° and $20^{\circ}C$, the egg volume after 24 hours increased by approximately two per cent for every $1^{\circ}C$ rise in temperature. Kobayashi (1954) studied the effect of temperature on the swelling of gelatinous capsules from *Bufo vulgaris formosus* eggs. The swelling of the jelly was faster at higher temperatures. After 27 hours jelly kept at $26.5^{\circ}C$ was 21 per cent heavier than jelly kept at $3^{\circ}C$.


500
400
300
200
100
0
EGG VOLUME, mm.3
5
10
15
20
TEMPERATURE, °C.

9.2.2 The effect of aeration on the swelling of the egg capsule

Batches of 16 eggs were placed in four plastic jars and 300ml of two millimolar $CaCl_2$ was added to each one. Two of the jars were aerated and two were not. The eggs were cultured for 24 hours at 15°C.

There was a five per cent difference between the mean egg volume of aerated and non-aerated eggs after 24 hours (Table 30). This difference was not significant ($t = 1.67$, d.f. = 62). This suggests that aeration does not affect the swelling of the gelatinous egg capsule surrounding a frog's egg.

Table 30. The mean volume $\pm$ 1 S.E. of two batches of eggs (32 eggs in a batch) after 24 hours in two millimolar $CaCl_2$. Batch (A) was aerated, batch (B) was not.

	Batch (A)	Batch (B)
Mean egg volume $\pm$ 1 S.E. after 24 hours (mm^3)	228.6 $\pm$ 3.65	216.7 $\pm$ 6.11

The difference in mean egg volume $\pm$ 1 S.E. between batch (A) and batch (B) after 24 hours in two millimolar $CaCl_2$ is 11.9 $\pm$ 7.13, $t = 1.67$, d.f. = 62, $P > 0.05$.

9.2.3 The effect of ionic concentration on the swelling of the egg capsule

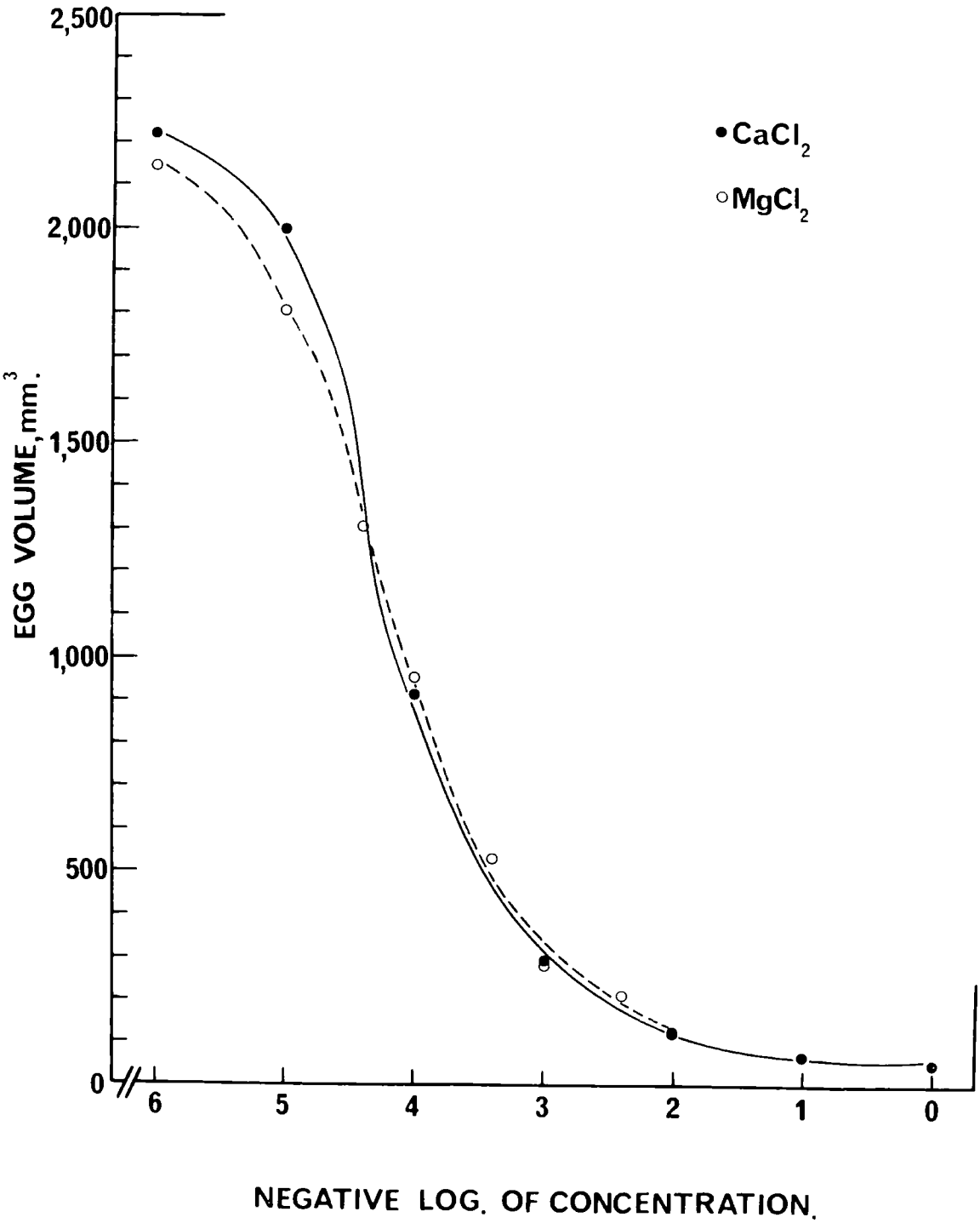
Batches of 16 eggs from the same female were placed in 15 plastic jars and 300ml of $CaCl_2$ solution of varying strengths was added to seven of these jars, and 300ml of $MgCl_2$ solution of varying strengths was added to the other eight jars. After 24 hours at 15°C the volume of all the eggs was measured (Tables 31 and 32). The eggs in the $MgCl_2$ solutions were also weighed so that a comparison could be made between a volumetric and a gravimetric method for measuring capsular swelling (Table 32).

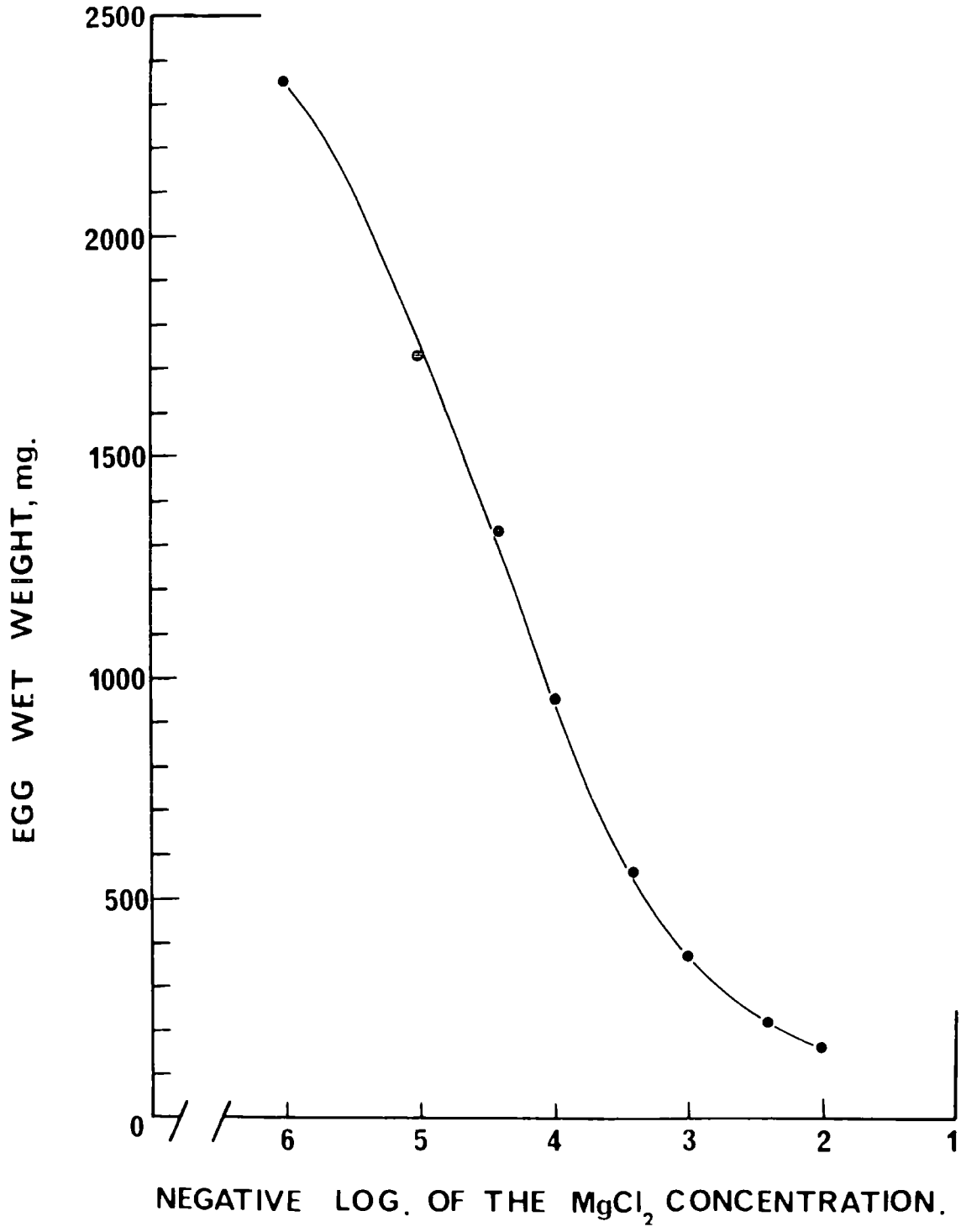
Table 31. Mean egg volume ± 1 S.E. after 24 hours in different strength solutions of $CaCl_2$; each sample was based on 16 eggs.

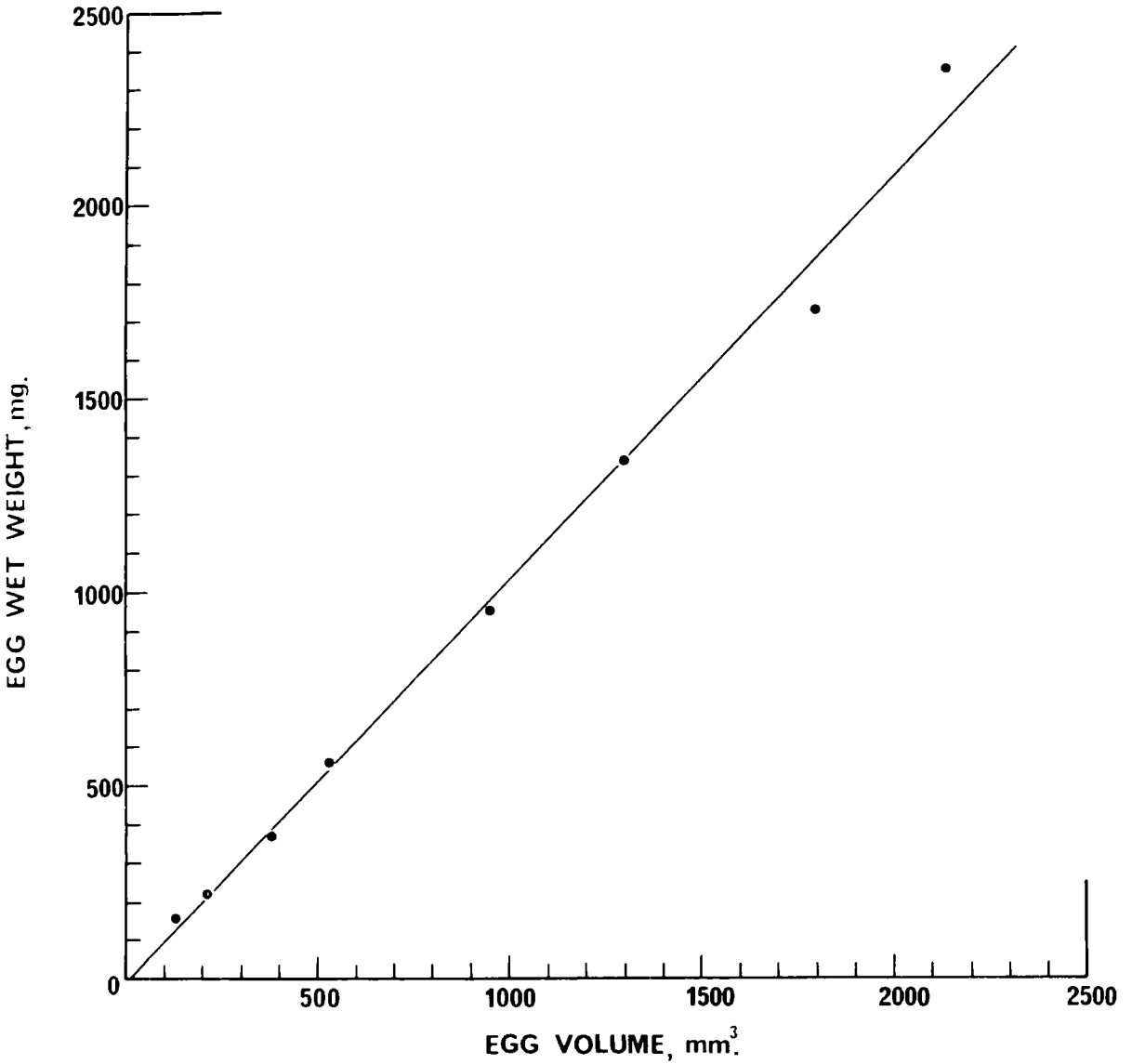
Concentration of the $CaCl_2$ solution (moles/litre)	Mean egg volume after 24 hours ± 1 S.E. (mm^3)
1M	57.1 ± 2.53
10^{-1}M	71.7 ± 3.80
10^{-2}M	130.1 ± 2.99
10^{-3}M	297.4 ± 11.01
10^{-4}M	910.5 ± 35.41
10^{-5}M	1,998.5 ± 118.18
10^{-6}M	2,220.3 ± 137.09

Table 32. Mean egg volume ± 1 S.E. and mean wet weight of eggs after 24 hours in $MgCl_2$ solutions of different concentration; each sample was based on 16 eggs

Concentration of the $MgCl_2$ solution (moles/litre)	Mean egg volume ± 1 S.E. after 24 hours (mm^3)	Mean egg wet weight after 24 hours (mg)
10^{-2}M	129 ± 9.9	160
4 x 10^{-3}M	216 ± 11.4	220
10^{-3}M	383 ± 20.5	370
4 x 10^{-4}M	533 ± 23.8	560
10^{-4}M	955 ± 72.3	950
4 x 10^{-5}M	1,303 ± 82.8	1,330
10^{-5}M	1,803 ± 95.1	1,720
10^{-6}M	2,143 ± 123.0	2,340


2,500
2,000
1,500
1,000
500
0
EGG VOLUME, mm.3
•CaCl2
○MgCl2
6
5
4
3
2
1
0
NEGATIVE LOG. OF CONCENTRATION.


2500
2000
1500
1000
500
0
EGG WET WEIGHT, mg.
6
5
4
3
2
1
NEGATIVE LOG. OF THE $MgCl_2$ CONCENTRATION.


2500
2000
1500
1000
500
0
EGG WET WEIGHT, mg.
500
1000
1500
2000
2500
EGG VOLUME, mm3.

There was a negative curvilinear relationship between egg volume and the negative logarithm of the ionic concentration. The sigmoidal curves for $MgCl_2$ and $CaCl_2$ were very similar; both tended to plateau at very high and very low concentrations (Fig. 17). A sigmoidal curve was also produced when the wet weight of eggs was plotted against the negative logarithm of the ionic concentration (Fig. 18). The relationship between the mean wet weight of eggs in milligrammes ($\underline{y}$) and the egg volume in cubic millimetres ($\underline{x}$) can be described by the linear relationship: $y = 1.04(\pm 0.04)x - 10.75$. The coefficient of correlation of regression, $r = +0.995$, was highly significant (d.f. = 6, $P < 0.001$).

There was a close correlation between egg swelling measured by volumetric and gravimetric methods (Fig. 19). Both methods showed a marked decrease in egg swelling with a rise in the ionic concentration. Lee (1964) studied the effect of various concentrations of sodium chloride on the swelling of *R. pipiens* jelly. As was found in the present study, swelling increased as the ionic concentration of the surrounding medium decreased.

9.2.4 The effect of pH on the swelling of the egg capsule

In the previous section ionic concentration was shown to affect the swelling of the gelatinous egg capsule. To study the effect of pH in isolation, therefore, acetate buffer solutions were used which had a constant salt content. Ninety six eggs taken from the same female were divided equally between six plastic jars and 300ml of a constant salt acetate buffer solution of known pH was added to each one. These buffer solutions were prepared by the method described by Palmer (1946). The volume of the eggs measured after 24 hours at 15°C (Table 33) was plotted against pH (Fig. 20).

Table 33. Mean egg volume ± 1 S.E. after 24 hours in constant salt acetate buffer solutions of different pH; each sample was based on 16 eggs

pH	Mean egg volume after 24 hours ± 1 S.E. (mm^3)
4.0	62.3 ± 2.50
4.4	80.7 ± 2.44
4.6	86.8 ± 2.81
4.8	90.7 ± 2.68
5.2	104.8 ± 4.62
5.6	152.7 ± 3.94

There was a positive curvilinear relationship between egg volume and pH. The swelling of the gelatinous capsule surrounding the frog's egg was progressively inhibited by increasing hydrogen ion concentration. Lee (1964) studied the effect of pH on the swelling of *R. pipiens* eggs and found that swelling was inhibited in solutions of low pH. Similarly, Kobayashi (1954) observed that the swelling of *Bufo vulgaris formosus* eggs was reduced in solutions of low pH.

9.2.5 The effect of different anions and cations on the swelling of the egg capsule

Batches of 16 eggs from the same female were placed in eighty plastic jars. Ten salt solutions, each at eight molar concentrations, were made up and 300ml of each one was placed in a jar of 16 eggs. Most of the ions used were those found commonly in pond water (Moore and Bellamy, 1974). The volume of the eggs was measured after 24 hours at 15°C (Table 34).

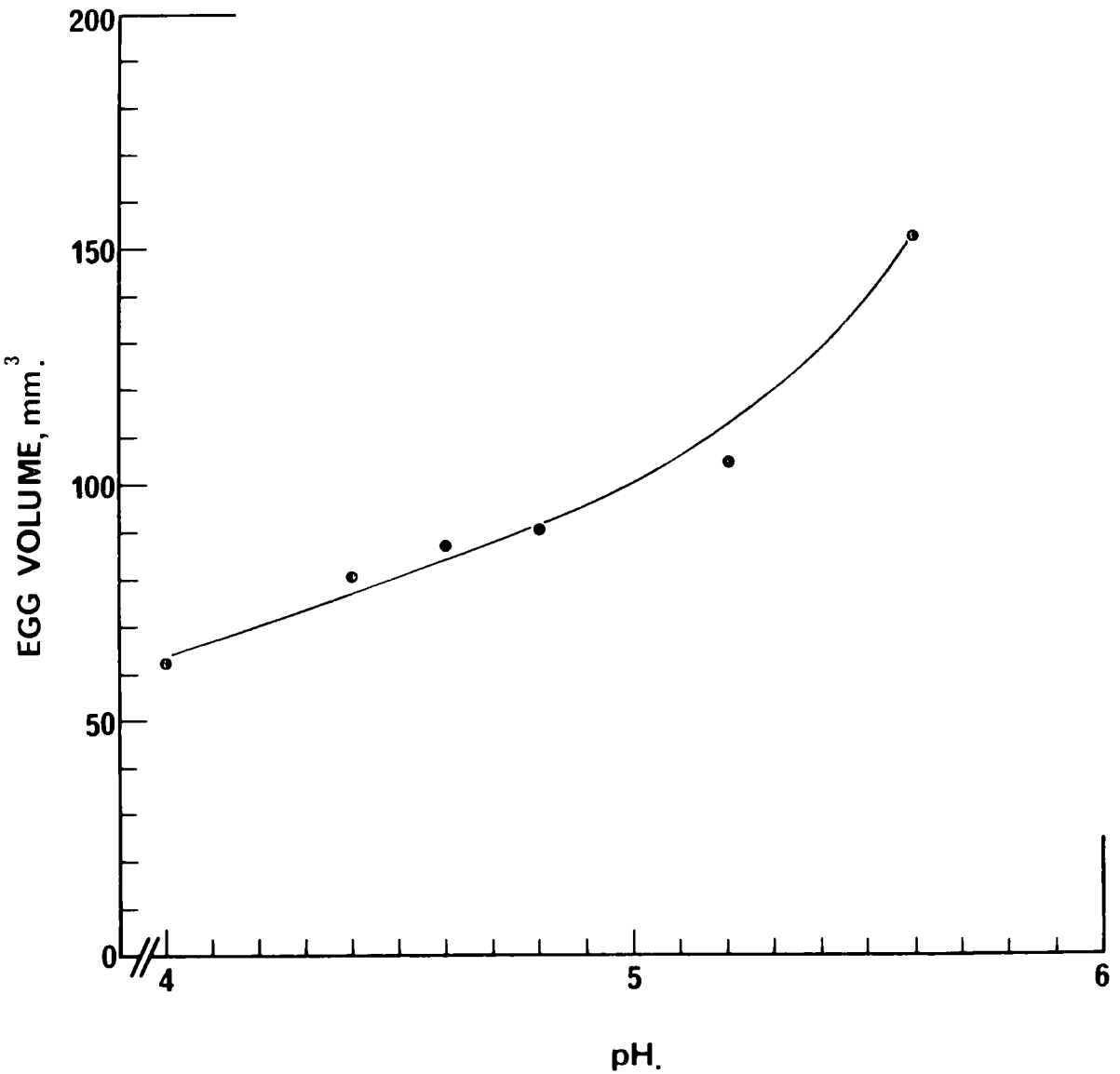

200
150
100
50
0
EGG VOLUME, mm.3
4
5
6
pH.

Table 34. The mean egg volume after 24 hours in salt solutions of varying concentration; each sample was based on 16 eggs

		Mean volume (mm^3) $\pm$ 1 S.E. after 24 hours in salt solutions at the following concentrations:							
Molar Concn.		10^{-2}M	$4x10^{-3}$M	10^{-3}M	$4x10^{-4}$M	10^{-4}M	$4x10^{-5}$M	10^{-5}M	10^{-6}M
Normal Concn.		10^{-2}N	$4x10^{-3}$N	10^{-3}N	$4x10^{-4}$N	10^{-4}N	$4x10^{-5}$N	10^{-5}N	10^{-6}N
NaCl		159±10.0	254±15.3	462±31.2	988±77.1	1,500±66.9	1,748± 74.5	1,949±120.0	2,206± 93.9
KCl		174±14.6	230±19.3	535±38.9	970±89.7	1,566±87.2	1,749± 79.5	2,010±148.8	2,200±104.2
$NaHCO_3$		173± 7.6	285±17.3	588±52.4	1,009±60.8	1,624±87.9	2,085±164.9	2,134±106.7	2,276±138.7
$NaNO_3$		151±10.8	268±20.4	556±31.0	1,033±90.1	1,522±80.9	1,745±134.3	1,946± 65.7	2,135±140.3
HCl		50± 1.6	84± 1.2	114±10.0	197±13.9	489±32.5	968± 46.0	1,521± 74.9	2,083±104.4
Molar Concn.		10^{-2}M	$4x10^{-3}$M	10^{-3}M	$4x10^{-4}$M	10^{-4}M	$4x10^{-5}$M	10^{-5}M	10^{-6}M
Normal Concn.		$2x10^{-2}$N	$8x10^{-3}$N	$2x10^{-3}$N	$8x10^{-4}$N	$2x10^{-4}$N	$8x10^{-5}$N	$2x10^{-5}$N	$2x10^{-6}$N
$CaCl_2$		137± 6.3	176± 5.9	294±10.7	481±19.4	984±54.3	1,153± 70.4	1,744± 58.0	2,147±102.3
$MgCl_2$		129± 9.9	216±11.4	383±20.5	533±23.8	955±72.3	1,303± 82.8	1,803± 95.1	2,143±123.0
$MgSO_4$		181± 7.3	211± 8.8	348±20.4	499±18.9	1,020±70.2	1,241±118.5	1,849± 79.5	2,095±106.1
Na_2SO_4		129± 9.2	172±12.0	343±30.8	621±35.4	1,294±71.1	1,752±126.6	2,073±128.1	2,165±174.7
H_2SO_4		27± 1.9	50± 6.2	108± 9.9	163±11.1	518±35.0	818± 46.3	1,537± 79.0	2,109± 88.9

Analysis of the data showed that the variance increased as the egg volume increased. To stabilize the variance the data were transformed by taking $\log_e$ of the egg volume and $\log_{10}$ of the concentration, plus six. By adding six, values of $\log_{10}$ concentration became positive. The relationship between egg volume and concentration after transformation was slightly sigmoidal, but over the concentration range, 4×10^{-3} to 10^{-5}M, there was a linear relationship between the two variables, with a correlation in excess of 0.91 in all cases. Excluding the maximum and minimum concentrations was justifiable, as they represent pond water concentrations absent in nature (Salthe, 1965). Transforming the data into logarithms also facilitated comparisons using molar and normal concentrations, because the slopes of the regression lines were the same and differences were expressed in terms of their intercepts.

The effect of different salts on egg capsule swelling, the concentration expressed as molarity.

Least squares lines were fitted to the data. The estimate of error variance was the error mean square after fitting individual lines to the data. The results of the statistical analyses are shown in Table 35. To distinguish the 23 values of P given in Table 35 each was assigned a number. Thus, when a value of P was quoted in the text, the test to which it referred could be identified. Ten hypotheses were examined to discover if there was any significant difference within and between four monovalent and four divalent salts with respect to their action on egg capsule swelling.

Table 35. The statistical analysis of the effects of different salts on egg capsule swelling

where (b = slope, a = intercept)

a_1b_1 = $NaCl$, a_2b_2 = KCl, a_3b_3 = $NaHCO_3$, a_4b_4 = $NaNO_3$, a_5b_5 = $CaCl_2$, a_6b_6 = $MgCl_2$, a_7b_7 = $MgSO_4$, a_8b_8 = Na_2SO_4, a_9b_9 = HCl, $a_{10}b_{10}$ = H_2SO_4.

N.B. The tests are not mutually independent.

	Hypothesis tested (Concentration expressed as molarity)	P value (approx.)	
1	$b_1=b_2=b_3=b_4=b_5=b_6=b_7=b_8$	$P_1 = 5\times10^{-4}$	sign
2	$b_5=b_6=b_7=b_8$	$P_2 = 3\times10^{-3}$	sign
3	$b_1=b_2=b_3=b_4$	$P_3 = 0.7$	insign
4	$b_5=b_6=b_7$	$P_4 = 0.3$	insign
5	$b_1=b_2=b_3=b_4=b_5=b_6=b_7$	$P_5 = 0.3$	insign
6	$b_1=b_2=b_3=b_4=b_8$	$P_6 = 0.9\times10^{-6}$	sign
7	$a_1=a_2=a_3=a_4$, $b_1=b_2=b_3=b_4$	$P_7 = 0.5$	insign
8	$a_5=a_6=a_7$, $b_5=b_6=b_7$	$P_8 = 1.00$	insign
9	$a_1=a_2=a_3=a_4=a_5=a_6=a_7$, $b_1=b_2=b_3=b_4=b_5=b_6=b_7$	$P_9 = 0.6\times10^{-9}$	sign
10	$a_1=a_2=a_3=a_4=a_5=a_6=a_7$	$P_{10} = 0.03$	sign
	(Concentration expressed as normality)		
11	$b_1=b_2=b_3=b_4=b_5=b_6=b_7=b_8$	$P_{11} = 5\times10^{-4}$	sign
12	$b_5=b_6=b_7=b_8$	$P_{12} = 3\times10^{-3}$	sign
13	$b_1=b_2=b_3=b_4$	$P_{13} = 0.7$	insign
14	$b_5=b_6=b_7$	$P_{14} = 0.3$	insign
15	$b_1=b_2=b_3=b_4=b_5=b_6=b_7$	$P_{15} = 0.3$	insign
16	$b_1=b_2=b_3=b_4=b_8$	$P_{16} = 0.9\times10^{-6}$	sign
17	$a_1=a_2=a_3=a_4$, $b_1=b_2=b_3=b_4$	$P_{17} = 0.5$	insign
18	$a_5=a_6=a_7$, $b_5=b_6=b_7$	$P_{18} = 0.96$	insign

Continued overleaf.....

Table 35. (Continued)

	(Concentration expressed as normality)	P value (approx.)	
19	$a_1=a_2=a_3=a_4=a_5=a_6=a_7$, $b_1=b_2=b_3=b_4=b_5=b_6=b_7$	$P_{19} = 0.79$	insign
20	$a_1=a_2=a_3=a_4=a_5=a_6=a_7$	$P_{20} = 0.92$	insign
21	$b_9=b_{10}$	$P_{21} < 0.001$	sign
22	$a_9=a_{10}$	$P_{22} < 0.001$	sign
23	$a_9=a_{10}$, $b_9=b_{10}$	$P_{23} < 0.001$	sign

Hypothesis 1 That the slopes of the linear regression lines for the four monovalent salts (b_1, b_2, b_3, b_4) and the four divalent salts (b_5, b_6, b_7, b_8) were not significantly different. This hypothesis was false, $P_1 < 0.001$.

Hypothesis 2 That the slopes of the linear regression lines for the four divalent salts (b_5, b_6, b_7, b_8) were not significantly different. This hypothesis was false, $P_2 < 0.001$.

Hypothesis 3 That the slopes of the linear regression lines for the four monovalent salts (b_1, b_2, b_3, b_4) were not significantly different. This hypothesis was true, $P_3 > 0.05$.

Hypothesis 4 That the slopes of the linear regression lines for three of the divalent salts, excluding Na_2SO_4, (b_5, b_6, b_7) were not significantly different. This hypothesis was true, $P_4 > 0.05$.

Hypothesis 5 That the slopes of the linear regression lines for the four monovalent salts and three of the divalent salts, excluding Na_2SO_4, (b_1, b_2, b_3, b_4, b_5, b_6, b_7) were not significantly different. This hypothesis was true, $P_5 > 0.05$.

Hypothesis 6 That the slopes of the linear regression lines for the four monovalent salts (b_1, b_2, b_3, b_4) and the divalent salt Na_2SO_4 (b_8) were not significantly different. This hypothesis was false, $P_6 < 0.001$.

Hypothesis 7 That the slopes and the intercepts of the linear regression lines for the four monovalent salts (b_1, b_2, b_3, b_4) (a_1, a_2, a_3, a_4) were not significantly different. This hypothesis was true, $P_7 > 0.05$.

Hypothesis 8 That the slopes and the intercepts of the linear regression lines for three of the divalent salts, excluding Na_2SO_4, (a_5, a_6, a_7) (b_5, b_6, b_7) were not significantly different. This hypothesis was true, $P_8 > 0.05$.

Hypothesis 9 That the slopes and the intercepts of the linear regression lines for the four monovalent salts and three of the divalent salts, excluding Na_2SO_4, (a_1, a_2, a_3, a_4, a_5, a_6, a_7) (b_1, b_2, b_3, b_4, b_5, b_6, b_7) were not significantly different. This hypothesis was false, $P_9 < 0.001$.

Hypothesis 10 That the intercepts of the linear regression lines for the four monovalent salts and three of the divalent salts, excluding Na_2SO_4, (a_1, a_2, a_3, a_4, a_5, a_6, a_7) were not significantly different. This hypothesis was false, $P_{10} < 0.03$.

From these tests the following conclusions can be drawn. There was no significant difference between the four monovalent salts (P_3, $P_7 > 0.05$). Similarly, there was no significant difference between three of the divalent salts (P_4, $P_8 > 0.05$), excluding $NaSO_4$, which was significantly different from all monovalent and divalent salts (P_1, P_2, $P_6 < 0.033$). The monovalent salts were significantly different from the divalent salts (P_{10}, $P_9 < 0.03$), the slopes of the regression lines, however, were the same ($P_5 > 0.05$). Three least squares lines were fitted to the transformed data:

Monovalent salts	$y = -0.84x + 8.75$
Divalent salts, excluding Na_2SO_4	$y = -0.84x + 8.38$
Na_2SO_4	$y = -1.02x + 8.91$

The effect of different salts on egg capsule swelling, the concentration expressed as normality.

Least squares lines were fitted to the data. The estimate of error variance was the error mean square after fitting individual lines to the data. Ten hypotheses were tested as before (Table 35). There was no significant difference between the monovalent salts and three of the divalent salts (P_{13}, P_{14}, P_{15}, P_{17}, P_{18}, P_{19}, $P_{20} > 0.05$), excluding Na_2SO_4, which was significantly different from all other salts (P_{11}, P_{12}, $P_{16} < 0.003$). Two least squares lines were fitted to the transformed data:

All salts except Na_2SO_4	$y = -0.85x + 8.73$
Na_2SO_4	$y = -1.02x + 9.22$

The effect of different acids on egg capsule swelling, the concentration expressed as normality.

Least squares lines were fitted to the data. The estimate of error variance was the error mean square after fitting individual lines to the data. Three hypotheses were examined (Table 35). There was a significant difference between the two acids (P_{21}, P_{22}, $P_{23} < 0.001$), and the following least squares lines were fitted to the transformed data:

HCl	$y = -1.22x + 8.59$
H_2SO_4	$y = -1.41x + 9.27$

There was a significant difference between the effect of monovalent and divalent salts (excluding Na_2SO_4) on egg capsule swelling when molar concentrations were used. This was probably because of the differential reactivity of monovalent and divalent ions. When concentrations

were converted to normality, however, the same number of equivalent weights of solute were compared (an equivalent weight of a solute being that quantity in grams which will react with one gram of hydrogen, or which will react with that amount of another substance which in turn will react with one gram of hydrogen), and there was no significant difference between the effect of monovalent and divalent salts. This suggests that one divalent ion is equivalent in effect to two monovalent ions. Krogh *et al.* (1938) found that a divalent calcium salt was more effective in inhibiting the swelling of *R. temporaria* egg jelly than a monovalent sodium salt. Kobayashi (1954), working with *Bufo vulgaris formosus* eggs, found that at low concentrations monovalent anions affected jelly swelling in accordance with the order of the lyotrophic series (Kruyt, 1949). No such trend existed in the present data.

The effect of Na_2SO_4 on capsular swelling was different from all the other salts tested. The reason for this anomaly was not known.

Hydrogen ions were more effective in inhibiting capsular swelling than other monovalent cations. The reason for this difference was not known.

Kruyt (1949) thought that the effect of neutral salts on macromolecular gels was due to the interaction of the salts with the water. Hey *et al.* (1976) produced a quantitative theory in an attempt to explain the action of anions and cations on a macromolecular system. They thought that it was the action of the ions on the 'empty' volume of the solvent that was important.

9.2.6 The reversibility of egg capsule swelling

Batches of 16 eggs from the same female were placed in four plastic jars. The eggs in the four jars were treated as follows:

The eggs in jar (1) were cultured in low conductivity water from pond (41) for the duration of the experiment.

The eggs in jar (2) were cultured in low conductivity water from pond (41) for 24 hours, then the water was replaced by high conductivity water from pond (2).

The eggs in jar (3) were cultured in high conductivity water from pond (2) for the duration of the experiment.

The eggs in jar (4) were cultured in high conductivity water from pond (2) for 24 hours, then the water was replaced by low conductivity water from pond (41).

All the jars were kept in a constant temperature room at 15^{o}C.

Egg volumes measured at different time intervals are shown in Table 36 and plotted in Fig. 21. After six days the eggs in jar (4) were only two per cent smaller than the eggs in jar (1). The eggs in jar (2) shrank, but were still 38 per cent larger than the eggs in jar (3) after six days. It may be that once the macromolecular gel framework has been stretched, it loses some of its elasticity. It was still possible, however, to reverse the swelling pattern to a large extent.

9.3 Egg volume predictions from pH and conductivity measurements of pond water

9.3.1 Introduction

The previous experiments showed that the temperature and ionic concentration of a solution had a marked effect on the swelling of the gelatinous egg capsule. The nature of the ions in solution appeared to be less important, with the exception of hydrogen ions which were considerably more effective swelling inhibitors than any other ions investigated.

Table 36. The mean volume at different intervals of eggs kept under the following conditions; each sample was based on 16 eggs

(1) Eggs were kept in low conductivity pond water (from pond 41) for the duration of the experiment.

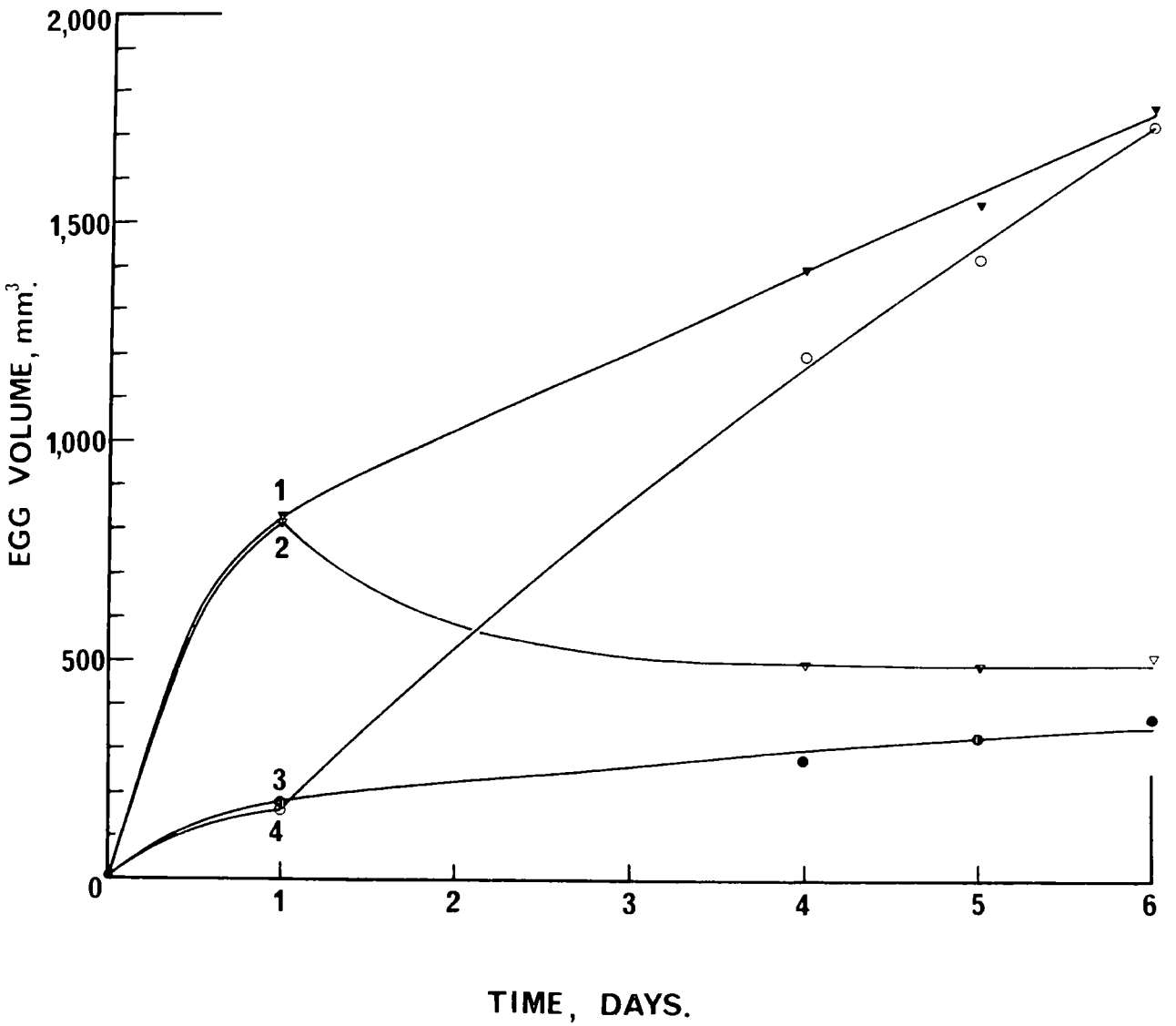
(2) Eggs were kept in low conductivity pond water (from pond 41) for 24 hours, then the water was replaced by pond water of high conductivity (from pond 2).

(3) Eggs were kept in high conductivity pond water (from pond 2) for the duration of the experiment.

(4) Eggs were kept in high conductivity pond water (from pond 2) for 24 hours, then the water was replaced by pond water (from pond 41) of low conductivity.

Time (Hours)	Mean egg volume ± 1 S.E. (mm^3)			
	(1)	(2)	(3)	(4)
0	9.8 ± 0.24	9.8 ± 0.30	9.5 ± 0.38	9.5 ± 0.36
24	832 ± 34.03	820 ± 21.66	173 ± 3.90	161 ± 3.56
96	1,394 ± 53.28	498 ± 22.13	277 ± 12.77	1,195 ± 74.13
120	1,547 ± 55.70	489 ± 18.99	323 ± 18.88	1,418 ± 91.52
144	1,766 ± 68.71	513 ± 23.81	373 ± 25.10	1,729 ± 116.38

It is relatively simple to study a single factor in relation to swelling, but the effect of pond water on the swelling process will obviously be more complex. Even with a detailed analysis of the ionic content of a specific pond water, the expected effect on swelling deduced from the sum of the parts may not necessarily equal the observed effect of the whole. An attempt was made, therefore, to find a relationship


2,000
1,500
1,000
500
0
EGG VOLUME, mm³.
1
2
3
4
1
2
3
4
5
6
TIME, DAYS.

between egg volume and some easily measurable characteristics of pond water, bearing in mind that the total ionic concentration, the hydrogen ion concentration and the temperature of the pond water are probably the critical factors involved.

A good estimate of the ionic concentration of a solution can be obtained from measurements of conductivity, which is a measure of the ability of a conductor to convey an electric current. In the case of pond water, conductivity is related to the concentration of the ions present in solution and to the temperature of the solution. The values obtained, however, give no indication of the nature of the ions in solution. The hydrogen ion concentration and temperature of pond water can be easily measured by means of a pH meter and thermometer respectively.

In the present section, experiments were performed to discover if pH and conductivity measurements of pond water could be used to predict the volume of frog's eggs.

9.3.2 Methods

Water samples were collected from 20 different ponds in 500ml polethylene bottles and promptly returned to the laboratory where they were placed in a 15^{o}C constant temperature room. When the temperature of the water samples reached 15^{o}C, pH and conductivity measurements were taken. Conductivity measurements were made with an Electrolytic Conductivity Measuring Set (model 1 MC-1, Mark V) manufactured by Electronic Switchgear Ltd. Measurements of pH were made with a mains operated Beckman Zeromatic pH meter, model 96.

A total of 20 jars with 16 eggs per jar were set up as described in section (9.1.1) and 300ml of pond water of known conductivity and pH was added to each one. The volume of the eggs was measured after 24 hours (Table 37).

Table 37. Mean egg volume after 24 hours in pond waters of different pH and conductivity; each sample was based on 16 eggs

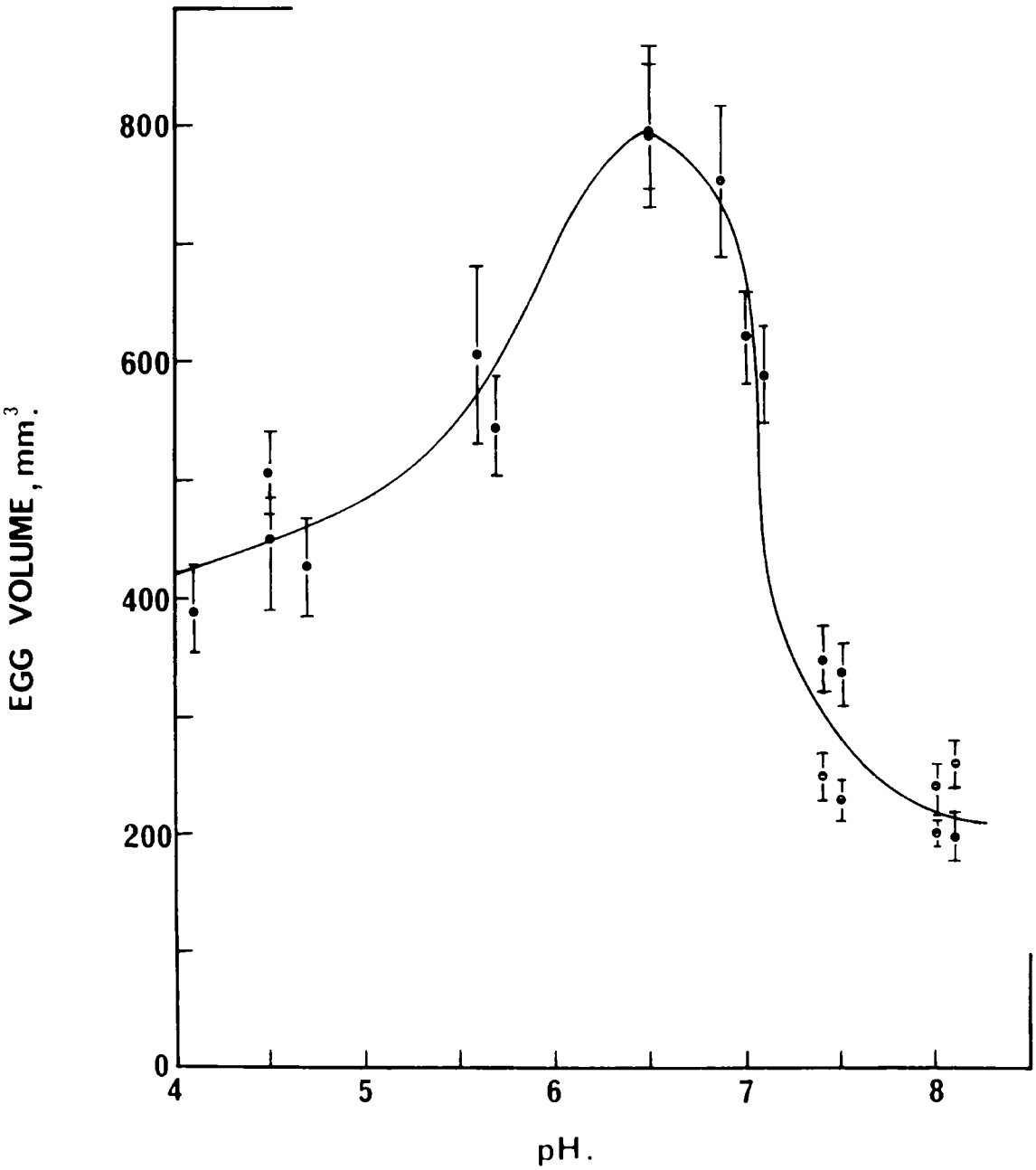
Pond altitude (m)	Conductivity of the pond water (micromhos/cm at 15°C)	pH of the pond water	Mean egg volume after 24 hours ± 1 S.E. (mm^3)
61	890	8.1	198 ± 10.8
137	760	8.0	204 ± 6.0
46	750	7.5	230 ± 8.7
183	670	8.1	260 ± 9.6
122	650	7.4	248 ± 10.5
411	550	8.0	236 ± 11.4
518	355	7.4	347 ± 15.3
373	290	7.5	337 ± 12.2
442	145	4.7	427 ± 20.3
488	135	7.1	587 ± 20.7
335	130	7.0	621 ± 19.3
602	130	3.9	284 ± 22.5
335	115	6.9	752 ± 31.5
617	105	4.1	390 ± 17.7
556	100	4.5	450 ± 30.5
610	90	6.5	790 ± 29.6
602	87	4.5	507 ± 18.3
602	80	5.7	542 ± 21.4
556	77	6.5	792 ± 37.5
617	75	5.6	605 ± 37.2

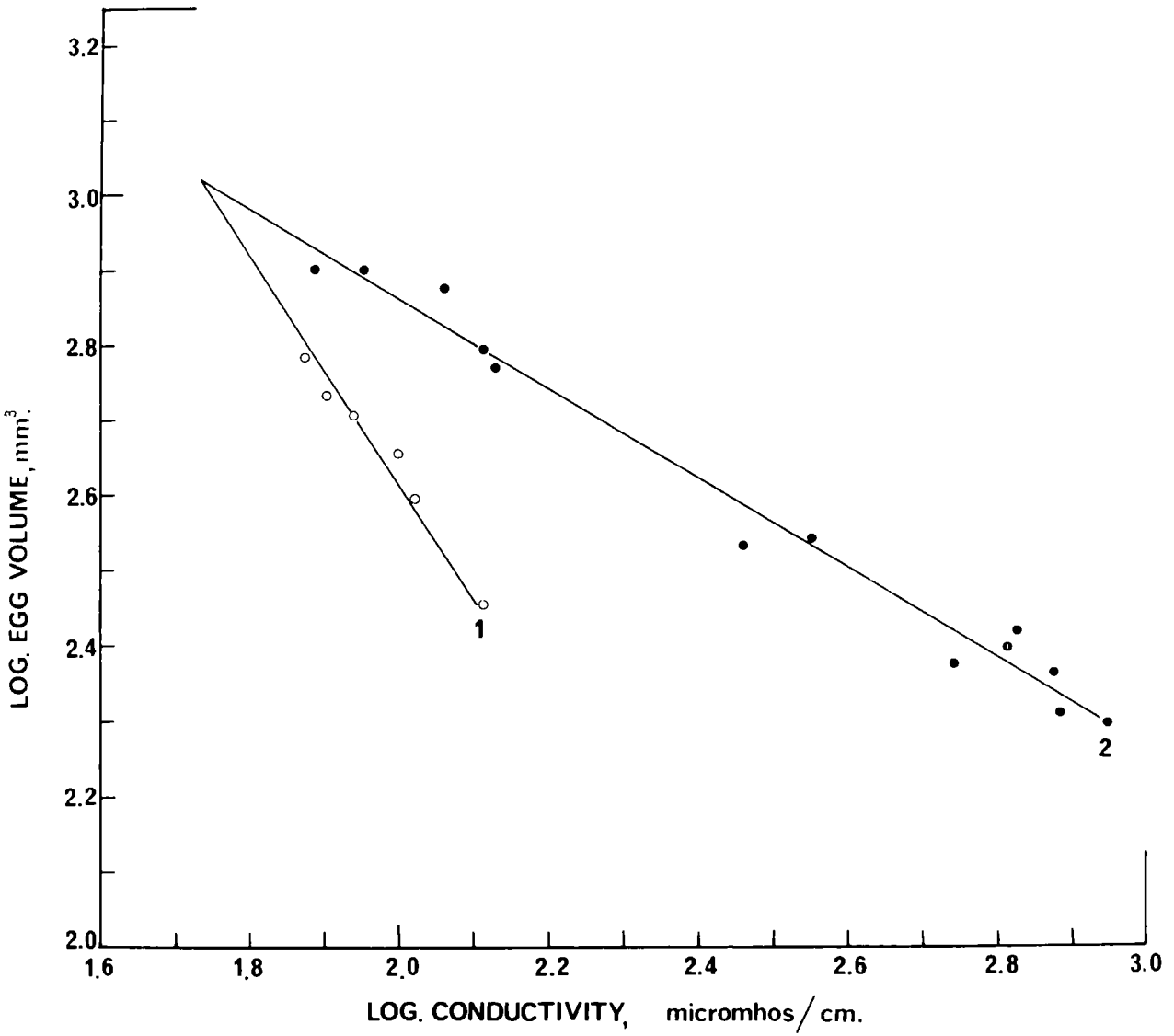
The mean volume ± 1 S.E. of eggs in water from ponds below 200m = 228±12.05.

The mean volume ± 1 S.E. of eggs in water from ponds above 200m = 511±46.41

9.3.3 Results

The relationship between egg volume and pH is shown in Fig. 22. The optimum pH for swelling was about 6.5, very close to the optimum pH of 6.4 for the swelling of *Bufo vulgaris formosus* egg jelly (Kobayashi, 1954).


EGG VOLUME, mm3.
800
600
400
200
0
4
5
6
7
8
pH.


3.2
3.0
2.8
2.6
2.4
2.2
2.0
LOG. EGG VOLUME, mm³.
1
2
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
LOG. CONDUCTIVITY, micromhos / cm.

The relationship between pond water conductivity and egg volume is shown in Fig. 23. The logarithm of mean egg volume in cubic millimetres ($\underline{y}$) was related to the logarithm of the pond water conductivity in micromhos/cm ($\underline{x}$) by the following linear relationships:

For pond water of pH > 6.5, $y = -0.60(\pm 0.02)x + 4.06$

$(r = -0.99, t = 23.35, d.f. = 11, P < 0.001)$

For pond water of pH < 6.5, $y = -1.52(\pm 0.09)x + 5.66$

$(r = -0.99, t = 15.71, d.f. = 5, P < 0.001)$

There was a significant difference between the slopes of the two regression lines ($t = 9.98$, d.f. = 6, $P < 0.001$).

9.3.4 Discussion

From pH and conductivity measurements of pond water a good prediction of whole egg volume can be made. The volume of the whole egg was inversely related to the conductivity of the pond water used as the culture medium. This was expected since conductivity is essentially a measure of ionic concentration.

As discussed in section 2.1, the conductivity of the water in a pond decreases by 126 micromhos/cm for each 100m increase in altitude. From Table 37 it can be seen that the mean volume of eggs after 24 hours in water from ponds below 200m was $228m^3$, less than half the mean volume of $511mm^3$ for eggs in water from ponds above 200m. The capsules of eggs in highland ponds, therefore, are larger than the capsules of eggs in lowland ponds ($t = 5.90$, d.f. = 5, $P < 0.002$), due to the decrease in the ionic concentration of pond water with altitude.

CHAPTER 10

General Discussion

A climatic gradient exists in northern England between the comparatively equable lowland regions and the harsher high moorland areas of the northern Pennines which are invariably colder (Manley, 1936) and subject to more violent climatic fluctuations (Cragg, 1961). Rainfall also increases with altitude. The mean annual rainfall for Moor House (altitude 561m) is 1,889mm, almost three times as much as the mean annual rainfall of 650mm for Durham (altitude 102m). Nevertheless, drought conditions can also occur in high moorland areas (Coulson, 1962). During April 1974 only 16mm of rain fell at Moor House compared with the average of 128mm for this month. The water level in most of the ponds dropped considerably, killing the eggs laid in the shallower areas. *R. temporaria* normally lay their eggs in the shallower water at the edge of a pond (Leutscher, 1953; Savage, 1961; Cooke, 1975), so high egg mortality may often occur in years with low spring rainfall.

Although spawning is later at higher altitudes, highland breeding ponds are probably colder during the embryonic development period. During 1976 the mean temperature between spawning and hatching in highland pond (42) (altitude 556m) was 6.0°C, 1.1°C colder than the eggs in lowland pond (4) (altitude 61m). Highland ponds are also subject to low temperatures for long periods of time. In 1975, for example, the temperature in pond (42) fell below 0.5°C for 36 hours, reaching a minimum temperature of 0.1°C. Although *R. temporaria* eggs can tolerate at least 12 hours at 0°C (Douglas, 1948), the surface layer of eggs in highland ponds is often killed by freezing. Greenhalgh (1974) observed many dead eggs during the spring of 1973 in the high-level tarns on Howgill Fells in northern England. He attributed the death of the eggs to the severity of the weather, but produced little data to justify this statement.

The pH of the breeding ponds decreased by 0.5 for every 100m increase in altitude. Some highland ponds were very acidic with pH values less than 4.0. There is evidence to suggest that the pH of the pond water may affect the success of fertilization. The hydrogen ion concentration is known to be of importance in determining optimum conditions for sperm activity in many animals (Rothschild, 1956). There is also evidence for a diffusible 'fertilizin'-like substance in amphibian egg mucoids which is important for sperm activation (Barbieri and Villeco, 1966). The activity of these substances is affected by pH (Nelsen, 1953; Brachet, 1950). Gosner and Black (1957) found that low pH caused abnormalities in the embryos of certain frogs from New Jersey. The abnormalities were produced by mechanical pressure due to the failure of the perivitelline space to absorb normal quantities of water.

During the course of the present work dead eggs were found in many of the highland ponds. For example, when nine clumps of spawn laid in pond (45) (altitude 602m) were examined on 30 April 1976, no living embryos were found. Similarly, out of 37 clumps of spawn examined in pond (41) (altitude 556m), only one per cent of the embryos were alive. Both pond (41) and pond (45) had low pH values of 4.5 and 3.9 respectively, but whether this caused or contributed to the death of the eggs was not known.

Most of the dead eggs in highland ponds were covered with a grey fungus, similar to that described by Fryer (1973) and identified as *Saprolegnia ferax*. This fungus may have killed the eggs, or perhaps were saprophytic, living on eggs killed by some other agent. None of the eggs in lowland ponds were infected with this fungus. Greenhalgh (1974) found *R. temporaria* eggs covered with this fungus in certain high-level tarns on Howgill Fells in northern England, but could find no infected eggs in several hundred clumps of spawn examined in ponds on the Lancashire coastal plain.

Desiccation, freezing, low pH and possibly fungal attack are some of the environmental pressures experienced by the eggs of highland frogs. Differences were found to exist between highland and lowland frogs and their eggs, which may have been the result of adaptation to these environmental pressures.

The spawning of frogs was delayed by six days for every 100m increase in altitude. Highland embryos will, therefore, avoid the lower temperatures at higher altitudes to some extent. The later spawning of highland frogs may simply be the effect of low environmental temperature on a 'physiological clock', or perhaps the date of spawning is under genetic control. Recording the date of spawning of lowland frogs transferred to a highland site, and *vice versa*, would help to resolve this problem. Low temperatures and later spawning at higher altitudes, however, cause a delay in metamorphosis. Larval *R. temporaria* from northern England metamorphose comparatively late in the year. Young frogs leave lowland ponds during June and July, and highland ponds during August and September. In most other parts of England juvenile frogs are leaving the ponds at the end of May or the beginning of June (Smith, 1969). Accordingly, in the highland areas of northern England in particular, the young frogs have little or no time to feed and store the energy reserves required to survive the first winter.

Embryos from highland areas in northern England developed about four per cent faster at $6^{\circ}C$ than lowland embryos between stage 3 (appearance of the furrow of the first division) and stage 20 (gill circulation). This will compensate to some extent for the later spawning and colder ponds at higher altitudes, and will also reduce the time that frail immobile embryos are at risk from freezing and desiccation. This difference in the development rate may extend beyond stage 20, but this is rather difficult to test because once the embryos have hatched, numerous additional factors can affect the rate of development and the time of metamorphosis.

Larvae deprived of food soon stop growing and differentiating. D'Angelo *et al.* (1941) found that *R. sylvatica* and *R. pipiens* tadpoles subjected to complete inanition were either retarded or accelerated in metamorphosis depending on the development stage at which food was withdrawn. Inanition caused atrophic and degenerative changes in the thyroid and pituitary gland, so it was concluded that failure of starved animals to metamorphose was directly related to a decreased production and release of thyotropic hormone from the anterior hypophysis.

Any inanition found in nature would probably be associated with crowding, which also affects the growth and development of amphibian larvae. Using *R. sylvatica* and *R. pipiens* larvae, Adolf (1931) showed that crowding retarded growth and metamorphosis. When tadpoles are crowded, some grow much larger and develop faster than others (Rose, 1960). Experiments with *R. pipiens* by Richards (1958) showed that large tadpoles produced a substance which inhibited the growth of smaller tadpoles. The inhibitory substance in *R. pipiens* was shown to be associated with a type of algal cell (Richards, 1962). In most cases this substance is species specific (Akin, 1966).

Lack of iodine in the environment and in the diet can also affect metamorphosis (Lynn and Brambel, 1935) but there is little evidence to suggest that iodine deficiency ever delays metamorphosis in nature. Metamorphosis is also affected by light. Guyétant (1964) found that the growth and metamorphosis of *R. temporaria* larvae was accelerated in constant light, but was retarded in complete darkness.

R. temporaria embryos from highland regions were found to have a lower lethal limit for 50 per cent normal development of 2.8°C between stage 3 (appearance of the furrow of the first division) and stage 20 (gill circulation), one Celsius degree lower than the limit for the normal development of lowland embryos. The temperature tolerance of these embryos, however, may vary at different developmental stages. For example, Zwölfer

(1935) found that the developmental null point of larvae of the Black Arches Moth, *Lymantria monacha* was higher at each developmental stage. This is consistent with the fact that larval *L. monacha* in central Europe develop from April to June when the environmental temperature is rising. The increased resistance of highland *R. temporaria* embryos to low temperatures almost certainly increases their chances of survival because, as previously mentioned, highland ponds are subject to temperatures near freezing for long periods of time. The physiological reason for the difference in lower lethal limit between highland and lowland frogs is not known. In wasps, the difference in the temperature tolerance between castes appears to be due to the differential resistance of the mitochondria (Schwalbach and Agostini, 1964).

Highland female frogs produced an average of 707 eggs, less than half the mean number of 1,586 eggs produced by lowland females. This is probably because highland female frogs are about five per cent smaller than lowland females, and smaller *R. temporaria* usually produce fewer eggs (Kozlowska, 1971). In addition, the short growing season and the possible lack of food in high moorland areas (Houston, 1973) limits the energy available for growth and reproduction, as well as for enduring the long winter. The difference in egg number and female size, therefore, is probably phenotypic, being the result of environmental variation. Although highland frogs have a lower reproductive potential than lowland frogs, they appear to be more numerous. This implies that at some stage in the life cycle, highland frogs have a higher percentage survival rate than lowland frogs. A decrease in reproductive potential with altitude is not uncommon. The rush, *Juncus squarrosus*, for example, often produces fewer seed capsules at higher altitudes than at lower altitudes (Reay, 1964). Similarly, Coulson (1956) found that the Meadow Pipit, *Anthus pratensis* produced fewer eggs at higher altitudes. The average clutch size near sea-level was 4.52, whereas at altitudes over 305m the average clutch size decreased to 4.07.

The mucopolysaccharide capsules covering the eggs of Common Frogs act as an insulating layer, keeping the embryos warmer than the surrounding water. Savage (1950) found that the eggs of *R. temporaria* were on average 0.63°C warmer than the surrounding water. This difference is small, but may be very important to a species breeding early in the year when pond temperatures are often close to the lower lethal limit. The volume of the egg capsules and their insulating efficiency varied in different pond waters. Egg capsules in low conductivity pond water from highland ponds (altitude > 200m) were 241 per cent larger than egg capsules in high conductivity pond water from lowland ponds (altitude < 200m). The size of the gelatinous capsules made little difference to the temperature of a clump of eggs heated by a batch of light bulbs. During the cooling phase, however, the eggs with large capsules were more effective in conserving heat. In nature clumps of eggs will warm up during the day and cool to their lowest point during the night and early morning. This is the time when eggs will be at greatest risk, and even if the gelatinous capsules keep them even a fraction of a degree warmer than the surrounding water, it may allow the eggs to survive. The fact that gelatinous capsules are larger in highland ponds may well increase egg survival. This is probably fortuitous, as there is no evidence to suggest that frogs choose their breeding ponds because of the effect that the pond water will have on the size of the egg capsule.

Using Prosser's definition given in the introduction, several of the differences between lowland and highland frogs would appear to be adaptive and favour survival in a stressful environment. As Maynard Smith (1966) pointed out, however, it is desirable to demonstrate the selective advantage of a particular characteristic in the field before describing it as adaptive. Because of the difficulties involved, this is seldom done, and the adaptive value of a characteristic has to be implied rather than proven.

There have been some demonstrations of a characteristic's adaptive value, as for example in the work by Dice (1947). In populations of Deer-mice, *Peromyscus maniculatus*, animals living on sandy soils have a lighter coat colour than animals living on darker soils. Dice found that owls took a greater proportion of mice whose colour differed from the background than mice whose colour merged with the background.

Highland Common Frogs have a low reproductive potential and although their eggs appear to have adapted to the lower environmental temperatures at higher altitudes, almost 100% mortality can still occur. Nevertheless, Common Frogs are abundant in the high moorland areas of the northern Pennines and there are few ponds not used by frogs for spawning. The reason for their success may be that highland tadpoles have fewer predators than lowland tadpoles. Savage (1961) found that 99 to 100 per cent of all tadpoles were lost in certain lowland ponds in southern England. Once the young frogs leave highland ponds and survive the first winter there is probably little or no mortality. Common Frogs are eaten by snakes, hawks, owls, crows, gulls, ducks, terns, herons, hedgehogs, stoats, weasels, badgers, otters and rats (Smith, 1969). Most of these predators are less numerous or absent at higher altitudes.

Man has had little impact on highland Common Frogs, but human activities are one of the major reasons for the general decline in frog numbers. The results of the survey carried out by Cooke (1972a) suggest that Common Frog numbers decreased slightly over most of the British Isles during the 1940s and 1950s and suffered considerable declines over most of England throughout the 1960s. Cooke thought that the main reason for the decline was the loss of suitable wetland habitat due to sites being filled in or drained. There are many frogs killed on roads during breeding migrations (Savage, 1961). Hodson (1966) found 409 dead frogs on two miles of road in Northamptonshire during 1959 and 1960. It is difficult to

assess the effect of road deaths on frog numbers, however, unless the number of frogs killed is expressed as a percentage of the total population. There is probably little mortality resulting from insecticides. Environmental concentrations required to produce hyperactivity in amphibian larvae (tissue levels of 2-4 p.p.m. DDT (Cooke, 1972b, 1973) are not normally encountered in the field in Britain (Cooke, 1972a).

Superficially, the cold wet moorland habitat seems to be unfavourable for Common Frogs, causing a reduction in reproductive potential, high egg mortality and a delay in the date of emergence of juvenile frogs. There are benefits, however, to be derived from living at higher altitudes. The high annual rainfall in highland regions means that there are numerous breeding ponds for Common Frogs which seldom dry out. In addition, young frogs emerging from the ponds are less likely to become desiccated. A wet environment is highly suitable for other animals. The Enchytraeid, *Cognettia sphagnetorum*, for example, is abundant at higher altitudes where it is relatively free from desiccation and it has a large supply of decaying organic matter (Peachey, 1959). Predation may also be less at higher altitudes. Coulson (1956) found that mortality in the Meadow Pipit decreased with altitude, probably due to less nest predation.

The Common Frog appears to be successful in the highland environment. This is fortunate, because if the decline in frog numbers over the last thirty years continues in lowland England, highland populations may well become an important reservoir.

SUMMARY

1. Aspects of the breeding biology of the Common Frog, *Rana temporaria temporaria* L. were studied in relation to altitude. Most of the research was carried out between 1974 and 1976, although spawn date records were obtained during the spring of 1977.

2. Work centred on 55 breeding ponds in northern England, ranging in altitude from 86 to 838m. All the ponds were within a 20km range of latitude, mostly in the Wear Valley to the west of Durham City.

3. The water chemistry of the ponds in this area varied with altitude. The pH and conductivity of the pond water decreased by 0.5 and 126 micromhos/cm respectively for every 100m increase in altitude.

4. Prior to spawning, a chemical temperature integration technique with a probable precision of $\pm 0.1^{\circ}C$ was used to measure pond and soil temperatures at a series of sites. Both pond temperatures measured at a depth of 10cm and soil temperatures measured at a depth of 5cm fell by approximately $0.4^{\circ}C$ for every 100m increase in altitude.

5. During 1976 two thermographs were used to measure the temperature of clumps of spawn in highland and lowland ponds. The mean temperature of the highland clump between spawning and hatching was $6.0^{\circ}C$, $1.1^{\circ}C$ lower than the mean temperature of the lowland clump. The lowland clump was at or below $5^{\circ}C$ for five per cent of the time, the minimum temperature being $4.5^{\circ}C$, while the highland clump was at or below $5^{\circ}C$ for 54 per cent of the time, the minimum temperature being $2.9^{\circ}C$.

6. Temperature data from the Moor House and Durham Meteorological Stations suggest that highland ponds are normally colder than lowland ponds during the embryonic development period.

7. Between 1974 and 1977 the date of spawning was always later at higher altitudes. On average, spawning was six days later for every 100m increase in altitude. Possible triggering mechanisms for emergence and spawning are discussed.

8. The time taken for *R. t. temporaria* embryos from northern England to develop from stage 3 (appearance of the furrow of the first division) to stage 20 (initiation of gill circulation) was measured at 6°, 8°, 10°, 15°, 16° and 20°C. These results are compared with similar data for French/Belgian and Japanese embryos. Embryos from northern England had a rapid development rate compared with the embryos from Europe and Japan, particularly at low temperatures. It is suggested that the rapid development rate in embryos from northern England was associated with the shorter growing season and colder environmental conditions at higher latitudes.

9. Highland embryos from northern England developed one per cent slower than lowland embryos at 16°C, but four per cent faster at 6°C. This is thought to be an adaptation to the colder breeding ponds at higher altitudes.

10. The lower lethal limit for 50 per cent normal development in highland embryos was 2.8°C, one Celsius degree lower than the limit for the normal development of lowland embryos. These values are below most of the lower limiting temperature estimates previously obtained for *R. t. temporaria*.

11. Highland female frogs had snout-vent lengths approximately five per cent smaller than lowland females, the reverse of that expected from zoo-geographical rules.

12. Highland females produced an average of 707 eggs, less than half the number of 1,586 eggs produced by lowland females.

13. Possible causes for the variation in female size and egg number with altitude are discussed.

14. The mucopolysaccharide capsule covering the eggs of the Common Frogs acted as an insulating layer, keeping the embryos warmer on average than the surrounding water. This is thought to be of importance as Common Frogs breed early in the year when pond temperatures are often close to the lower lethal limit.

15. The volume and insulating efficiency of egg capsules varied in different pond waters. Egg capsules in low conductivity pond water were larger and more efficient insulators than egg capsules in high conductivity pond water.

16. There was no significant difference between the swelling of fertile and infertile egg capsules.

17. Temperature had an effect on the swelling of the gelatinous egg capsule. Over the temperature range 2° to $20^{\circ}C$ the volume of the egg capsule after 24 hours increased by two per cent for every $1^{\circ}C$ rise in temperature.

18. Variation in the oxygen concentration of the culture medium had no significant effect on the swelling of the egg capsule.

19. The ionic concentration of the culture medium had a marked effect on the swelling of the egg capsule. The whole egg volume decreased as the ionic concentration increased.

20. There was a negative curvilinear relationship between the whole egg volume after 24 hours and the pH of the culture medium.

21. In general, the effect on capsular swelling of two monovalent ions is the same as the effect of one divalent ion. Hydrogen ions, however, are more effective in inhibiting swelling than other monovalent cations. The effect of sodium sulphate on the swelling of the egg capsule was different from all the other salts tested. The reason for this anomaly is not known.

22. The swelling of the gelatinous egg capsule was reversible to a large extent.

23. A prediction of the whole egg volume could be made from pH and conductivity measurements of the pond water used as the culture medium.

24. The mean volume of eggs after 24 hours in low conductivity water from highland ponds (altitude > 200m) was 511 mm^3, more than twice the mean volume of 218 mm^3 for eggs in high conductivity water from lowland ponds (altitude < 200m). The larger gelatinous capsules surrounding eggs in highland ponds may affect their chances of survival.

25. The differences found between lowland and highland frogs are discussed in terms of their potential adaptive significance.

REFERENCES

(Abbreviations for the Journals were taken from 'World List for Scientific Periodicals', London, Butterworths)

ADOLPH, E.F. 1931. Body size as a factor in the metamorphosis of tadpoles. Biol. Bull., 61:376-386.

AEBLI, H. 1966. Rassenunterschiede in Bezug auf Entwicklungsgeschwindigkeit und Geschlechtsdifferenzierung bei *Rana temporaria* in den Tälern des Kantons Glarus (Schweiz). Revue suisse Zool., 73:1-35. (In German with English and French summaries)

AKIN, G.C. 1966. Self-inhibition of growth in *Rana pipiens* tadpoles. Physiol. Zool., 39:341-356.

ALLISON, R.M. 1956. Failure of enforced hibernation to inhibit breeding in the frog (*Rana temporaria*). Nature, 177:342.

ANGELIER, E. and M.L. ANGELIER. 1968. Observations sur le développement embryonnaire et larvaire de *Rana temporaria* L. Ann. Limnol., 4:113-131. (In French with English and German summaries)

BACHMANN, K. 1969. Temperature adaptation of amphibian embryos. Am. Nat., 103:115-130.

BAILEY, N.T.J. 1973. Statistical methods in Biology. The English Universities Press Ltd.

BALCELLS, E.R. 1956. Estudio morfológico, biológico y ecologico de *Rana temporaria* L. Publnes. Inst. Biol. apl., 24:81-121. (In Spanish)

BALINSKY, B.I. 1969. The reproductive ecology of amphibians of the Transvaal highveld. Zool. Afr., 4:37-93.

BANTA, A.M. and R.A. GORTNER. 1914. A milky white amphibian egg jell. Biol. Bull., 27:259-261.

BARBIERE, F.D. and E.I. VILLECO. 1966. A fertilizin-like substance in the egg jelly of the toad, *Bufo arenarum*. Archo zool. ital., 51:227-237.

BAXTER, G.T. 1952. Notes on growth and the reproductive cycle of the Leopard Frog, *Rana pipiens* Schreber, in southern Wyoming. J. Colo.-Wyo. Acad. Sci., 4:91.

BERTHET, P. 1960. La mésure écologique de la température par détermination de la vitesse d'inversion du saccharose. Vegetatio acta geobotanica, 9:197-207. (In French)

BOLOGNANI, L., A.M. BOLOGNANI FANTIN, R. LUSIGNANI and L. ZONTA. 1966. Presence of sialopolysaccharidic components in egg gelatinous mantle of *Rana latastei* and *Bufo vulgaris*. Experienta, 22:601-603.

BOULENGER, G.A. 1897. The Tailless Batrachians of Europe. Ray Society, London, 2 vols.

BRACHET, J. 1950. Chemical Embryology. New York, Interscience Publishers.

BRAGG, A.N. 1964. Lens action by jelly of frog's egg. Proc. Okla. Acad. Sci., 44:23.

BRENNER, F.J. 1969. The role of temperature and fat deposition in hibernation and reproduction in two species of frogs. Herpetologica, 25:105-113.

BULLOCK, T.H. 1955. Compensation for temperature in the metabolism and activity of poikilotherms. Biol. Rev., 30:311-342.

CLARKE, F.W. 1930. Data of Geochemistry. U.S. Geol. Survey Bull., 770.

CLYMO, R.S. 1964. The origin of acidity in *Sphagnum* bogs. Bryologist, 67:427-431.

COOKE, A.S. 1972a. Indications of recent changes in status in the British Isles of the frog *Rana temporaria* and the toad *Bufo bufo*. J. Zool., Lond., 167:161-178.

COOKE, A.S. 1972b. The effects of DDT, dieldrin and 2,4-D on amphibian spawn and tadpoles. Environ. Pollut., 3:51-68.

COOKE, A.S. 1973. Responses of *Rana temporaria* tadpoles to chronic doses of pp'-DDT. Copeia, 1973, No.4, :647-652.

COOKE, A.S. 1975. Spawn site selection and colony size of the frog *Rana temporaria* and the toad *Bufo bufo*. J. Zool., Lond., 175:29-38.

CORNMAN, I. and N. GRIER. 1941. Refraction of light by amphibian egg jelly. Copeia 1941, No. 3, pg. 180.

COULSON, J.C. 1956. Mortality and egg production of the Meadow Pipit with special reference to altitude. Bird Study, 3:119-132.

COULSON, J.C. 1962. The biology of *Tipula subnodicornis* Zetterstedt, with comparative observations on *T. paludosa* Meigen. J. Anim. Ecol., 31:1-21.

COULSON, J.C., J.C. HOROBIN, J. BUTTERFIELD and G.R.J. SMITH. 1976. The maintenance of the annual life-cycles in two species of Tipulidae (Diptera); a field study relating development, temperature and altitude. J. Anim. Ecol., 45:215-233.

CRAGG, J.B. 1961. Some aspects of the ecology of moorland animals. J. Anim. Ecol., 30:205-234.

CRISP, D.T. 1966. Input and output of minerals for an area of Pennine moorland: the importance of precipitation, drainage, peat erosion and animals. J. appl. Ecol., 3:327-348.

D'ANGELO, S.A., A.S. GORDON and H. A. CHARIPPER. 1941. The role of the thyroid and pituitary glands in the anomalous effect of inanition on amphibian metamorphosis. J. exp. Zool., 87:259-277.

DARWIN, C. 1859. Origin of Species. New York.

DAVIES, L. and C.D. SMITH. 1958. The distribution and growth of *Prosimulium* larvae (Diptera : Simulidae) in hill streams in northern England. J. Anim. Ecol., 27:335-348.

DICE, L.R. 1947. Effectiveness of selection by owls of Deer-mice *Peromyscus maniculatus* which contrast in colour with their background. Contr. Lab. vertebr. Biol. Univ. Mich., 34:1-20.

DOUGLAS, R. 1948. Temperature and rate of development of the eggs of the British Anura. J. Anim. Ecol., 17:189-192.

FALCONER, K. 1971. The ecology of the Common Frog (*Rana temporaria* Linn.) in County Durham. M.Sc. Thesis, Durham.

FOLKES, B.F., R.A. GRANT and J.K.N. JONES. 1950. Frog-spawn mucin. J. chem. Soc., 440:2136-2140.

FOX, H. MUNRO. 1938. The Activity and Metabolism of Poikilothermal Animals in Different Latitudes. - III. Proc. zool. Soc. Lond., 108:501-505.

FREEMAN, S.B. 1968. A study of the jelly envelopes surrounding the egg of the amphibian, *Xenopus laevis*. Biol. Bull., 135:501-513.

FRY, F.E.J. 1958. Temperature compensation. A. Rev. Physiol., 20:207-224.

FRYER, G. 1973. Unusual egg spoilage in the Common Frog. Naturalist, 926:105-106.

GABAYEVA, N.S. 1962. On the antibiotic properties of the jelly-like membrane of the eggs of *Rana temporaria*. Vest. leningr. gos. Univ., 15:25-32. (In Russian with English summary)

GADOW, H. 1901. Amphibia and Reptiles. The Cambridge Natural History, Macmillan.

GALLIEN, L. 1959. Endocrine basis for reproductive adaptations in Amphibia. Symposium on Comparative Endocrinology. A. Gorbman ed. 479-487.

GHIARA, G. 1960. Ricerche intorno alla struttura microscopica, submicroscopica ed istochimica e alle funzioni degli involucri ovulari di *Discoglossus pictus* Otth e di altre specie di Anfibi. Archo zool. ital., 45:9-92. (In Italian)

GIACOSA, P. 1882. Études sur la composition chimique de l'oeuf et de ses enveloppes chez la grenouille commune. Z. physiol. Chem., 7:40-56. (In French)

GORE, A.J.P. 1968. The supply of six elements by rain to an upland peat area. J. Ecol., 56:483-495.

GOSNER, K.L. and I.H. BLACK. 1957. The effects of acidity on the development and hatching of New Jersey Frogs. Ecology, 38:256-262.

GRAINGER, J.N.R. 1959. The effect of constant and varying temperatures on the developing eggs of *Rana temporaria*. L. Zool. Anz., 163:267-277.

GREENHALGH, M.E. 1974. Egg spoilage in the Common Frog in high-level Pennine tarns. Naturalist, 928:39.

GUYÉTANT, R. 1964. Actions comparées de la lumière et de l'obscurité sur la croissance et la métamorphose des têtards de *Rana temporaria* L. Note préliminaire. Annls scient. Univ. Besançon, 2 série, Zool. et Physiol., 19:77-97. (In French)

GUYÉTANT, R. 1969. Influence du facteur température sur le développement embryonnaire de *Rana temporaria* L. et *Rana dalmatina* B. B. vie. Milieu, 20(c):231-241. (In French with English and German summaries.

HASSINGER, D.D. 1970. Notes on the thermal properties of frog eggs. Herpetologia, 26:49-51.

HERREID, C.F., II, and S. KINNEY. 1967. Temperature and development of the Wood Frog, *Rana sylvatica*, in Alaska. Ecology, 48:579-590.

HEUSSER, H. 1968. Die Lebensweise der Erdkröte, *Bufo bufo* (L.); Wanderungen und Somerquartiere. Revue suisse Zool., 75:927-982. (In German with English and French summaries)

HEUSSER, H. and J. OTT. 1968. Wandertrieb und populationsspezifische Sollzeit der Laichwanderung bei der Erdkröte, *Bufo bufo* (L.) Revue suisse Zool., 75:1005-1022. (In German with English and French summaries)

HEY, M.J., J.M. CLOUGH and D. J. TAYLOR. 1976. Ion effects on macromolecules in aqueous solution. Nature, 262 (No. 5571):807-809.

HIYAMA, N. 1949a. Biochemical studies on carbohydrates. CII. On the jelly of toad and frog eggs. First report. Tohoku J. exp. Med., 50:373-378.

HIYAMA, N. 1949b. Biochemical studies on carbohydrates. CIII. On the jelly of toad and frog eggs. Second report. Hexosamine in the toad mucin. Tohoku J. exp. Med., 50:379-383.

HIYAMA, N. 1949c. Biochemical studies on carbohydrates. CIV. On the jelly of toad and frog eggs. Third report. Hexosamines in the frog mucin. Tohoku J. exp. Med., 50:385-387.

HODKINSON, I.D. 1973. The population dynamics and host plant interactions of *Strophingia ericae* (Curt.)(Homoptera:Pslloidae). J. Anim. Ecol., 42:565-584.

HODSON, N.L. 1966. A survey of road mortality in mammals (and including data for the Grass Snake and Common Frog). J. Zool., Lond., 148:576-579.

HOUSTON, W.W.K. 1973. The food of the Common Frog, *Rana temporaria*, on high moorland in northern England. J. Zool., Lond., 171:153-165.

HUMPHRIES, A.A., Jr. 1966. Observations on the deposition, structure, and cytochemistry of the jelly envelopes of the egg of the newt, *Triturus viridescens*. Devl. Biol., 13:214-230.

HUMPHRIES, A.A., Jr. and W. N. HUGHES. 1959. A study of the polysaccharide histochemistry of the oviduct of the newt, *Triturus viridescens*. Biol. Bull., 116:446-451.

JUSZCZYK, W. and W. ZAMACHOWSKI. 1965. Terms of ovulation and oviposition of the Grass Frog (*Rana temporaria* L.) in conditions of an artificial and prolonged hibernation. Acta biol. cracov., Ser. Zool., 8:211-223.

KAMBARA, S. 1953. Role of jelly envelope of toad eggs in fertilization. Annotnes zool. jap., 26:78-84.

KAMBARA, S. 1956a. Histochemical studies on the distribution of phosphatases in the oviduct of the newt, *Triturus pyrrhogaster*. Annotnes. zool. jap., 29:86-90.

KAMBARA, S. 1956b. Histochemical studies on the distribution of mucopolysaccharides in the oviduct of the newt, *Triturus pyrrhogaster*. Annotnes. zool. jap., 29:146-150.

KAMBARA, S. 1957. Metachromatically staining material in epithelial cells of the oviduct of the newt, *Triturus pyrrhogaster*. J. Fac. Sci. Tokyo Univ., Sect. IV, 8:145-148.

KATAGIRI, C. 1961. On the fertilizability of the frog egg, 1. J. Fac. Sci. Hokkaido Univ. (Zool.), 14:607-613.

KATAGIRI, C. 1966. Fertilization of dejellied uterine toad eggs in various experimental conditions. Embryologia (Nagoya), 9:159-169.

KAWAMURA, T. 1962. On the names of some Japanese Frogs. J. Sci. Hiroshima Univ., Ser. B, Div. 1, 20:181-193.

KAWAMURA, T. and M. KOBAYASHI. 1960. Studies on hybridization in amphibians. VII. Hybrids between Japanese and European Brown Frogs. J. Sci. Hiroshima Univ., Ser. B, Div. 1, 18:221-238.

KAWAMURA, T. and M. NISHIOKA. 1962. Hybridization between European and Japanese populations of *Rana t. temporaria*. Zool. Mag. (Tokyo), 71(11.12):395. (In Japanese)

KELLY, J.W. 1954. Metachromasy in the eggs of fifteen lower animals. Protoplasma, 43:329-346.

KOBAYASHI, H. 1954. Hatching mechanism in the toad, *Bufo vulgaris formosus*. 3. Extrusion of embryos from the jelly string and colloidal nature of the jelly. J. Fac. Sci. Tokyo Univ., 7, Sect. IV:97-105.

KOBAYASHI, M. 1962a. Studies on reproductive isolation mechanisms in Brown Frogs. 1. Development and inviability of hybrids. J. Sci. Hiroshima Univ., Ser. B, Div. 1, 20:147-156.

KOBAYASHI, M. 1962b. Studies on reproductive isolation mechanisms in Brown Frogs. II. Hybrid sterility. J. Sci. Hiroshima Univ., Ser. B, Div. 1, 20:157-179.

KOSKELA, P. 1973. Duration of the larval stage, growth and migration in *Rana temporaria* L. in two ponds in northern Finland in relation to environmental factors. Ann. Zoo. Fenn., 10:414-418.

KOZLOWSKA, M. 1971. Differences in the reproductive biology of mountain and lowland Common Frogs, *Rana temporaria* L. Acta biol. cracov., 14:17-32.

KROGH, A., K. SCHMIDT-NIELSEN and E. ZEUTHEN. 1938. The osmotic behaviour of frog's eggs and young tadpoles. Z. vergl. Physiol., 26:230-238.

KRUYT, H.R. 1949. Colloid Science. Elsevier Publishing Co., Inc. New York, Amsterdam, London and Brussels.

KURAMOTO, M. 1975. Embryonic temperature adaption in development rate of frogs. Physiol. Zoöl., 48:360-366.

KUSA, M. and E. OZU. 1961. Monosaccharide pattern of egg jellies of three species of *Hynobius*. Proc. Japan Acad., 37:223-226.

LAWTON, J.E. 1969. Studies on the ecological energetics of Damselfly Larvae (Odonata : Zygoptera). Thesis, Durham University Library.

LEE, P.A. 1967. Studies of frog oviducal jelly secretions. 1. Chemical analysis of secretory product. J. exp. Zool., 166:99-106.

LEE, R. 1969. Chemical temperature integration. J. appl. Met., 8:423-430.

LEE, T.W. 1964. Maintenance of the unswollen state of the egg jelly in the *Rana pipiens* reproductive tract. J. Elisha Mitchell scient. Soc., 80:1-8.

LEUTSCHER, A. 1953. Note on the spawning site of the Common Frog. Br. J. Herpet., 1:149-150.

LICHT, L.E. 1969. Comparative breeding biology of the Red-legged Frog (*Rana aurora aurora*) and the Western Spotted Frog (*Rana pretiosa pretiosa*) in south-western British Columbia. Can. J. Zool., 47:1287-1299.

LIU, C.-C. 1950. Amphibians of western China. Fieldiana, Zool. Mem., Chicago Nat. Hist. Museum, 2:1-400.

LIVEZEY, R.L. 1953. Late breeding of *Hyla regilla* Baird and Girard. Herpetologica, 9:73.

LYNN, W.G. and C. E. BRAMBEL. 1935. Effects of lack of iodine upon amphibian growth and metamorphosis. Anat. Rec., 64:46.

MANLEY, G. 1936. The climate of the northern Pennines; the coldest part of England. Q. Jl. R. met. Soc., 62:103-115.

MARTIN, A.A. 1969. Emergence from hibernation in an Australian Burrowing Frog. Copeia, 1969:176-178.

MARTOF, B.S. and R. L. HUMPHRIES. 1959. Variation in *Rana sylvatica*. Am. Midl. Nat., 61:350-389.

MASAMUNE, H., Z. YOSIZAWA and M. MAKI, 1951. Biochemical studies on carbohydrates. CXXXV. Paper partition chromatograms of sugar components in glucidamines and glycoproteins. II. Tohoku J. exp. Med., 53:237-241.

MASAMUNE, H. and Z. YOSIZAWA. 1953. Biochemical studies on carbohydrates. CLXI. Paper partition chromatography of sugars, in particular hexosamines and hexuronic acids. Tohoku J. exp. Med., 59:1-9.

McLAREN, I.A. and J. M. COOLEY. 1972. Temperature adaption of embryonic development rate among frogs. Physiol. Zoöl. 45:223-228.

MICHNIEWSKA-PREDYGIER, Z. and A. PIGOŃ. 1957. Wczesne stadia rozwojowe u *Rana temporaria* L., *Rana terrestris* Andrz., *Rana esculenta* L. i *Bufo bufo* L. Studia Soc. Sci. torun., Sect. E, 3, 147-157. (In Polish with English and Russian summaries)

MIKULSKI, J.S. 1938. Experimental studies in thermal ecology of developing eggs of some amphibians (*Rana, Bufo, Pelobates*). Acad. Pol. Sci. Let. Cracovie, series B, 2:23-45.

MINGANTI, A. 1955. Chemical investigations on amphibian egg jellies. Expl. Cell Res., Suppl., 3:248-251.

MINGANTI, A. and T. D'ANNA. 1957. Richerche sulla mucina ovulare di *Triton cristatus*. Ricerca scient., 27:3052-3054. (In Italian with French, English and German summaries)

MINGANTI, A. and T. D'ANNA. 1958. Sulla composizione della mucina ovulare di *Discoglossus pictus*. Ricerca scient., 28:2090-2094. (In Italian with French, English and German summaries)

MONROY, A. 1965. Biochemical aspects of fertilization. In: The Biochemistry of Animal Development. R. Weber, ed. Vol. 1, pgs. 82-85. Academic Press, New York, N.Y.

MOORE, J.A. 1939. Temperature tolerance and rate of development in the eggs of amphibia. Ecology, 20:459-478.

MOORE, J.A. 1940. Adaptive differences in the egg membranes of frogs. Am. Nat., 74:89-93.

MOORE, J.A. 1942. Embryonic temperature tolerance and rate of development in *Rana catesbeiana*. Biol. Bull., 83:375-388.

MOORE, J.A. 1943. Study of embryonic temperature tolerance and rate of development in *Rana pipiens* from different latitudes. Yb. Am. phil. Soc., 1942:175-178.

MOORE, J.A. 1949a. Geographic variation of adaptive characters in *Rana pipiens* Schreber. Evolution, 3:1-24.

MOORE, J.A. 1949b. Patterns of evolution in the genus *Rana*. In: Jepsen, G.L.; Simpson, G.G.; and Mayr, E. (eds.), Genetics, Paleontology, and Evolution. Princetown Univ. Press, New Jersey. 315-338.

MOORE, J.A. 1951. Hybridization and embryonic temperature adaption studies of *Rana temporaria* and *Rana sylvatica*. Nat. Acad. Sci., 37:862-866.

MOORE, P.D. and D. J. BELLAMY. 1974. 'Peatlands' Elek Science, London.

MORIYA, K. 1960. Studies on the five races of the Japanese Pond Frog, *Rana nigromaculata* Hallowell. II. Differences in character of development. J. Sci. Hiroshima Univ., Ser. B, Div. 1, 18:109-124.

MULLALLY, D.P. and J. D. CUNNINGHAM. 1956. Ecological relations of *Rana mucosa* at high elevations in the Sierra Nevada. Herpetologica, 12:189-197.

NELSEN, O.E. 1953. Comparative Embryology of the Vertebrates. New York: Blakiston.

NOBLE, G.K. 1954. The biology of the Amphibia. Dover Publications, Inc. New York.

PALLMANN, H., E. EICHENBERGER and A. HASLER. 1940. Eine neue Methode der Temperaturmessung bei ökologischen oder bodenkundlichen Untersuchungen. Ber. schweiz. bot. Ges., 50:337-362. (In German)

PALLMANN, H. and E. FREI. 1943. Beitrag zur Kenntnis der Lokalklimate einiger kennzeichnender Waldgesellschaften des schweizerischen Nationalparkes. Ergebn. wiss. Unters. schweiz. Natn. Parks, 1:436-464. (In German)

PALMER, W.G. 1946. Experimental Physical Chemistry. Cambridge.

PEACHEY, J.E. 1959. Studies on the Enchytraeidae of moorland soils. Thesis, Durham University Library.

PENGILLEY, R. 1973. Breeding biology of some species of *Pseudophryne (Anura: Leptodactylidae)* of the southern highlands, New South Wales. Aust. Zool., 18(1):15-30.

PETTUS, D. and G. M. ANGLETON. 1967. Comparative reproductive biology of montane and piedmont Chorus Frogs. Evolution, 21:500-507.

POLLISTER, A.W. and J. A. MOORE. 1937. Tables for the normal development of *Rana sylvatica*. Anat. Rec., 68:489-496.

PRECHT, H., J. CHRISTOPHERSEN, H. HENSEL and W. LARCHER. 1973. Temperature and Life. Springer-Verlag Berlin, Heidelberg, New York, 1973.

PROKOFIEVA, A. 1935. On the chromosome morphology of certain amphibia. Cytologia, 6:148-164.

PROSSER, C.L. 1955. Physiological variation in animals. Biol. Rev., 30:229-262.

PROSSER, C.L. 1964. Perspectives of adaptation: theoretical aspects. In: Adaptation to the Environment, Handbook of Physiology 4 (ed. D. B. Dill). Am. Physiol. Soc., Washington, D.C.

RAY, C. 1960. The application of Bergmann's and Allen's rules to the poikilotherms. J. Morph., 106:85-108.

REAY, R.C. 1964. The numbers of eggs and larvae of *Coleophora alticolella* Zell. (Lep.). J. Anim. Ecol., 33:117-128.

RICHARDS, C.M. 1958. The inhibition of growth in crowded *Rana pipiens* tadpoles. Physiol. Zoöl., 31:138-151.

RICHARDS, C.M. 1962. The control of tadpole growth by alga-like cells. Physiol. Zoöl., 35:285-296.

ROSE, S.M. 1960. A feedback mechanism of growth control in tadpoles. Ecology, 41:188-199.

ROTHSCHILD, LORD. 1956. The physiology of sea-urchin spermatozoa. Action of pH, dinitrophenol, dinitrophenol and versene and usnic acid on O_2 uptake. J. exp. Biol., 33:155-173.

RUGH, R. 1934. Induced ovulation and artificial fertilization in the frog. Biol. Bull., 66:22-29.

RUGH, R. 1951. The frog, its reproduction and development. McGraw-Hill, N.Y.C.

RUIBAL, R. 1955. A study of altitudinal races in *Rana pipiens*. Evolution, 9:322-338.

RUIBAL, R. 1957. An altitudinal and latitudinal cline in *Rana pipiens*. Copeia 1957, (3) 212-222.

RUIBAL, R. 1962. Ecology and genetics of a desert population of *Rana pipiens*. Copeia 1962, 189-195.

RUNNSTRÖM, S. 1936. Die Anpassung der Fortpflanzung und Entwicklung mariner Tiere an die Temperaturverhältnisse verschiedener Verbreitungsgebiete. Bergens Mus. Årb., 3:1-36. (In German)

SALTHE, S.N. 1963. The egg capsules in the Amphibia. J. Morph., 113:161-171.

SALTHE, S.N. 1965. Increase in volume of the perivitelline chamber during development of *Rana pipiens* SCHREBER. Physiol. Zoöl., 38:80-98.

SAVAGE, R.M. 1950. A thermal function of the envelope of the egg of the Common Frog, *Rana temporaria temporaria* (Linn.) with observations on the structure of the egg clusters. Br. J. Herpet., I, No.3, 57-66.

SAVAGE, R.M. 1961. The ecology and life history of the Common Frog *(Rana temporaria temporaria)*. Pitman, London.

SAVAGE, R.M. 1965. External stimulus for the natural spawning of *Xenopus laevis*. Nature (London), 205:618-619.

SAVAGE, R.M. 1971. The natural stimulus for spawning in *Xenopus laevis* (Amphibia). J. Zool., Lond., 165(2):245-260.

SCHULZ, F.N. and M. BECKER. 1935. Über die Kohlenhydrate der Eiweissdrüse von *Rana esculenta*. Biochem. Z., 280:217-226. (In German)

SCHWALBACH, G. and B. AGOSTINI. 1964. Über beziehungen zwischen kälteresistenz und mitochondrienmorphologie bei der wespe *(Vespa germanica* F.). Z. Zellforsch., 62:113-120. (In German)

SHAVER, J.R. 1966. Immunobiological studies of the jelly-coats of anuran eggs. Am. Zool., 6:75-87.

SMITH, C.L. 1955. Reproduction in female Amphibia. Mem. Soc. Endocr., 4:39-56.

SMITH, J.M. 1966. The Theory of Evolution. Penguin Books Inc.

SMITH, M. 1969. The British Amphibians and Reptiles. Collins, London.

STEINKE, J.H. and D. G. BENSON, Jr. 1970. The structure and Polysaccharide Cytochemistry of the jelly envelopes of the egg of the frog, *Rana pipiens*. J. Morph., 130:57-66.

TAYLOR, B.J., I. C. BURGESS, D. H. LAND, D. A. C. MILLS, D. B. SMITH and P. T. WARREN. 1971. British Regional Geology, Northern England. H.M.S.O.

TERENTIEV, P.W. 1950. Liaguszka. Gos. Izdat. Sowietskaja. Nauka Moskva. (In Russian)

TERENTJEV, P.V. 1960. Some quantitative peculiarities of frog eggs and tadpoles. Zool. Zh. Ukr., 39:779-781. (In Russian with an English summary)

VAN OORDT, G.J. and P.G.W.J. VAN OORDT. 1955. The regulation of spermatogenesis in the frog. Mem. Soc. Endocr., 4:25-38.

VAN OORDT, P.G.W.J. 1956. The role of temperature in regulating the spermatogenetic cycle in the Common Frog (*Rana temporaria*). Acta endocr., 23:251-264.

VERNBERG, F.J. 1962. Comparative physiology: latitudinal effects on physiological properties of animal populations. A. Rev. Physiol., 24:517-546.

VOLPE, E.P. 1953. Embryonic temperature adaptions and relationships in toads. Physiol. Zoöl., 26:344-354.

VOLPE, E.P. 1954. Hybrid inviability between *Rana pipiens* from Wisconsin and Mexico. Tulane Stud. Zool., 1:111-123.

VOLPE, E.P. 1955. Intensity of reproductive isolation between sympatric and allopatric populations of *Bufo americanus* and *Bufo fowleri*. Am. Nat., 89:303-318.

VOLPE, E.P. 1957a. Embryonic temperature tolerance and rate of development in *Bufo valliceps*. Physiol. Zoöl. 30:164-176.

VOLPE, E.P. 1957b. Embryonic temperature adaptions in highland *Rana pipiens*. Am. Nat. 91:303-309.

WICKBOM, A. 1945. Cytological studies on Dipnoi, Urodela, Anura and Emys. Hereditas, 31:241-346.

WITSCHI, E. 1922a. Chromosomen und Geschlecht bei *Rana temporaria* L. Z. indukt. Abstamm.-u. VererbLehre, 27:253-255. (In German)

WITSCHI, E. 1922b. Vererbung und Zytologie des Geschlechts nach Untersuchungen an Fröschen. Z. indukt. Abstamm.-u. VererbLehre, 29:31-68.

WITSCHI, E. 1924. Die Entwicklung der Keimzellen der *Rana temporaria* L. I. Urkeimzellen und Spermatogenese. Z. Zell.-u. Gewebelehre, Apt. B, 1:523-561.

WITSCHI, E., M. KODANI and K. MIKAMO. 1958. Comparative study of the chromosomes of European, American and Japanese frogs. Anat. Rec., 131:610.

ZWEIFEL, R.G. 1968. Reproductive biology of Anurans of the arid southwest, with emphasis on adaption of embryos to temperature. Bull. Am. Mus. nat. Hist., 140:1-64.

ZWÖLFER, W. 1935. Die Temperaturabhängigkeit der Entwicklung der Nonne (*Lymantria monacha* L.) und ihre bevölkerungswissenschaftliche Auswertung. Z. Angew Entomol., 21:333-384. (In German)

APPENDIX

The method used to calculate integrated temperature values from the sucrose inversion technique

The velocity of sucrose hydrolysis is related to temperature by means of an Arrhenius-type equation:

$$\log K = C_1 - \frac{C_2}{T} \qquad \text{----------(1)}$$

where:

K = the velocity coefficient

C_1 = constant depending on the pH of the solution

C_2 = constant independent of pH

T = absolute temperature

The constant C_1 varies with pH and has to be calculated for each batch of sucrose solution. Since C_2 is independent of pH, Berthet's value for this constant of 5,854 was used in the calculations.

Equation 1 can be written:

$$T = \frac{5,854}{C_1 - \log K} \qquad \text{----------(2)}$$

This equation can be used to calculate the environmental temperature T in degrees absolute. Pallmann *et al.* (1940) showed that for a sucrose solution:

$$K = \frac{1}{t} \log \left(\frac{A}{(A - X)} \right) \text{----------(3)}$$

where:

t = time in days

A = initial concentration of sucrose

X = amount of sucrose that inverts to glucose and fructose in time t.

All the sugars are optically active in solution so their effective concentrations can be determined polarimetrically and a rotation angle ratio substituted for the concentration ratio in (3):

$$K = \frac{1}{t} \log \left(\frac{a_o - b_o}{a - b_o} \right) \text{----------(4)}$$

where:

a_o = initial rotation of the solution before inversion

a = rotation angle after being in the field for time t

b_o = rotation angle at complete inversion: it is a constant given by Berthet as -9.17^o.

C_1 is calculated from the following equation, applied to the solutions from the constant temperature rooms:

$$C_1 = \frac{5,854}{T} + \log \left(\frac{1}{t} \log \frac{a_o - b_o}{a - b_o} \right) \text{--------(5)}$$

where:

t = time in days in the constant temperature room.

T = temperature in degrees absolute of the constant temperature rooms

a_o = initial rotation of the solution before inversion

b_o = rotation angle at complete inversion

Specimen Calculation

The results are summarized in Table 38

Table 38. Rotation angles after time t.

Location		Time period: Start time	Start date	Finish time	Finish date	t (days)	Angle of rotation (a)
Sucrose tubes	5°C	12.15	24:12:75	12.15	15:1:76	22	32.85°
in constant	10°C	12.15	24:12:75	12.15	15:1:76	22	14.60°
temperature rooms	20°C	12.15	24:12:75	12.15	29:12:76	5	13.40°
Sucrose tube in the soil near pond (2)		10.15	1:1:76	17.00	3:3:76	63.28	16.00°

Initial rotation values measured from three different samples of the same sucrose solution:

a_o = 51.25, 51.15, 51.25 (mean = 51.22)

Calculation of the constant C_1

This is calculated using equation (5), i.e.

$$C_1 = \frac{5,854}{T} + \log \left(\frac{1}{t} \log \frac{a_o - b_o}{a - b_o} \right)$$

For the sucrose tube in the 5°C constant temperature room

$$C_1 = \frac{5,854}{278} + \log \left(\frac{1}{22} \log \frac{51.22 + 9.17}{16.00 + 9.17} \right)$$

$$C_1 = \underline{18.9126}$$

A similar calculation for the sucrose tubes in the 10°C and 20°C constant temperature rooms gave C_1 values of 18.9510 and 18.9115 respectively.

$$\underline{\text{Mean } C_1 = 18.9250}$$

Calculation of the velocity coefficient K

This is calculated using equation (4), i.e.

$$K = \frac{1}{t} \log \left(\frac{a_o - b_o}{a - b_o} \right)$$

$$K = \frac{1}{63.28} \log \frac{51.22 + 9.17}{16.00 + 9.17}$$

$$\underline{K = 0.006006} \quad (\log K = \bar{3}.7786 \text{ or } -2.2214)$$

Calculation of the environmental temperature T

This is calculated using equation (2), i.e.

$$T = \frac{5,854}{C_1 - \log K}$$

$$T = \frac{5,854}{18.9250 + 2.2214}$$

$$\underline{T = 276.83^{\circ} \text{ absolute}, 3.83^{\circ} \text{ centigrade}}$$

STATISTICAL ABBREVIATIONS USED IN THE TEXT

The statistical analyses used in the present study were based on Bailey (1973), and the following symbols have been used:

S.E.	=	Standard Error
t	=	the "Student's t" modified for small samples
d.f.	=	degrees of freedom
P	=	significance level actually achieved by data
r	=	coefficient of correlation of regression

A LIST OF SPECIES MENTIONED IN THE TEXT WITH THEIR AUTHORITIES

Acris crepitans	BAIRD
Ambystoma maculatum	SHAW
Anthus pratensis	LINNAEUS
Bufo americanus	HOLBROOK
Bufo bufo	LINNAEUS
Bufo fowleri	HINCKLEY
Bufo terrestris	BONNATERRE
Bufo valliceps	WIEGMAN
Bufo vulgaris formosus	BOULENGER
Bufo woodhousie	GIRARD
Cognettia sphagnetorum	VEDJ.
Hyla regilla	BAIRD & GIRARD
Juncus squarrosus	LINNAEUS
Limnodynastes dorsalis	GRAY
Lymantria monacha	LINNAEUS
Molophilus ater	MEIGEN
Peromyscus maniculatus	WAGNER
Prosimulium hirtipes	FRIES
Pseudacris triseriata	WEID
Pseudophryne corroboree	MOORE
Rana aurora	BAIRD & GIRARD
Rana catesbeiana	SHAW
Rana clamitans	LATREILLE
Rana esculenta	LINNAEUS
Rana japonica	GUENTHER
Rana mucosa	CAMP
Rana nigromaculata	HALLOWELL
Rana pipiens	SCHREBER
Rana ridibunda	PALLAS
Rana sylvatica	LE CONTE
Rana temporaria chensinensis	DAVID
Rana temporaria ornativentris	WERNER *
Rana temporaria parvipalmata	SEOANE
Rana temporaria temporaria	LINNAEUS
Saprolegnia ferax	(GRUITH) THURET
Strophingia ericae	CURT
Tipula subnodicornis	ZETTERSTEDT
Xenopus laevis	DAUDIN

*Note there is a good deal of evidence to suggest that *Rana temporaria ornativentris* WERNER should be changed to *Rana ornativentris* WERNER (Kawamura, 1961).