Intraspecific life-history and morphological variations in *Lymnaea peregra* (Müller) (Gastropoda: Pulmonata); environmental or genetic variance?

Thesis submitted for the degree

of

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by

P.K.S. Lam

1987

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Contents

A summary of the thesis entitled "Intraspecific life-history and morphological variations in *Lymnaea peregra* (Müller) (Gastropoda: Pulmonata); environmental or genetic variance?" by Paul Kwan Sing Lam.

Three neighbouring populations of *Lymnaea peregra* were studied between January 1985 and December 1986. The populations differed in egg sizes, juvenile growth rates, survival regimes, breeding patterns, and total reproductive efforts. Differences in water temperatures were largely responsible for the annual variation in the timing of the breeding seasons while interpopulation divergence in growth rates, and consequently number of breeding bouts per year, was attributed mainly to varying food availability.

Snails at the Don site had lower winter mortalities than the Sheaf and the Rivelin individuals, partly due to the higher winter temperatures at the former site. Moreover, faster current speeds might also be responsible for the higher mortalities at the Sheaf and the Rivelin than the Don site.

The lower reproductive efforts exhibited by the Don snails as compared with the other two populations were ascribed to the low food availability, high population density and shortage of oviposition sites at the Don. The Don snails consistently started breeding earlier than the Sheaf and the Rivelin individuals. Mass-culture and controlled breeding experiments revealed that the early-breeding trait of the Don snails was heritable, and probably genetically fixed. It is postulated that the early breeding of the Don snails is an adaptation to exploit a longer breeding season. Quantitative genetical analyses indicated a genetic trade-off between the age at first reproduction and the hatchling size of the Don snails. Similar analyses also showed significant genetic variances for the juvenile

growth rates and the hatchling size in the Sheaf and the Rivelin populations respectively. There is some suggestion that the snail populations showed varying reproductive investment per individual offspring as predicted by the Sibly & Calow model.

This study shows that *L. peregra*, though primarily phenotypically plastic, can evolve local adaptations according to specific ecological circumstances.

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1. Introduction

The theory of life-history tactics predicts the evolution of specific sets of coadapted traits as a result of varying selection regimes under different ecological circumstances [see review by Stearns (1976)]. Studies based on comparisons between organisms of different taxa have yielded data which are, in general, consistent with the theory [Browne & Russell-Hunter 1978, Calow 1978, also see reviews by Stearns (1977) and Calow (1983)]. However, Stearns (1976) and Brown (1983) pointed out that organisms of different taxa were likely to be subject to particular phylogenetic constraints, and hence the adaptive basis of life-history and/or morphological variations should be best studied at the intraspecific level.

It is well documented that freshwater snails exhibit extensive intraspecific variations in their life-history and morphological characters (see reviews by Russell-Hunter 1964, 1978, 1983 and Hubendick 1951). It is often inferred that the intraspecific variability is a result of different populations adopting particular sets of traits in response to the diverse environmental challenges prevailing in various freshwater habitats. The above evolutionary inference, attributing interpopulation differences to local adaptations resulting from natural selection, contains the important assumption that such variations have a genetic basis.

A survey of the literature on freshwater snails reveals that, with a few exceptions (e.g. Calow 1981b, McMahon 1983 and references therein), a vast majority of the intraspecific variations among different populations is predominantly phenotypic (i.e. little or no genetic variance). Some evolutionary ecologists maintained that it is only legitimate to attach adaptive significance to such interpopulation differences if the corresponding genetic divergence

can be demonstrated (e.g. Berven & Gill 1983, Gill *et al.* 1983, Charlesworth 1984). By contrast, others argued that the absence of genetic fixation and the resulting phenotypically plastic traits can be adaptive by virtue of their developmental flexibility (e.g. Russell-Hunter 1964, 1978, Bradshaw 1965, Caswell 1983).

The present study on the intraspecific variations among three populations of *Lymnaea peregra* was initiated to examine whether the populations exhibit life-history and/or morphological differences in habitats with markedly contrasting environmental conditions, and to distinguish between phenotypic responses to proximal environmental factors and evolutionary adaptations involving genetic divergence as a result of selection.

I shall start by reviewing the life-history theory and models that predict 'optimal strategies' on the basis of specific sets of selection pressures (chapter 2). This provides the theoretical framework which helps to pinpoint the potentially important environmental parameters (i.e. possible causes) and the expected life-history and/or morphological responses (i.e. possible effects) that may be of relevance in the present investigation. I shall, then, compare the physico-chemical features of the three study sites to identify various ecological factors that may constitute the unique selection regimes in each habitat (chapter 3). The first part of chapter 4 will consider the life-history data of the three populations of *L. peregra*, and the extent to which the interpopulation differences can be ascribed directly to environmental variabilities in space and time. The second part of chapter 4 will address specifically the variations in shell morphometrics (shell shape) among the three populations, and their possible adaptive significance in lotic (fast-flowing) and lentic (still-water)

habitats. This will be followed by an investigation into the genetic basis of the interpopulation differences by a combination of controlled-breeding and mass-culture experiments (chapter 5). The relationship and, in particular, possible trade-offs between fitness traits will be examined at both phenotypic and genotypic levels. Chapter 6 will compare the life-history tactics exhibited by the three populations in the field with the 'optimal strategies' predicted by the appropriate life-history model chosen from among those reviewed in chapter 2.

Since natural selection is only effective if the appropriate genetic variability is available, a study of the evolution of life-history and/or morphological adaptations will be incomplete without the necessary information on the nature and extent of the genetic variation of the traits involved (Charlesworth 1984). Likewise, it is invalid to infer that a trait with significant genetic variance is adaptive unless the ecological importance of such a trait can be established in the natural habitat where selection occurs. The following study on the intraspecific variation of the freshwater snail, *L. peregra*, is aimed at embracing both the ecological and genetical aspects of life-history and morphological evolution.

2. Literature review on life-history models and evolution

2.1 Introduction

Life-history theory predicts that natural selection should favour organisms possessing a set of ontogenetic traits that maximize the number of offspring capable of surviving to breeding within the shortest period of time. Within an organism, total resources (E_t) are partitioned into the reproductive (E_r), and somatic (E_s) components i.e.

$$E_t = E_r + E_s \qquad 2.1$$

As offspring production is likely to be a function of E_r, all other things being equal, natural selection should act to maximize E_r. It is obvious from equation 2.1 that E_r could be raised in at least two ways:

(1) By increasing E_t (Tuomi *et al.* 1983)

E_t available within an organism is affected by its intrinsic foraging and conversion efficiency (Townsend & Hughes 1981, Parker & Begon 1986), and extrinsic factors such as intra- and inter-specific competition (Brockelman 1975, Credland *et al.* 1986, Parker & Begon 1986). Most organisms have a distinct growth phase during which surplus energy (i.e. energy not required for vital processes) is stored (Calow 1981a), resulting in an increased E_t at the time of gamete production.

(2) By increasing E_r at the expense of E_s (Williams 1966a, 1966b, Gadgil & Bossert 1970)

If the resources within an organism (E_t) are limited, raising E_r means reducing E_s. Such a reduction in energy available for somatic processes e.g. growth and maintenance, is often referred to as the 'cost of reproduction', and often expressed as an increase in post-reproductive mortality (Calow 1973). Indeed, organisms that invest

heavily in reproduction often breed once and then die [described as semelparous by Cole (1954)] while others, presumably by not investing exhaustively during each breeding session, can achieve several successive breeding bouts [described as iteroparous by Cole (1954)].

2.2 Semelparity versus iteroparity

Cole (1954) examined the population consequences of semelparity and iteroparity, by comparing the growth of two hypothetical populations; one semelparous and the other iteroparous. In his model, he assumed that both the semelparous and iteroparous populations had zero juvenile mortality, and that adults of the latter population could survive indefinitely.

Assuming exponential population growth, a population of initial size N_0 would, after a time interval of t, be of size N_t:

$$N_t = N_0 e^{rt} \qquad 2.2$$

where e is the base of natural logarithms, and r is the instantaneous rate of natural increase (also known as the innate capacity for increase, the intrinsic rate of natural increase, and often used as a measure of 'fitness').

In the case of a hypothetical semelparous population with zero juvenile mortality, and a generation time of one year (i.e. $t = 1$), equation 2.2 can be simplified as

$$N_t = N_0 e^{r} \qquad 2.3$$

Substituting e^r by B_s which is equivalent to the average female birth rate per capita, equation 2.3 becomes

$$N_t = B_s N_0 \qquad 2.4$$

For the immortal iteroparous population, all the individuals from the initial population survive, and therefore

$$N_t = B_i N_0 + N_0 \qquad 2.5$$

where B_i is the average female birth rate per year starting at year

one.

Rearranging equation 2.5

$$N_t = N_0(B_i + 1) \qquad 2.6$$

From equations 2.4 and 2.6, the same final population size (N_t) could be attained if

$$B_s = B_i + 1 \qquad 2.7$$

This result lead Cole (1954) to conclude that "for an annual species, the absolute gain in intrinsic population growth which could be achieved by changing to the perennial reproductive habit would be exactly equivalent to adding one individual to the average litter size". The above statement suggested that iteroparity should be of negligible selective value. However, it should be stressed that Cole's conclusion contained the unrealistic assumption of zero mortality for the juveniles of both the semelparous and iteroparous populations, as well as the adults of the latter population.

Cole's conclusion has been re-examined, and in some cases refined, by other authors. Gadgil & Bossert (1970) assumed some mortality for juveniles (i.e. < one year old) but none beyond age one, and concluded that a semelparous annual could attain the same increase in fitness as an iteroparous perennial if the former doubled its average litter size. Bryant (1971), accepting that organisms are mortal, assumed equal mortality for both juveniles and adults, and arrived at the same conclusion as Cole (1954) did. The reason for this convergence was demonstrated by Charnov & Schaffer (1973) who incorporated juvenile and adult mortality into equations 2.4 and 2.5 as variables.

Assuming that offspring of both the semelparous, annual and iteroparous, perennial populations have the same survival probability (C) in the first year, and that the adults of the latter population

had a survival probability (P) between years.

Equation 2.4 becomes

$$N_t = B_s C N_0 \qquad 2.8$$

Equation 2.5 becomes

$$N_t = B_i C N_0 + P N_0 \qquad 2.9$$

To attain the same N_t,

$$B_s C N_0 = B_i C N_0 + P N_0 \qquad 2.10$$

Cancelling N_0 and rearranging, equation 2.10 becomes

$$B_s = B_i + (P/C) \qquad 2.11$$

It is obvious that both Cole's and Bryant's results are special cases (i.e. P = C) of the Charnov & Schaffer model. Equation 2.11 shows that in situations where adults and juveniles have vastly different survival rates i.e. $P \gg C$ or $C \gg P$, B_s and/or B_i will have to be drastically changed to attain the same rate of growth (r) for both populations. The relative merits of semelparity and iteroparity were also examined by Young (1981) who concluded that high juvenile relative to adult survivorship would favour semelparity and *vice versa*.

2.3 *r*- and K-selection, bet-hedging model and the balanced-mortality model

MacArthur & Wilson (1967) argued that natural selection will act to maximize r (referred to as *r*-selection) only when population density is low, resources non-limiting, and individuals suffer density-independent mortality. A typical '*r*-strategy' consists of high maximum rate of natural increase, early reproduction, large clutch size, small body size, short life span and semelparity (MacArthur & Wilson 1967, Pianka 1970, Southwood 1977). In a stable environment, where catastrophes are rare and population density close to the carrying capacity (K), individuals may be expected to

experience density-dependent mortality as a result of keen competition. Under these circumstances, natural selection should favour traits that increase K (referred to as K-selection) (MacArthur 1962, MacArthur & Wilson 1967). A 'K-strategy' is characterized by low maximum rate of natural increase, delayed reproduction, small clutch size, large body size, long life span and iteroparity. The *r*-K theory is dichotomous in nature, and thus is useful in providing falsifiable hypotheses. Early attempts to test the theory, involving comparisons across taxa , yielded generally supportive results (e.g. Cody 1966, Landahl & Root 1969, Abrahamson & Gadgil 1973).

The *r*-K theory, being deterministic, fails to accommodate situations where mortality and fecundity fluctuate stochastically. Schaffer (1974) modelled life-history strategies in a fluctuating environment, and concluded that in situations where adult mortality experiences wider fluctuations than juvenile mortality or recruitment, traits constituting a typical *r*-strategy will evolve. Conversely, if juvenile mortality or recruitment fluctuates while adult mortality remains relatively stable, traits similar to those predicted by K-selection will prevail, bet-hedging. A comparison between the *r*-K selection and the bet-hedging model has been reviewed by Stearns (1976, 1977). Price (1974) proposed that in a hostile environment, natural selection should act to balance high mortality levels by increasing egg production - the 'balanced-mortality model'. This model describes with some success the behaviour of certain parasitoids (Price 1974, 1975).

2.4 Trade-offs

It is generally assumed in all life-history models that there are trade-offs between various life-history components, and that variation in life history is a result of differences in resource

allocation. This would mean, for example, that the optimal strategy to produce maximum number of offspring under *r*-selection can only be achieved by reducing investment per individual progeny. By the same argument, various trade-offs, which form the basis of the life-history theory, can be postulated e.g.

(1) reproductive investment versus parental survivorship,

(2) clutch size versus offspring size (or offspring survival)

Assuming a stable age-distribution, fitness (F), measured as the innate capacity for increase (*cf.* equation 2.2), can be defined by the following equation (Charlesworth 1980):

$$1 = 1/2 \sum_{t=1}^{\infty} e^{-Ft} S_t n_t \qquad 2.13$$

where S_t and n_t are the survival and fecundity at time t respectively. Assuming that the organisms are absorption-costing (*sensu* Sibly & Calow 1984), and annual breeders, equation 2.13 can be simplified (Sibly & Calow 1986, chapter 4) thus:

$$F = \ln [(1/2)(S_j n + S_a)] \qquad 2.14$$

where n is the number of offspring produced, S_j and S_a are the survival of juveniles and adults respectively.

Assuming that a trade-off exists between S_a and n [section 2.1 (2), also see Calow 1979, Bell 1983, 1984a, 1984b, Reznick 1985], F can be maximized by selecting a combination of n and S_a that will give the highest F at a fixed S_j - optimization modelling. This approach is feasible only if the specific form of the trade-off curve is known. Unfortunately, data on the sign (i.e. positive or negative) and the form (i.e. the shape of the curve) of the relationship between n and S_a are difficult to collect and thus, scarce. The main problem in the investigation of trade-offs lies in

the difficulty of designing experiments that allow only the components under investigation e.g. S_a and n, to vary whilst holding all other possible confounding factors constant. Also, it is likely that natural selection might have restricted the possible options of S_a and n to an optimal segment of the trade-off curve in a particular population (Calow 1984). In the absence of empirical evidence, trade-off curves are constructed according to qualitative biological intuition rather than quantitative experimental results. Three general forms of trade-off curves can readily be visualized namely linear (I), concave downwards (II) and convex downwards (III) (Fig. 2.1). The optimal predictions in each case depend on S_j since this determines the slope of the fitness contours i.e. lines joining points of equal fitness (F). Three possible cases can be identified using the linear trade-off [i.e. (I) in Fig. 2.1] as a reference line (RL):

(1) When the fitness contours are parallel to RL [Fig. 2.2 (a)], the organisms will have constant fitness irrespective of S_a and n if the former is linearly related to the latter. The concave downwards curve (II) predicts no reproduction or, equally likely, maximum investment. The convex downwards curve (III), however, predicts an optimum (intermediate n) where the curve makes contact with the contour of highest fitness.

(2) When the slope of the fitness contours is greater than that of RL i.e. S_j relatively high [Fig. 2.2 (b)], both the linear (I) and the concave downwards (II) trade-off relationships predict maximum investment. The convex downwards curve (III) predicts an optimum to the right (high n).

(3) When the slope of the fitness contours is smaller than that of RL i.e. S_j relatively low [Fig. 2.2 (c)], both I and II predict no

Fig. 2.1. Possible forms of trade-offs between adult survivorship (S_a) and number of offspring (n); (I) linear, (II) concave downwards, and (III) convex downwards.

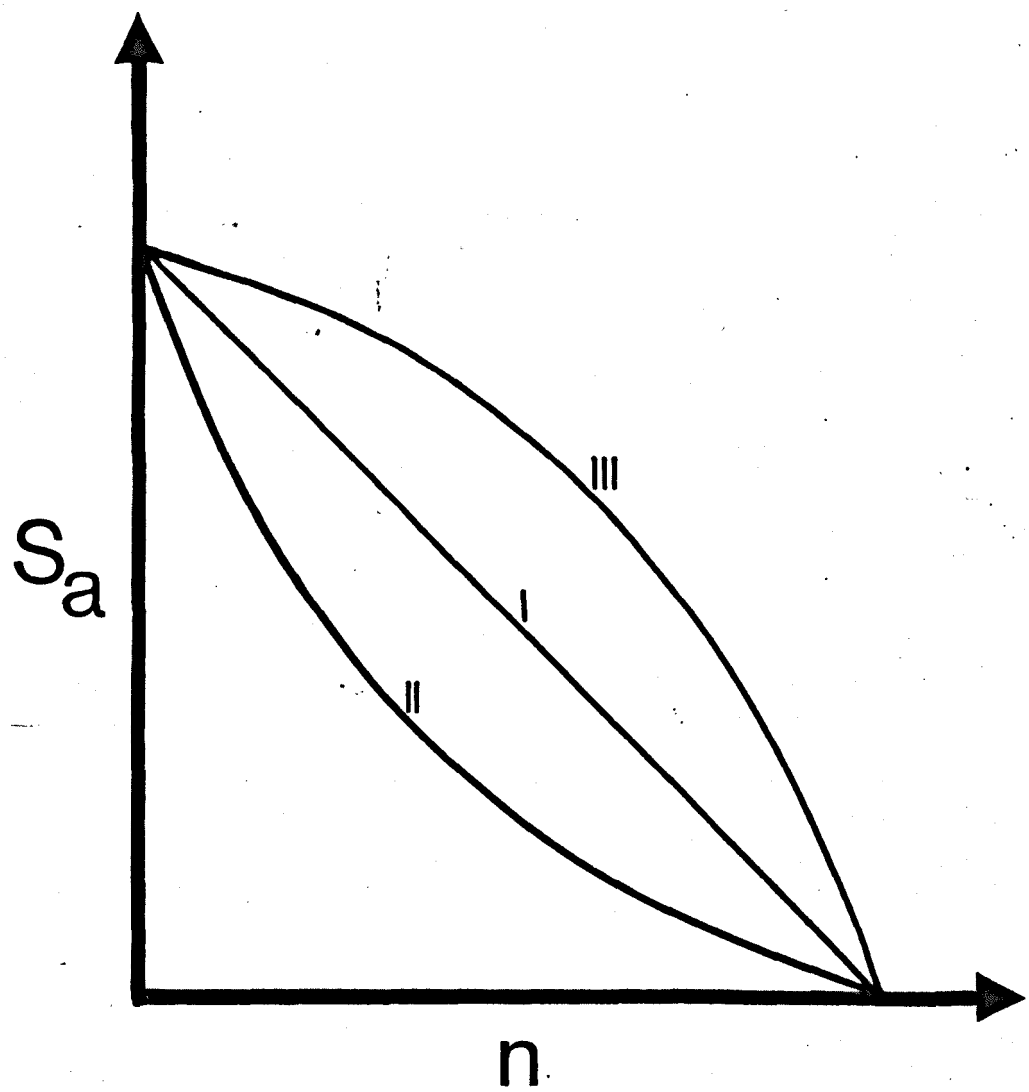
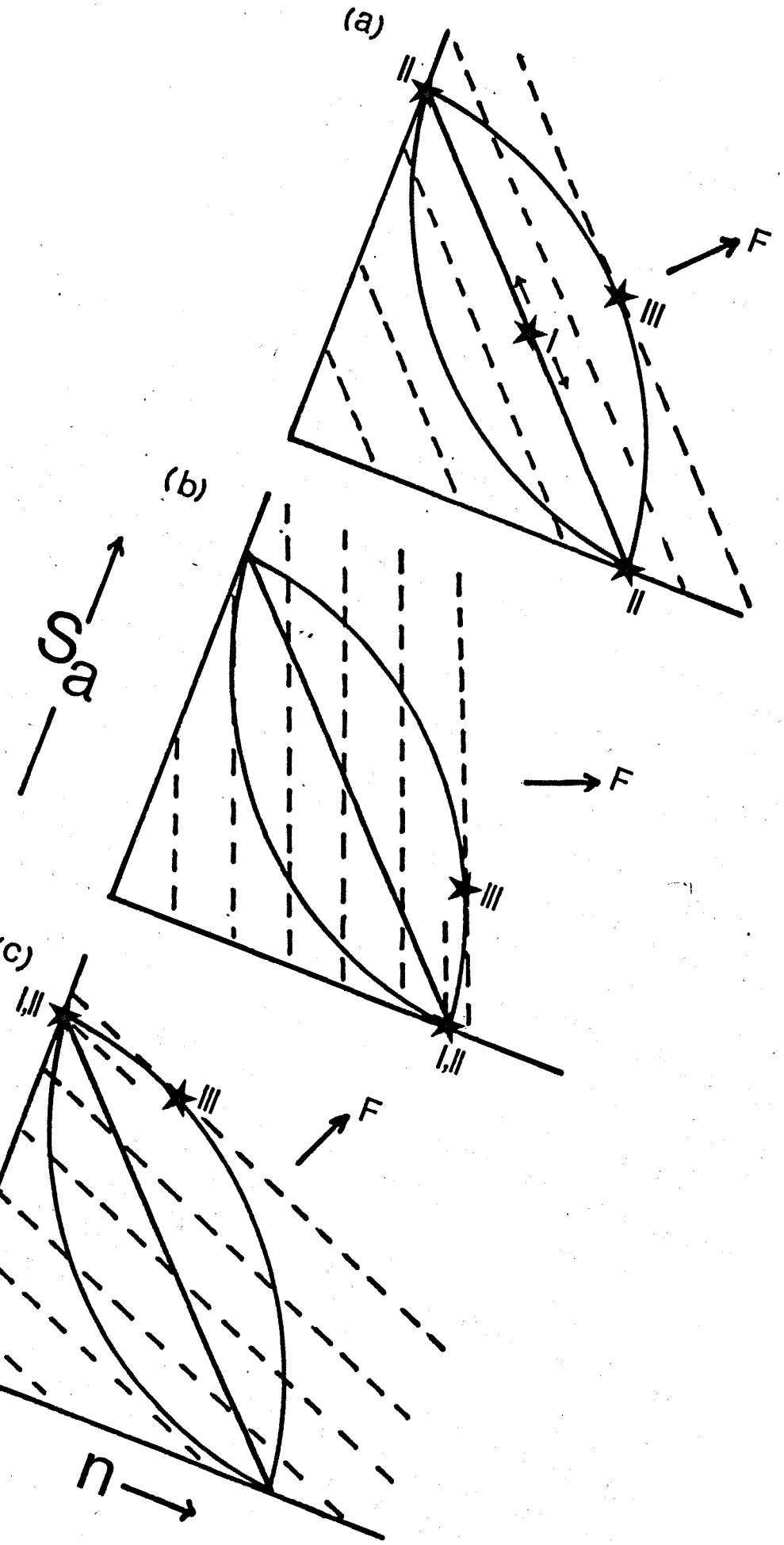

S_a
III
I
II
n

Fig. 2.2. Relationships between the fitness (F) contours (broken lines) and the possible trade-offs (solid lines) between adult survivorship (S_a) and number of offspring (n) when the slope of the fitness contours is (a) equal to, (b) higher than, and (c) lower than that of the linear trade-off (I). The optimal strategies are denoted by stars.


(a)
II
III
I
F
II
(b)
S_a
F
III
I,II
(c)
I,II
III
F
n

reproduction while curve (III) predicts an optimum shifted to the left (low n).

There are speculations in the literature that the relationship between S_a and n follows the form of a convex downwards curve i.e. III in Fig. 2.1 (e.g. Calow 1983, Sibly & Calow 1984). The argument is based on physiological considerations in that as more resources are invested in reproduction, the less there are available for maintenance, and the mortality is expected to increase. A sharper increase in mortality is expected, at a later stage, when essential resources are diverted to gamete production (Calow 1984).

2.5 The Sibly & Calow model

Until this point, all the life-history models mentioned above involve only one key selection pressure (KSP). For example, the *r*- and K- selection uses population density or resource availability as the KSP while the bet-hedging strategy recognizes the relative predictability of mortality and fecundity regimes as the KSP. Sibly & Calow (1985) proposed a model based on two, rather than one KSPs, namely an index of age-specific survivorship (S) and an index of juvenile growth rate (G). The model is based on two important principles:

(1) When S is high, total reproductive investment (RI_t) is high [Fig. 2.3 (a)] [no specific expectation concerning reproductive investment per individual offspring (RI_i)],

(2) When G is high, RI_i is low [Fig. 2.3 (b)] (no specific expectation concerning RI_t).

Superimposing Figs. 2.3 (a) and 2.3 (b), a matrix of four categories of selection with specific qualitative predictions can be generated (Fig. 2.4). The number of offspring (n) is given by the equation:

$$n = RI_t/RI_i \qquad 2.15$$

Fig. 2.3. Qualitative life-history predictions on (a) the total reproductive investment (RI_t) under high and low S, an index of age-specific survivorship, and (b) the reproductive investment per individual offspring (RI_i) under high and low G, an index of juvenile growth rate.

(a)

Index of age-specific survivorship (S)		
	HIGH	RI_t: high
	LOW	RI_t: low

(b)

	Index of juvenile growth rate (G)	
	LOW	HIGH
	RI_i: high	RI_i: low

Fig. 2.4. A matrix of four sets of life-history predictions under varying conditions of S, an index of age-specific survivorship and G, an index of juvenile growth rate.

Index of age-specific survivorship (S)		Index of juvenile growth rate (G)	
		LOW	HIGH
HIGH		RI_t: high RI_i: high n: intermediate	RI_t: high RI_i: low n: very high
LOW		RI_t: low RI_i: high n: low	RI_t: low RI_i: low n:intermediate

2.6 Testing predictions of life-history models

Predictions of a life-history model can be tested by comparing the predicted (or theoretical) optimum with the observed (or empirical) data (e.g. Maltby & Calow 1986b, Sibly & Monk 1987) - i.e. the *a priori* approach (Calow & Townsend 1981). Alternatively, the predictions can be tested by comparing the life-history patterns of conspecific populations occurring in different environments. This *a posteriori* approach (Calow & Townsend 1981) attempts to explain, often qualitatively, phenotypic variation as a consequence of natural selection. The challenge for ecologists adopting the *a posteriori* approach is to relate the observed divergence in life history to different selection pressures resulting from environmental heterogeneity (Calow 1986). I suggest that this problem can be alleviated, though not solved, by experiments designed to establish the genetic basis of the variation - a necessary requirement for invoking an evolutionary explanation.

2.7 Phenotype versus genotype

The possible genetic basis of phenotypic variations can be examined in at least four ways:

(1) by culturing organisms from different populations through one or preferably more generations under 'identical' conditions in the laboratory (e.g. Noland & Carriker 1946, Calow 1981b),

(2) by transferring reciprocally cohorts of different origins, and comparing their performance in the home and alien environments (e.g. Mackie *et al.* 1976a, 1976b, Berven 1982a, 1982b, Brown 1985a, 1985b),

(3) by partitioning phenotypic variations into environmental and genetic components by quantitative genetics (e.g. Falconer 1981, Lawrence 1984, Ennos 1985),

(4) by directional selection experiments (Parsons 1983, p. 226). If a

quantitative trait is heritable, continuous directional selection of extreme phenotypes would mean selection of extreme genotypes, and should result in the phenotypes of the progeny converging to the selected types (e.g. Marinković *et al.* 1980, Rose & Charlesworth 1981b).

If results of the above exercises indicate genetic variance, then the difference is *more likely* to be adaptive. If, however, the variation is found to be purely environmental (often referred to as phenotypic plasticity), the interpretation is less clear. Some evolutionary biologists (e.g. Bradshaw 1965, Caswell 1983, Lynch & Gabriel 1987) argue that phenotypic plasticity *per se* could be heritable, and may be adaptive. It is important to note that, by definition, the genetic basis of a phenotypically plastic character is difficult to establish. Consequently, one may have to accept that any phenotypic variation is adaptive (or of an evolutionary origin) provided that a reasonable explanation can be put forward. This problem still remains as one of the major challenges in the study of life-history evolution.

3. Study sites

3.1 General characteristics of the study sites

The three study sites were situated within a 2.5 mile (4 km) radius of the city centre of Sheffield (Fig. 3.1): 1) River Sheaf, 2) River Rivelin and 3) a backwater connected to River Don via a sluice gate. The three habitats will be referred to as 'Sheaf', 'Rivelin' and 'Don' respectively:

3.1.1 The Sheaf site

The site (National Grid Reference SK435385) was a 80 m stretch of the River Sheaf, situated to the south of the city centre of Sheffield. The river bed was covered with stones ranging in size from pebbles (longest length *c.* 3cm) to large boulders (longest length > 40 cm). The vegetation along the river banks consisted mainly of short shrubs (< 1 m), thus allowing considerable light penetration.

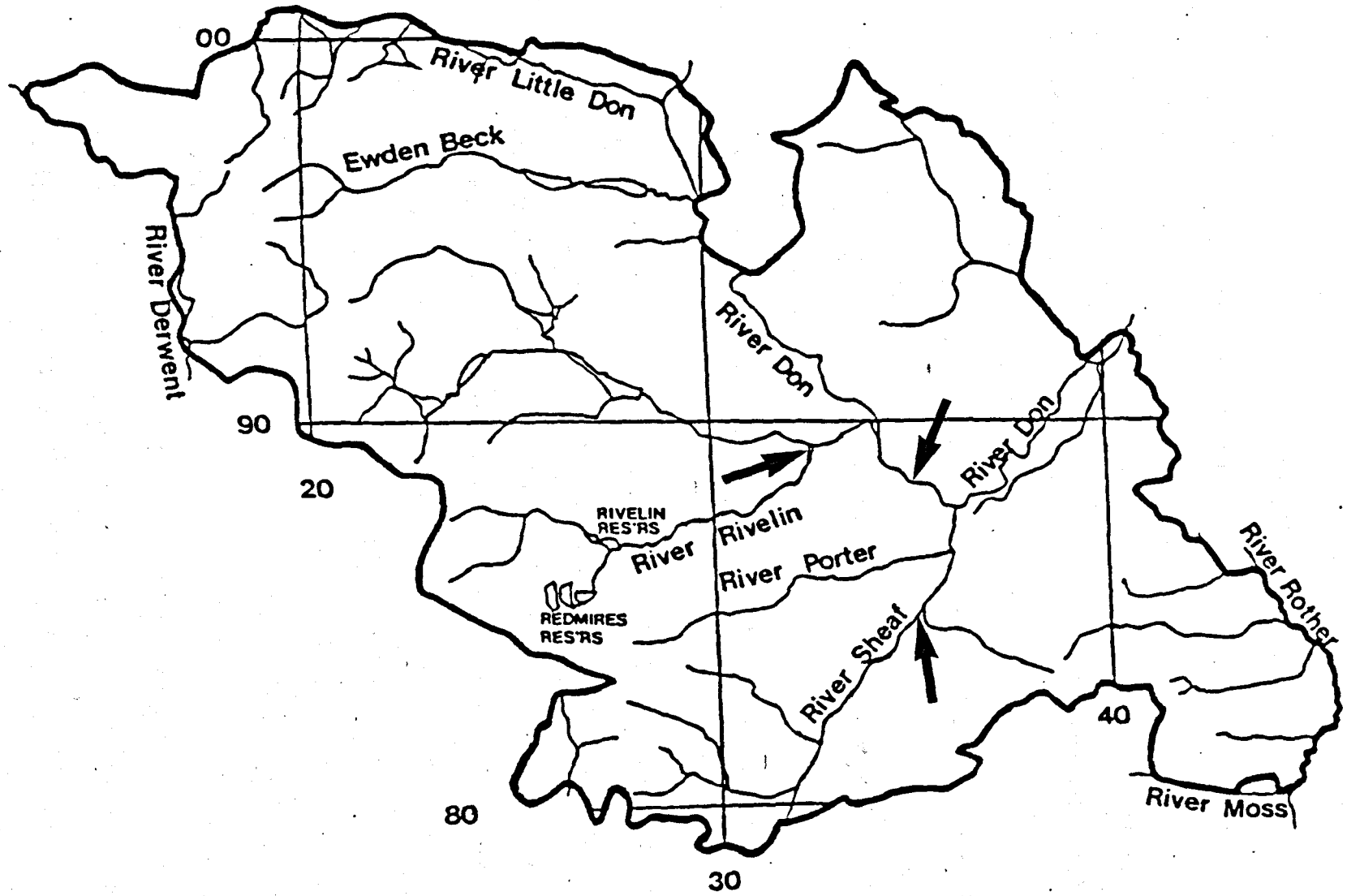
3.1.2 The Rivelin site

This sampling site (National Grid Reference SK433389) was a 80 m stretch of the River Rivelin, downstream from the outflow of the Rivelin Reservoirs. The water level and current speed of the river were directly affected by the water level of the reservoirs, which was regulated by sluice gates. The substrate was sandy with pebbles and boulders (longest lengths ranging from 3 to 30 cm). The site was well sheltered by canopies of deciduous trees (> 3 m tall) on both banks which also reduced ambient light reaching the river bed.

3.1.3 The Don site

Sampling was confined to a 80 m length of a backwater (National Grid Reference SK435388) connected to the west bank of the River Don. The backwater (*c.* 5 m wide) was rectangular in cross section. A brick wall of a steel factory formed one side of the backwater, while wooden panels sunk vertically into the muddy substrate (sludge)

Fig. 3.1. Map of Sheffield Metropolitan District showing the locations of the study sites (indicated by arrows).


00
River Little Don
Ewden Beck
River Derwent
River Don
90
20
RIVELIN RES'RS
River Rivelin
River Porter
REDMIRES RES'RS
River Don
River Rother
River Sheaf
40
80
River Moss
30

constituted the other bank. The vertical panels were held in position by wooden poles (10 cm x 10 cm in cross section) running horizontally (or lengthwise). Pipes from the adjacent steel factory were submerged for cooling, and a sluice gate maintained the water level at about 0.5 m. Every three years, the site is drained for two weeks (last week in July and the first week in August when the factory closed down during the traditional holidays for steel workers in Sheffield), and the sludge pumped out. Domestic sewage was also discharged from the factory. Riparian vegetation consisted of grasses, sedges and a few shrubs (mostly < 1 m).

3.2 Physico-chemical conditions during the study period

3.2.1 Introduction

It is well documented that temperature has an important influence on the metabolism and life history of poikilothermic animals (Brown 1979, Hardy 1979). Water chemistry, particularly water hardness, has also been shown to be an important factor influencing snail distribution (Boycott 1936, Williams 1970, Dussart 1976), physiology and life histories (Dussart 1979, Dussart & Kay 1980), and shell morphology (Boycott 1938, Diver *et al.* 1939, McMahon & Whitehead 1987). Other factors that can influence the expression of life-history traits in freshwater snails include the stability or permanency of the habitat (Brown 1979, 1985a) and food availability or habitat productivity (Brown 1985a, 1985b).

During the study period between January 7 1985 and December 8 1986, I monitored a number of environmental parameters that are potentially important in shaping the life histories and the shell morphology of freshwater snails.

3.2.2 Materials and methods

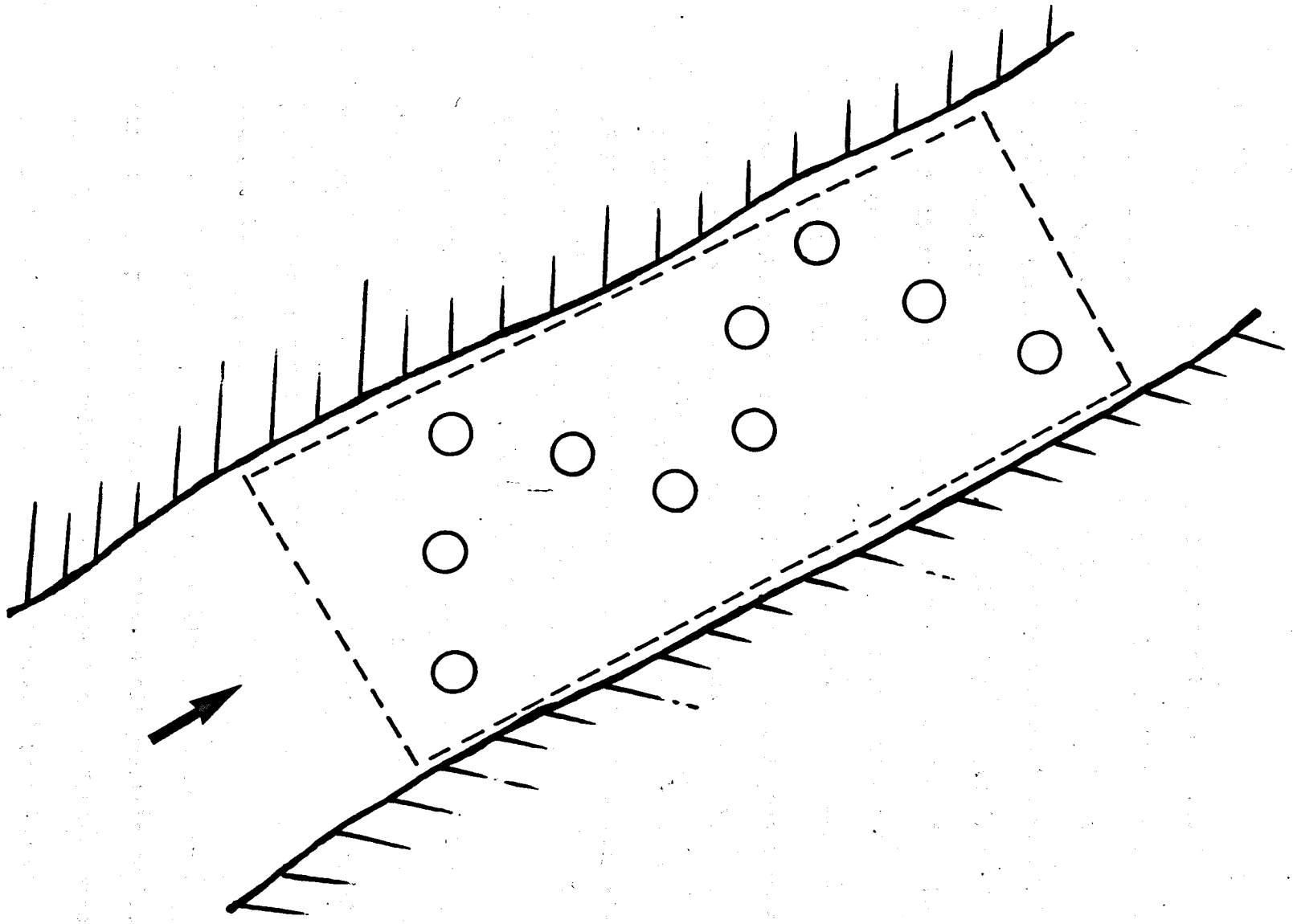
The three sites were visited weekly, and the maximum and minimum water temperatures (at 30 cm depth) recorded at each using a submerged maximum/minimum thermometer. Initially, conductivity and pH were determined using a salinity-conductivity-temperature meter (Yellow Springs Instrument Co. model 33) and a PTI-11 pH/mV/^{o}C meter (FSA Laboratory Supplies) respectively. This equipment was later replaced by a Harris environmental test centre (Philip Harris Ltd.) which was also used to measure the concentration of dissolved oxygen (June 2 to December 8 1986).

Upon each visit to the sites, the widths of the Sheaf and the Rivelin sites were measured on a fixed transect at roughly the widest part of the sampled reach while depth was determined at the Don.

Calcium concentrations of monthly water samples were measured using a calcium electrode (Russell ion-selective electrode model ISE 310).

Food availability, in terms of periphyton (*Aufwuchs*) biomass, was estimated by a method similar to that of Douglas (1958). Ten stones were collected from each of the Sheaf and the Rivelin sites, and algae scrubbed off with a tooth brush. Each stone was then wrapped in a sheet of aluminium foil with a 5 cm x 5 cm square cut out of it, thus limiting the exposed area to 25 cm^{2}. The stones were placed, exposed surface upwards, on their respective river beds at the start of each season: March (spring), June (summer), September (autumn) and December (winter). The stones were arranged in a zig-zag fashion (Fig. 3.2) to minimize any possible bias arising from heterogeneous conditions prevailing at different parts of the river beds. The stones were retrieved after 28 days, and were carefully rinsed to remove any loose debris attached to the exposed surfaces.

Fig. 3.2. The zig-zag arrangement of the stones at the Sheaf and Rivelin sites, for determination of rates of *Aufwuchs* accumulation. The dotted line represents the boundary of the sampling area. The circles represent the positions of the stones while the arrow indicates the direction of flow.



The exposed surface of each stone was then scrubbed as previously, and the algal cover washed into a beaker with distilled water. This was oven dried to constant weight at 60 ^{o}C. The ash-free dry weight of the algal material was measured from the loss of weight upon ignition in a muffle furnace maintained at 500 ^{o}C for one hour (Allen *et al.* 1974).

The above procedure was modified for the Don site due to its different physical conditions (section 3.1.3). At the start of each season, algae were scrubbed from 10 patches (*c.* 1 m apart) on the horizontal surface of the wooden poles supporting the wooden wall of the backwater (section 3.1.3). Each cleared patch (10 cm x 10 cm) was marked by four drawing pins. The site was revisited after 28 days. The neck (4.5 cm in diameter) of a 50 ml polythene bottle with the bottom cut off was pressed, neck downwards, onto the previously cleared patch. River water trapped inside the bottle was removed by a 50 ml syringe. The inverted bottle, while held tightly in position, was filled with distilled water, and the algae scrubbed off the area (15.90 cm^2) delimited by the neck. The algal suspension was then collected using another 50 ml syringe. The ash-free dry weight of the algae was determined as previously.

In situ observations during the study period revealed that the three sites differed markedly in their current speeds. Typical current speeds were estimated from the distance travelled by a plastic float per unit time when the water level at each site was close to its annual mean.

To detect any between-site differences in environmental parameters, pairwise comparisons involving all possible combinations i.e. Sheaf versus Rivelin, Sheaf versus Don and Rivelin versus Don were performed using paired *t* tests. As repeated pairwise comparisons

increase the probability of obtaining a significant result by chance, the critical probability for the rejection of the null hypothesis was lowered in each group of tests to attain the conventionally accepted levels e.g. 0.05, 0.01 and 0.001. The adjustment was achieved by Šidák's multiplicative inequality, $P' = 1 - (1 - P)^{1/k}$ where P' was the adjusted probability level for k comparisons and P was the conventional significance level (Sokal & Rohlf 1981). A few examples of the conventional and adjusted probability levels are given in Table 3.1.

Table 3.1 Conventional and adjusted significance levels for pairwise comparisons involving three combinations (i.e. k = 3).

Significance levels	
Conventional (P)	Adjusted (P')
0.05	0.0170
0.01	0.0033
0.001	0.0003

3.2.3 Results

Maximum and minimum water temperatures recorded at the three sites showed similar seasonal trends from February 1985 to December 1986 (Fig. 3.3). Maximum temperatures (19 - 21 ^{o}C) were recorded in July. Minimum temperatures reached 0 ^{o}C in January and February at the Sheaf and the Rivelin sites, while the Don site maintained temperatures above 0 ^{o}C throughout the study period. At each sampling occasion, the field temperature recorded at the Don was significantly higher than those of the Sheaf and the Rivelin sites (Fig. 3.3, Table 3.2). Pairwise comparison also revealed that the Sheaf site experienced a significantly higher temperature than the Rivelin site (Table 3.2).

Fig. 3.3. Monthly mean temperatures at the Sheaf (solid squares), Rivelin (open triangles) and Don (solid triangles) sites over the period February 1985 to December 1986. Mean maximum and mean minimum temperatures are plotted.
(Missing data: November 85 and February 86 for the Sheaf and Rivelin sites; March 86 for the Rivelin site)

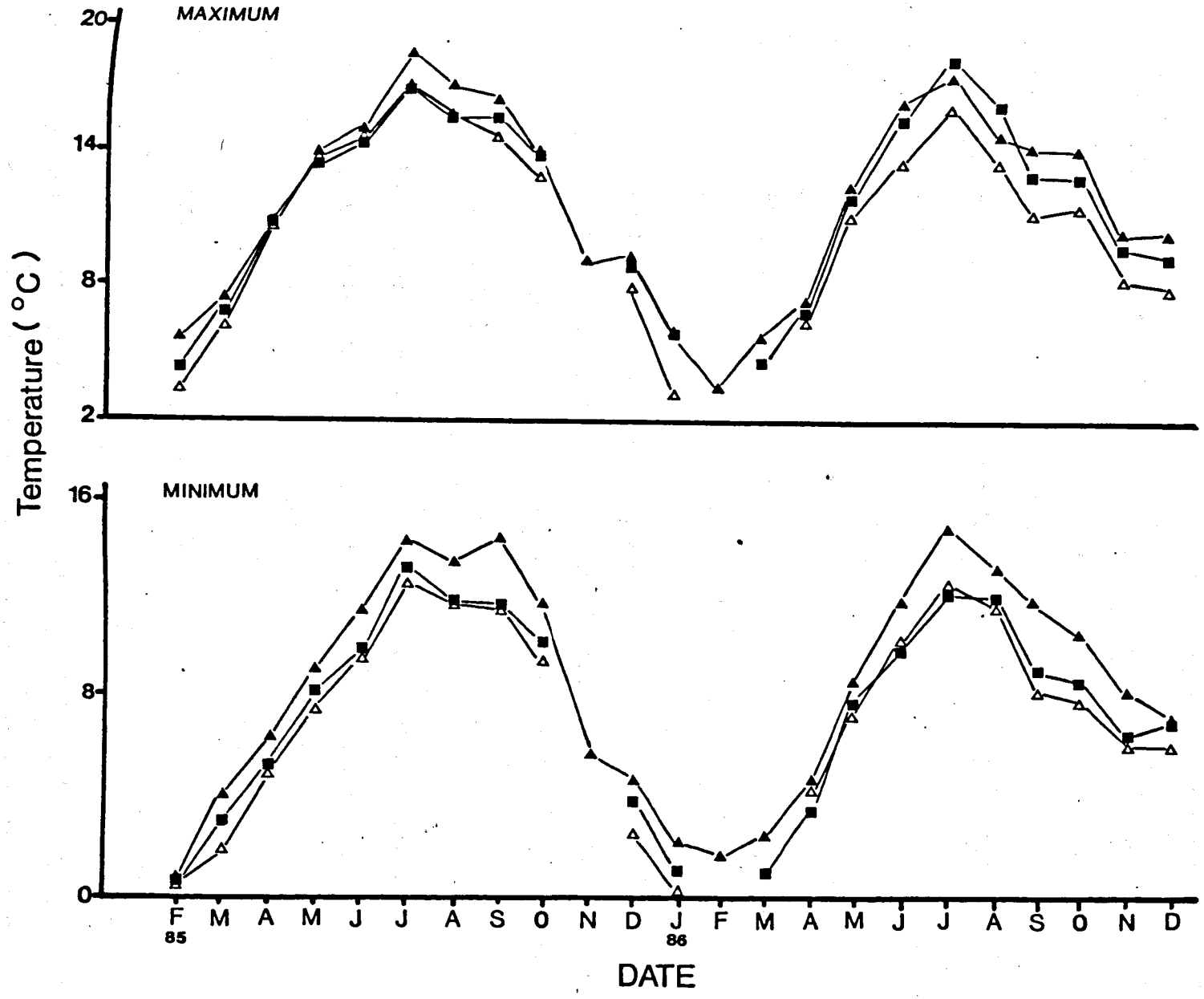

MAXIMUM
20
14
8
2
MINIMUM
16
8
0
Temperature (°C)
F M A M J J A S O N D J F M A M J J A S O N D
85
86
DATE

Table 3.2 Between-site pairwise comparisons of maximum and minimum temperatures (^{o}C) by paired t tests. Significance levels: * = $P < 0.05$, *** = $P < 0.001$.

	Maximum temperatures	
	Sheaf	Don
Rivelin	$t = 7.02$***	$t = 9.16$***
Don	$t = 2.87$*	

	Minimum temperatures	
	Sheaf	Don
Rivelin	$t = 5.22$***	$t = 18.23$***
Don	$t = 13.06$***	

Conductivity readings (μohm^{-1}) showed no apparent seasonal trends (Fig. 3.4) but exhibited significant between-site variations; Don (mean $\pm$ S.E.: 339 $\pm$ 7.9) > Sheaf (290 $\pm$ 6.4) > Rivelin (225 $\pm$ 6.5) (Table 3.3).

Table 3.3 Between-site pairwise comparisons of conductivity (μohm^{-1}) by paired t tests. Significance level: *** = $P < 0.001$.

	Sheaf	Don
Rivelin	$t = 11.63$***	$t = 21.66$***
Don	$t = 7.71$***	

Lowest pH was recorded consistently at the Don (mean $\pm$ S.E.: 7.2 $\pm$ 0.04), followed by the Rivelin (7.4 $\pm$ 0.04) and then the Sheaf (7.6 $\pm$ 0.04) sites (Fig. 3.5). Pairwise comparisons confirmed that the differences were significant (Table 3.4).

Fig. 3.4. Monthly mean conductivity (μohm^{-1}) recorded at the Sheaf (solid squares), Rivelin (open triangles) and Don (solid triangles) sites over the period February 1985 to December 1986.

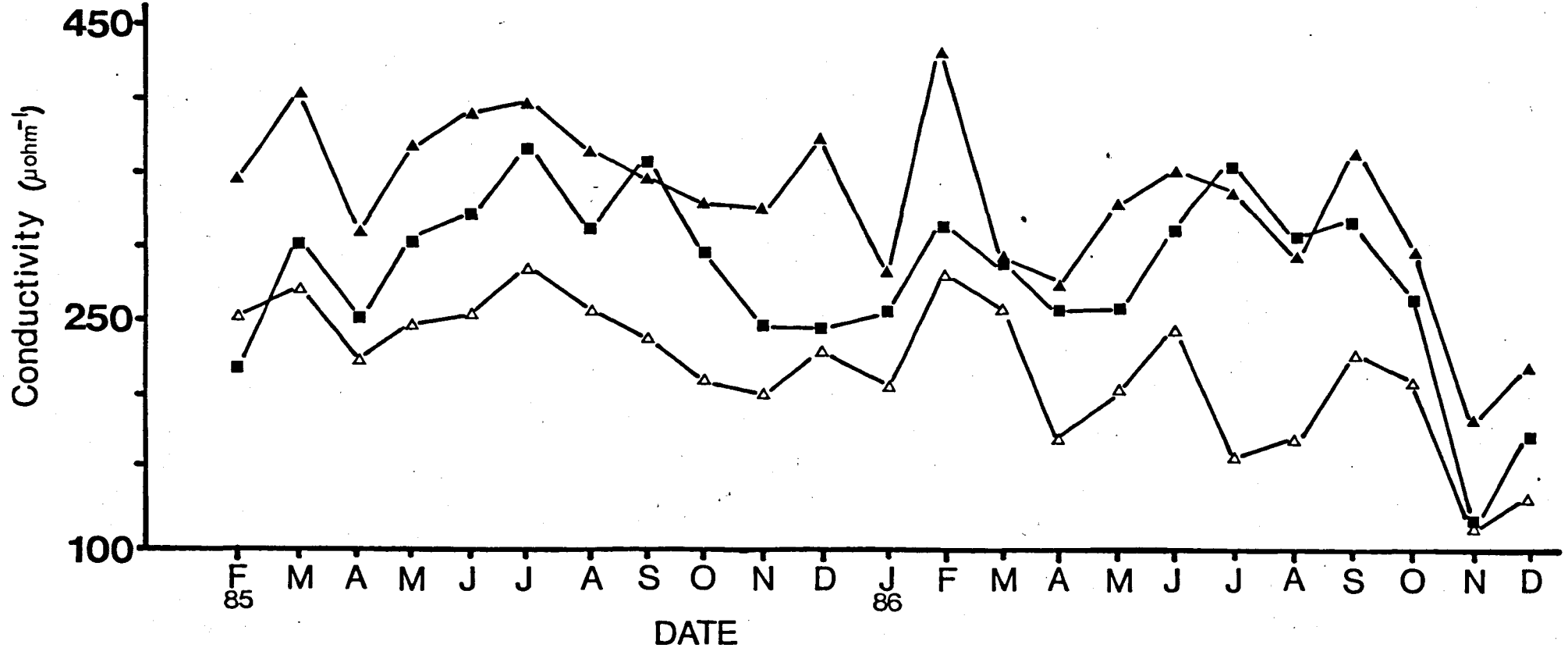

450
250
100
Conductivity (μohm⁻¹)
F
85
M
A
M
J
J
A
S
O
N
D
J
86
F
M
A
M
J
J
A
S
O
N
D
DATE

Fig. 3.5. Monthly mean pH recorded at the Sheaf (solid squares), Rivelin (open triangles) and Don (solid triangles) sites over the period January 1985 to December 1986.
(No data for January 85 at the Don)

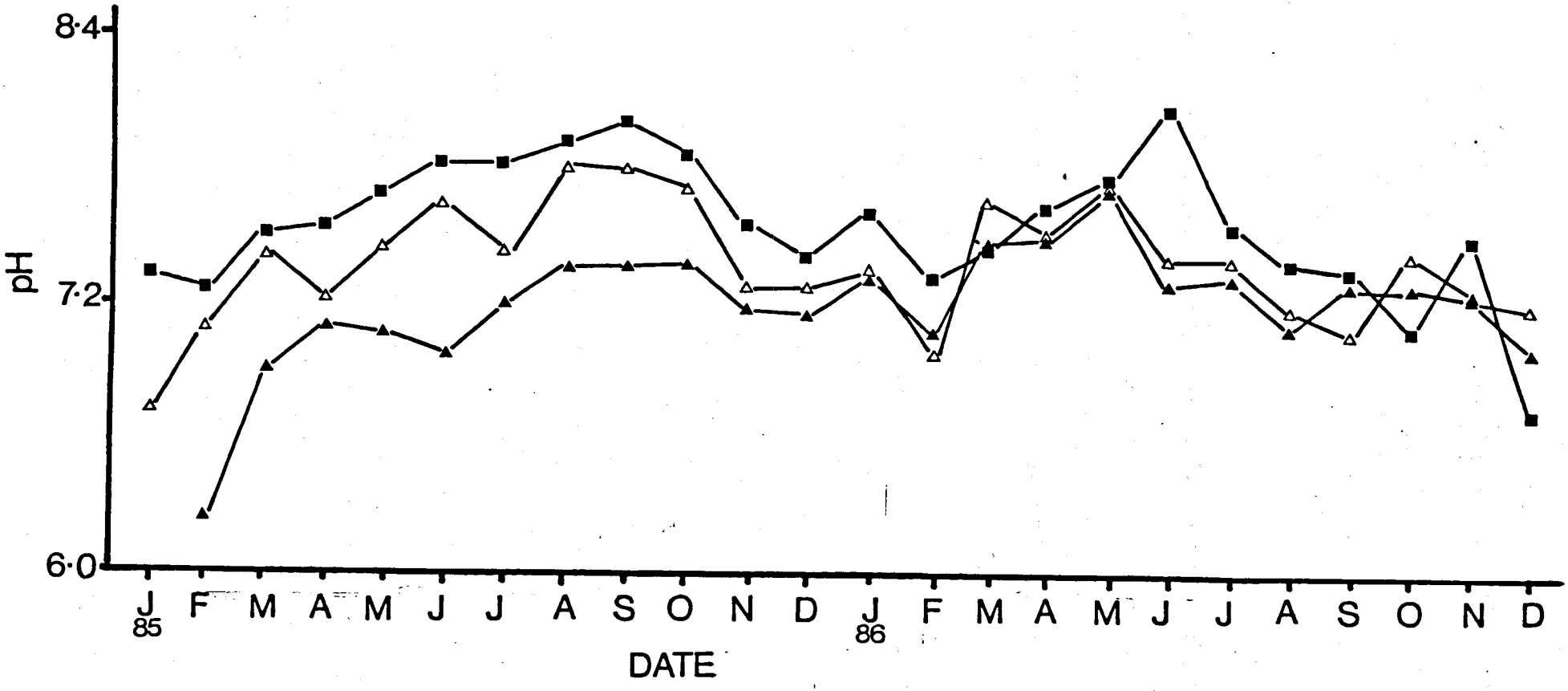

8·4
7·2
6·0
pH
J
85
F
M
A
M
J
J
A
S
O
N
D
J
86
F
M
A
M
J
J
A
S
O
N
D
DATE

Table 3.4 Between-site pairwise comparisons of pH by paired t tests. Significance level: *** = $P < 0.001$.

	Sheaf	Don
Rivelin	$t = 4.65$***	$t = 5.05$***
Don	$t = 8.28$***	

Concentrations of dissolved oxygen (mg l^{-1}) recorded at the 'river' sites (Sheaf and Rivelin) were similar ($t = 0.10$, $d.f. = 20$, $P > 0.05$), but higher than those of the Don site ($t > 7.35$, $d.f. > 19$, $P < 0.001$) (Fig. 3.6). The mean oxygen concentrations are given in Table 3.5.

Table 3.5 Mean concentrations of dissolved oxygen (mg l^{-1}) at the study sites from June 2 to December 8 1986.

	Sheaf	Rivelin	Don
mean	12.7	12.9	9.6
S.E.	0.4	0.5	0.4
n	23	22	22

Calcium concentrations (mg l^{-1}) showed no consistent fluctuations according to seasons (Fig. 3.7). Water samples from the Sheaf and the Don sites had similar Ca^{2+} concentrations ($t = 1.64$, $d.f. = 18$, $P > 0.05$) while those from the Rivelin site were characterized by significantly lower Ca^{2+} levels ($t > 11.69$, $d.f. > 18$, $P < 0.001$). The mean Ca^{2+} concentrations at the three sites are summarized in Table 3.6.

Fig. 3.6. Concentrations of dissolved oxygen (mg l^{-1}) recorded at the Sheaf (solid squares), Rivelin (open triangles) and Don (solid triangles) sites between June 2 and December 8 1986. (Missing data: 2/6/86 at the Rivelin site and 11/8/86 at the Don site)

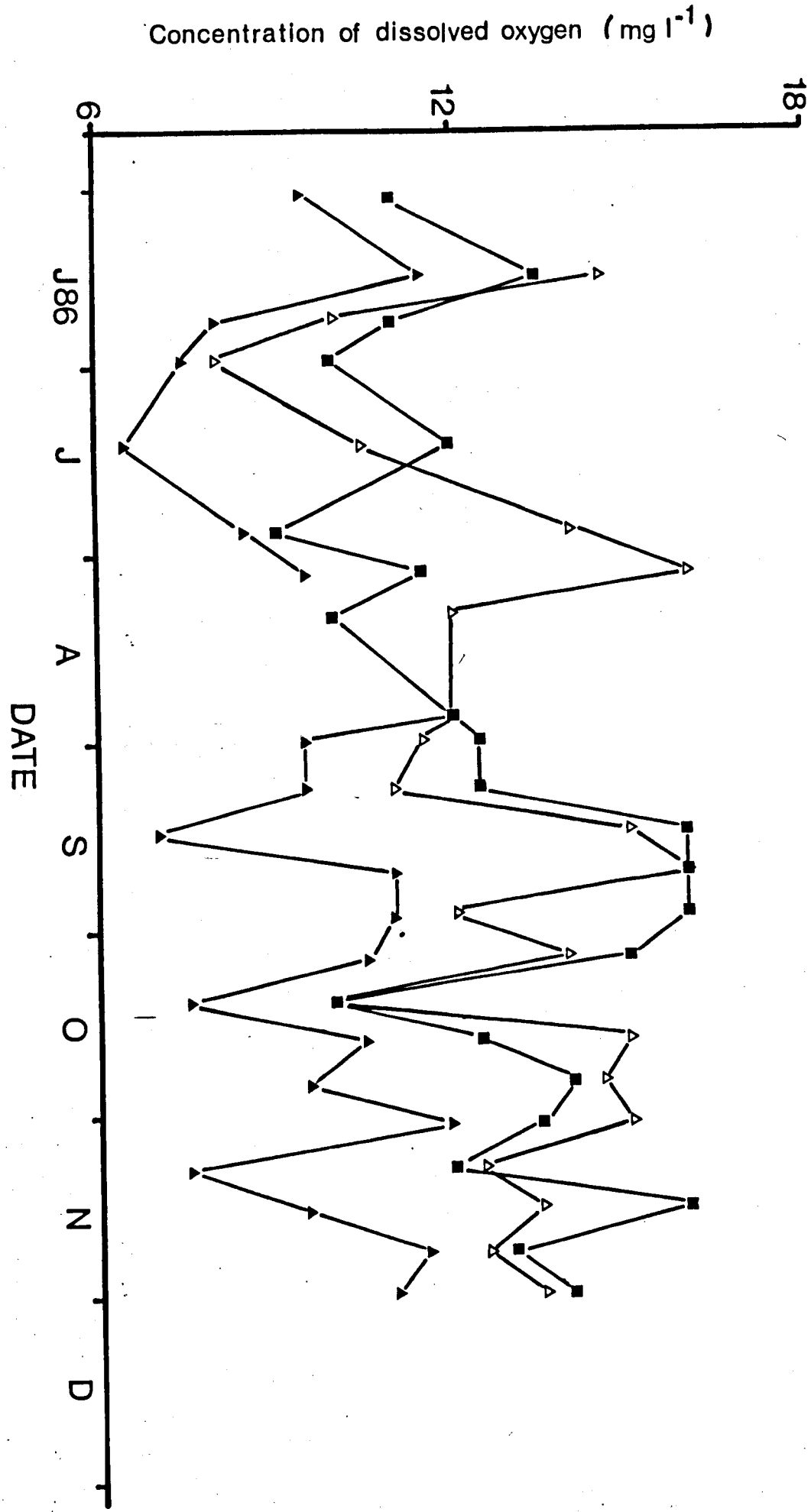

Concentration of dissolved oxygen (mg l-1)
6
12
18
J86
J
A
S
O
N
D
DATE

Fig. 3.7. Calcium concentrations (mg l^{-1}) of monthly water samples collected at the Sheaf (solid squares), Rivelin (open triangles) and Don (solid triangles) sites over the period April 85 to December 1986.

(No data for April 85 at the Sheaf site)

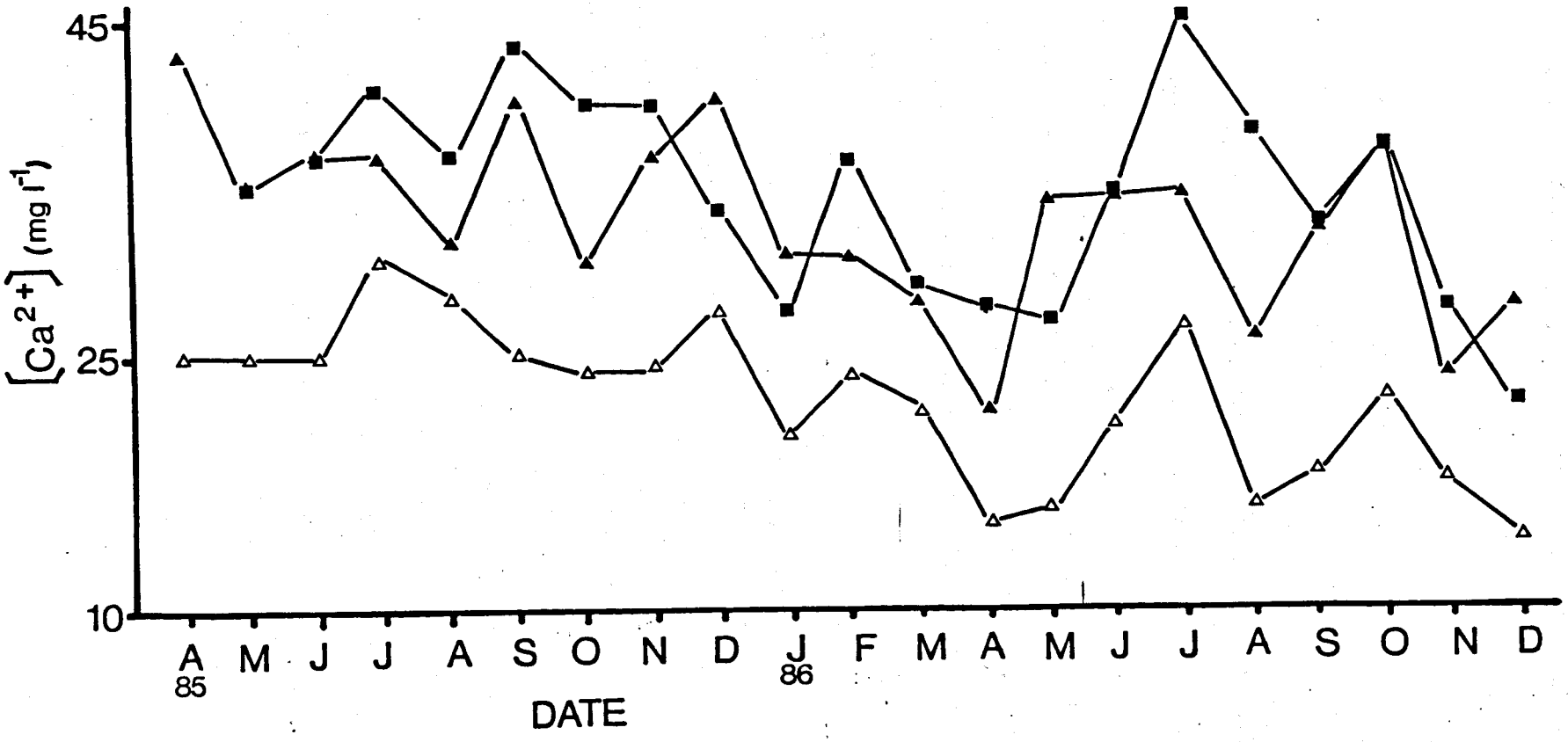

45
25
10
[Ca2+] (mg l-1)
A
85
M
J
J
A
S
O
N
D
J
86
F
M
A
M
J
J
A
S
O
N
D
DATE

Table 3.6 Mean Ca^{2+} concentrations (mg l^{-1}) at the three study sites.

	Sheaf	Rivelin	Don
mean	34.8	22.4	33.3
S.E.	1.4	1.1	1.2
n	20	21	21

A measure of the changes in the widths of the rivers or the depth of the backwater could be used as an indicator of habitat stability. However, extent of fluctuation *per se* could be misleading as a drop in the water level of a wider river would likely be less important than a change of a similar magnitude occurred in a narrower river. Changes in water level (L) in each month were therefore expressed as follows:

$$\text{Monthly \% fluctuation} = [(L_{max} - L_{min})/L_{mean}] \times 100\%$$

where L_{max}, L_{min} and L_{mean} were respectively the maximum, minimum and mean widths or depths recorded in a month.

The Sheaf site represented the most stable habitat in terms of water level while the Rivelin and the Don sites experienced wider fluctuations during the study period (Fig. 3.8). However, paired *t* tests on the arcsine transformed data revealed that only the water regimes at the Sheaf and the Don sites were significantly different (Table 3.7). It should be noted that the clearing of the Don site in August 1986 (section 3.1.3) was responsible for the August peak (*c.* 100%) in Fig. 3.8.

Fig. 3.8. Percentages fluctuation of water level recorded at the Sheaf (solid squares), Rivelin (open triangles) and Don (solid triangles) sites over the period January 1985 to December 1986. (No data for January 85 at the Don site)

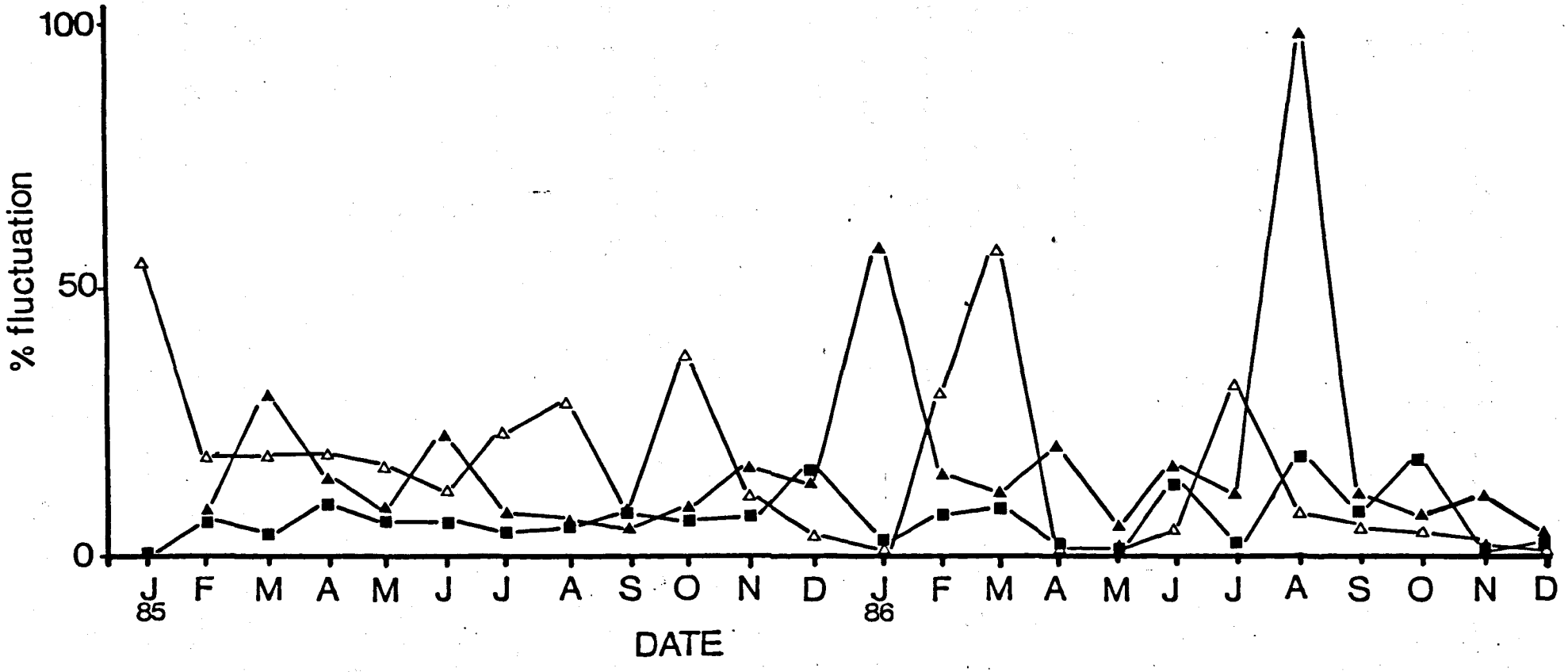

100
50
0
% fluctuation
J
85
F
M
A
M
J
J
A
S
O
N
D
J
86
F
M
A
M
J
J
A
S
O
N
D
DATE

Table 3.7 Between-site pairwise comparisons of the % fluctuation in water levels at the three study sites by paired *t* tests following arcsine transformation. Significance levels: * = $P < 0.05$, NS = Not significant.

	Sheaf	Don
Rivelin	$t = 2.37$ NS	$t = 1.06$ NS
Don	$t = 3.04$*	

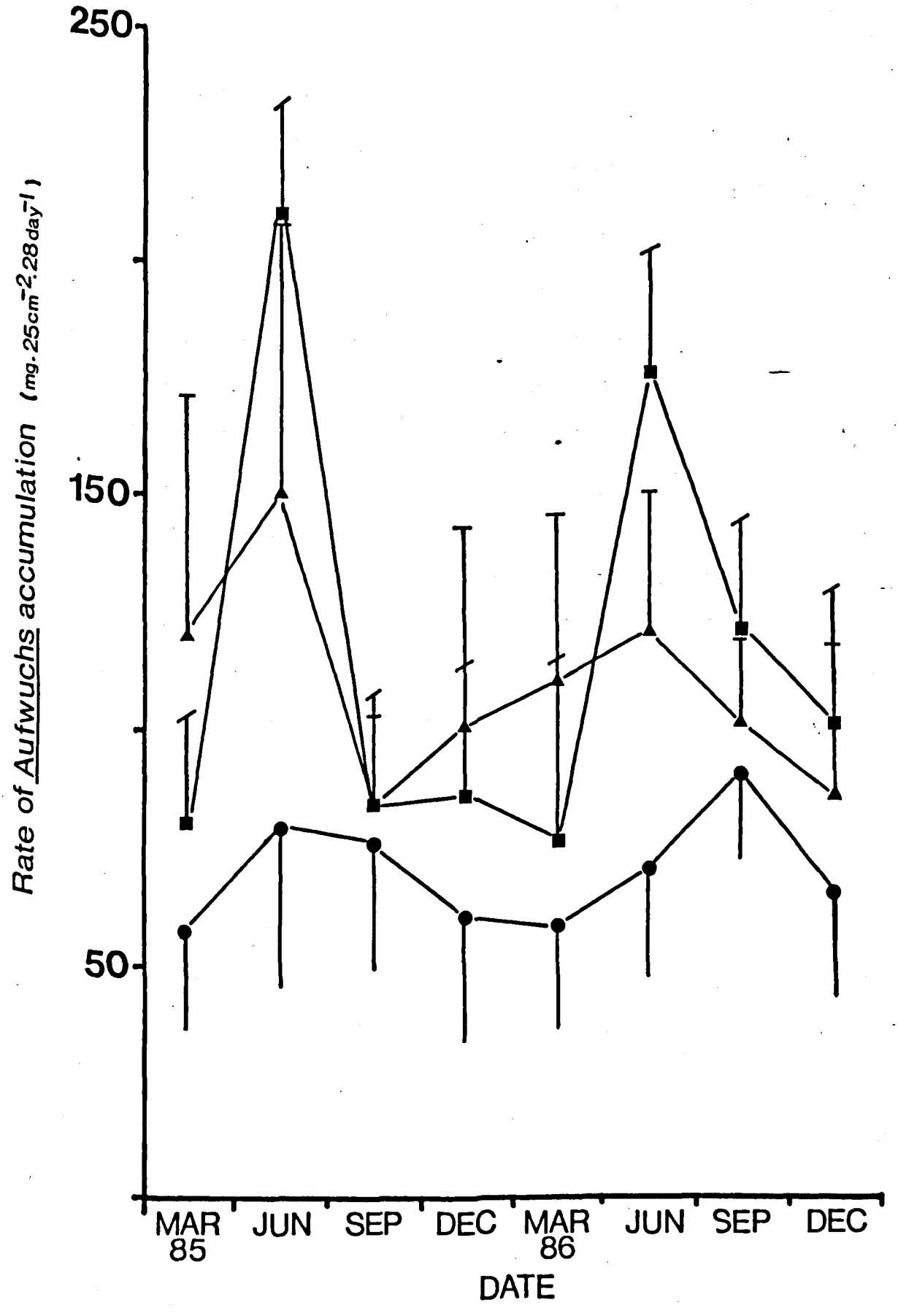
Rates of accumulation of *Aufwuchs* (algal material) were standardized to ash-free dry weight in $mg.25cm^{-2}.28day^{-1}$. These exhibited clear seasonal variations with highest peaks in the summer (June), and followed the general order Sheaf and Don > Rivelin (Fig. 3.9). However, the difference was significant only between the Rivelin and the Don sites ($t = 4.61$, $d.f. = 6$, $P < 0.001$).

3.2.4 Summary

The higher temperatures (maximum and minimum) recorded at the Don site were probably resulted from the warming effects of the submerged pipes (section 3.1.3). The cause (or causes) of the temperature differences between the Sheaf and the Rivelin sites was less clear. Although the differences might appear to be small (only 1 - 2 ^{o}C at any one time), the pattern of variation was very consistent amongst the three sites. This would mean that substantial differences in terms of degree-days (Munn 1970) could be accumulated, especially in winter.

The high Ca^{2+} level at the Don and to a lesser extent the Sheaf sites, paralleled the high conductivity readings. Conductivity measured the concentration of charged solutes, some of which would likely be important elements required for *Aufwuchs* growth. It is, therefore, not surprising to find that the Don and the Sheaf sites

Fig. 3.9. Seasonal changes of the rates of *Aufwuchs* accumulation ($mg.25cm^{-2}.28day^{-1}$) at the Sheaf (solid squares), Rivelin (solid circles) and Don (solid triangles) sites. Vertical bars represent 1 S.E..


250
150
50
Rate of Aufwuchs accumulation (mg.25cm-2.28day-1)
MAR 85
JUN
SEP
DEC
MAR 86
JUN
SEP
DEC
DATE

had higher rates of *Aufwuchs* accumulation. Low *Aufwuchs* productivity at the Rivelin sites could also be a result of dense canopies of deciduous trees reducing light penetration, especially in the summer. It should be noted that rates of *Aufwuchs* accumulation, when used as an indicator of food availability, had to be adjusted for the number of potential consumers i.e. snail density (see chapter 4.1).

The amount of dissolved oxygen could be considerably influenced by water movement such as current speed which, coupled with the temperature differences mentioned above, could account for the higher concentrations of dissolved oxygen recorded at the river sites (current speed > 0.3 ms^{-1}). Typical current speeds at the Don site were below 0.04 ms^{-1}. The Don site, characterized by a lower pH, also experienced relatively high fluctuations in water level, especially when the site was cleared in August 1986. There was enough evidence to suggest that the three sites differed considerably in their physical and chemical characteristics, with the Don site showing many unique features.

4. Intraspecific variation - field study

4.1 Intraspecific life-history variation of *Lymnaea peregra*

4.1.1 Introduction

Intraspecific life-history variation has been widely observed in animals such as leeches (e.g. Maltby & Calow 1986a, 1986b), molluscs (e.g. Russell Hunter 1961b, Hunter 1975a, Mackie *et al.* 1976a, 1976b, Browne 1978, Hornbach *et al.* 1980, Way *et al.* 1980, Aldridge 1982, Brown 1985a, 1985b), insects (e.g. Baldwin & Dingle 1986, Lillehammer 1987), amphibians (e.g. Berven 1982a, 1982b, Howard & Wallace 1985, Cummins 1986) and lizards (e.g. Tinkle & Ballinger 1972). The diversity of phenotypes amongst populations is often attributed to varying selection pressures as a result of environmental heterogeneity. The life-history studies mentioned above are often concerned with predicting the optimal strategy under a specific set of environmental conditions (the *a priori* approach, section 2.6), and/or correlating the observed strategy with the particular ecological circumstances (the *a posteriori* approach, section 2.6). Ecologists adopting the latter approach are inevitably faced with the crucial task of identifying the physical and/or biological parameters that constitute the key selection pressures (KSPs, section 2.5). Significantly, factors such as water temperature (e.g. Russell Hunter 1961b), environmental periodicity (e.g. Aldridge 1982), habitat productivity (e.g. Brown 1985a, 1985b), and current speed (e.g. Calow 1981b) have been considered as KSPs in the study of life-history evolution of freshwater snails.

Data presented in chapter 3 indicated important physico-chemical differences amongst the three sites chosen for this study (Sheaf, Rivelin and Don). Parameters such as temperature, pH, oxygen tension and water level fluctuations can act as important selection pressures

because of their potential impact on individual survival (l), while food availability and temperature are key determinants of growth rates (G). Survivorship (l), time to first breeding (t_j) [t_j is likely to be related to growth rate (G)], and fecundity (n) are the key fitness components for a semelparous population (section 2.4). Therefore, studies on the spatial and temporal variations of l, n and t_j amongst populations inhabiting vastly different environments should provide some insights into the cause(s) of the evolution of various life-history strategies.

This chapter compares and contrasts the population dynamics (changes in l and G) and reproductive patterns (changes in n and t_j) of three neighbouring populations (chapter 3) of a freshwater snail *Lymnaea peregra* (Müller) (Gastropoda: Pulmonata: Basommatophora: Lymnaeidae), and has three major aims:

(1) to examine the life-history strategies exhibited by the three populations,

(2) to identify the key selection pressure(s) that may be responsible for any interpopulation life-history variation,

(3) to discuss the possible significance of various life-history tactics as adaptations to local circumstances.

4.1.2 Materials and methods

4.1.2.1 Population density

The Sheaf and Rivelin sites. The sites were sampled every four weeks from January 1985 to December 1986, except during the breeding season when densities were estimated biweekly. The densities of snails were determined following the stratified sampling method developed by Wrona *et al.* (1986). This method was used instead of the conventional quadrat technique because it took into account factors such as habitat heterogeneity, dispersion pattern of the snails, and

caused less disturbance to the site (Wrona *et al.* 1986). The stratified sampling technique involves estimating 2 parameters:

(a) Stone profile of the habitat

Stones were assigned to five size classes [(1) < 5 x 5 cm^2, (2) 5 x 5 - 9 x 9 cm^2, (3) 10 x 10 - 14 x 14 cm^2, (4) 15 x 15 - 20 x 20 cm^2, (5) > 20 x 20 cm^2] on the basis of plan area. The number of stones belonging to each of the five size classes enclosed by a quadrat of 0.5 m x 0.5 m was counted. Fifteen such samples were taken randomly at each site, and the mean number of stones of each size classes per 0.25 m^2 (X) calculated.

(b) Number of snails per stone for each size category

At each site, 100 stones (20 of each size class) were sampled 'randomly', and the number of snails present on each stone recorded. This information allowed the mean number of snails per stone for each size category (Y) to be calculated.

The number of snails per 0.25 m^2 of the river bed was calculated by combining (a) and (b):

$$D = \sum_{i=1}^{5} X_i Y_i \qquad 4.1$$

where D is the weighted mean number of snails per 0.25 m^2, X_i is the mean number of stones of the *i*th size class per 0.25 m^2, and Y_i is the mean number of snails per stone of the *i*th size class.

The weighted variance, V_D, of the mean density estimate was calculated by the equation:

$$V_D = \sum_{i=1}^{5} V_{XiYi} \qquad 4.2$$

where

$$V_{XiYi} = [V_{Xi} + (X_i)^2][V_{Yi} + (Y_i)^2] - (X_i)^2(Y_i)^2 \qquad 4.3$$

where V_{Xi} is the mean variance of the number of stones of the *i*th size class per 0.25 m^2 and V_{Yi} is the mean variance of the number of snails per stone of the *i*th size class.

The standard error (S.E.) of the mean density estimate was given by:

$$S.E. = [V_D]^{1/2} \qquad 4.4$$

The above procedure was also used to determine the density of egg capsules during the breeding season. Detailed statistical treatment of the above method was given by Wrona *et al.* (1986). A worked example is included in Appendix 1.

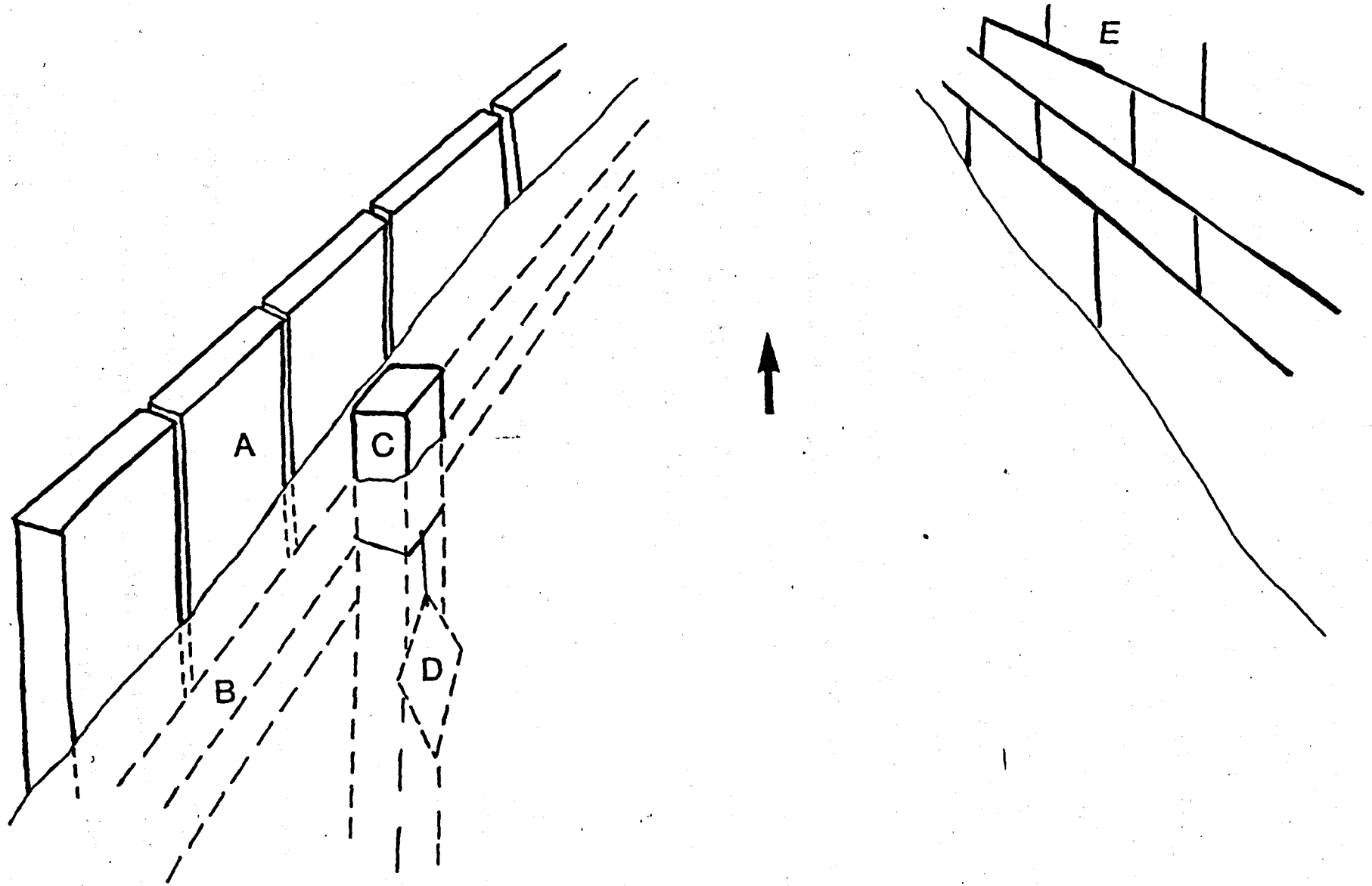
The stratified sampling technique described above was, however, not applicable to small juveniles (shell length of hatchlings < 1.3 mm) which were difficult to detect visually in the field. Consequently, during the recruitment period (from the time when egg capsules were first observed to one month after the last capsule had been recorded), 25 more stones (5 of each size class) were sampled by washing them separately in a bucket. The content collected from individual stone, after sieving through a net of mesh size less than 0.8 mm, was brought back to the laboratory. Juvenile snails in each sample were counted under a stereomicroscope, and then returned to the rivers.

The Don site. This site was sampled from February 1985 to December 1986 at intervals similar to those of the Sheaf and the Rivelin. Due to the absence of removable items such as stones or boulders (*cf.* Sheaf and Rivelin), the density of snails was determined by planting artificial substrates (Southwood 1978). Thirty plastic templates of 15 cm x 15 cm were tied to vertical wooden poles (*c.* 1 m apart) with nylon string (one template per pole), and suspended at 25 cm above the bottom of the backwater to ensure

constant submersion (Fig. 4.1.1). Upon each visit to the site, every individual template was carefully lifted above the water, and the number of snails attached to the colonizable surface (i.e. the outward-facing surface not in direct contact with the wooden pole) counted. Each template was then returned to its original position. To account for young juveniles that could not be detected visually, an additional procedure was carried out at the time of recruitment (see above). Colonizable surfaces of ten randomly chosen templates were brushed, and the content from each template washed into a separate beaker. Juvenile snails in each sample were counted as previously, and then returned to the backwater.

The gastropod community at the Don was co-dominated by *L. peregra* and *Physa fontinalis* (Linnaeus) (Pulmonata: Physidae). The density of *P. fontinalis* was monitored in view of the potential importance of interspecific competition between the two species. Egg capsules of *P. fontinalis* differ from those of *L. peregra* by the eggs having well developed external membranes (Bondesen 1950), and hence could be identified by examination. However, the two species of egg capsules could not be readily separated in the field, and thus it was possible to estimate only the combined densities of egg capsules of both species. The egg capsule density of individual species was then determined from the combined estimate according to the relative abundance of *L. peregra* and *P. fontinalis* capsules in the field. This information was obtained from the relative abundance of the two species of egg capsules in three random samples of egg capsules (n = 50) collected at regular intervals (*c.* 5 weeks) during each breeding season. Eggs in the *P. fontinalis* capsules were counted, and all capsules were returned to the backwater.

Fig. 4.1.1. Arrangement of plastic templates (artificial substrates) at the Don site. The arrow indicates the direction of flow. (A: vertical wooden panel, B: horizontal wooden pole, C: vertical wooden pole, D: plastic template and E: brick wall).


A
B
C
D
E

4.1.2.2 Growth rates of *L. peregra*

A random sample of snails ($38 < n < 55$) was collected from each of the three sites in January 1985. The shell length and shell width (breadth) (*sensu* Hubendick 1951) of snails were measured with a graticuled eyepiece on a stereomicroscope. The shell of each snail was then separated from its soft part, and both oven dried to constant weight at 60 ^{o}C. Regression analyses revealed that shell length was a good predictor of shell dry weight ($r > 0.83$, $P < 0.001$), tissue dry weight ($r > 0.88$, $P < 0.001$), and total dry weight ($r > 0.89$, $P < 0.001$). Hence, increase in shell length with time was used as a measure of growth rate in this study. A random sample of snails ($n > 30$, except for the Sheaf on May 26 and June 23 1986) was collected from each site monthly, except at the time of recruitment when snail samples were collected biweekly. Adult snails were collected by hand, while juveniles were collected by washing them off stones or templates (section 4.1.2.1). The shell length (mm) of snails was measured as previously.

4.1.2.3 Egg capsules of *L. peregra*

Random samples of egg capsules ($40 \leq n \leq 50$) were collected from each site in May 1985 and June 1986. Eggs in each capsule were counted, and the volume of 10 of the typically ellipsoidal eggs calculated by:

$$v_e = 4/3(\pi)(a/2)(b/2)^2 \qquad 4.5$$

where v_e mm^3 is the egg volume, a and b mm are the longer and shorter axes respectively.

The above egg capsules were then incubated individually at 15 ($\pm$1) ^{o}C in plastic pots containing 25 ml of reconstituted water (Appendix 2). The mean percentage egg hatchability for each population was given by:

$$h_e = (1/n) \sum_{i=1}^{n} x_i/y_i \times 100\% \qquad 4.6$$

where h_e is the mean percentage egg hatchability, x_i and y_i are respectively the number of eggs hatched and the total number of eggs in the *i*th egg capsule, and *n* is the total number of egg capsules monitored.

4.1.2.4 Life tables of *L. peregra*

A life table was constructed for the 1985 cohort of each population based on field density estimates. Each life table consists of the following columns (Deevey 1947):-

x is the pivotal age (in four week periods),

a_x is the number per 0.25 m^2 observed at the start of age interval *x*,

l_x is the standardized number per 0.25 m^2 surviving at the start of age interval *x*,

d_x is the standardized number per 0.25 m^2 dying between *x* and *x* + 1,

q_x is the mortality rate,

e_x is the expectation of life remaining for individuals of age *x*.

a_x is the only column that contains field data. All other columns are derived from the a_x column (Southwood 1978) as follows:

$$l_x = a_x(1000)/a_0 \qquad 4.7$$

where a_0 0.25 m^{-2} is the initial density i.e. $x = 0$.

$$d_x = l_x - l_{x+1} \qquad 4.8$$

$$q_x = d_x/l_x \qquad 4.9$$

The number per 0.25 m^2 surviving between age *x* and *x* + 1 (L_x) is given by:

$$L_x = (l_x + l_{x+1})/2 \qquad 4.10$$

and

$$e_x = (L_x + L_{x+1} + L_{x+2} + \ldots . L_w)/l_x \qquad 4.11$$

where w is the last age.

Initial density (a_0) was calculated by:

$$a_0 = E_{max} n_e h_e \qquad 4.12$$

where E_{max} 0.25 m^{-2} is the peak density of egg capsules, n_e is the mean number of eggs per capsule, and h_e is the mean percentage egg hatchability. Subsequent a_x values were determined by [Fig. 4.1.2 (a)]:

$$a_x = (D_{x-0.5} + D_x + D_{x+0.5})/3 \qquad 4.13$$

where D_x 0.25 m^{-2} is the field density at the start of age interval x, $D_{x-0.5}$ and $D_{x+0.5}$ 0.25 m^{-2} are the field densities two weeks before and after the start of age interval x respectively.

In the case where one of the values ($D_{x-0.5}$, D_x or $D_{x+0.5}$) was missing (e.g. when snail density was monitored every four weeks), the missing value was estimated by assuming that density estimates decreased linearly with time. For example, when $D_{x+0.5}$ was not available from field data [Fig. 4.1.2 (b)], it was calculated by:

$$D_{x+0.5} = (D_x + D_{x+1})/2 \qquad 4.14$$

where D_{x+1} 0.25 m^{-2} is the field density four weeks after the start of age interval x.

Pairwise comparison of life tables was carried out using the log-rank test (Anderson *et al.* 1980, Pyke and Thompson 1986). The principle is that if there were no survival difference between two groups, the total deaths occurring at any one time should split between the two in the ratio of the numbers surviving in the two groups at that time. The 'observed' number of deaths in each group was then compared to the 'expected' number of deaths by a chi-square test. A worked example is given in Appendix 3.

Fig. 4.1.2. Diagram illustrating the calculation of the density of snails surviving at the start of age interval x (a_x) from field density estimates (e.g. $D_{x-0.5}$, D_x, $D_{x+0.5}$ and D_{x+1}).

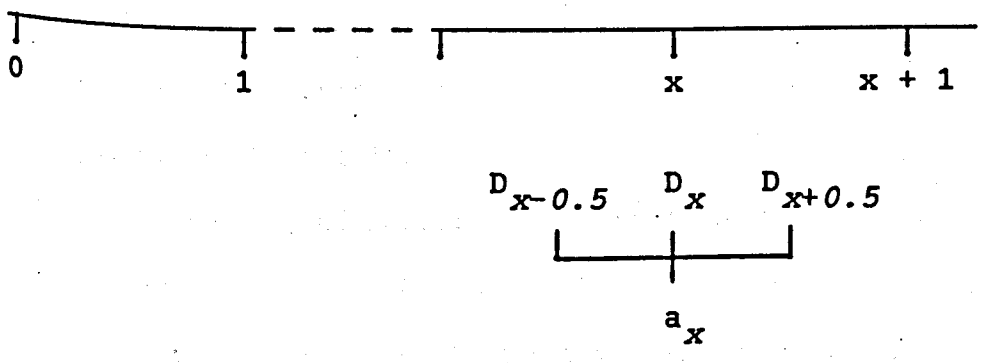
(a) $a_x = (D_{x-0.5} + D_x + D_{x+0.5})/3$

(b) In the case where one of the values, for example, $D_{x+0.5}$ is not available from field data, $D_{x+0.5}$ is obtained by:

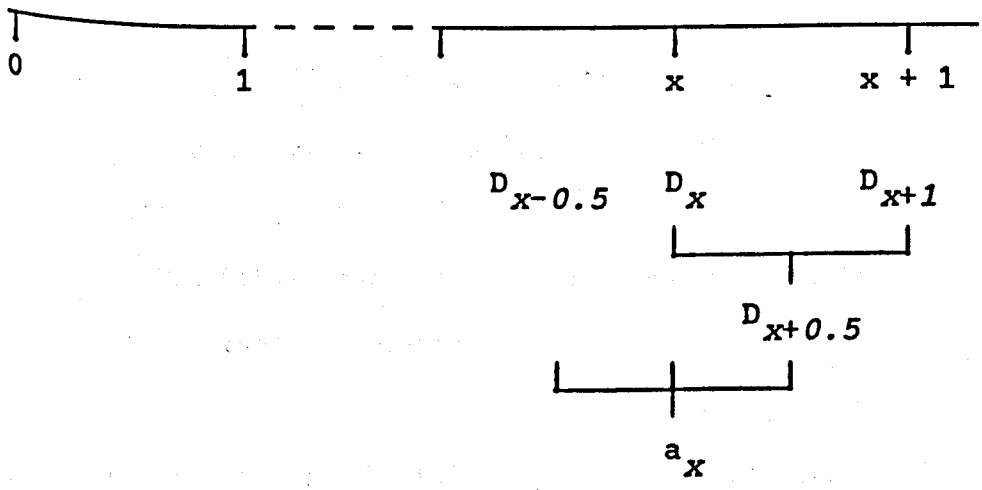
$$D_{x+0.5} = (D_x + D_{x+1})/2$$

a_x is subsequently calculated as in (a).

(a)



(b)



Nomenclature:

0,1,....x and x + 1 are pivotal ages.

$D_{x-0.5}$, D_x, $D_{x+0.5}$ and D_{x+1} are field density estimates.

a_x is the density surviving at the start of age interval x.

4.1.2.5 Other reproductive parameters

Hatching time (T weeks) in the field was estimated independently as the time interval between the peak of egg capsule density and the peak of juvenile density (Method 1), and the time interval between the first occurrence of egg capsules and the first emergence of juveniles (Method 2).

Fecundity of snails in the field was estimated by the graphical method similar to that described by Southwood (1978). Successive estimates of egg-capsule densities were plotted against time (weeks) and the area under the line (A) estimated by numerical integration:

$$A = \sum_{i=1}^{n} D_i t_i \qquad 4.15$$

where D_i 0.25 m^{-2} is the density of egg capsules recorded on one sampling date, t_i weeks is the sampling interval and n is the total number of egg-capsule density estimates.

*The total number of egg capsules produced in one breeding season (n_t) was then calculated by:

$$n_t = A/T \qquad 4.16$$

where T weeks is the hatching time (see above).

The total number of egg capsules produced per individual snail (n_c) was given by:

$$n_c = n_t/D_{mean} \qquad 4.17$$

where D_{mean} 0.25 m^{-2} is the mean density of adult snails during the breeding period.

The total fecundity per individual snail (n) was obtained by:

$$n = n_c n_e \qquad 4.18$$

where n_e is the mean number of eggs per capsule (as defined in equation 4.12).

* Assuming negligible egg-capsule mortality

4.1.3 Results

4.1.3.1 Egg capsules of *L. peregra*

The hatching time (T) at different sites was similar in 1985 (*c.* 8 weeks), but varied between sites in 1986 (Table 4.1.1). T was longer in 1985 than in 1986.

Table 4.1.1 Hatching time (T weeks) at the three sites, estimated by Method 1 [denoted by (1)] and Method 2 [denoted by (2)] (see section 4.1.2.5). (Data for Sheaf refer only to egg capsules produced in the summer).

	Hatching time (T weeks)					
	1985			1986		
	(1)	(2)	mean	(1)	(2)	mean
Sheaf	7	10	8.5	4	6	5.0
Rivelin	6	10	8.0	4	8	6.0
Don	8	8	8.0	6	8	7.0

Table 4.1.2. Interpopulation variation in mean egg volume (v_e mm^3), mean number of eggs per capsule (n_e) and mean percentage egg hatchability (h_e). The figure in parenthesis represents the number of egg capsules sampled.

	$v_e \pm$ S.E. (mm^3)		$n_e \pm$ S.E.		$h_e \pm$ S.E.
	1985	1986	1985	1986	1985
Sheaf	0.4275 ± 0.014 (43)	0.4087 ± 0.011 (40)	57.2 ± 5.26 (43)	39.4 ± 1.98 (40)	94.78 ± 0.92 (43)
Rivelin	0.4396 ± 0.009 (46)	0.4147 ± 0.007 (40)	43.9 ± 2.90 (46)	42.4 ± 1.66 (40)	94.88 ± 0.63 (46)
Don	0.4425 ± 0.008 (40)	0.4483 ± 0.010 (50)	15.9 ± 0.36 (40)	31.9 ± 1.75 (50)	95.80 ± 0.83 (40)

Mean egg volume (v_e), mean number of eggs per capsule (n_e) and mean percentage egg hatchability (h_e) recorded for each population are summarized in Table 4.1.2. Multiple pairwise comparisons of v_e and n_e were carried out using the Tukey test for unequal sample sizes (Zar 1984, p. 189). The Don snails produced significantly larger eggs than the Sheaf individuals in 1986 (Tukey test: $q_{127,3} = 3.83$, $P < 0.05$), while the difference between the volume of eggs produced by the Don and the Rivelin snails was significant at the 90% but not the 5% level (Tukey test: $q_{127,3} = 3.30$). Although egg sizes were not statistically different between the Sheaf and the Rivelin samples in 1986 (Tukey test: $q_{127,3} = 0.55$), nor amongst all three samples in 1985 (analysis of variance: $F = 0.53$, $d.f. = 2, 126$), the means followed consistently the same order Don > Rivelin > Sheaf in the two years sampled. No significant difference in egg size was observed

between years in the Sheaf and the Don populations (two-sample *t* tests: $t < 1.03$, $d.f. < 89$), and the between-year difference in egg size of the Rivelin population was significant only at the 5% level (two-sample *t* test: $t = 2.22$, $d.f. = 84$). Egg capsules produced by the Don snails contained fewer eggs than their Sheaf and Rivelin counterparts (Tukey tests: $q_{126,3} > 7.80$, $P < 0.05$) in 1985, while the Sheaf capsules were not different from either the Rivelin or the Don capsules in 1986 (Tukey tests: $q_{127,3} < 2.90$). Number of eggs per capsule showed significant variation between years in the Sheaf and the Don populations (two-sample *t* tests: $t > 3.16$, $d.f. > 81$, $P < 0.001$), but not the Rivelin (two-sample *t* test: $t = 0.45$, $d.f. = 84$). There was no significant difference in the percentage egg hatchability amongst the three populations (analysis of variance on arcsine transformed data: $F = 1.23$, $d.f. = 2, 126$).

4.1.3.2 Population dynamics of *L. peregra*

Snail densities peaked in summer (June and July) at all three sites (Figs. 4.1.3, 4.1.4 and 4.1.5). At the Sheaf site, in addition to the summer recruitment, a second peak of egg capsule production was evident in autumn (August and September) (Fig. 4.1.3), suggesting that the Sheaf population was bivoltine. This was confirmed by examining the size-frequency histograms (Fig. 4.1.6) which indicated a second recruitment of juveniles in October 1985, and in September 1986. Adults from the overwintering population bred in April and May with recruitment in June and July; some of those recruits grew sufficiently rapidly and reproduced in August, with recruitment in September and October. Overwintering adults invariably died but "summer adults" probably did not, and might breed again in the following year (Fig. 4.1.6). By contrast, both the Don and the Rivelin populations were univoltine with single recruitment per year

Fig. 4.1.3. Seasonal variations of the density of *Lymnaea peregra* snails and egg capsules at the Sheaf site between January 1985 and December 1986. Vertical lines are ± 1 S.E..

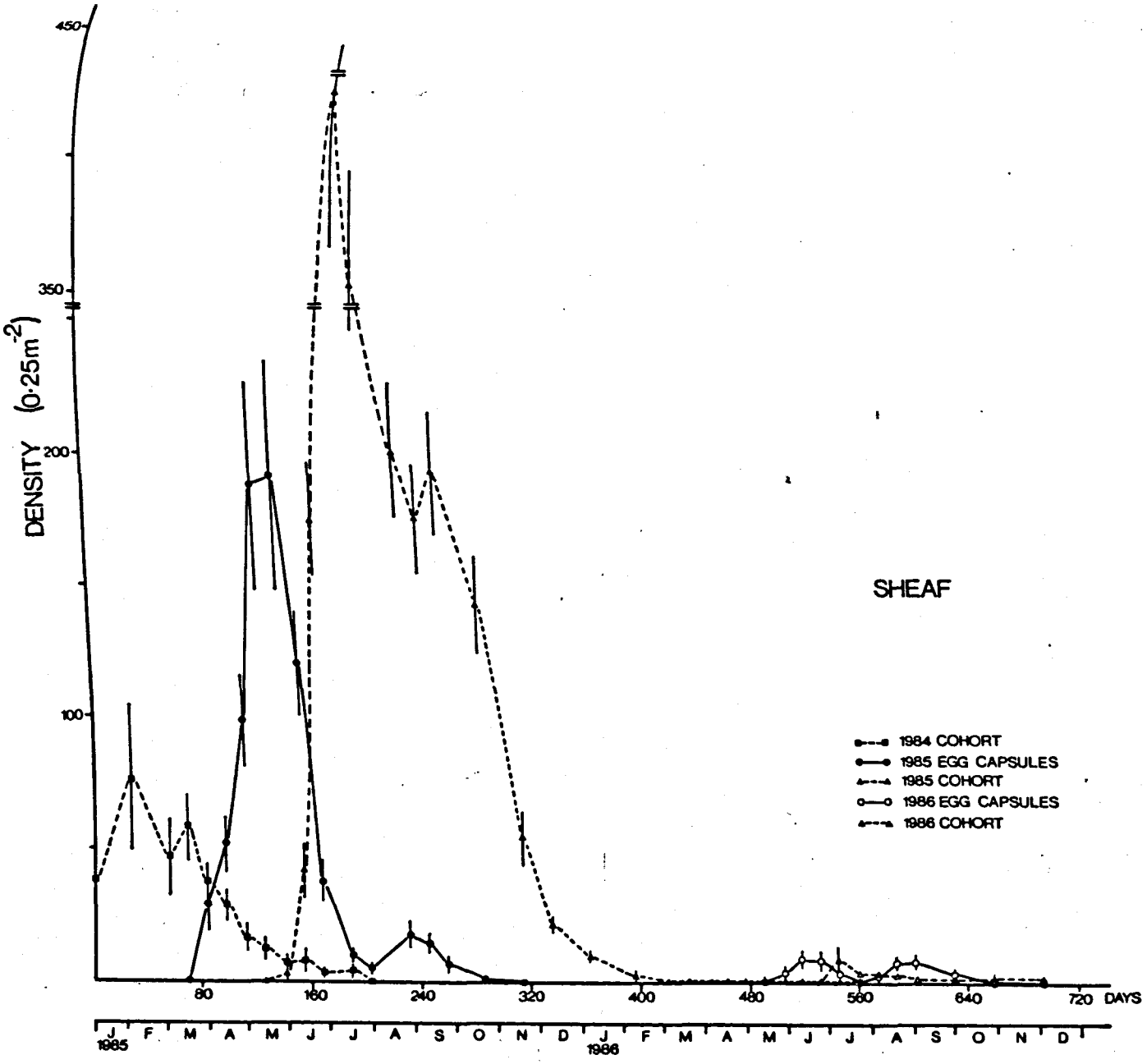

DENSITY (0·25m⁻²)
450
350
200
100
SHEAF
1984 COHORT
1985 EGG CAPSULES
1985 COHORT
1986 EGG CAPSULES
1986 COHORT
80
160
240
320
400
480
560
640
720 DAYS
J F M A M J J A S O N D J F M A M J J A S O N D
1985
1986

Fig. 4.1.4. Seasonal variations of the density of *Lymnaea peregra* snails and egg capsules at the Don site between February 1985 and December 1986. Vertical lines are ± 1 S.E.. The arrow indicates that the site was drained.

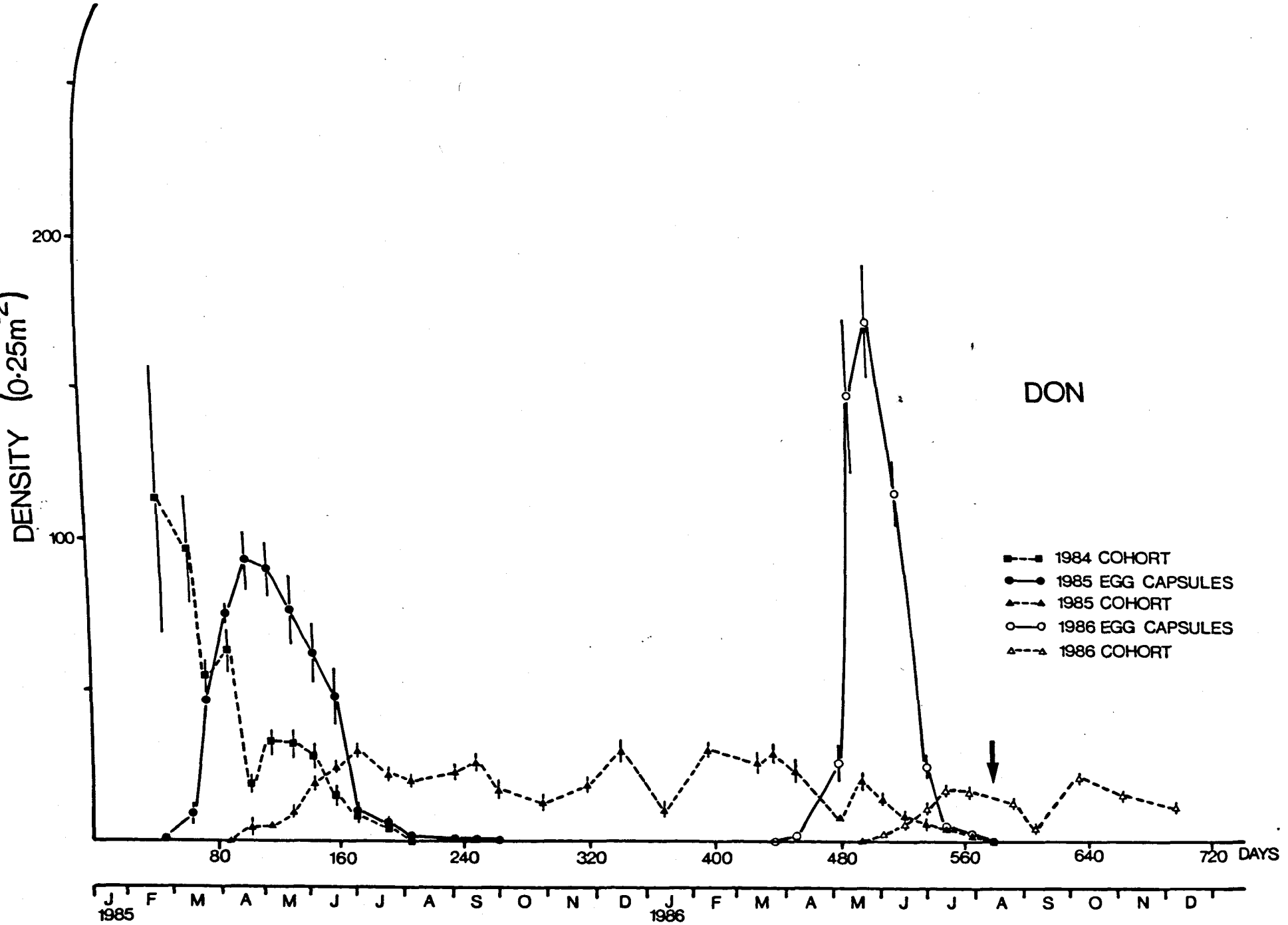

DENSITY (0·25m^{-2})
200
100
DON
1984 COHORT
1985 EGG CAPSULES
1985 COHORT
1986 EGG CAPSULES
1986 COHORT
80
160
240
320
400
480
560
640
720 DAYS
J F M A M J J A S O N D J F M A M J J A S O N D
1985
1986

Fig. 4.1.5. Seasonal variations of the density of *Lymnaea peregra* snails and egg capsules at the Rivelin site between January 1985 and December 1986. Vertical lines are ± 1 S.E..

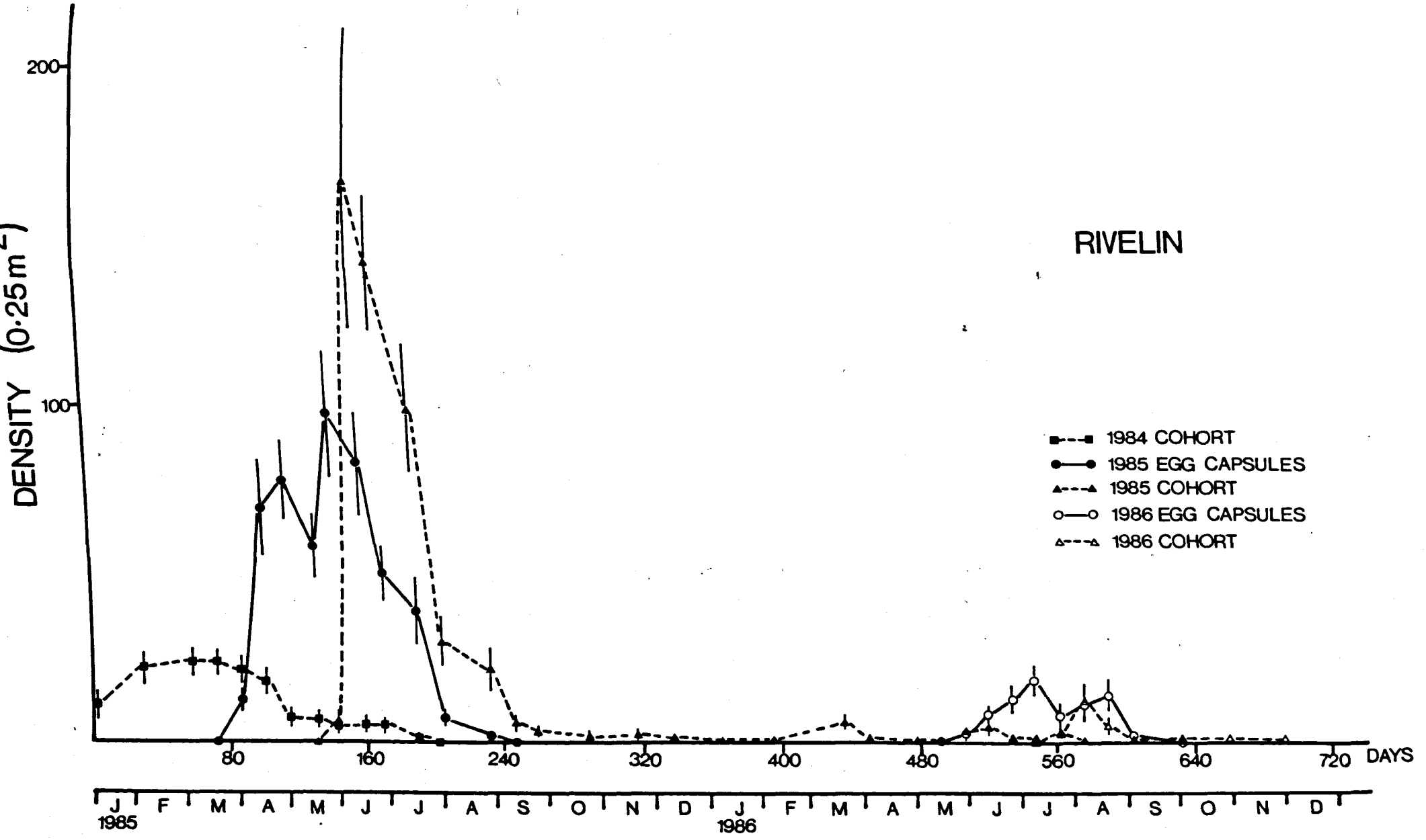
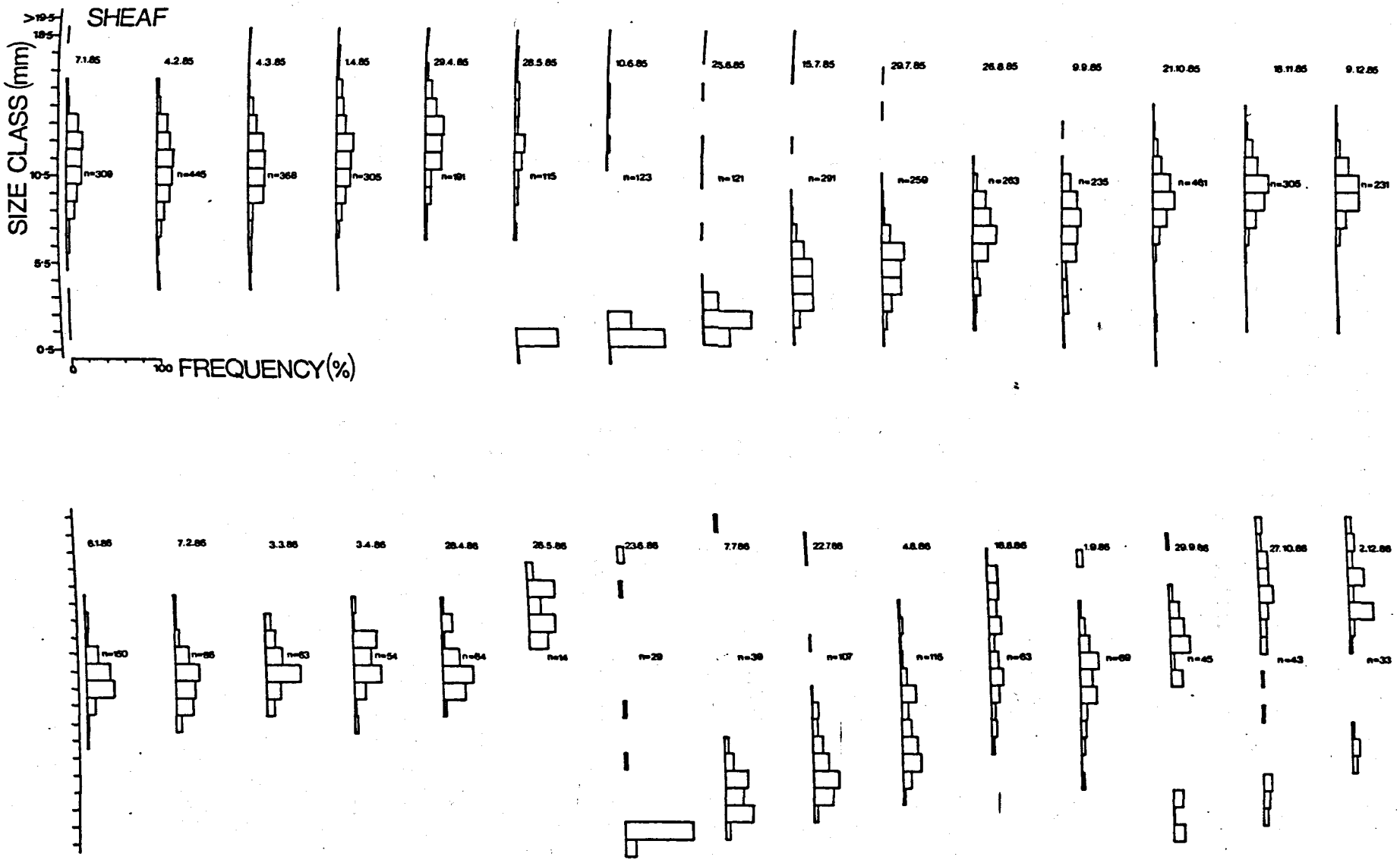

RIVELIN
DENSITY (0·25 m-2)
200
100
1984 COHORT
1985 EGG CAPSULES
1985 COHORT
1986 EGG CAPSULES
1986 COHORT
80
160
240
320
400
480
560
640
720
DAYS
J F M A M J J A S O N D J F M A M J J A S O N D
1985
1986

Fig. 4.1.6. Changes in population size-frequency distribution of *Lymnaea peregra* at the Sheaf site between January 1985 and December 1986. Sampling date and sample size (n) are given for each histogram.


SHEAF
SIZE CLASS (mm)
>19·5
18·5
10·5
5·5
0·5
0
100
FREQUENCY(%)
7.1.85
n=309
4.2.85
n=445
4.3.85
n=368
1.4.85
n=305
29.4.85
n=191
28.5.85
n=115
10.6.85
n=123
23.6.85
n=121
15.7.85
n=291
29.7.85
n=259
26.8.85
n=263
9.9.85
n=235
21.10.85
n=461
18.11.85
n=305
9.12.85
n=231
6.1.86
n=150
7.2.86
n=86
3.3.86
n=63
3.4.86
n=54
28.4.86
n=64
26.5.86
n=16
23.6.86
n=29
7.7.86
n=39
22.7.86
n=107
4.8.86
n=115
18.8.86
n=63
1.9.86
n=69
29.9.86
n=45
27.10.86
n=43
2.12.86
n=33

(Figs. 4.1.7 and 4.1.8). Snail densities at the Sheaf and the Rivelin sites were higher in 1985 than those in 1986, while such annual fluctuations were less apparent at the Don.

Life tables of the 1985 cohorts at the Sheaf and the Rivelin sites are given in Tables 4.1.3 and 4.1.4.

Table 4.1.3 Life table of the summer cohort at the Sheaf site in 1985.

x	a_x	l_x	d_x	q_x	e_x
0	10425.4	1000.0	973.4	0.973	0.582
1	277.5	26.6	8.3	0.312	2.597
2	190.6	18.3	2.1	0.115	2.545
3	168.9	16.2	6.7	0.414	1.808
4	99.3	9.5	2.1	0.221	1.724
5	77.0	7.4	4.3	0.581	1.065
6	32.4	3.1	2.5	0.806	0.833
7	6.6	0.6	0.4	0.667	1.136
8	1.7	0.2	0.1	0.550	1.408
9	0.9	0.09	0.04	0.444	1.461
10	0.5	0.05	0.03	0.600	1.230
11	0.2	0.02	0.012	0.600	1.075
12	0.08	0.008	0.005	0.625	0.938
13	0.03	0.003	0.003	1.000	0.500

x = 0 was set to the time when juvenile density was maximum

Fig. 4.1.7. Changes in population size-frequency distribution of *Lymnaea peregra* at the Don site between February 1985 and December 1986. Sampling date and sample size (n) are given for each histogram.


DON
SIZE CLASS (mm)
15·5
10·5
5·5
0·5
0
100
FREQUENCY(%)
22.2.85
n=239
12.3.85
n=109
4.4.85
n=145
2.5.85
n=209
30.5.85
n=97
13.6.85
n=86
27.6.85
n=249
17.7.85
n=245
1.8.85
n=148
29.8.85
n=239
12.9.85
n=183
24.10.85
n=158
21.11.85
n=109
12.12.85
n=175
9.1.86
n=126
6.2.86
n=89
10.3.86
n=82
3.4.86
n=133
1.5.86
n=91
29.5.86
n=85
26.6.86
n=135
9.7.86
n=117
24.7.86
n=136
7.8.86
n=134
21.8.86
n=72
4.9.86
n=32
2.10.86
n=76
30.10.86
n=66
2.12.86
n=56

Fig. 4.1.8. Changes in population size-frequency distribution of *Lymnaea peregra* at the Rivelin site between January 1985 and December 1986. Sampling date and sample size (n) are given for each histogram. ☒ indicates that sample size was too small for analysis.

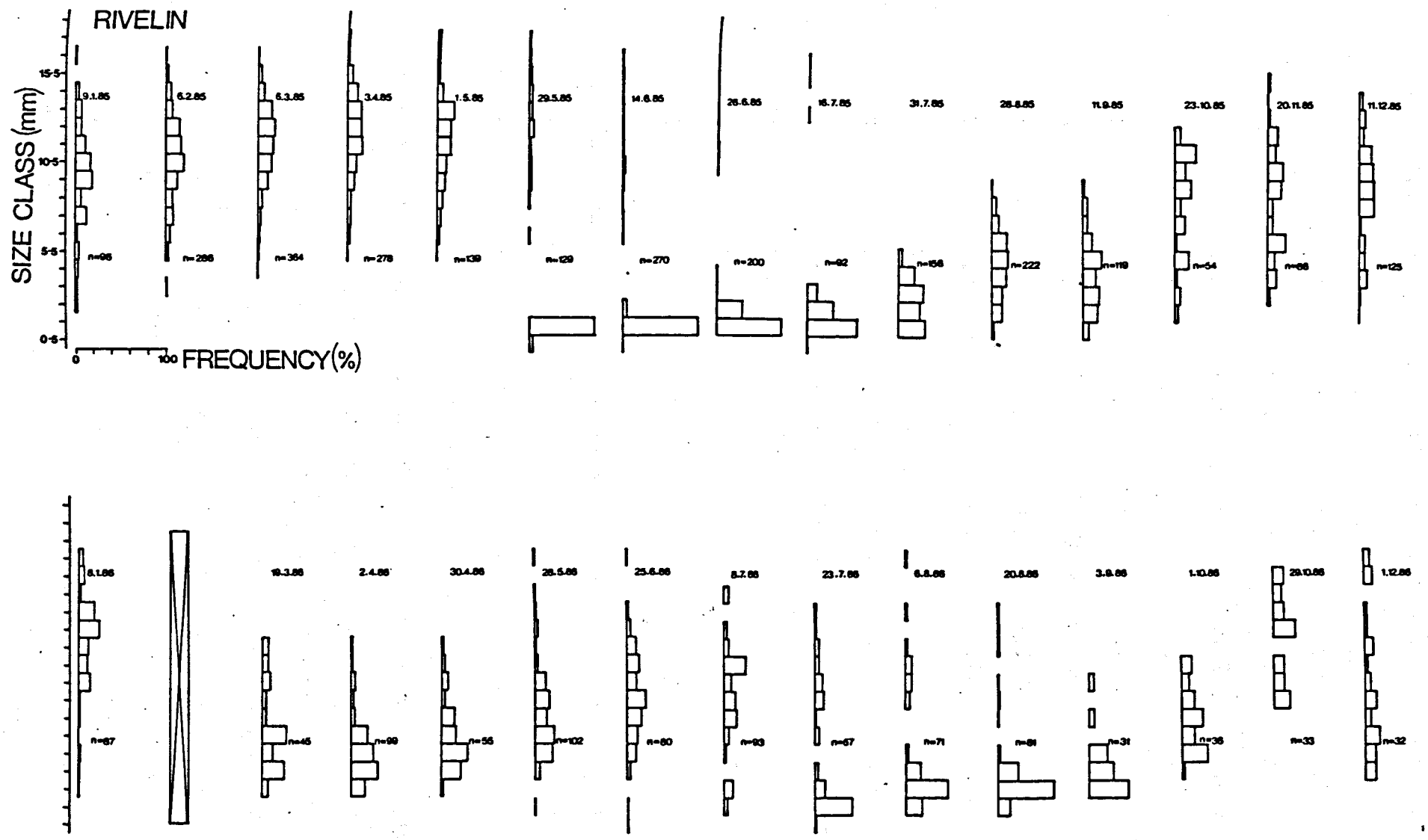

RIVELIN
SIZE CLASS (mm)
15·5
10·5
5·5
0·5
0
100
FREQUENCY(%)
9.1.85
n=96
6.2.85
n=286
6.3.85
n=364
3.4.85
n=278
1.5.85
n=139
29.5.85
n=129
14.6.85
n=270
26.6.85
n=200
16.7.85
n=92
31.7.85
n=158
28.8.85
n=222
11.9.85
n=119
23.10.85
n=54
20.11.85
n=66
11.12.85
n=125
8.1.86
n=67
19.3.86
n=45
2.4.86
n=99
30.4.86
n=56
28.5.86
n=102
25.6.86
n=60
8.7.86
n=93
23.7.86
n=67
6.8.86
n=71
20.8.86
n=61
3.9.86
n=31
1.10.86
n=36
29.10.86
n=33
1.12.86
n=32

Table 4.1.4 Life table of the 1985 cohort at the Rivelin site.

x	a_x	l_x	d_x	q_x	e_x
0	3257.2	1000.0	968.1	0.968	0.562
1	103.9	31.9	18.3	0.574	1.434
2	44.3	13.6	9.2	0.677	1.692
3	14.3	4.4	3.3	0.756	1.144
4	3.5	1.1	0.5	0.458	2.140
5	1.9	0.6	0.09	0.160	2.526
6	1.6	0.5	0.18	0.367	1.898
7	1.0	0.3	0.19	0.613	1.710
8	0.4	0.12	0.028	0.233	2.625
9	0.3	0.092	0.000	0.000	2.272
10	0.3	0.092	0.031	0.337	1.272
11	0.2	0.061	0.055	0.902	0.656
12	0.02	0.006	0.003	0.500	1.000
13	0.01	0.003	0.003	1.000	0.500

The snail densities recorded at the Don site (*cf.* a_x of Table 4.1.5) showed considerable fluctuations which would result in negative d_x. These irregularities were probably due to the ephemeral and intermittent growth of macrophytes (mainly *Elodea canadensis* Michx.) in the backwater during the summer. The macrophytes provided extra surface for snail colonization (*cf.* Lodge 1985), and thus affected density estimates in the field. Consequently, the l_x column between $x = 1$ and $x = 12$ was 'smoothed' by the technique of Lowe (1969) which assumed linear decrease of l_x with time. l_x was regressed against x (regression equation: $l_x = 47.18 - 0.49\,x$, $r = -0.20$, $n = 12$), and an extra column of steadily declining l'_x was created in Table 4.1.5.

Table 4.1.5 Life table of the 1985 cohort at the Don site (l'_x is the column created by 'smoothing' l_x).

x	a_x	l_x	l'_x	d_x	q_x	e_x
0	457.5	1000.0	1000.0	953.3	0.953	1.045
1	21.2	46.3	46.7	0.5	0.010	11.181
2	22.5	49.2	46.2	0.5	0.011	10.294
3	21.7	47.4	45.7	0.5	0.011	9.399
4	15.1	33.0	45.2	0.5	0.011	8.493
5	18.7	40.9	44.7	0.5	0.011	7.580
6	21.6	47.2	44.3	0.5	0.011	6.659
7	17.4	38.0	43.8	0.5	0.011	5.727
8	24.2	52.9	43.3	0.5	0.011	4.787
9	27.4	59.9	42.8	0.5	0.011	3.836
10	22.6	49.4	42.3	0.5	0.011	2.874
11	14.8	32.4	41.8	0.5	0.012	1.902
12	14.4	31.5	41.3	28.4	0.688	0.918
13	5.9	12.9	12.9	8.5	0.661	0.839
14	2.0	4.4	4.4	4.4	1.000	0.500

Snails at all three sites suffered high mortalities immediately after hatching ($q_0 > 0.9$), and during the breeding season (Fig. 4.1.9). The mortality rates of the Sheaf snails reached a second peak in winter (December 1985, $x = 6$). The Rivelin snails experienced relatively high mortalities at the early stages ($x = 0$ to $x = 3$) and in December 1985 ($x = 7$), but had reduced mortality rates in November 1985 ($x = 5$) and February 1986 ($x = 9$). The Don snails were characterized by high mortalities only during the first four and the last eight weeks.

An examination of the survivorship curves in Fig. 4.1.10

Fig. 4.1.9. Age-specific mortality rates (q_x) of the 1985 cohorts at the Sheaf (solid squares), Rivelin (open triangles) and Don (solid triangles) sites. The arrows indicate the onset of breeding (S: Sheaf, R: Rivelin, and D: Don).

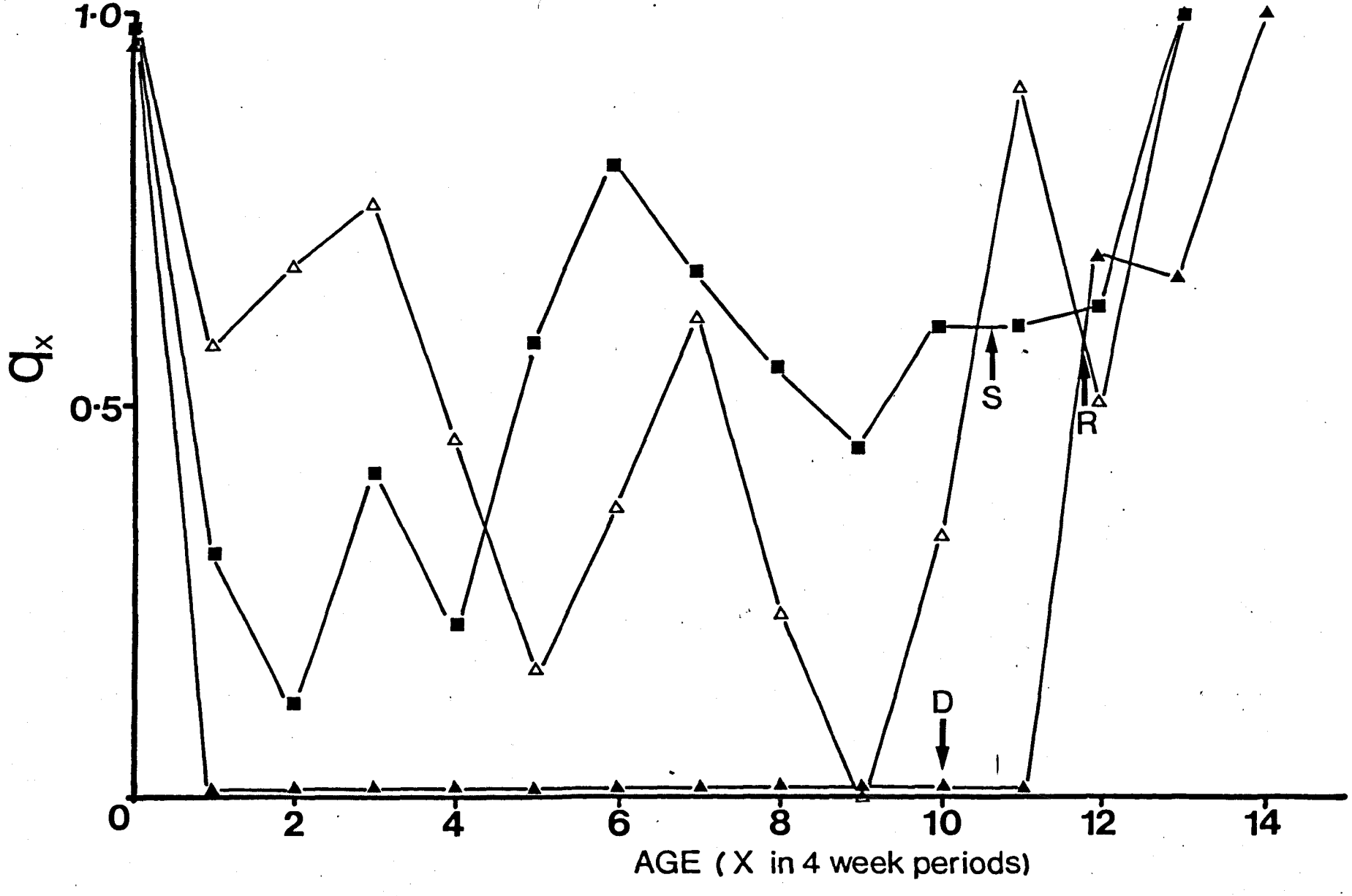
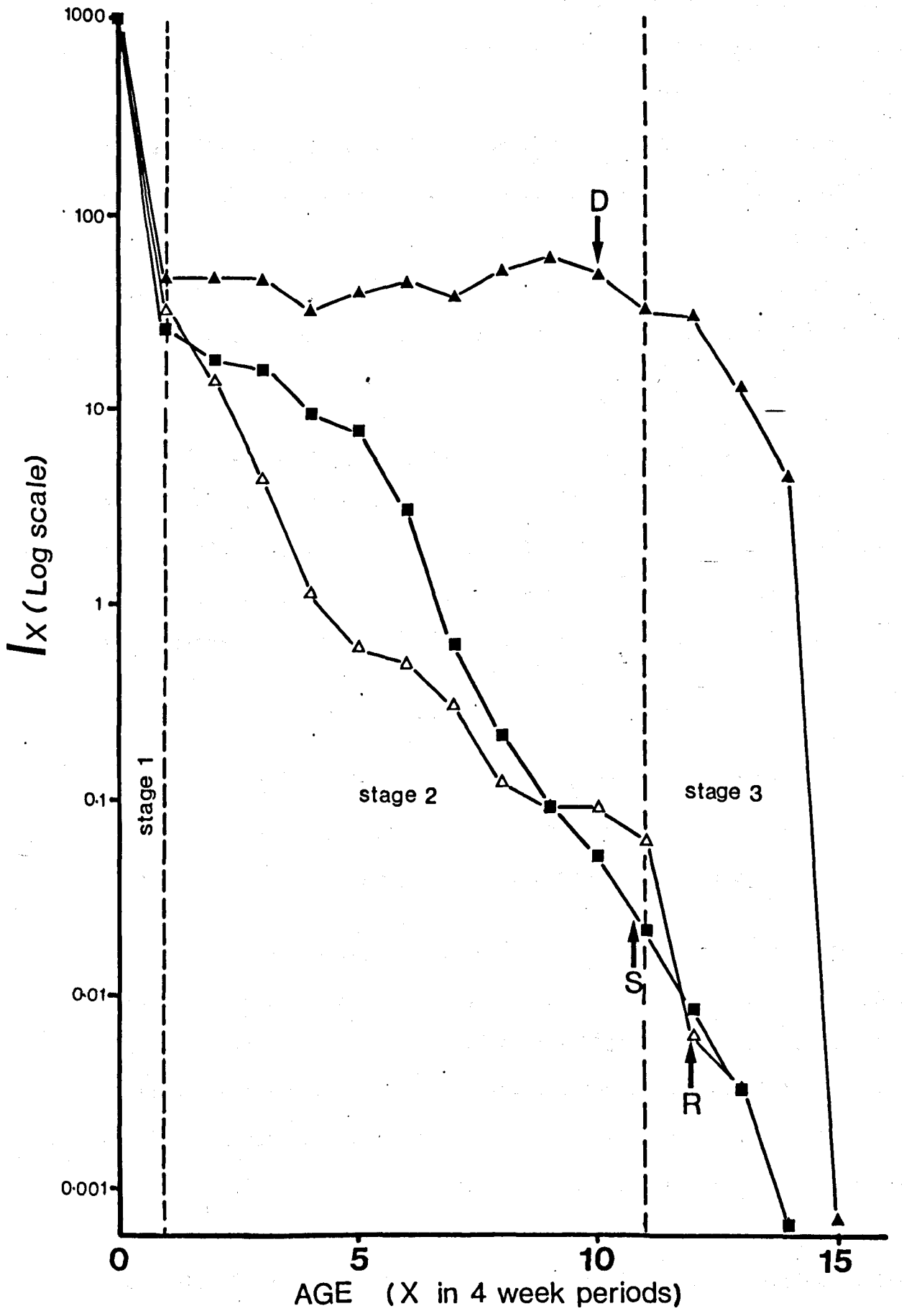

1·0
0·5
0
q_x
2
4
6
8
10
12
14
AGE (X in 4 week periods)
S
R
D

Fig. 4.1.10. Age-specific survivorship (l_x) of the 1985 cohorts at the Sheaf (solid squares), Rivelin (open triangles) and Don (solid triangles) sites. The arrows indicate the onset of breeding (S: Sheaf, R: Rivelin and D: Don).


1000
100
10
1
0·1
0·01
0·001
l_X (Log scale)
stage 1
stage 2
stage 3
D
S
R
0
5
10
15
AGE (X in 4 week periods)

revealed that the life history of *Lymnaea peregra* could be divided into three separate stages according to its mortality regimes: stage 1 (first four weeks) during which maximum mortalities were observed, stage 2 [between the end of the fourth week and the onset of breeding (taken as the 44th week for comparative purposes, see Table 4.1.9)], and stage 3 (from the start of breeding to the end of the maximum life span). The survivorship of different populations during the three stages was compared separately by the log-rank tests (Table 4.1.6).

Table 4.1.6 Pairwise comparisons of the survivorship at different stages of the life histories of the Sheaf, Rivelin and the Don cohorts (data from Tables 4.1.3, 4.1.4 and 4.1.5) using the log-rank test. Significance levels after adjustment by Sidak's multiplicative inequality: *** = $P < 0.001$, NS = Not significant.

Stage	Sheaf vs Rivelin	Sheaf vs Don	Rivelin vs Don
1	$X^2 = 0.014$ NS	$X^2 = 0.76$ NS	$X^2 = 0.57$ NS
2	$X^2 = 5.94$ NS	$X^2 = 68.03$***	$X^2 = 63.21$***
3	$X^2 = 0.001$ NS	$X^2 = 0.045$ NS	$X^2 = 0.42$ NS

There was no significant difference in mortality amongst the three populations during stages 1 and 3 (Table 4.1.6). The Don cohort, however, showed significantly higher survivorship than the Sheaf and the Rivelin cohorts during the second stage. Although the constant mortality rates at the Don (between $x = 1$ and $x = 11$) shown in Fig. 4.1.9 were an artefact as a result of the smoothing procedure, the general pattern of low mortalities during that period was real. Indeed, the proportion of the 1985 cohort surviving to the start of the breeding season (P) was highest for the Don population,

and lowest for the Rivelin population (Table 4.1.7).

Table 4.1.7 Percentages of the 1985 cohorts surviving at the start of the breeding season (P x 100%) at the Sheaf, Rivelin and Don sites.

	Percentage survival at breeding		
	Sheaf	Rivelin	Don
1985 cohort	0.002	0.0006	4.23

The Sheaf snails produced highest number of eggs per individual (522 in 1985 and 2914 in 1986) while the Don snails had the lowest fecundities (50 in 1985 and 421 in 1986) (Table 4.1.8). Both the Sheaf and the Don populations showed great between-year variations in fecundity (Table 4.1.8).

Table 4.1.8 Fecundity estimates of field populations at the Sheaf, Rivelin and Don sites. Nomenclature as defined in section 4.1.2.5. A, area enclosed by the plot of egg-capsule densities against time; T, hatching time; n_t, total number of egg capsules produced in one breeding season; D_{mean}, mean density of adult snails during the breeding period; n_c, total number of egg capsules produced per individual snail; n, total number of eggs produced per individual snail. (Estimates for Sheaf refer only to the summer cohort).

	1985			1986		
	Sheaf	Rivelin	Don	Sheaf	Rivelin	Don
A	1533.69	1075.75	1052.12	54.28	163.23	993.53
T	8.5	8	8	5	6	7
n_t	180.43	134.47	131.52	10.86	27.21	141.93
D_{mean}	19.79	10.52	41.81	0.15	2.28	10.76
n_c	9.12	12.78	3.15	73.95	11.96	13.19
n	521.50	561.02	50.02	2913.59	507.04	420.69

The breeding season in 1986 started later, and was shorter than that in 1985 (Table 4.1.9). The Don population consistently commenced breeding earlier, and had longer breeding seasons than the Sheaf and the Rivelin populations in both years (Table 4.1.9).

Table 4.1.9 A summary of the breeding patterns of the Sheaf, Rivelin and Don populations. (Unless otherwise indicated, information for Sheaf refers only to the summer cohort).

			Sheaf	Rivelin	Don
Time to first breeding in weeks (based on 1985 cohort)			43	48	40
Breeding season	1985	summer cohort	mid-March to mid-July	mid-March to mid-July	late February to mid-July
		autumn cohort	late August to late October		
	1986	summer cohort	mid-May to late July	mid-May to late July	early April to late July
		autumn cohort	early August to early December		
Length of breeding season (weeks)	1985		17	17	21
	1986		10	10	16

4.1.3.3 Growth rates of *L. peregra*

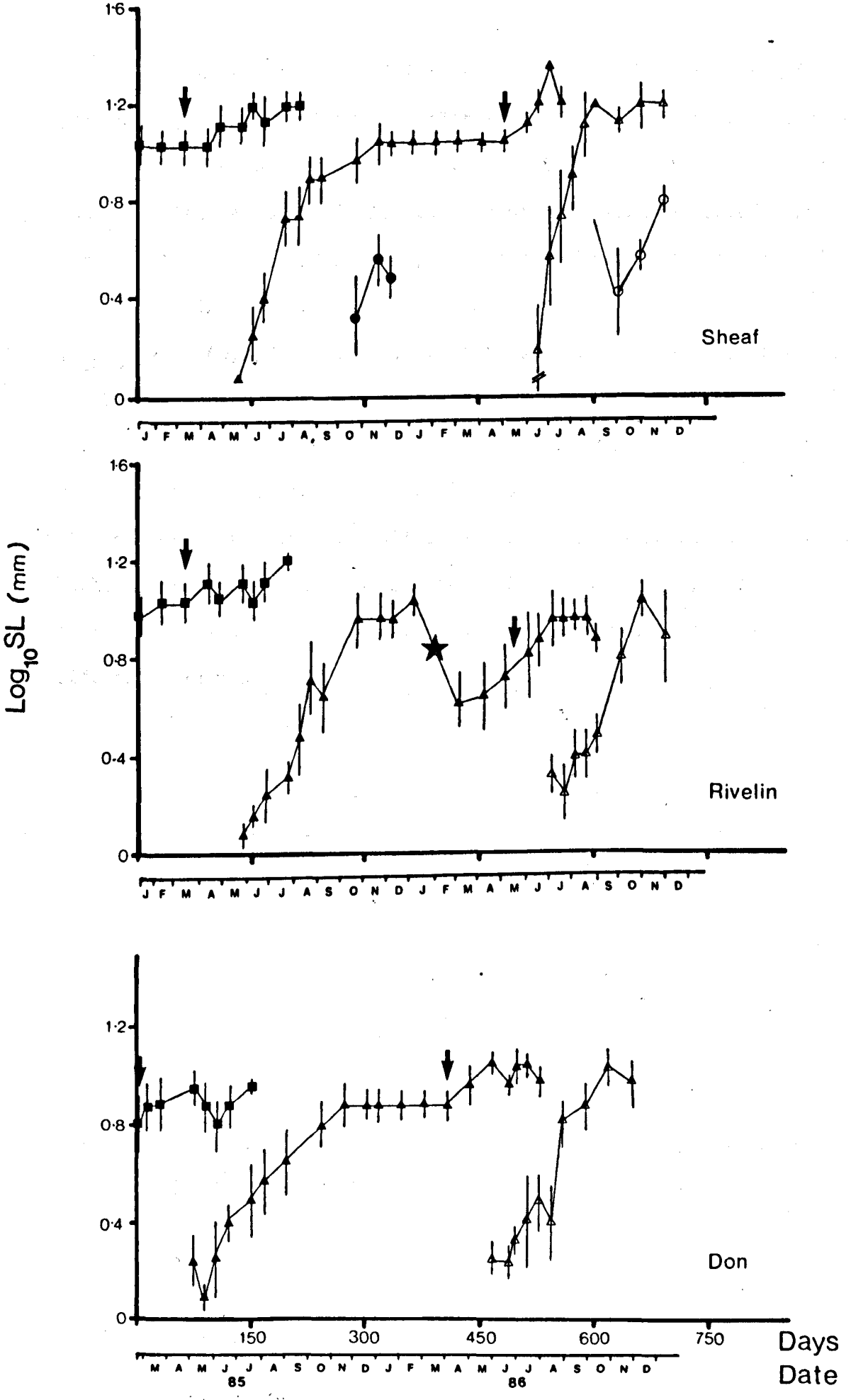
The initial linear relationship between the logarithms of shell length (SL mm) and time (t days) (Fig. 4.1.11) indicated that SL of juveniles increased exponentially with time. Growth corresponding to the linear part of the curves was described by a log-linear model:

$$\text{Log}_{10} SL = a + bt \qquad 4.19$$

where a and b are constants, and b (regression coefficient) was used as an index of juvenile growth rate (G) (section 2.5).

Multiple comparisons between growth rate indices were carried

Fig. 4.1.11. Graphs of shell length (Log_{10}SL mm) against time (days) for the Sheaf, Rivelin and Don cohorts. The arrows indicate the onset of breeding. (Solid squares: 1984 cohort, solid triangles: 1985 cohort, open triangles: 1986 cohort, solid circles: second cohort of 1985 and open circles: second cohort of 1986). The star indicates the sudden drop in the mean SL of the Rivelin samples in 1986.


1·6
1·2
0·8
0·4
0
Log10SL (mm)
Sheaf
Rivelin
Don
J F M A M J J A S O N D J F M A M J J A S O N D
150
300
450
600
750
Days
M A M J J A S O N D J F M A M J J A S O N D
85
86
Date

out by the technique designed for testing the equality of individual pair of regression coefficients (Zar 1984, p. 302 - 303). Growth rate indices of the autumn cohorts at the Sheaf were not included in the analysis because of their low degrees of freedom. The Sheaf snails born in the summer had higher growth rates than their contemporaries at the Rivelin and the Don sites in both 1985 and 1986 (Tukey tests: $q_{>18,3} > 4.60$, $P < 0.01$) (Table 4.1.10). Growth rates of the Don snails were not significantly different from those of the Rivelin snails (Tukey tests: $q_{<24,3} < 2.80$). Growth rates of the autumn cohort at the Sheaf site were similar to those recorded in the summer at the other two sites. All three populations appeared to have higher growth rates in 1986 than in 1985 (Table 4.1.10), but only the summer cohorts at the Sheaf site exhibited a significant between-year difference ($t = 3.78$, $d.f. = 9$, $P < 0.001$).

Table 4.1.10 Indices of juvenile growth rate (G) at the Sheaf, Rivelin and Don sites. *r* and *n* are the correlation coefficient and number of pairs of readings respectively.

	1985		1986	
	summer cohort	autumn cohort	summer cohort	autumn cohort
Sheaf	G = 0.0080 *r* = 0.9642 *n* = 7	G = 0.0039 *r* = 0.8906 *n* = 3	G = 0.0142 *r* = 0.9880 *n* = 6	G = 0.0063 *r* = 0.9999 *n* = 3
Rivelin	G = 0.0045 *r* = 0.9700 *n* = 11		G = 0.0058 *r* = 0.9433 *n* = 9	
Don	G = 0.0034 *r* = 0.9398 *n* = 11		G = 0.0050 *r* = 0.9346 *n* = 10	

Table 4.1.11 Mean sizes at reproduction (SL_{rep} mm) of the Sheaf, Rivelin and Don populations. Figures in parenthesis are sample sizes.

		Sheaf	Rivelin	Don
mean size at reproduction (mean SL_{rep} ± S.E. mm)	1985	10.94 ± 0.12 (368)	11.55 ± 0.12 (364)	6.19 ± 0.11 (239)
	1986	10.99 ± 0.18 (64)	6.81 ± 0.23 (101)	7.99 ± 0.14 (133)

The mean size at reproduction (SL_{rep}) of the Sheaf population was significantly larger than that of the Don in both 1985 and 1986

(two-sample *t* tests: $t > 12.95$, $d.f. > 139$, $P < 0.001$). SL_{rep}s were similar between years in the Sheaf, and in the Don populations (Table 4.1.11). By contrast, the Rivelin snails had the largest SL_{rep} amongst the three populations in 1985, but the smallest in 1986 (Table 4.1.11). This discrepancy was due to a sudden drop in the mean SL of samples of the 1985 cohort (indicated by a star in Fig. 4.1.11) just before the breeding season, and this decline in mean SL coincided with a period of high precipitation which caused frequent spates in the river. It is possible that the decrease in mean SL was a consequence of larger snails being selectively washed away.

4.1.3.4. Population dynamics and fecundity of *Physa fontinalis*

The breeding pattern of *P. fontinalis* (Table 4.1.12) was very similar to that of the sympatric *L. peregra* (*cf.* Tables 4.1.8 and 4.1.9). A comparison of Fig. 4.1.12 with Fig. 4.1.4 revealed that the breeding seasons of *P. fontinalis* and *L. peregra* exactly coincided with each other in both 1985 and 1986.

Fig. 4.1.12. Seasonal variations of the density of *Physa fontinalis* snails and egg capsules at the Don site between February 1985 and December 1986. Vertical lines are ± 1 S.E.. The arrow indicates that the site was drained.

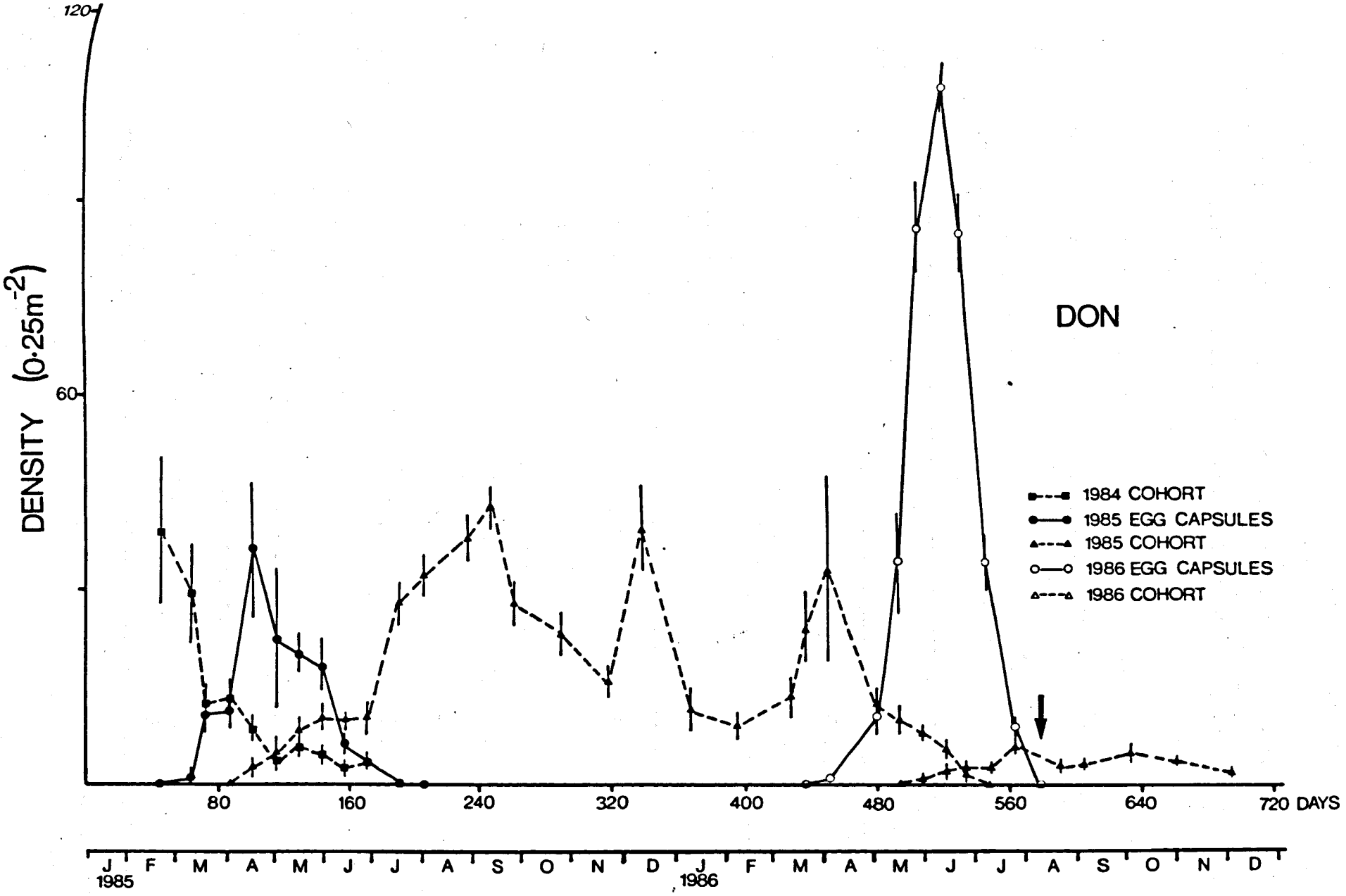

120
60
DENSITY (0·25m^{-2})
DON
1984 COHORT
1985 EGG CAPSULES
1985 COHORT
1986 EGG CAPSULES
1986 COHORT
80
160
240
320
400
480
560
640
720 DAYS
J F M A M J J A S O N D J F M A M J J A S O N D
1985
1986

Table 4.1.12 A summary of the life-history characteristics of *Physa fontinalis* at the Don site. A, area enclosed by the plot of egg-capsule densities against time; T, hatching time; n_t, total number of egg capsules produced in one breeding season; D_{mean}, mean density of adult snails during the breeding period; n_c, total number of egg capsules produced per individual snail; n_e, mean number of eggs per capsule; n, total number of eggs produced per individual snail.

Time to first breeding in weeks (based on 1985 cohort) *c.* 45

	1985	1986
Breeding season	late February to late June	early April to late June
Length of breeding season (weeks)	18	12
A	258.28	727.55
T	7	7
n_t	36.90	103.94
D_{mean}	11.99	11.67
n_c	3.08	8.91
n_e	18.02	14.67
n	55.50	130.71

In situ observations indicated that the combined densities of egg capsules from both *L. peregra* and *P. fontinalis* were very high at the Don, with egg capsules covering almost all surfaces, except the muddy bottom of the backwater. Indeed, the maximum combined densities of egg capsules at the Don were 130.0 and 258.3 per 0.25 m^2 in 1985

and 1986 respectively, with *L. peregra* capsules accounting for *c.* 70%. By contrast, maximum densities of egg capsules recorded at the Sheaf site were 188.6 and 8.5 per 0.25 m^2, while those at the Rivelin site were 98.6 and 18.9 per 0.25 m^2. It is important to note that the density estimates at the Don site were measures of the numbers per 0.25 m^2 of colonizable surfaces (e.g. wooden panels, wooden poles or the brick wall) whereas estimates at the river sites are numbers per 0.25 m^2 of the river bed. Since the total surface area of the stones within 0.25 m^2 of the river bed will be considerably greater than 0.25 m^2, the densities of egg capsules at the Sheaf and the Rivelin given above will be significantly reduced when converted to numbers per 0.25 m^2 of stone surface. Clearly, this means that the combined density of egg capsules from both *L. peregra* and *P. fontinalis* at the Don is substantially greater than the densities of *L. peregra* capsules prevailing at the Sheaf and the Rivelin sites.

4.1.3.5 Estimates of reproductive efforts

The reproductive efforts of the three populations were estimated by an indirect index (IEI) as defined by Calow (1978).

$$IEI = (n)(v_e)/v_a \qquad 4.20$$

where n is the total number of eggs produced per individual snail as defined in equation 4.18 of section 4.1.2.5, v_e mm^3 is the egg volume as defined in equation 4.5 of section 4.1.2.3, and v_a mm^3 is the parent volume [calculated from $1/3(\pi)(SW)^2(SL)$ by assuming that snail shape approximates to a cone, where SW mm = shell width and SL mm = shell length].

The Sheaf snails showed higher reproductive efforts than the Rivelin snails in both 1985 and 1986, while the Don snails had lowest IEI (Table 4.1.13).

Table 4.1.13 Indirect indices of reproductive effort (IEI) of *L. peregra* at the Sheaf, Rivelin and Don sites. n, total number of eggs produced per individual snail; v_e, mean egg volume; SW, mean shell width; SL, mean shell length; v_a, mean parent volume; IEI, indirect index of reproductive effort.

	1985			1986		
	Sheaf	Rivelin	Don	Sheaf	Rivelin	Don
n	521.50	561.02	50.02	2913.59	507.04	420.69
v_e	0.4275	0.4396	0.4425	0.4087	0.4147	0.4483
SW	6.97	7.98	3.95	7.02	4.36	5.11
SL	10.94	11.55	6.19	10.99	6.81	7.99
v_a	556.56	770.22	101.14	567.15	135.57	218.48
IEI	0.40	0.32	0.22	2.10	1.55	0.86

4.1.4 Discussion

The mean temperatures in March and April of 1986 were consistently lower than those recorded during the same months of the previous year (Table 4.1.14), and it is possible that the lower water temperatures in 1986 were responsible for the delayed breeding season in that year. Indeed, the direct inhibitory effect of low temperatures on oviposition has been demonstrated for various species of freshwater pulmonates such as *Lymnaea palustris* (Müller) (Cheatum 1951), *Physa gyrina* Say (De Witt 1954a, 1954b) and *L. stagnalis* (Linnaeus) (Van der Steen 1967). As a consequence of the late breeding season, the 1986 recruitment at the Sheaf and the Rivelin sites occurred in July and August when temperatures (means ranged from 13.3 to 15.3 ^{o}C) were higher than those experienced by the 1985 recruits (12.2 - 15.5 ^{o}C), that hatched during June and July. Similarly, the 1986 recruits at the Don were subjected to June and

July temperatures (14.3 - 16.4 °C) higher than those recorded during the 1985 recruitment period (11.7 - 13.4 °C), which occurred in May and June. The longer hatching time in 1985 than in 1986 also reflects the lower field temperatures during the recruitment period of the former (Al-Habbib & Grainger 1981).

Table 4.1.14 A summary of the monthly mean spot temperatures (°C) during March and April in 1985 and 1986.

	Monthly mean spot temperatures (°C)					
	MARCH			APRIL		
	Sheaf	Rivelin	Don	Sheaf	Rivelin	Don
1985	4.8	3.8	5.8	7.5	7.4	8.4
1986	3.9	3.4	4.5	5.4	4.7	6.0

Despite the generally higher water temperatures experienced by the 1986 recruits, there was no significant annual variation in growth rates for the Rivelin and the Don juveniles. This suggests that, although temperature may have an effect on the timing of breeding, it is less important in influencing growth rates in this instance. This suggestion is supported by the fact that the Don snails, which experienced significantly highest temperatures amongst all three populations (section 3.2.3), had growth rates that were consistently less than half of those of the Sheaf individuals. The above findings indicate that other factors such as food availability might be more important than temperature in accounting for the interpopulation variations in growth rates.

To compare food availability (FA) at various sites, I quantified FA by the ratio of the amount of food present (rates of *Aufwuchs* accumulation, section 3.2.3) to the number of potential consumers (density of snails). Although *P. fontinalis* at the Don site may have

a dietary preference different from that of *L. peregra*, the fact that both species are indiscriminate grazers (Boycott 1936, Calow 1970) would mean that the feeding actions of *P. fontinalis* and *L. peregra* will produce the same overall effect of removing *Aufwuchs*. I therefore used the combined density of *L. peregra* and *P. fontinalis* in estimating FA at the Don site. Food availability was higher at the Sheaf and the Rivelin than at the Don (Table 4.1.15), suggesting that low FA might have been limiting snail growth at the latter. It should be noted that snail density estimates at the Sheaf and the Rivelin are numbers per 0.25 m^2 of the river bed while the rates of *Aufwuchs* accumulation at all three sites and the snail densities at the Don are estimates per 0.25 m^2 of surface area (discussed in section 4.1.3.4). Hence, the FA estimates for the Sheaf and the Rivelin sites in Table 4.1.15 are underestimates. This, however, only strengthens the conclusion that food availability at the Don is indeed the lowest. The above findings strongly suggest that food availability is one of the key determinants of growth rate in the three populations studied. The high estimates of food availability recorded at the Sheaf and the Rivelin in 1986 were partly a result of the relatively low snail densities in that year.

Table 4.1.15 A summary of the inter-site variation of food availability (g individual^{-1} 28 day^{-1}) from June to December in 1985 and 1986.

Mean estimates of food availability from June to December

	Sheaf	Rivelin	Don
1985	0.021	0.17	0.020
1986	7.75	5.41	0.086

The three populations of *L. peregra* showed a clear difference in the number of breeding bouts per year; the Sheaf population was bivoltine while the other two were univoltine. These reproductive patterns have been previously observed among other populations of *L. peregra* (Young 1975, Walter 1977, Dussart 1979). In particular, Russell Hunter (1961b) reported that separate populations of Scottish *L. peregra* exhibited different life-history patterns, while the same species inhabiting a eutrophic, permanent pond in southern England was found to be univoltine in one year, but multivoltine in another (Lodge & Kelly 1985).

Ribi & Gebhardt (1986) proposed that variation in habitat productivity was important in explaining the difference in the number of reproductive peaks per season between two populations of *Viviparus ater* (Crist. & Jan). Similarly, Hunter (1975a, 1975b) studied three populations of *Lymnaea palustris*, and concluded that differences in trophic conditions were largely responsible for the variations in the number of generations per year. In this study, the divergence in growth rates, and consequently reproductive patterns (number of breeding bouts per year) between the Sheaf and the Don populations could at least partly be ascribed to differences in food availability, while other physico-chemical parameters such as the higher oxygen tensions and pHs at the Sheaf than at the Don could also have been important contributing factors (Dussart 1976, Russell-Hunter 1978). Although the Sheaf and the Rivelin sites had similar levels of food availability, they differed markedly in concentrations of calcium ions (section 3.2.3). This factor might partly account for the difference in growth rates between the two populations as snails inhabiting softer waters might have to invest more energy in the

active transport of calcium ions for shell formation, and hence less in growth *per se*, than those living in harder waters (Van der Borght & Van Puymbroeck 1964, 1966, Hunter 1975b). The study sites might also differ in other environmental factors that could have an effect on growth, such as quality of food (Eisenberg 1966, 1970, McMahon *et al.* 1974, Skoog 1978). Although the high variabilities of the life-history patterns of *L. peregra* have often been considered as phenotypic responses (Russell Hunter 1961a, Hunter 1975a, Lodge & Kelly 1985), the possibility of genetic variances could not be ruled out, and this will be investigated in chapter 5.

The high mortalities recorded immediately after hatching are common to freshwater snails (Calow 1978), suggesting that juveniles are probably more vulnerable to environmental stresses such as starvation or predation than adult snails. High adult mortality rates observed during the breeding seasons were in line with the basic assumption of the life-history theory that there is a trade-off between reproductive investment and parental survivorship (section 2.4). Of course, it is also possible that the life span of *L. peregra* is genetically fixed so that a snail will die once its 'maximum' life span has lapsed, and hence may not be related to the cost of reproduction (section 2.1) *per se*. Clearly, it is important to distinguish between the two possibilities by determining the genetic basis of snail longevity, and the genetic correlation between fecundity and longevity (chapter 5). The shape of the survivorship curves of the Sheaf and the Rivelin cohorts was similar to that observed by Calow (1978) on Scottish *L. peregra*. The survivorship curve of the Don snails was characterized by high mortalities early in life and towards the end of the life span. The relatively low winter mortalities at the Don could be a result of the less severe

low temperature stress due to the warming effects of the submerged pipes (sections 3.1.3 and 3.2.3). Significantly, the Sheaf population which experienced higher minimum temperatures also had higher winter survival than the Rivelin population. Hence, there is correlative evidence to suggest that temperature is one of the key factors influencing snail survival especially during winter.

Another important distinction among the three study sites is that the Sheaf and the Rivelin sites are lotic (fast-flowing) habitats, and are more prone to disturbances resulting from spates than the lentic (slow-flowing) Don site (section 3.2.4). The above feature might also be important in accounting for the observed difference in survival regimes between the two habitat types. The suggestion that water current could be an important mortality factor in *L. peregra* is supported by the differential loss of larger individuals at the Rivelin during spates (section 4.1.3.3). Obviously, it can be argued that, if the disappearance of larger individuals from the Rivelin population is due to size-specific susceptibility to water current, a similar phenomenon should also occur at the Sheaf. Importantly, *in situ* observations during the regular sampling programme revealed that the current speeds at the Sheaf were consistently high whereas rates of water movement at the Rivelin varied considerably during the two-year study period, as current flow at the Rivelin is directly affected by the outflow from the Rivelin Reservoirs (section 3.1.2). The consistent, rapid movement of water at the Sheaf might have resulted in snails better adapted to strong currents than the Rivelin individuals, especially during spates. This will be considered further in chapter 4.2.

The Don snails, with highest juvenile survivorship (measured by the percentage of offspring surviving to first breeding), showed

lowest total reproductive efforts (measured as IEI) among the three populations. A similar pattern was also observed by Browne (1978) who studied four populations of a prosobranch *Viviparus georgianus* (Lea), and found that the population with the highest fecundity also had the highest juvenile mortality. Previous studies on freshwater pulmonates, *Lymnaea elodes* (Say) and *Physa gyrina*, have indicated that proximal factors such as low food (or resource) availability and high population density could result in reduced snail fecundities (Brown 1982, 1985a). Similar density effects on reproductive output have also been demonstrated in *Lymnaea stagnalis* (Mooij-Vogelaar *et al.* 1970). These findings suggest the possibility that the low fecundities of the Don snails may be partly due to the high population density and low food availability at this site.

It is also worth noting that *in situ* observations during routine sampling visits to the Don suggested that almost all possible oviposition sites (= colonizable surfaces) were covered with egg capsules during the breeding season, indicating strongly the possibility that there is severe intraspecific and interspecific (with *P. fontinalis*) competition for oviposition sites (*cf.* section 4.1.3.4). If the lack of oviposition sites and/or other proximal factors such as low food availability are indeed limiting the reproductive output of the Don snails to a level below its physiological maximum, the observed low fecundity should not be genetically fixed (see chapter 5). If the above consideration is valid, there should be a strong selection to start breeding at an earlier age at the Don, possibly at the expense of a smaller reproductive size, so as to attain a higher total fecundity by exploiting a longer breeding season. The breeding strategy of the Don snails was consistent with the above expectations in both 1985 and

1986. Of course, there is a distinct possibility that the earlier breeding season at the Don is a result of higher prevailing temperatures (i.e. a phenotypic response) rather than an adaptation to the particular ecological circumstances (i.e. a genotypic response). I shall distinguish between the two possibilities by estimating the genetic variance of age at first reproduction by controlled breeding experiments (see chapter 5).

Clearly, the three *L. peregra* populations showed significant differences in their life histories, especially in terms of juvenile growth rates (G), egg sizes (v_e), proportions of snails surviving to breeding (P), and total reproductive efforts (IEI). These data will be used for testing the Sibly & Calow model (section 2.5) in chapter 6.

4.2 Intraspecific variation in the shell shape of *Lymnaea peregra*

4.2.1 Introduction

I have proposed, in chapter 4.1, that *L. peregra* at the Sheaf and the Rivelin sites was subjected to higher current speeds than its conspecifics at the Don, and that more rapid water movement was partly responsible for the higher pre-reproductive mortalities evident at the lotic (fast-flowing) than the lentic (slow-flowing to still-water) sites. If water current is indeed an important selection pressure, as suggested above, it would be reasonable to expect the Sheaf and the Rivelin snails to show adaptations to withstand strong water currents. It is well documented that the shell shape of aquatic gastropods can vary intraspecifically according to the intensity of wave action or current flow. For example, *Nucella lapillus* (Linnaeus) was typically shorter and broader on exposed as compared with sheltered shores (e.g. Crothers, 1973, 1974, 1975, 1979, 1980, 1981). Similarly, Balaparameswara Rao & Subba Rao (1985) reported that a tropical freshwater snail, *Bellamya dissimilis* (Müller), characteristic of flowing waters, had shorter, but broader shells with relatively larger apertures than the related *B. bengalensis* (Lamarck) which was common in standing waters. Intraspecific morphological variation was also observed in a tropical estuarine snail, *Neritina violacea* (Gmelin) by Murty & Balaparameswara Rao (1978), and it was suggested that the larger aperture was an adaptation to increase adhesive power to withstand water current. Indeed, it was demonstrated for *Nucella* that individuals with wider apertures and larger feet adhered more tightly to the substrate than those with smaller apertures (Kitching *et al.* 1966). By contrast, Ibarra & McMahon (1981), after examining 31 populations of American *Physa virgata* Gould, concluded that snails from lentic populations

had significantly larger apertures relative to shell size than lotic ones.

Lymnaea peregra is well known for its variable shell shape (e.g. Boycott & Diver 1930, Boycott *et al.* 1930, Boycott 1938, Diver *et al.* 1939). Indeed, Taylor (1890) described 29 varieties of *L. peregra* based entirely on variations in shell forms. Diver *et al.* (1939) found evidence that certain shell forms of *L. peregra* persisted through 10 laboratory generations, suggesting genetic control. Conversely, Arthur (1982) reported that the difference in the shell shape of two populations of Irish *L. stagnalis* (Linnaeus) was mainly due to direct environmental effects. Hubendick (1951), in his review of lymnaeid systematics, examined the shell morphometrics of 69 populations of Northern Scandinavian *L. peregra* and noted considerable interpopulation variation. Mozley (1935) considered the shell shape of *L. palustris* (Müller) and *L. emarginata* Say to be genetically controlled, and interpreted the observed interpopulation divergence in terms of adaptations to specific microenvironments.

In this context, I test firstly the general hypothesis that *L. peregra* living in lotic habitats should possess larger apertures (relative to total shell size) than their lentic counterparts as an adaptation to withstand water current (hypothesis 1), and secondly the specific hypothesis that the apertures of the Sheaf and the Rivelin snails should be proportionally bigger than those of the Don individuals (hypothesis 2). The differences in aperture sizes might either be due to environmental influences on development (i.e. phenotypic) or to different selection pressures between habitats (i.e. genotypic). I shall distinguish between these possibilities using laboratory culture experiments (see chapter 5).

4.2.2 Materials and methods

4.2.2.1 Choice of habitats

Apart from the three study sites in Sheffield, snails were also collected from a range of lotic and lentic habitats in Britain. Since the classification of lotic and lentic habitats was not always straightforward (i.e. sites did not naturally fall into two distinct categories), only sites that could be classified readily with confidence were chosen. Such habitats included fast-flowing rivers (current speed $>$ 0.3 ms^{-1}) and bodies of standing water with no appreciable water movement (current speed $<$ 0.04 ms^{-1}). Intermediate habitats, such as slow-flowing streams and wave-swept shores of large lakes, were excluded.

4.2.2.2 Collection of field data

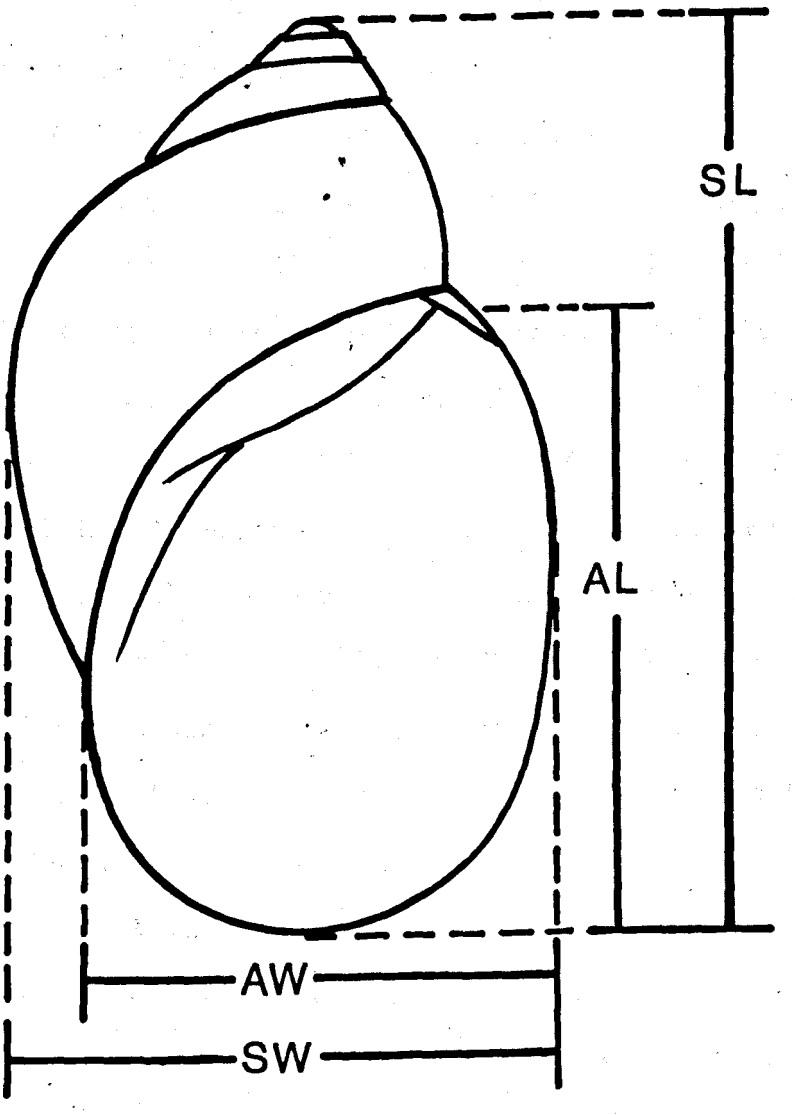
Eighteen populations (6 lotic and 12 lentic, Table 4.2.1) were studied. Snail samples were collected in June 1985 after the breeding season had started. Four shell parameters, namely maximum shell length (SL), maximum shell width (SW), maximum aperture length (AL) and maximum aperture width (AW) (Fig. 4.2.1) were measured on each snail with a graticuled eyepiece on a stereomicroscope. Since SL and AL were highly correlated with SW and AW respectively ($r > 0.95$, $n > 30$, $P < 0.001$), the shell shape was summarized as the ratio AL/SL following Hubendick (1951). Hereafter the above ratio will be referred to as the Relative Aperture Size Ratio (RASR).

Eight populations (3 lotic and 5 lentic, Table 4.2.1) were revisited and sampled ($n > 26$) in June 1986. SL, AL and RASR of individual snails were determined as previously.

4.2.2.3 Statistical analysis

RASR is by definition the ratio of AL/SL, and so is not independent of SL. It is also possible that aperture length increases

Fig. 4.2.1. Dimensions used in shell measurements: SL = maximum shell length, SW = maximum shell width, AL = maximum aperture length and AW = maximum aperture width.


SL
AL
AW
SW

at a different rate from shell length, so that RASR might change with SL (Wilbur & Owen 1964). Therefore, variation in the sizes of snails rather than adaptive effects *per se* could account for the difference in shell shape between habitats (McMahon & Whitehead 1987). The crucial test here is therefore to examine whether the variations in RASR between habitat types could be explained adequately by differences in SL alone.

It has been reported that individuals of *L. peregra* from lotic sites are generally smaller than those from lentic habitats (Boycott 1938, Bondesen 1950, Russell Hunter 1961a, 1961b, Calow 1981b). Additionally, Peters (1938) observed a negative correlation between RASR and SL in *L. palustris*. The above studies suggested that RASR might, indeed, be related to SL in *L. peregra*. I investigated this possibility by performing correlation analysis on Hubendick's (1951) data for *L. peregra*, and discovered a significant positive relationship between RASR and SL ($r = 0.32$, $n = 66$, $P < 0.02$). Similar analysis, based on population means across the 18 populations sampled in 1985, also gave a positive correlation ($r = 0.63$, $n = 18$, $P < 0.01$). To remove the effect of the confounding variable SL in all subsequent tests, analyses of covariance (with SL as covariate) were employed (Snedecor & Cochran 1967). This technique assumed that RASR was normally distributed, and I confirmed that it was by the graphical method of Harding (1949).

4.2.3 Results

4.2.3.1 Hypothesis 1

Analysis of covariance revealed that the regression lines of mean AL against mean SL corresponding to the lotic and the lentic populations (Fig. 4.2.2) had similar residual variances ($F = 0.70$, $d.f. = 4, 10$), and slopes ($F = 1.81$, $d.f. = 1, 14$), but the lotic

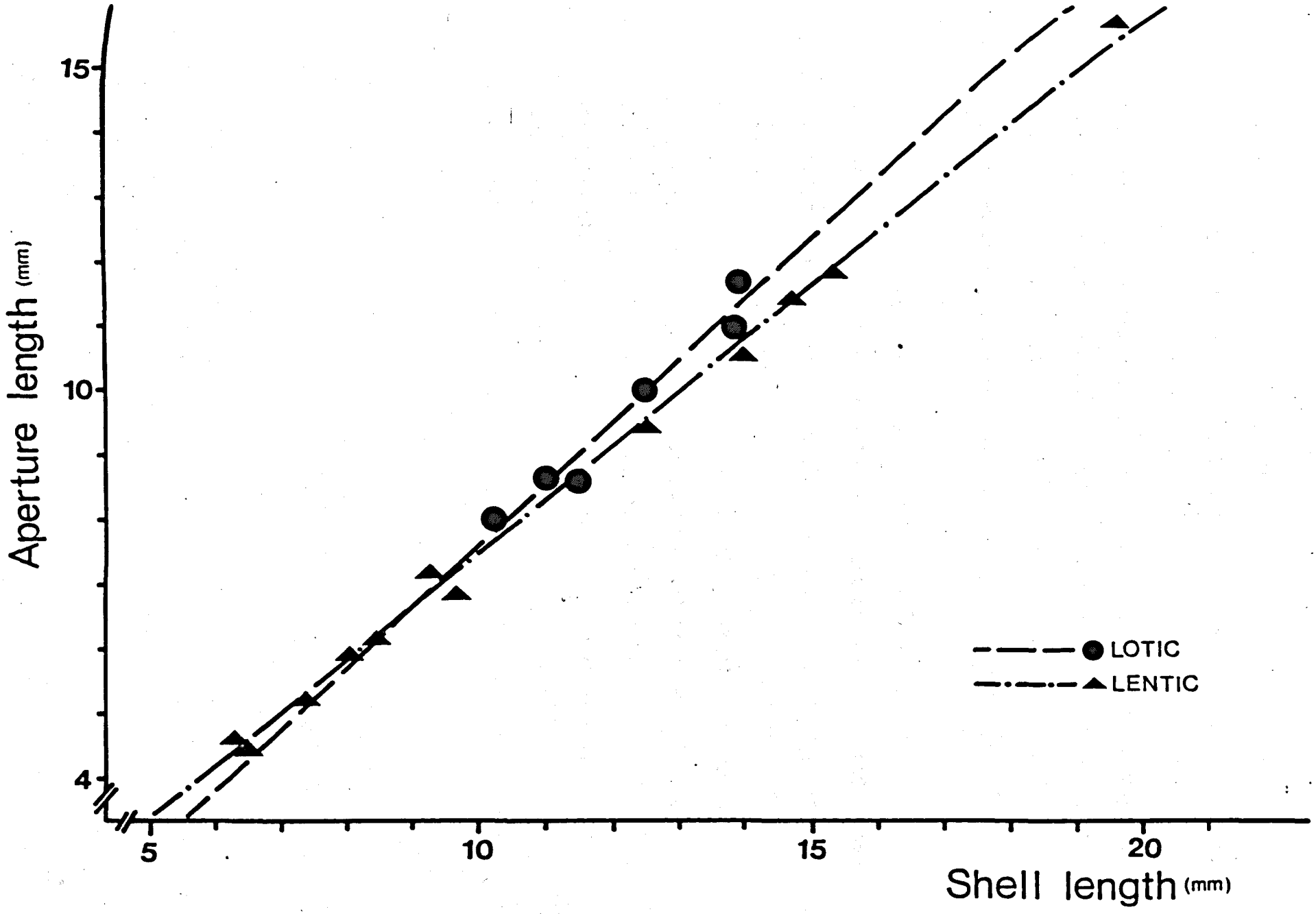
Fig. 4.2.2. The relationship between aperture length (AL mm) and shell length (SL mm) for lotic and lentic populations in the 1985 sample.

(Regression equation based on lotic populations:

AL = -2.01 + 0.96 SL, r^2 = 0.96,

regression equation based on lentic populations:

AL = -0.81 + 0.83 SL, r^2 = 0.99).


15
10
4
Aperture length (mm)
5
10
15
20
Shell length (mm)
LOTIC
LENTIC

line had a significantly higher adjusted mean than the lentic line (F = 5.29, $d.f.$ = 1, 15, $P < 0.05$). This means that lotic snails had larger apertures than their lentic counterparts. It should be noted that in this instance a test of adjusted means is more appropriate than a test of Y-intercepts for making comparisons among the dependent variables (AL). The adjusted means are the values of AL at mean SL whereas the Y-intercepts correspond to values obtained from extrapolations.

The mean aperture sizes of the 1985 samples (18 populations) are summarized in Table 4.2.1. The lotic populations generally had higher mean RASRs than the lentic ones (Fig. 4.2.3). Analysis of covariance indicated that the two regression lines, predicting mean RASR from mean SL, corresponding to lotic and lentic populations (Fig. 4.2.4) did not differ in residual variances (F = 1.31, $d.f.$ = 4, 10), nor in slopes (F = 0.85, $d.f.$ = 1,14), but the lotic line had a significantly higher adjusted mean than the lentic line (F = 6.46, $d.f.$ = 1,15, $P < 0.05$). These findings further suggest that there was a real difference in aperture size (shell shape) between snails from the two contrasting habitat types.

Fig. 4.2.3. Histograms showing the distribution of mean Relative Aperture Size Ratio (RASR) of lotic and lentic populations in the 1985 and 1986 samples (data from Table 4.2.1). Each circle represents one population. Plates of typical shells for each class interval are included.

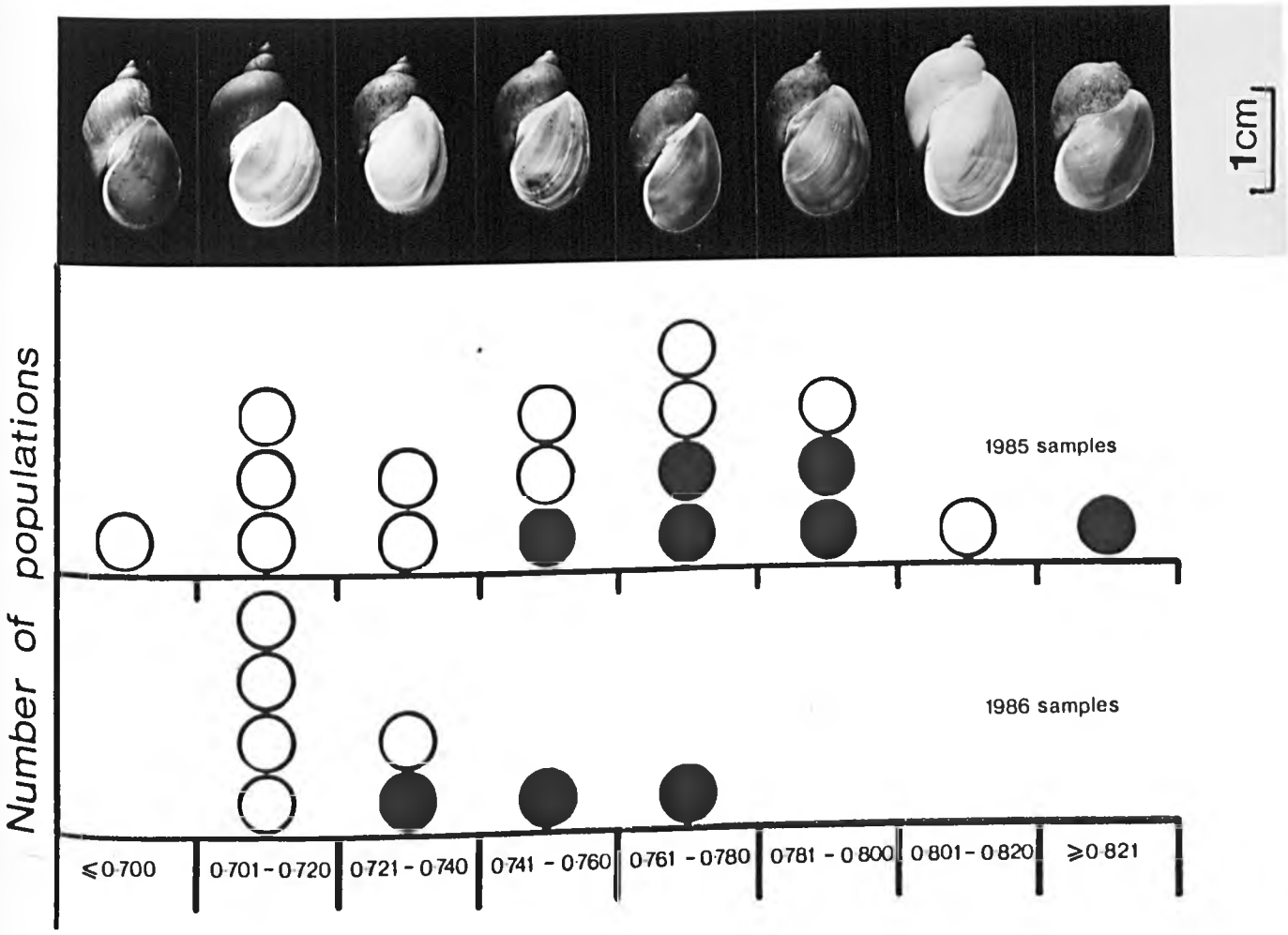

1cm
Number of populations
1985 samples
1986 samples
≤0·700
0·701 - 0·720
0·721 - 0·740
0·741 - 0·760
0·761 - 0·780
0·781 - 0·800
0·801 - 0·820
≥0·821
RASR
LENTIC
LOTIC

Fig. 4.2.4. The relationship between shell shape (as measured by the Relative Aperture Size Ratio, RASR) and shell size (as measured by shell length, mm) for lotic and lentic populations in the 1985 sample.

(Regression equation based on lotic populations:

RASR = 0.62 + 0.014 SL, r^2 = 0.48,

regression equation based on lentic populations:

RASR = 0.68 + 0.006 SL, r^2 = 0.45).

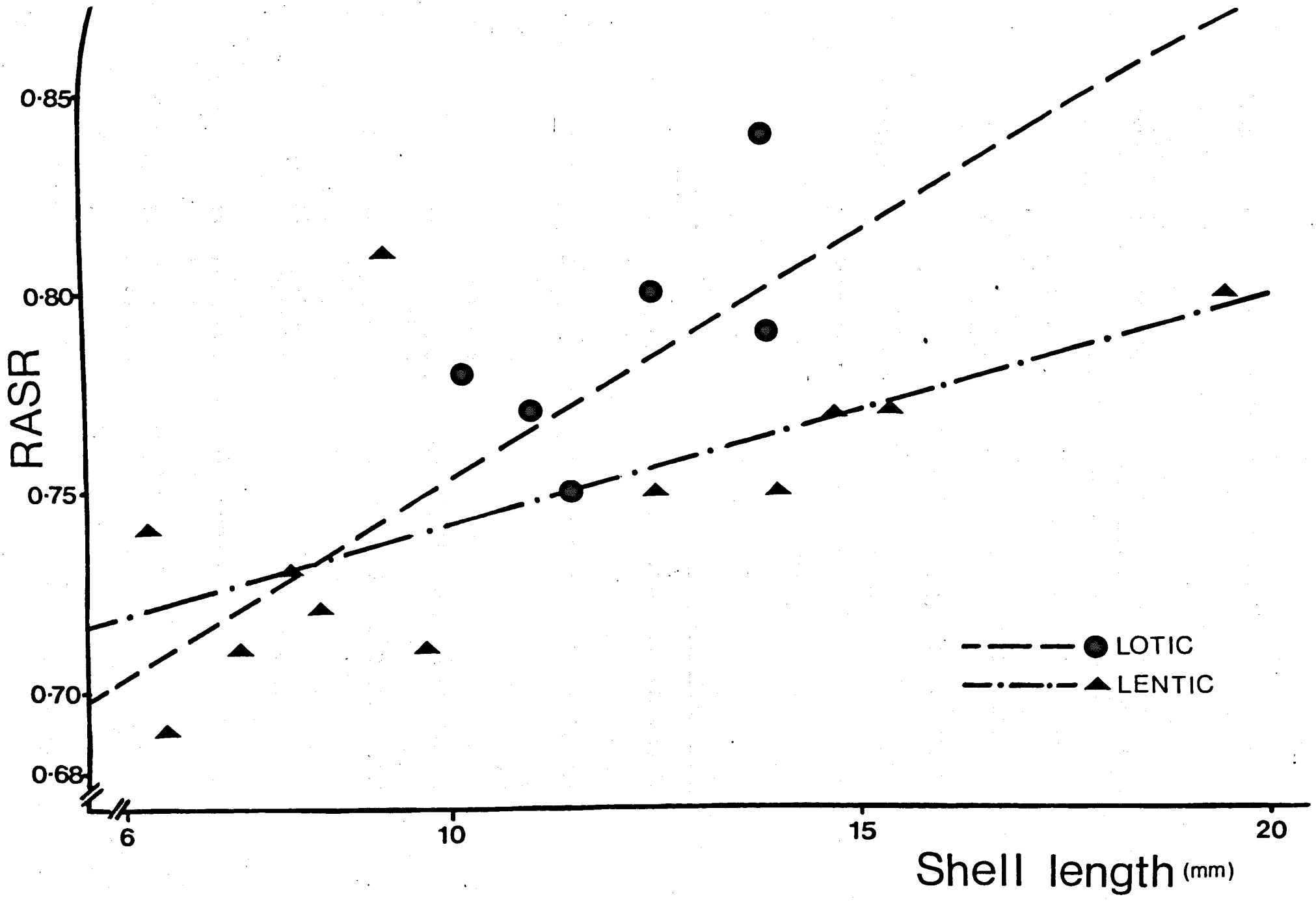

0·85
0·80
0·75
0·70
0·68
RASR
6
10
15
20
Shell length (mm)
LOTIC
LENTIC

Table 4.2.1 List of sites showing habitat details and mean Relative Aperture Size Ratios (RASRs).

Habitat type	site no.	location	mean RASR of 1985 samples mean (S.E., *n*)	mean RASR of 1986 samples mean (S.E., *n*)
LOTIC	1	River Sheaf, Sheffield	0.75 (0.0062,55)	0.78 (0.0064,50)
	2	River Rivelin, Sheffield	0.78 (0.0066,55)	0.76 (0.0079,26)
	3	River Don, Sheffield	0.77 (0.0034,40)	0.74 (0.0076,37)
	4	Belmedie, Aberdeen	0.80 (0.0069,45)	
	5	River Don, Aberdeen	0.84 (0.0093,28)	
	6	Gordale Beck, Yorkshire	0.79 (0.0075,24)	
LENTIC	7	Don backwater, Sheffield	0.69 (0.0057,64)	0.73 (0.0076,50)
	8	Stoney Middleton, Derbyshire	0.75 (0.0075,11)	0.72 (0.0066,32)
	9	Monyash, Derbyshire	0.77 (0.0074,10)	0.71 (0.0068,30)
	10	Over Haddon, Derbyshire	0.73 (0.0053,55)	0.72 (0.0053,40)
	11	Monyash, Derbyshire	0.72 (0.0058,34)	0.71 (0.0055,40)
	12	Wheston, Derbyshire	0.75 (0.0073,28)	
	13	Stoney Middleton, Derbyshire	0.71 (0.0062,51)	
	14	Wheston, Derbyshire	0.80 (0.026,3)	
	15	Wheston, Derbyshire	0.74 (0.011,20)	

16	Wheston, Derbyshire	0.77 (0.011,23)
17	Tideswell, Derbyshire	0.71 (0.0080,45)
18	Middlewich, Cheshire	0.81 (0.0069,40)

[Note: Sites 1-6 are fast-flowing rivers or streams.

Site 7 is the backwater connected to River Don, Sheffield (referred to as Don in this study).

Sites 8-17 are cattle ponds of size of 60-70 m^2.

Site 18 is a pond beside a canal.

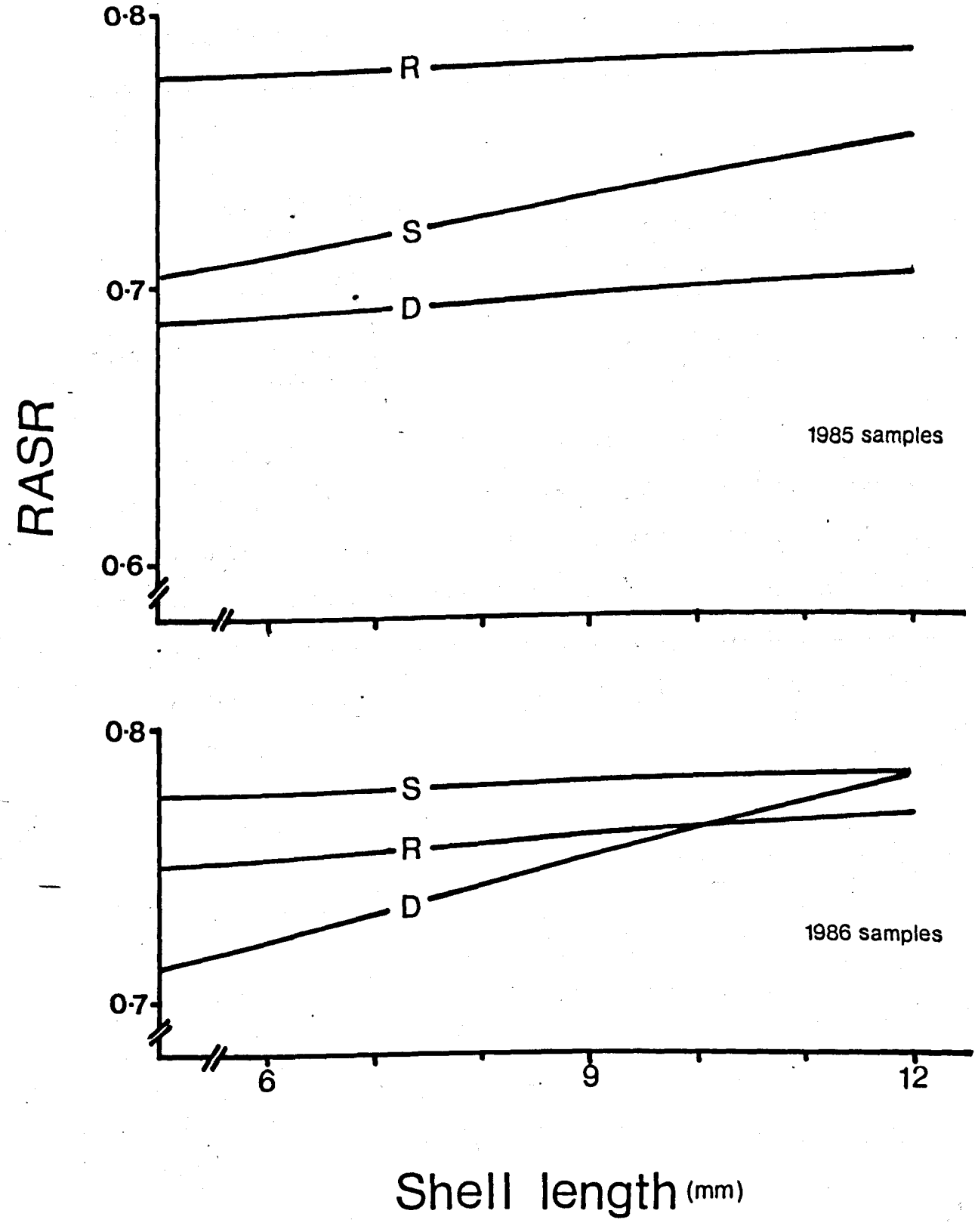
S.E. and *n* are standard error and sample size respectively.]

The mean RASRs of the eight samples taken in 1986 were compared with the 8 corresponding population means of the data set from 1985 (Table 4.2.1). Analysis of covariance (SL as covariate) indicated no between-year difference (Test of slopes: $F = 0.33$, $d.f. = 1, 12$; Test of adjusted means: $F = 0.0011$, $d.f. = 1, 13$). Within the data from 1986, analysis of covariance (SL as covariate) revealed no difference in slopes between the lotic and lentic regression lines ($F = 0.17$, $d.f. = 1, 4$). The lotic regression line had, however, a significantly higher adjusted mean than the lentic one ($F = 10.75$, $d.f. = 1, 5$, $P < 0.03$). These results showed that the difference between lotic and lentic populations was again apparent in 1986.

4.2.3.2 Hypothesis 2

The regression lines predicting RASR from SL corresponding to the Sheaf, Rivelin and Don populations (Fig. 4.2.5) were compared by an analysis of covariance designed for testing the equality of slopes and adjusted means of more than two lines (Zar 1984, p. 300 - 301). In each of the 1985 and 1986 samples, the regression lines did not differ in slopes, but had significantly different adjusted means

Fig. 4.2.5. The relationship between shell shape (as measured by the Relative Aperture Size Ratio, RASR) and shell size (as measured by shell length, mm) for the Sheaf (S), Rivelin (R) and Don (D) populations in the 1985 and 1986 samples.


0·8
0·7
0·6
RASR
R
S
D
1985 samples
0·8
0·7
S
R
D
1986 samples
6
9
12
Shell length (mm)

(Table 4.2.2).

Table 4.2.2 Testing the difference between the regression lines predicting RASR from SL corresponding to the Sheaf, Rivelin and Don populations. Significance levels: *** = $P < 0.001$, * = $P < 0.05$, NS = Not significant.

	test for difference between slopes	test for difference between adjusted means
1985	$F = 0.67$ NS ($d.f. = 2,168$)	$F = 28.83$ *** ($d.f. = 2,170$)
1986	$F = 0.85$ NS ($d.f. = 2,120$)	$F = 3.69$ * ($d.f. = 2,122$)

The Tukey test for multiple comparisons was employed to detect differences between the adjusted means of individual pairs of regression lines (Zar 1984, p. 188 - 189). In both the 1985 and 1986 samples, the Sheaf snails were found to have larger RASRs than the Don individuals (Table 4.2.3). The RASRs of the Rivelin snails were greater than those of the Don individuals in 1985, but not in 1986 (Table 4.2.3).

Table 4.2.3 Multiple comparisons by Tukey test among adjusted means of the regression lines predicting RASR from SL corresponding to the Sheaf, Rivelin and Don populations. Significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, NS = Not significant.

(a) 1985 samples

comparison	$q_{171,3}$	$q_{0.05,120,3}$	P
Sheaf vs. Don	4.240	3.356	**
Sheaf vs. Rivelin	5.035	3.356	**
Rivelin vs. Don	9.196	3.356	***

(b) 1986 samples

comparison	$q_{123,3}$	$q_{0.05,120,3}$	P
Sheaf vs. Don	3.420	3.356	*
Sheaf vs. Rivelin	1.965	3.356	NS
Rivelin vs. Don	1.256	3.356	NS

4.2.4 Discussion

Lotic snails had significantly larger apertures relative to total shell size than lentic snails, and this was recorded in two independent samples taken in separate years. Significantly, Dussart (1987) demonstrated for *L. peregra* that the ability to adhere to a substrate (measured by the critical current velocity at which a snail becomes detached) was positively related to the size (area) of the foot. Assuming that a larger aperture is associated with a larger foot, the difference in shell shape of *L. peregra* between lotic and lentic populations supports the hypothesis that larger apertures are adaptations to withstand water current in this species. However, it

is not inconceivable that producing a big foot is costly; a greater investment in somatic processes will mean less resources available for gamete production (section 2.1), which may result in reduced fitness. Hence, a trait for producing a large aperture (assumed to be related to a big foot) should only evolve in situations where water current constitutes a major source of mortality.

The fact that Sheaf snails possess consistently larger apertures than their contemporaries at the Don suggests that rapid current flow might have been, and probably still is, an important factor influencing snail survival at the former. Data from the lotic Rivelin population were less conclusive; the Rivelin snails exhibited the predicted phenotypes, namely having larger apertures than the Don individuals, in 1985, but not in 1986. These results cast doubts on the ability of the Rivelin snails to withstand water current, and this is in line with the observation that larger individuals, presumably more prone to strong currents, were differentially lost from the Rivelin population during the spates in 1986 (section 4.1.3.3) while such a phenomenon was not apparent at the Sheaf.

Both hypotheses 1 and 2 claiming an adaptive significance for aperture-length/shell-length ratios (RASRs) assume that these traits have a genetic component. This is not to say that they must be genetically fixed, for phenotypic plasticity (section 2.7), for example in the capacity of the foot to grow larger in fast-flowing habitats, could be genetically determined (Bradshaw 1965, Lynch & Gabriel 1987). I will address the genetic basis of differences in shell shape using culture experiments under controlled laboratory conditions in chapter 5.

In conclusion, therefore, the circumstantial evidence from this study suggests that water current is an important selection pressure,

and probably a major mortality factor, at the Sheaf and Rivelin sites, and may partly account for the observed difference in survival regimes between the lotic (Sheaf and Rivelin) and lentic (Don) populations (section 4.1.3.2). The Sheaf snails consistently produced shells with apertures bigger than those of the Don individuals in both 1985 and 1986, while the Rivelin snails did not. These findings indicated the possibility that the Rivelin snails were less well adapted to high rates of water movement than the Sheaf snails, and this difference in ability to adhere to the substrate under lotic conditions might explain the relatively higher survivorship of the Sheaf than the Rivelin snails (Table 4.1.7, chapter 4.1).

5. An investigation of the genetic basis of intraspecific variation

5.1 Introduction

In previous chapters (4.1 and 4.2), I reported significant variations in traits such as egg size, growth rate, age at the onset of breeding, reproductive pattern, fecundity and shell shape among three neighbouring populations of *L. peregra*. There is empirical evidence to suggest that at least some of the above differences could be *adaptive*. However, Gill *et al.* (1983) maintained that any claim that a specific trait has an adaptive significance is in effect shorthand for saying that that trait is heritable, has been subject to natural selection in the past, and represents the optimal solution to the particular ecological challenges in the current environment. The above statement clearly underlines the necessity to establish the availability of genetic variance for the specific trait concerned before invoking any evolutionary explanation.

The presence of genetic variation can be demonstrated by a quantitative genetical parameter known as heritability which estimates the proportion of genetic to total or phenotypic variance (Falconer 1981). Although heritability estimates are useful and instructive, they do not give any indication as to ~~whether a trait is genetically fixed nor~~ the direction of selection (e.g. large or small size, early- or late-breeding). The above problems can, however, be tackled by culturing snails under the same, controlled conditions in the laboratory, and observing the persistence of any interpopulation variation over one or more generations (section 2.7).

It has also been suggested that morphological or life-history traits can be adaptive by virtue of their flexibility or variability, referred to as phenotypic plasticity (e.g. Bradshaw 1965, Marshall & Jain 1968, Caswell 1983). The above suggestion contains the implicit

assumption that the ability for a trait to vary according to environmental conditions is genetically determined (Lynch & Gabriel 1987).

The existence of genetic trade-offs between key fitness components is vital to all life-history models [including the Sibly & Calow model (1985)] which seek to predict optimal tactics under a particular set of environmental conditions (Reznick 1985, Sibly & Calow 1986). I shall study these trade-offs by measuring the genetic correlations between the fitness components concerned.

This chapter attempts to reveal the genetic nature of the variations in the quantitative traits of *L. peregra* based on observations on the phenotypes by statistical analyses, and has three major aims:

(1) to estimate the heritabilities of various morphological and life-history (fitness) traits within each population,

(2) to investigate the persistence of variations among different populations when cultured under the same laboratory conditions,

(3) to study the relationships between key fitness components on both phenotypic and genotypic levels.

5.2 Materials and methods

5.2.1 Controlled breeding experiments

F_1 generation. Fifty adult snails were collected from each of the three study sites in Sheffield (Sheaf, Rivelin and Don) in June 1985. The animals from each population were maintained at 15 ($\pm$1)^{o}C in separate 40 litre glass tanks containing 20 litres of aerated, reconstituted water (RW) (Appendix 2) (conductivity = 600$\mu\Omega^{-1}$, pH = 7.4 and $[Ca^{2+}]$ = 80 mgl^{-1}). Egg capsules were collected daily, and incubated individually at 15 ($\pm$1)^{o}C in plastic pots containing 25 ml of RW. Shell length of at least 20 hatchlings ($\leq$ 24 hr after

hatching) from each egg capsule was determined using a graticuled eyepiece on a stereomicroscope, and the hatchlings were divided equally among four temperatures; 2, 10, 15 and 20 ^{o}C. Following a random block design (Sokal & Rohlf 1981 p. 348 - 354), individuals within each temperature group were transferred to culture chambers of 5 x 5 compartments (2 cm x 2 cm x 2cm) containing 3 ml of RW. All cultures were maintained on a 12 hr light: 12 hr dark cycle. Every three days snails were fed *ad libitum* with boiled lettuce, and the water in each compartment replenished. Shell length was measured every ten days for the first 100 days after which time four shell parameters namely shell length (SL), shell width (SW), aperture length (AL) and aperture width (AW) (as defined in section 4.2.2.2) were measured for each snail as previously. Growth of individual snails was described by the log-linear model, Log_{10} SL = a + bt, where a and b are parameters estimated using least squares regression (*cf.* section 4.1.3.3). The regression coefficient (b) was used as an index for juvenile growth rate. Only data for snails that survived at least 50 days were fitted to the model. The snail cultures at 2 and 10 ^{o}C were terminated after 100 days due to high mortalities.

Each snail at 15 and 20 ^{o}C (hereafter referred to as the F_1 generation) was then paired randomly with another snail of the same population by referring to a random number table, and each pair was transferred to a 250 ml plastic beaker containing 100 ml of RW. The snails were fed, and the water in the beakers changed every three days as previously. The shell morphometrics (SL, SW, AL and AW) were sufficiently unique to enable individual snails to be recognized in each pairs. Upon the onset of breeding, the pair was separated, and each snail maintained individually in a beaker as above. The following parameters were measured for each partner:

(1) shell length at the onset of breeding [SL_{rep} (mm)],

(2) age at the onset of breeding [t_j (days)],

(3) maximum shell length attained [SL_{max} (mm)],

(4) longevity [t_{max} (days)],

(5) total number of eggs produced (n),

(6) total number of egg capsules produced (n_c),

(7) mean number of eggs per capsule (n_e),

(8) length of breeding period [t_e (days)],

(9) mean rate of egg capsule production [r_c (day^{-1})],

(10) mean rate of egg production [r_e (day^{-1})],

(11) mean size of eggs produced [v_e (mm^3)],

(12) post-reproductive survivorship [$t_{prep} = t_{max} - t_j$ (days)],

(13) Relative Aperture Size Ratio (RASR = aperture-length/shell-length).

Although *L. peregra* is typically semelparous, a number of snails in this experiment stopped breeding well before they died. Hence, length of breeding period (t_e, defined as the time interval between the production of the first and the last egg capsules), and post-reproductive survivorship (t_{prep}, defined as $t_{max} - t_j$) were estimated independently. Over 80% of the cultures at 15 °C failed to breed after more than two years, and these cultures were terminated after 800 days. All breeding data of this experiment, therefore, referred exclusively to cultures at 20 °C.

F_2 generation. Thirty-six egg capsules (one egg capsule per snail, 12 snails per population) were collected from the breeding F_1 snails at 20 °C. These egg capsules were incubated separately at 20 ($\pm$1) °C in plastic pots as previously. Hatchlings (hereafter referred to as the F_2 generation) were maintained individually in culture chambers following a random block design, and then cultured in pairs

in plastic beakers following the same procedure described above. Hatchling size, juvenile growth rate, as well as the other thirteen traits (SL_{rep}, t_j, SL_{max}, t_{max}, n, n_c, n_e, t_e, r_c, r_e, v_e, t_{prep} and RASR) were determined as previously.

5.2.2 Mass culture experiments

The adult snails of the Sheaf, Rivelin and Don populations that served as the parents for the F_1 generation in the previous experiment (5.2.1) also provided hatchlings for this experiment. In addition, adult snails were collected from five other populations (Table 5.1) in June 1985, and were kept separately at 15 ($\pm$1)^{o}C in 40 litre glass tanks containing 20 litres of aerated RW as described in section 5.2.1. At least 30 egg capsules were collected from each tank, and were individually reared at 15 ($\pm$1)^{o}C in plastic pots containing 25 ml of RW. The hatchlings from these capsules, together with those collected from the Sheaf, Rivelin and Don populations, provided the initial stocks for this mass culture experiment.

Table 5.1 List of populations chosen for the mass culture experiment.

Habitat type	site no.	location	number of founding adults
LOTIC	1	River Sheaf, Sheffield	50
	2	River Rivelin, Sheffield	50
	3	River Don, Sheffield	40
LENTIC	7	Don backwater, Sheffield	50
	8	Stoney Middleton, Derbyshire	11
	9	Monyash, Derbyshire	10
	10	Over Haddon, Derbyshire	50
	11	Monyash, Derbyshire	34

(Note: Site numbers are the same as those used in Table 4.2.1. The Sheaf, Rivelin and Don populations used in experiment 5.2.1 correspond to sites 1, 2 and 7)

To reduce the chance of hatchlings having identical parents, less than four hatchlings were collected from each egg capsule, and these were kept in RW under a continuous-flow system (flow-rate *c.* 4 mls^{-1}) consisting of eight tanks (with 100 hatchlings per population per tank initially) (Fig. 5.1). The snails were cultured under natural lighting, and were fed *ad libitum* twice a week with boiled lettuce. Water temperature in the tanks was allowed to fluctuate naturally with the ambient temperature. The mean water temperature in the tanks over the experimental period was 16 ^{o}C, while the minimum

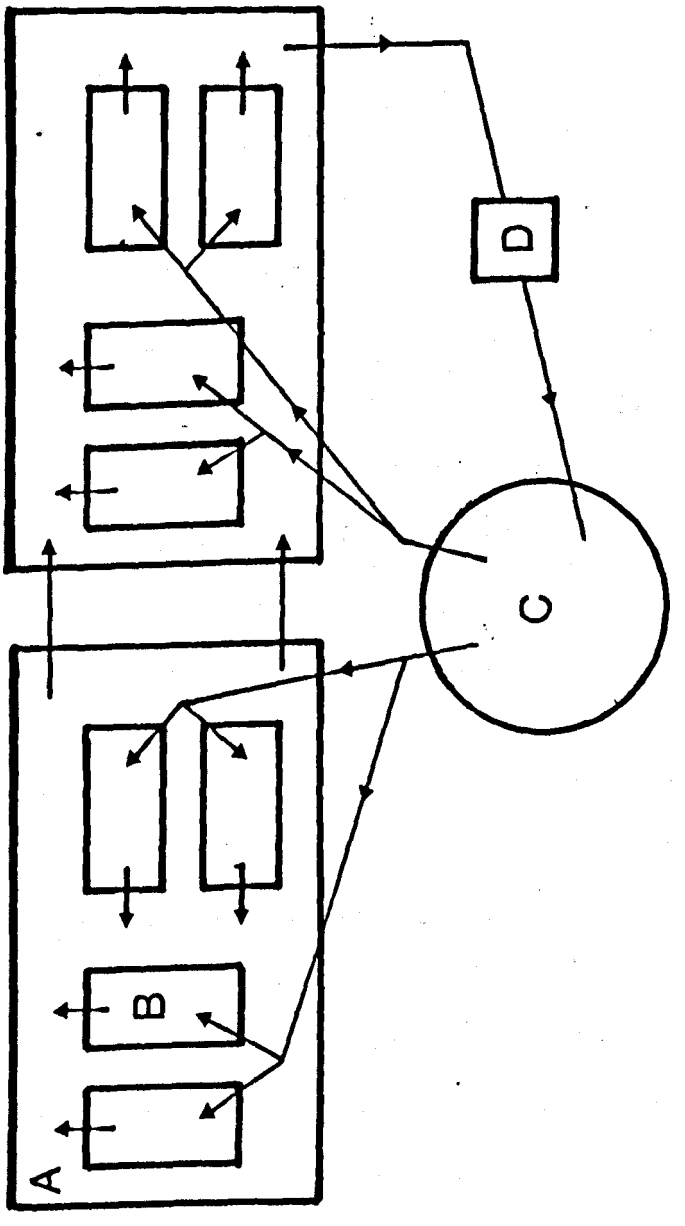
Fig. 5.1. A schematic diagram of the continuous-flow system used in the mass-culture experiment (viewed from above). Arrows indicate direction of water flow.

A. 300 litre fibre glass tank,

B. 40 litre glass tank,

C. 30 litre polystyrene tank,

D. filter pump with activated charcoal and glass wool.


A
B
C
D

and the maximum were 7 and 22 °C respectively. RW was replenished monthly. The density of snails in each tank was adjusted to 50 individuals per tank after 50 days, to reduce the potential effect of density on the life history and the shell shape of individual snails.

A sample of snails ($40 \leq n \leq 46$) was collected from each of the eight tanks after the onset of breeding, and the shell length (SL), aperture length (AL) and Relative Aperture Size Ratio (RASR = AL/SL) of individual snail measured. In addition, the tanks corresponding to the Sheaf, Rivelin and Don populations (site no. 1, 2 and 7) were inspected twice a week for egg capsules which were then isolated. The following data were collected from each of the three populations:

(1) mean shell length at the tenth week [SL_{10} (mm)],

(2) mean shell length at the onset of breeding [SL_{rep} (mm)],

(3) age at the onset of breeding [t_j (weeks)],

(4) length of breeding period [t_e (weeks)],

(5) total number of egg capsules produced per individual (n_c)

$$(= \frac{\text{total number of egg capsules produced}}{\text{density of snails in the tank at the onset of breeding,}})$$

(6) mean number of eggs per capsule (n_e),

(7) total number of eggs produced per individual (n),

(8) rate of egg capsule production per individual per week (r_c),

(9) rate of egg production per individual per week (r_e),

(10) mean size of eggs produced [v_e (mm^3)].

5.2.3 Quantitative genetical analysis

Heritability. Assuming negligible genotype-environment interaction, the amount of variation of a given trait (defined as the phenotypic variance, V_P) can be divided into two variance components: genetic (V_G) and non-genetic or environmental (V_E). By definition, therefore,

$$V_P = V_G + V_E \qquad 5.1$$

The ratio V_G/V_P [termed the *heritability in the broad sense* (h^2_B), or the *degree of genetic determination*] measures the extent to which phenotypes are determined by genotypes. It is important to note that there are two types of genotypic variations, one of which is determined directly by genes transmitted from parents to offspring, and is equivalent to considering each gene occurring as a single entity. This type of genetic variance is described as *additive* (V_A). The other type of genetic variation results from the pairing of genes to form diploid genotypes, and is called *nonadditive* variance (V_{NA}). V_{NA} is caused by dominance and interaction (epistasis) between different loci, thus,

$$V_{NA} = V_D + V_I \qquad 5.2$$

where V_D and V_I are the variance components due to dominance and interaction respectively.

Clearly, additive genetic variance is more relevant in this study as it reflects the genetic influence that can be transmitted from parents to offspring. The heritability estimate that measures the proportion of the total variance made up by the additive component is called the *heritability in the narrow sense* (h^2_N):

$$h^2_N = V_A/V_P \qquad 5.3$$

The estimation of heritability is based on a measure of the degree of resemblance between relatives, and two methods were used in this study:

(1) Correlation (intraclass) of half sibs and full sibs

This technique uses a simple one-way analysis of variance to partition total variance into a within-capsule (6^2_w) and a between-capsule (6^2_b) component (Becker 1975). The intraclass correlation coefficient (t) is then given by:

$$t = \sigma^2_b/(\sigma^2_b + \sigma^2_w) \qquad 5.4$$

The degree of resemblance between snails that hatched from the same egg capsule obviously depends on how close they are related to each other, and if the exact relationship is known, h^2_N can be estimated from t as shown in Table 5.2 (Falconer 1981, Lawrence 1984). A worked example is given in Appendix 4.

Table 5.2 A summary of the relationship between intraclass correlation coefficient (t) and narrow-sense heritability (h^2_N) when snails from the same egg capsule are related to different degrees.

Relationship between snails hatched from the same egg capsule	Relationship between t and h^2_N
offspring produced by self-fertilization (i.e. homozygous parents)	$h^2_N = t$
full sibs	$h^2_N = 2t$
half sibs	$h^2_N = 4t$

As mating of the field-collected snails (parents of F_1) was not controlled, the exact relationships between F_1 snails that hatched from the same egg capsule were not known. This is further complicated by the fact that *L. peregra* is a hermaphrodite, and capable of self-fertilization. Assuming, however, that self-fertilization is of minor importance in the field, and during the time when the snails were kept in the tanks, it is reasonable to assume that F_1 snails that hatched from the same egg capsule were related either as half sibs or full sibs. Hence, the lower and upper limits of the heritability of a specific trait could be calculated. The F_2 snails, on the other hand, were produced under controlled breeding, and if mating did occur between the pair of F_1 snails, individuals from the same egg capsule would be related as full sibs. However, the chance of self-

fertilization was likely to be higher when each snail was restricted to one partner. Indeed, in a number of pairs, one or both partners appeared to be 'sterile' as no egg capsule was produced throughout their entire lifetime. Consequently, the lower and upper limits of the heritability estimates of the F_2 snails were based on the assumption that the F_2 snails were either produced by self-fertilization or as a result of mating between the pair (i.e. they would be related as full sibs).

(2) Regression of offspring on one parent

This method involves fitting the measurements of one parent (the independent variables) and the means of their offspring (the dependent variables) to a linear model by least squares regression technique (Becker 1975). The slope (regression coefficient) of the linear regression of offspring on one parent estimates the heritability if the offspring are produced as a result of self-fertilization. If, however, the offspring are products of cross-fertilization between the pair of F_1 snails, the regression coefficient estimates half of the heritability (Falconer 1981).

As a result of mortality over the course of the experiment, the numbers of offspring from different egg capsules were not the same. This raises a problem for the computation of the parent-offspring regression as certain mean offspring values were more reliable than others. The estimation of a more accurate regression coefficient requires that the mean offspring values be weighted according to their sample sizes (Kempthorne & Tandon 1953, Falconer 1981). However, Falconer (1981) pointed out that, unless the number of offspring in each group varies substantially, the regression coefficient based on unweighted mean offspring values should not deviate significantly from the 'weighted one'. I, therefore,

performed initial parent-offspring regression analyses on all traits using unweighted mean offspring values. In the case where a *positive* regression coefficient was found to be significantly different from zero, a regression analysis was repeated using mean offspring values weighted according to Sokal & Rohlf (1981). Only 'weighted regression coefficients' were used in the calculation of heritabilities.

Genetic correlation. The correlation between two traits based on direct measurements on a number of individuals within a group is known as the *phenotypic correlation,* which can be partitioned into environmental and genetic components. The genetic component of the total phenotypic correlation (or simply *genetic correlation*) provides a quantitative measure of the extent to which two traits are controlled by the same gene (or genes). The estimation of genetic correlation is, in many ways, analogous to the computation of heritability described above. The difference is that heritability is concerned with the variance of one character whereas genetic correlation deals with the covariance of two traits (Falconer 1981). Similar to the computation of heritability, genetic correlation is estimated on the basis of resemblance between relatives. The technique involves calculating four covariances of two traits (e.g. 1 and 2) between the mother and the mean of her offspring (Becker 1975):

(1) cov_{12} = covariance of trait 1 in parent
and trait 2 in offspring,

(2) cov_{21} = covariance of trait 2 in parent
and trait 1 in offspring,

(3) cov_{11} = covariance of trait 1 in parent
and trait 1 in offspring,

(4) cov_{22} = covariance of trait 2 in parent

and trait 2 in offspring,

The genetic correlation (r_G) is given by

$$r_G = (cov_{12} + cov_{21})/2[(cov_{11})(cov_{22})]^{1/2} \quad 5.5$$

The standard error (S.E.) of the genetic correlation is estimated according to Falconer (1981):

$$S.E. = [(1-r_G^2)/1.41]\{[S.E.(h^2_1)][S.E.(h^2_2)]/(h^2_1)(h^2_2)\}^{1/2} \quad 5.6$$

where h^2_1 and $S.E.(h^2_1)$ are the heritability of trait 1 and its standard error, while h^2_2 and $S.E.(h^2_2)$ are the heritability of trait 2 and its standard error. All four parameters are estimated from regression analysis (see the section on heritability).

5.3 Results

5.3.1 Quantitative genetical analysis

Intraclass correlation analyses were carried out on both the F_1 and the F_2 snails for each of the 15 traits measured to detect heritable variations. Thirty such analyses were applied to each of the three populations, thus giving a total of 90 tests. At the critical 5% significance level, at least 4 out of the 90 tests might be expected to be significant due to chance alone. To deal with a similar problem, Stearns (1984) suggested that the critical significance level (5%) be divided by the total number of tests made. However, due to the relatively small sample sizes used in this experiment, lowering the significance level would make the tests too conservative. For example, an adjusted significance level of, say, 0.001 will require an unrealistically high *F*-ratio of 10.48 when the degrees of freedom of the numerator and the denominator are 5 and 10 respectively. Therefore, instead of adjusting the significance level, I compared the overall pattern of the results for the F_1 and the F_2 generations, and considered a trait to be heritable only if

consistent results (i.e. a significant *F*-ratio at the 5% level) were obtained in both generations.

Sheaf population. Analyses on the F_1 snails revealed that the between-capsule variances were significantly larger than the within-capsule variances for hatchling size, juvenile growth rate, rate of egg capsule production, and post-reproductive survivorship (Table 5.3). However, similar analyses of variance on the F_2 snails indicated a significant *F*-ratio for juvenile growth rate only.

Table 5.3 Analyses of variance within- and between-capsule (*F*) for various traits of the Sheaf population cultured at 20 °C. v_1 and v_2 are degrees of freedom in the numerator and denominator respectively. Significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, NS = Not significant.

Trait	F_1 generation $F(v_1,v_2)$	F_2 generation $F(v_1,v_2)$
hatchling size	2.97(9,44)**	1.85(6,17)NS
juvenile growth rate	5.00(9,38)***	3.17(6,17)*
shell length at the onset of breeding	1.53(7,17)NS	1.24(5,11)NS
age at the onset of breeding	1.65(8,19)NS	0.66(5,11)NS
maximum shell length	1.32(9,30)NS	0.81(6,16)NS
longevity	0.76(9,32)NS	0.27(6,16)NS
Relative Aperture Size Ratio	0.45(9,30)NS	0.79(6,17)NS
rate of egg capsule production	4.08(8,19)**	0.75(5,11)NS
rate of egg production	0.42(8,19)NS	0.35(5,11)NS
total number of egg capsules produced	1.96(8,19)NS	1.95(5,11)NS

number of eggs per capsule	1.06(8,19)NS	0.83(5,11)NS
total number of eggs produced	2.21(8,19)NS	3.07(5,11)NS
size of eggs produced	0.59(8,19)NS	2.42(5,11)NS
length of breeding period	2.25(8,19)NS	1.20(5,11)NS
post-reproductive survivorship	3.73(8,19)**	1.01(5,11)NS

Rivelin population. In the F_1 generation, the within-capsule variances were found to be significantly smaller than the between-capsule variances for hatchling size, juvenile growth rate, rate of egg capsule production, and rate of egg production (Table 5.4). However, analyses of variance on the F_2 snails showed evidence of heritable variation for hatchling size only.

Table 5.4 Analyses of variance within- and between-capsule (F) for various traits of the Rivelin population cultured at 20 ^{o}C. v_1 and v_2 are degrees of freedom in the numerator and denominator respectively. Significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, NS = Not significant.

Trait	F_1 generation $F(v_1,v_2)$	F_2 generation $F(v_1,v_2)$
hatchling size	5.44(9,40)***	8.29(5,10)**
juvenile growth rate	3.11(8,27)*	0.89(5,10)NS
shell length at the onset of breeding	0.24(3,5)NS	0.38(3,7)NS
age at the onset of breeding	0.41(3,5)NS	0.92(3,7)NS
maximum shell length	0.57(7,18)NS	0.40(4,8)NS
longevity	0.65(7,18)NS	1.34(4,8)NS

Relative Aperture Size Ratio	0.69(7,21)NS	0.27(5,10)NS
rate of egg capsule production	8.63(3,5)*	1.51(3,7)NS
rate of egg production	25.01(3,5)**	2.35(3,7)NS
total number of egg capsules produced	1.14(3,5)NS	1.01(3,7)NS
number of eggs per capsule	5.29(3,5)NS	3.15(3,7)NS
total number of eggs produced	1.86(3,5)NS	1.55(3,7)NS
size of eggs produced	4.68(3,5)NS	1.44(3,7)NS
length of breeding period	1.44(3,5)NS	3.17(3,7)NS
post-reproductive survivorship	0.82(3,5)NS	3.39(3,7)NS

Don population. Analyses of variance indicated that the between-capsule variances were consistently larger than the within-capsule variances for hatchling size, and age at the onset of breeding in both the F_1 and the F_2 generations (Table 5.5).

Table 5.5 Analyses of variance within- and between-capsule (F) for various traits of the Don population cultured at 20 ^{o}C. v_1 and v_2 are degrees of freedom in the numerator and denominator respectively. Significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, NS = Not significant.

Trait	F_1 generation $F(v_1,v_2)$	F_2 generation $F(v_1,v_2)$
hatchling size	3.50(13,49)**	11.82(9,21)***
juvenile growth rate	0.85(9,31)NS	8.77(9,21)***
shell length at the onset of breeding	0.70(8,16)NS	0.48(9,21)NS

age at the onset of breeding	3.89(8,16)**	3.61(9,21)*
maximum shell length	1.62(10,28)NS	0.82(9,21)NS
longevity	1.03(10,29)NS	0.76(9,21)NS
Relative Aperture Size Ratio	0.49(10,30)NS	0.28(9,21)NS
rate of egg capsule production	1.95(8,16)NS	2.49(9,21)*
rate of egg production	2.02(8,16)NS	1.22(9,21)NS
total number of egg capsules produced	1.49(8,16)NS	0.96(9,21)NS
number of eggs per capsule	3.68(8,16)*	1.89(9,21)NS
total number of eggs produced	1.03(8,16)NS	2.33(9,21)NS
size of eggs produced	1.23(8,16)NS	0.65(9,21)NS
length of breeding period	0.71(8,16)NS	2.01(9,21)NS
post-reproductive survivorship	0.46(8,16)NS	3.06(9,21)*

Regression analyses between the maternal and the mean offspring values revealed that, of all the 15 traits examined, none had a positive regression coefficient significantly different from zero at the 5% level (Table 5.6). However, the regression coefficient corresponding to the age at the onset of breeding of the Don snails was very close to significance ($0.05 < P < 0.10$) (Fig. 5.2). Indeed, when the mean offspring values were weighted according to their sample sizes (Sokal & Rohlf 1981), the analysis yielded a regression coefficient significantly different from zero ($t = 2.35$, $P < 0.05$).

Fig. 5.2 Estimation of the heritability of age at the onset of breeding of the Don snails by regression: a plot of mean offspring value against maternal value.

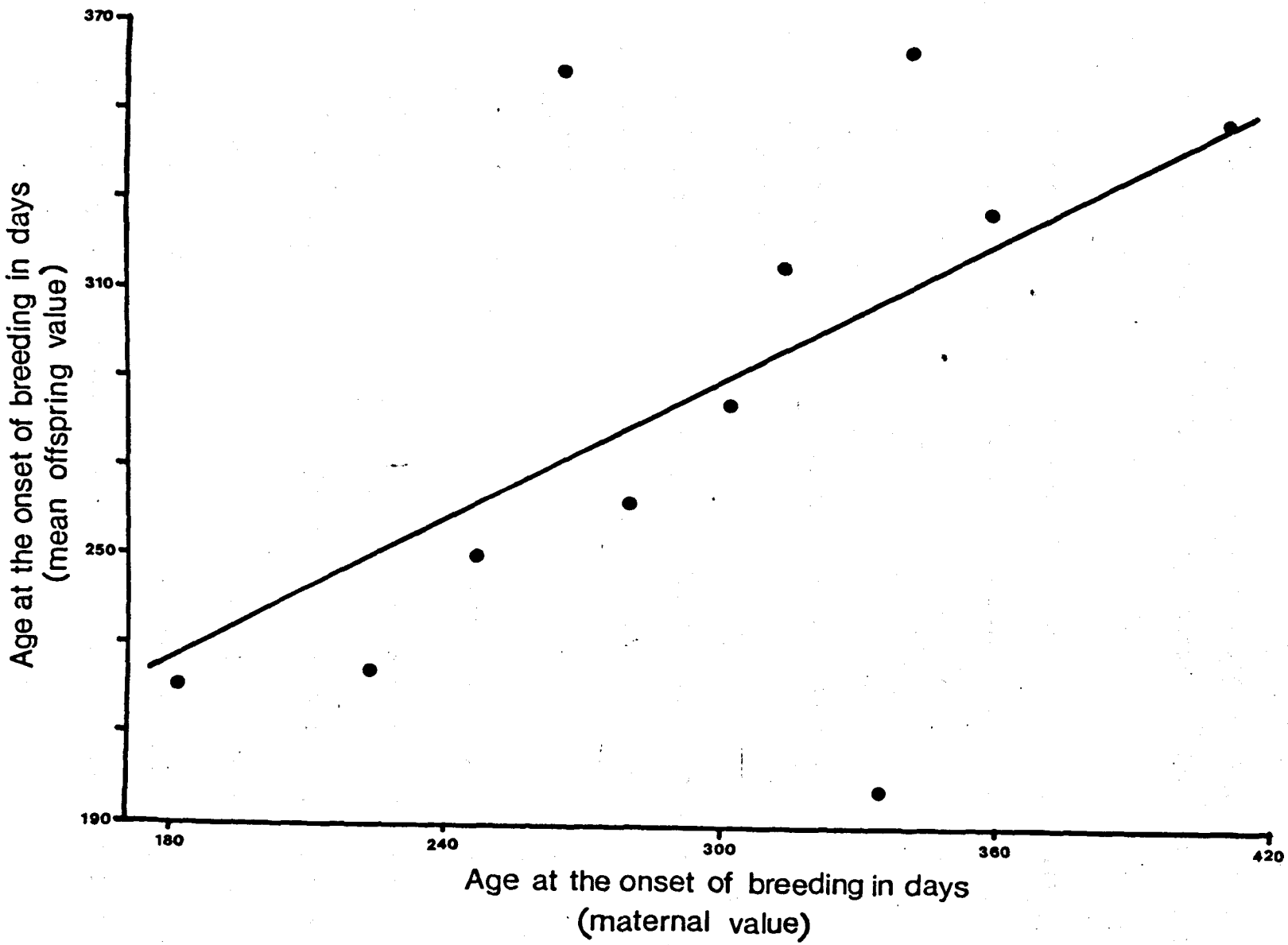

370
310
250
190
180
240
300
360
420
Age at the onset of breeding in days
(mean offspring value)
Age at the onset of breeding in days
(maternal value)

Table 5.6 Regression between the maternal and the mean offspring values for the Sheaf, Rivelin and Don populations. b is the regression coefficient with the number of parent-offspring pairs in parenthesis. The *t*-statistic is used to test the null hypothesis that b is zero. Significance levels: * = $P < 0.05$, NS = Not significant.

Trait	Sheaf (n = 7)		Rivelin (n = 6)		Don (n = 11)	
	b	*t*	b	*t*	b	*t*
hatchling size	-0.02	0.25NS	0.23	0.40NS	1.07	0.78NS
juvenile growth rate	-0.03	0.06NS	-0.63	3.75*	-0.05	0.23NS
shell length at the onset of breeding	-0.57	1.85NS	0.18	0.74NS	-0.03	0.42NS
age at the onset of breeding	-0.004	0.02NS	-0.34	0.71NS	0.53	2.10+
maximum shell length	-0.09	0.44NS	0.29	2.01NS	-0.18	1.44NS
longevity	0.04	0.47NS	0.15	0.60NS	0.01	0.10NS
Relative Aperture Size Ratio	-0.08	0.30NS	-0.20	1.73NS	-0.03	0.71NS
rate of egg capsule production	-1.14	3.41*	0.59	0.67NS	-1.82	2.87*
rate of egg production	0.07	0.19NS	-0.29	1.07NS	-0.16	1.68NS
total number of egg capsules produced	0.11	0.62NS	-0.28	1.63NS	0.002	0.01NS
number of eggs per capsule	0.31	1.00NS	-0.01	0.06NS	-0.07	0.86NS
total number of eggs produced	0.07	0.65NS	-0.17	2.43NS	-0.01	0.04NS
size of eggs produced	0.23	0.92NS	0.40	0.57NS	-0.04	0.34NS
length of breeding period	-0.10	0.94NS	-0.53	1.31NS	-0.12	1.02NS
post-reproductive survivorship	-0.09	0.68NS	-0.08	0.37NS	0.03	0.24NS

+ $t_{0.05[9]} = 2.26$

Heritability. Traits that consistently exhibited evidence of genetic variation in both the F_1 and the F_2 generations include juvenile growth rate of the Sheaf snails, hatchling size of the Rivelin population, as well as the hatchling size and age at the onset of breeding of the Don snails. The heritabilities (h^2_N) of the above traits were estimated, and are summarized in Tables 5.7, 5.8 and 5.9. In principle, h^2_N should not exceed unity (*cf.* equation 5.3) but in practice, due to maternal and common environmental effects (Falconer 1981), within-capsule variances could be substantially underestimated, thus resulting in estimates of h^2_N greater than one. I have, however, presented such values for comparative purposes.

Table 5.7 Heritability estimates of juvenile growth rate of the Sheaf snails based on independent intraclass correlation analyses on the F_1 and the F_2 generations. Standard errors are given in parenthesis.

	intraclass correlation coefficient (t)	heritability	
		lower limit	upper limit
F_1 generation[1]	0.46 (±0.17)	0.91 (±0.33)	1.82 (±0.65)
F_2 generation[2]	0.39 (±0.23)	0.39 (±0.23)	0.79 (±0.47)

[1] the lower and upper limits of heritabilities were calculated by assuming that snails from the same egg capsules were related as full sibs and half sibs respectively.

[2] the lower and upper limits of heritabilities were calculated by assuming that snails from the same egg capsules were produced as a result of self-fertilization and cross-fertilization (i.e. full-sibs) respectively.

Table 5.8 Heritability estimates of hatchling size of the Rivelin snails based on independent intraclass correlation analyses on the F_1 and the F_2 generations. Standard errors are given in parenthesis.

	intraclass correlation coefficient (t)	heritability lower limit	heritability upper limit
F_1 generation[1]	0.47 (±0.16)	0.94 (±0.32)	1.88 (±0.64)
F_2 generation[2]	0.74 (±0.17)	0.74 (±0.17)	1.48 (±0.34)

[1] the lower and upper limits of heritabilities were calculated by assuming that snails from the same egg capsules were related as full sibs and half sibs respectively.

[2] the lower and upper limits of heritabilities were calculated by assuming that snails from the same egg capsules were produced as a result of self-fertilization and cross-fertilization (i.e. full-sibs) respectively.

Table 5.9 Heritability estimates of (a) hatchling size and (b) age at the onset of breeding of the Don snails. Standard errors are given in parenthesis.

(a) Hatchling size	intraclass correlation coefficient (t)	heritability lower limit	heritability upper limit
F_1 generation[1]	0.36 (±0.14)	0.72 (±0.28)	1.44 (±0.56)
F_2 generation[2]	0.79 (±0.10)	0.79 (±0.10)	1.58 (±0.20)

(b) Age at the onset of breeding	intraclass correlation coefficient (t)	heritability lower limit	heritability upper limit
F_1 generation[1]	0.51 (±0.21)	1.02 (±0.42)	2.04 (±0.84)
F_2 generation[2]	0.47 (±0.19)	0.47 (±0.19)	0.94 (±0.38)

	regression coefficient (b)	heritability lower limit	heritability upper limit
regression of mean offspring values on one-parent (mother)[2]	0.61 (±0.26)	0.61 (±0.26)	1.22 (±0.52)

[1] the lower and upper limits of heritabilities were calculated by assuming that snails from the same egg capsules were related as full sibs and half sibs respectively.

[2] the lower and upper limits of heritabilities were calculated by assuming that snails from the same egg capsules were produced as a result of self-fertilization and cross-fertilization (i.e. full-sibs) respectively.

Phenotypic and genetic correlations. I performed two separate sets of correlations, one of which investigates the relationship between total number of eggs produced and post-reproductive survivorship. The other set of correlations uses rates of egg production as the measure of reproductive investment. Phenotypic correlations suggested that post-reproductive survivorship was positively related to total number of eggs produced, but negatively associated with rate of egg production (Table 5.10).

Table 5.10 Phenotypic correlation coefficients between post-reproductive survivorship and total number of eggs produced, as well as rate of egg production in both the F_1 and the F_2 generations. *n* is the number of pairs of readings. Significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, NS = Not significant.

F_1 generation		total number of eggs produced	rate of egg production	*n*
post-reproductive survivorship	Sheaf	0.69***	-0.28NS	29
	Rivelin	-0.35NS	-0.09NS	13
	Don	0.36[+]	-0.44*	29
	overall	0.53***	-0.25*	71

F_2 generation		total number of eggs produced	rate of egg production	*n*
post-reproductive survivorship	Sheaf	0.61**	-0.57*	18
	Rivelin	0.35NS	-0.59*	13
	Don	0.44*	-0.56***	32
	overall	0.43***	-0.54***	63

[+] The critical value of *r* at the 5% significance level is 0.37

Two traits can only be genetically correlated if both are heritable (i.e. cov_{11} and cov_{22} in equation 5.5 should both be positive). I estimated the four covariance terms namely cov_{12}, cov_{21}, cov_{11} and cov_{22} (*cf.* equation 5.5) required for the determination of the genetic correlations between post-reproductive survivorship and total number of eggs produced, as well as between post-reproductive survivorship and rate of egg production (Table 5.11). In all cases, at least one of the two covariance terms, cov_{11} and cov_{22}, was negative, suggesting the absence of genetic variation in at least one of the two traits. Data of the present study, therefore, provided no evidence of any genetic correlation between reproductive investment (measured separately as total number of eggs produced and rate of egg production) and post-reproductive survivorship.

Table 5.11 The covariance terms required for the estimation of genetic correlations between post-reproductive survivorship (trait 1) and total number of eggs produced (trait 2), as well as rate of egg production (trait 3). Examples of the nomenclature: cov_{12}, covariance of trait 1 in parent and trait 2 in offspring; cov_{13}, covariance of trait 1 in parent and trait 3 in offspring.

	cov_{12}	cov_{21}	cov_{11}	cov_{22}
Sheaf	1486.84	-20712.44	-6208.05	28446.50
Rivelin	-10505.39	-11084.19	-3547.20	-41224.59
Don	30025.44	-15539.00	5348.03	-1173.75

	cov_{13}	cov_{31}	cov_{11}	cov_{33}
Sheaf	150.90	-1.44	-6208.05	0.33
Rivelin	-287.01	-32.62	-3547.20	-8.77
Don	296.28	51.40	5348.03	-10.62

The fact that both hatchling size and age at the onset of breeding showed genetic variations in the Don snails suggested the possibility that the two traits might be genetically correlated in the Don population. Estimates of both phenotypic and genetic correlations revealed that the two traits were, indeed, negatively correlated (Table 5.12), providing strong evidence that there is a genetic trade-off between hatchling size and age at first reproduction in the Don snails.

Table 5.12 Phenotypic and genetic correlations between hatchling size and age at the onset of breeding of the Don snails. Sample size (*n*) and standard errors (S.E.) are given in parenthesis. Significance levels: ** = $P < 0.01$, *** = $P < 0.001$.

phenotypic correlations r_P (*n*)		genetic correlation
F_1 generation	F_2 generation	r_G(*n*,S.E.)
-0.49 (29)**	-0.57 (32)***	-0.89 (11,0.085)***

5.3.2 Direct observations of snail cultures

The possible genetic basis of the intraspecific differences reported in chapters 4.1 and 4.2 was also investigated by examining the persistence of the variations when snails were cultured under the same, controlled conditions in the laboratory (section 2.7).

Snails cultured in pairs. Analyses of variance of the F_1 generation revealed significant interpopulation differences in 10 out of the 15 traits examined (Table 5.13), while only three showed such divergence in the F_2 generation (Table 5.14). Age at the onset of breeding and longevity exhibited significant interpopulation variations in both the F_1 and the F_2 snails.

Table 5.13 Interpopulation comparisons of various traits of the F_1 snails cultured in separate pairs. Population means, standard errors (S.E.) and sample size (n) are presented. $F(v_1,v_2)$, F-ratio with v_1 and v_2 as the degrees of freedom of the numerator and denominator respectively. Significance levels: *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$, NS = Not significant.

Trait	Sheaf mean (S.E., n)	Rivelin mean (S.E., n)	Don mean (S.E., n)	F (v_1,v_2)
hatchling size (mm)	1.28 (0.012,54)	1.34 (0.015,50)	1.23 (0.006,63)	29.01*** (2,164)
juvenile growth rate (b x 10000)	96 (1.8,49)	66 (3.3,37)	86 (3.4,41)	27.31*** (2,124)
shell length at the onset of breeding (mm)	10.42 (0.31,27)	10.20 (0.74,13)	11.30 (0.51,29)	1.44NS (2,66)
age at the onset of breeding (days)	437.0 (26.1,29)	365.5 (14.4,13)	354.8 (18.7,29)	4.22* (2,68)
maximum shell length (mm)	11.07 (0.33,41)	9.19 (0.46,28)	12.12 (0.37,42)	13.75*** (2,108)
longevity (days)	560.5 (23.2,44)	464.7 (26.7,28)	543.9 (22.3,43)	3.84* (2,112)
Relative Aperture Size Ratio	0.75 (0.006,41)	0.72 (0.012,31)	0.72 (0.008,44)	2.35NS (2,113)
rate of capsule production (day^{-1})	0.22 (0.014,29)	0.29 (0.041,13)	0.23 (0.015,29)	2.96NS (2,68)
rate of egg production (day^{-1})	1.59 (0.13,29)	2.74 (0.56,13)	2.79 (0.34,29)	5.11** (2,68)
total number of egg capsules produced	24.69 (2.63,29)	17.38 (3.44,13)	28.00 (2.95,29)	2.37NS (2,68)
number of eggs per capsule	7.38 (0.59,29)	9.40 (1.89,13)	12.48 (1.29,29)	5.86** (2,68)
total number of eggs produced	192.7 (31.4,29)	168.0 (47.2,13)	316.9 (33.9,29)	4.97* (2,68)
size of eggs produced (mm^3)	0.4257 (0.016,29)	0.4384 (0.024,13)	0.4677 (0.016,29)	1.86NS (2,68)

length of breeding period (days)	133.4 (18.1,29)	66.7 (10.4,13)	136.6 (15.7,29)	3.50* (2,68)
post-reproductive survivorship (days)	149.9 (19.0,29)	89.7 (20.2,13)	193.9 (21.6,29)	4.60* (2,68)

Table 5.14 Interpopulation comparisons of various traits of the F_2 snails cultured in separate pairs. Population means, standard errors (S.E.) and sample size (n) are presented. $F(v_1,v_2)$, F-ratio with v_1 and v_2 as the degrees of freedom of the numerator and denominator respectively. Significance levels: *** = $P < 0.001$, NS = Not significant.

Trait	Sheaf mean (S.E., n)	Rivelin mean (S.E., n)	Don mean (S.E., n)	F (v_1,v_2)
hatchling size (mm)	1.10 (0.010,24)	1.11 (0.021,16)	1.11 (0.016,32)	0.22NS (2,69)
juvenile growth rate (b x 10000)	92 (3.5,24)	87 (3.3,16)	83 (3.4,32)	1.73NS (2,69)
shell length at the onset of breeding (mm)	10.54 (0.38,18)	9.82 (0.52,13)	10.09 (0.22,32)	0.96NS (2,60)
age at the onset of breeding (days)	241.0 (15.0,18)	206.7 (13.0,13)	292.2 (11.9,32)	9.72*** (2,60)
maximum shell length (mm)	10.82 (0.32,23)	10.28 (0.38,14)	10.85 (0.24,32)	0.85NS (2,66)
longevity (days)	335.1 (14.7,23)	288.6 (18.9,14)	404.7 (8.5,32)	20.14*** (2,66)
Relative Aperture Size Ratio	0.74 (0.007,24)	0.74 (0.009,16)	0.71 (0.004,32)	9.70*** (2,69)
rate of capsule production (day^{-1})	0.26 (0.026,18)	0.29 (0.050,13)	0.29 (0.032,32)	0.15NS (2,60)
rate of egg production (day^{-1})	2.16 (0.34,18)	2.57 (0.62,13)	2.03 (0.21,32)	0.60NS (2,60)
total number of egg capsules produced	17.39 (2.29,18)	16.92 (1.63,13)	22.03 (1.70,32)	2.27NS (2,60)
number of eggs per capsule	7.98 (0.82,18)	8.20 (0.81,13)	7.60 (0.49,32)	0.21NS (2,60)

total number of eggs produced	131.4 (20.3,18)	132.8 (15.1,13)	160.9 (13.9,32)	1.12NS (2,60)
size of eggs produced (mm^3)	0.4242 (0.014,18)	0.4332 (0.015,13)	0.4402 (0.011,32)	0.41NS (2,60)
length of breeding period (days)	76.0 (12.2,18)	69.8 (12.5,13)	97.3 (9.4,32)	1.76NS (2,60)
post-reproductive survivorship (days)	87.1 (13.3,18)	72.5 (12.8,13)	112.5 (10.5,32)	2.72NS (2,60)

Multiple pairwise comparisons revealed that the F_1 Don snails started breeding at a significantly younger age than the F_1 Sheaf snails (Tukey test: $q_{68,3} = 3.91$, $P < 0.05$). However, this pattern was reversed in the F_2 generation in which the Sheaf and the Rivelin snails commenced breeding significantly earlier than the Don individuals (Tukey tests: $q_{60,3} > 3.90$, $P < 0.05$). The life span of the Rivelin snails was significantly shorter than that of the Sheaf individuals in the F_1 generation (Tukey test: $q_{112,3} = 3.79$, $P < 0.05$), while the Don snails exhibited significantly higher longevity than both the Sheaf and the Rivelin snails in the F_2 generation (Tukey tests: $q_{66,3} > 5.90$, $P < 0.001$). In the F_1 generation, the Rivelin snails produced largest hatchlings among the three populations, but such a divergence was not observed in the F_2 generation. The F_2 Don snails produced significantly smaller apertures (relative to total shell size) than their Sheaf and Rivelin counterparts (Tukey tests: $q_{69,3} > 4.60$, $P < 0.01$). However, such a difference was not apparent in the F_1 generation. I used analyses of variance, instead of analyses of covariance (*cf.* section 4.2.2.3), in this instance to compare RASRs as the potential confounding variable of shell length (measured at the onset of breeding) did not differ among populations in both the F_1 and the F_2 generations.

Mass cultures. The life-history parameters of the Sheaf, Rivelin and Don snails reared in tanks (Table 5.15), followed a general pattern very similar to that of the F_1 snails cultured in pairs. For example, the growth rate of the Sheaf snails (measured as the mean shell length at the tenth week) was higher than that of the Rivelin individuals (Tukey test: $q_{57,3} = 3.45$, $P < 0.05$). The Don population started breeding three weeks earlier, and at a smaller average size than the Sheaf and the Rivelin populations (Tukey tests: $q_{57,3} > 6.60$, $P < 0.001$). There was no significant interpopulation differences in either the mean number of eggs per capsule or the rate of egg production per individual per week. The Rivelin snails produced significantly larger eggs than both the Sheaf and the Don individuals (Tukey tests: $q_{297,3} > 5.40$, $P < 0.001$). The Don snails had the longest breeding period, and highest fecundities.

Table 5.15 Interpopulation comparisons of life-history traits of the Sheaf, Rivelin and Don snails cultured in tanks. Population means, standard errors (S.E.) and sample size (n) are presented. $F(v_1,v_2)$, F-ratio with v_1 and v_2 as the degrees of freedom of the numerator and denominator respectively. Significance levels: *** = $P < 0.001$, * = $P < 0.05$, NS = Not significant.

Trait	Sheaf mean (S.E., n)	Rivelin mean (S.E., n)	Don mean (S.E., n)	F (v_1,v_2)
shell length at the tenth week (mm)	8.47 (0.27,20)	7.16 (0.37,20)	7.46 (0.47,20)	3.29* (2,57)
shell length at the onset of breeding (mm)	10.21 (0.27,20)	9.99 (0.38,20)	7.46 (0.47,20)	16.18*** (2,57)
age at the onset of breeding (weeks)	13	13	10	

rate of capsule production ($individual^{-1}week^{-1}$)	0.29 (0.041,21)	0.34 (0.050,21)	0.33 (0.041,24)	0.33NS (2,63)
rate of egg production ($individual^{-1}week^{-1}$)	8.56 (1.22,21)	11.21 (1.66,21)	11.35 (1.41,24)	1.16NS (2,63)
total number of capsules produced ($individual^{-1}$)	6.86	7.66	8.52	
number of eggs per capsule	29.58 (1.38,100)	33.26 (2.29,100)	34.35 (1.87,100)	1.76NS (2,297)
total number of eggs produced ($individual^{-1}$)	202.92	254.77	292.66	
size of eggs produced (mm^3)	0.4537 (0.0077, 100)	0.4992 (0.0091, 100)	0.4378 (0.0080, 100)	14.77*** (2,297)
length of breeding period (weeks)	22	22	26	

The mean RASRs of the eight tank populations are summarized in Table 5.16. An analysis of covariance (Shell length as covariate) between the mean RASRs of the laboratory-cultured populations and those of their corresponding field populations (1985 samples, chapter 4.2) revealed that laboratory conditions had a significant effect on the shell shape (RASR) of the snails (Test of slopes: $F = 3.49$, $d.f. = 1, 12$, $P > 0.05$; Test of adjusted means: $F = 5.74$, $d.f. = 1, 13$, $P < 0.05$). Lotic populations tended to have higher RASRs than lentic ones (Fig. 5.3). However, an analysis of covariance on the mean RASRs of the cultured snails suggested that, under laboratory (similar) conditions, populations of a lotic origin did not differ significantly from the lentic ones (Test of slopes: $F = 7.62$, $d.f. = 1, 4$, $P = 0.05$; Test of adjusted means: $F = 3.27$, $d.f. = 1, 5$, $P = 0.13$).

Fig. 5.3. Histograms showing the distribution of mean Relative Aperture Size Ratio (RASR) of lotic and lentic populations in the 1985 samples and laboratory cultures (data from Table 5.16). Each circle represents one population.

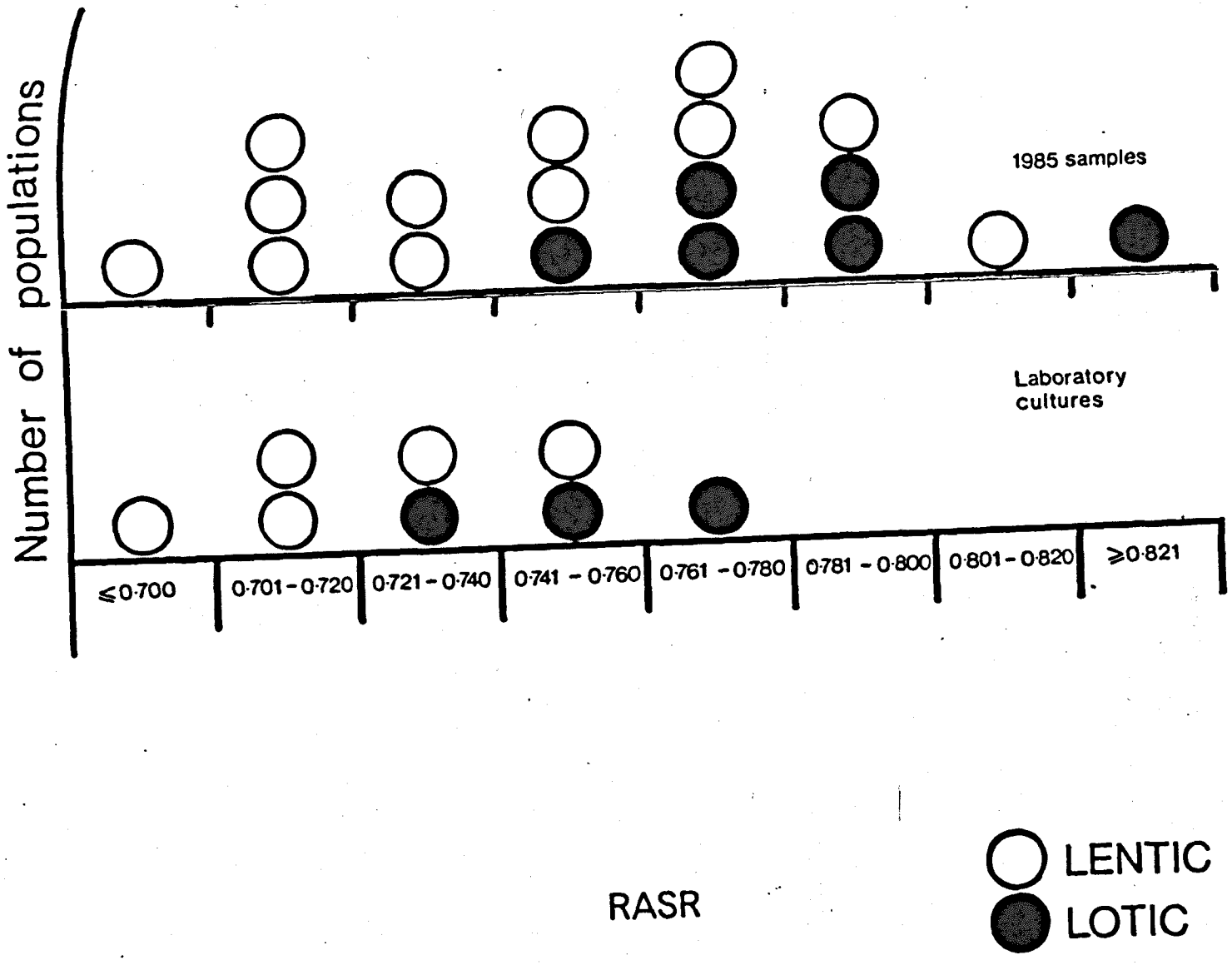

Number of populations
1985 samples
Laboratory cultures
≤0·700
0·701 - 0·720
0·721 - 0·740
0·741 - 0·760
0·761 - 0·780
0·781 - 0·800
0·801 - 0·820
≥0·821
RASR
LENTIC
LOTIC

Table 5.16 Summary of the mean Relative Aperture Size Ratios (RASRs) of the laboratory-cultured populations. Mean RASRs of their corresponding field populations (1985 samples, chapter 4.2) are included for comparison. Standard errors (S.E.) and sample size (*n*) are given in parenthesis.

Habitat type	site no.	location	mean RASR of laboratory cultures mean (S.E., *n*)	mean RASR of 1985 samples mean (S.E., *n*)
LOTIC	1	River Sheaf, Sheffield	0.74 (0.0060,45)	0.75 (0.0062,55)
	2	River Rivelin, Sheffield	0.77 (0.0047,45)	0.78 (0.0066,55)
	3	River Don, Sheffield	0.76 (0.0058,45)	0.77 (0.0034,40)
LENTIC	7	Don backwater, Sheffield	0.72 (0.0066,45)	0.69 (0.0057,64)
	8	Stoney Middleton, Derbyshire	0.76 (0.0060,45)	0.75 (0.0075,11)
	9	Monyash, Derbyshire	0.71 (0.0054,45)	0.77 (0.0074,10)
	10	Over Haddon, Derbyshire	0.73 (0.0063,46)	0.73 (0.0053,55)
	11	Monyash, Derbyshire	0.70 (0.0051,40)	0.72 (0.0058,34)

(Note: Site numbers are the same as those used in Table 4.2.1. Life-history data were collected from populations corresponding to sites 1, 2 and 7)

5.4 Discussion

5.4.1 Reliability of the experimental techniques

Heritabilities are estimated based on the assumption that the resemblance between relatives is entirely due to genetic factors. However, relatives might resemble each other due to non-additive genetic causes (e.g. dominance and epistasis), or even, non-genetic causes such as

(1) Common environmental effect

This arises from the fact that snails that hatched from the same egg capsule are likely to have experienced more similar conditions than snails that hatched from a different capsule. In the context of the present experiment, the critical stages include the development from eggs to hatchlings inside the same egg capsule and the post-hatching period ($\leq$ 24 hours) spent in the same incubation pot before being isolated.

(2) Maternal effect

Falconer (1981) considered that there are two types of maternal effect, the first of which results from the direct influence of maternal phenotype on the phenotypic values of the offspring. For example, bigger snails might be able to invest more resources per individual offspring, resulting in bigger snails in the next generation, than smaller parents. The second type of maternal effect arises as a result of similar rearing conditions provided by the mother, and is particularly important in mammals (Falconer 1981). This causes resemblance between offspring of the same mother (i.e. snails from the same egg capsule). However, this second type of maternal effect, probably more important in ovoviviparous snails, is likely to have a lesser influence on the oviparous *L. peregra*. In the

following discussion, I refer only to the first type of maternal effect which may partly be responsible for the resemblance between a mother and her progeny.

The relative importance of different non-genetic (environmental) causes of resemblance in various methods of heritability estimation is summarized in Table 5.17.

Table 5.17 Sources of non-genetic causes of resemblance in various methods of heritability estimation used in this study.

Type of correlation or regression	Major sources of non-genetic causes of resemblance
correlation between full sibs or half sibs in the F_1 generation	common environmental and maternal
correlation between full sibs or half sibs in the F_2 generation	common environmental
regression between offspring and one parent	maternal

The common environmental effect tends to reduce within-capsule variance, and thus could have the effect of overestimating t in the intraclass correlation analyses, whereas the maternal effect adds a non-genetic component to the covariance between the offspring and their mother, and this could result in an overestimation of b in the regression analyses. The maternal effect is probably more important in the F_1 than in the F_2 generation, as part of the variations observed in the F_1 could potentially be the remnants of the phenotypic differences exhibited by the parental snails under field conditions. However, this type of maternal effect is likely to diminish with generations when the animals are cultured under the same, carefully controlled laboratory conditions. This is supported by two observations. Firstly, 10 out of the 15 traits in the F_1

generation showed significant interpopulation variations, while similar analyses on the F_2 snails revealed significant differences in only 3 out of 15 traits. Secondly, for the same trait, heritability estimates based on the F_1 progeny are generally higher than those obtained from the F_2 snails. Although it is evident that maternal effect is less important in the F_2 generation, the common environmental effect still prevails (Table 5.17). For the regression of mean offspring value on one parent, as the offspring and their mother belong to two separate generations, and hence are more likely to be subject to different conditions in the cultures, maternal influence is probably more important than the common environmental effect in this case (Table 5.17).

Clearly, the correlation analyses based on the F_2 progeny and the regression analyses between the offspring and one parent are more conservative in revealing heritable variations than the correlation analyses involving the F_1 snails. Indeed, traits that had significantly bigger between-capsule than within-capsule variances in the F_2 also showed the same type of differences in the F_1 generation for both the Sheaf and the Rivelin populations, but not *vice versa*. Although three of the 15 traits of the Don snails showed significantly greater between-capsule than within-capsule variances in the F_2, but not the F_1 generation, the probability of encountering 3 significant tests out of 90 such analyses is still less than 5%. The above findings, therefore, suggest that intraclass correlation analyses of the F_1 snails are useful in refuting the null hypothesis that a specific trait is heritable, and thus can be used for screening non-heritable traits. Regression analyses of mean offspring value on one parent indicated genetic variance for only one trait, out of the four that showed evidence of heritable variation from the

two intraclass correlation analyses performed separately on the F_1 and the F_2 snails. Overall, a combination of all the three methods employed in this study (Table 5.17) proved to be a useful experimental approach for investigating the genetic basis of quantitative characters.

5.4.2 Heritable variations

Life-history traits. There is evidence to suggest that juvenile growth rate of the Sheaf population, hatchling size of the Rivelin population, as well as hatchling size and age at the onset of breeding of the Don population are heritable. Heritability estimates were generally high with all lower limits $\geq$ 0.39. However, due to the problems of common environmental and maternal effects (see above), the heritabilities probably have been overestimated (the upper limits of 7 out of the 9 estimates exceeded one), and hence should not be taken at face value.

It is also worth noting that all the heritable traits reported in this study are either directly or indirectly related to fitness. For example, assuming that there is a minimum reproductive size for *L. peregra*, a higher growth rate or a bigger size at hatching would mean that a snail can reach that critical size faster than a more slow-growing or a smaller hatchling. Alternatively, if age, rather than size, is the determining factor, a faster growth rate or a larger hatchling size would imply a larger reproductive size, which is often associated with a higher fecundity (e.g. Spight & Emlen 1976, Calow 1983, Ribi & Gebhardt 1986). Moreover, bigger juveniles are likely to have better chances of surviving to breeding than smaller ones.

It has been proposed that a continuous, strong selection pressure would have the effect of 'weeding out' genetic variability,

and this could result in an inverse relationship between the heritability of a trait and its influence on fitness (e.g. Law 1979, Falconer 1981, Rose 1983, Gustafsson 1986). Contrary to the above proposal, the present study revealed relatively high heritability for age at first reproduction. Similarly, considerable additive genetic variance in life-history traits have been reported in natural populations (e.g. Istock 1978, Van Noordwijk *et al.* 1980, Berven & Gill 1983). Charlesworth (1984) pointed out that high additive genetic variances could be maintained in fitness components provided that there are negative genetic correlations between some of the components. It is possible that the genetic trade-off between reproductive timing and hatchling size in the Don population represents a case in point. The fact that age at the onset of breeding is heritable in the Don snails supported the hypothesis that the early-breeding trait is an adaptation to exploit a longer breeding season at the Don as a result of strong competition for oviposition sites (chapter 4.1).

The observations that the Don snails started to breed earlier than both the Sheaf and the Rivelin individuals in the F_1 generation and in the mass cultures further suggest that age at the onset of breeding is, to a certain extent, genetically fixed in the Don population. Although the reversed pattern in the F_2 generation appeared to contradict the above suggestion, the discrepancy could probably be explained by the fact that the F_1 snails were hatched at 15 ^{o}C while the F_2 snails at 20 ^{o}C. The higher temperature experienced by the F_2 juveniles during their early stages of development might have had a long term effect on the phenotypes such as a forward shift in the age at the onset of breeding. It is possible that since the above trait is genetically fixed in the Don

snails, but environmentally determined in the Sheaf and Rivelin individuals, the temperature effect was less pronounced in the former than the latter two populations. The proposal that a higher temperature during the hatching period can reduce the time taken to first breeding is supported by the fact that the F_2 snails of all three populations started breeding at a younger age than their F_1 ancestors.

Although there is little doubt that age at the onset of breeding is genetically determined in the Don snails, the mechanism by which this could have arisen is not clear. There are at least two possibilities. First, natural selection may have acted directly upon the genes that control reproductive timing. Alternatively, the trait could have been modified indirectly as a result of natural selection acting upon a genetically correlated character. Although my data did not allow a conclusive test of the above alternatives, the presence of a genetic trade-off between hatchling size and age at first breeding suggests that natural selection may have acted upon both traits to balance the cost of producing large hatchlings and the benefit of early reproduction. This in fact is one of the major assumptions of the Sibly & Calow (1985) model, and this point will be further considered in chapter 6.

Shell shape. I have proposed, in chapter 4.2, that the larger apertures (relative to total shell size) possessed by *L. peregra* living in lotic habitats as compared with their lentic counterparts are an adaptation to withstand water current. An important assumption of the above hypothesis is that shell shape [measured as aperture-length/shell-length ratios (RASRs)] has a genetic component. In general, the lotic populations in the mass cultures tended to have larger RASRs than the lentic ones (Fig. 5.3), and in particular, the

mean RASRs of the Don snails were consistently lower than those of the Sheaf and the Rivelin populations in all cultures (i.e. F_1 and F_2 generations, and mass cultures). Since all the above observations were made in slow-flowing or still-water conditions, this does suggest that shell shape was, to a certain extent, genetically fixed in at least some of the lotic populations. This is supported by the fact that the Sheaf and the Rivelin snails produced significantly larger apertures than the Don individuals in the F_2 generation. However, the difference in RASRs between lotic and lentic populations was not statistically significant in the mass cultures (Test of slopes: $P = 0.05$, Test of adjusted means: $P = 0.13$). This might have been due to the small number of populations used.

There is also evidence to show that, when cultured under laboratory conditions, snails could develop a shell shape different from that of their ancestors in the field, indicating some degrees of developmental flexibility. This, however, does not refute the hypothesis that the variations in the shell shape of *L. peregra* can be adaptive as phenotypic plasticity, for example in the capacity of the foot to grow larger in fast-flowing habitats, could be genetically determined (Bradshaw 1965, Lynch & Gabriel 1987).

5.4.3 Cost of reproduction

I tested the hypothesis of reproductive cost (Calow 1979, Bell 1983, 1984a, 1984b) by calculating the correlation between reproductive investment (measured in terms of total fecundity and rate of egg production) and post-reproductive survivorship. It was found that post-reproductive survivorship is positively related to total number of eggs produced, but negatively associated with rate of egg production. In the literature, reproductive efforts are most commonly measured as the proportion of energy (or resources)

allocated to reproductive processes (e.g. Browne & Russell-Hunter 1978, Calow 1978, Calow 1979). However, the results of the present study suggest that the rate of egg production might be a more accurate measure of reproductive investment than total fecundity in *L. peregra*. Clearly, the time period over which a snail invests its resources in reproduction is crucial as, for example, investing a fixed amount of resources over a longer period would allow the snail to replenish its store of resources. This will have an overall effect of minimizing, at any one time, the diversion of important resources from vital processes which is often considered as the major cost of reproduction (Calow 1984). A lower rate of egg production would, therefore, enable the animal to live longer, and produce a higher total number of eggs. Indeed, the above strategy can be expected to evolve in situations where the survival chances of breeding adults are relatively high.

All life-history models that predict optimal tactics under specific ecological circumstances assume that the above negative correlation (trade-off) between reproductive investment and parental survivorship has a genetic basis (Williams 1966b, Gadgil & Bossert 1970, Reznick 1985). This kind of genetic trade-off has been demonstrated in *Drosophila melanogaster* by Gowen & Johnson (1946) and Rose & Charlesworth (1981a, 1981b). Contrary to the expectation of the hypothesis, Giesel (1979) reported strong positive genetic correlations between fecundity and longevity in the same *Drosophila* species. Bell (1983, 1984a, 1984b) tested the hypothesis of reproductive cost by examining the genetic correlations between present and expected future reproduction in six freshwater invertebrates including rotifers, oligochaetes, ostracods and cladocerans, and found that the correlations were generally zero or

positive. In the present study, I failed to establish any genetic correlations between post-reproductive survivorship and either total number of eggs produced or rate of egg production.

It is worth mentioning that the experimental design in the present study assesses a reproductive cost which is restricted almost entirely to the drain of important resources from vital processes. Under field conditions, an increase in reproductive investment could result in a reduction in competitive ability, and/or an increase in susceptibility to external mortality factors such as disease, parasitic infection and predation, and these potential risks could incur a substantial cost to the survival of an organism. It is possible that the incomplete assessment of reproductive costs under experimental conditions might be responsible for the unexpected correlative relationships between fecundity and longevity (Bell 1984a, also see review by Reznick 1985).

It has been hypothesized that freshwater habitats are transient in nature, with each separate population existing only for a limited number of generations, and thus do not generally allow long term genetic divergence and/or fixation (Hubendick 1952, 1954, 1960, Russell-Hunter 1978). Russell-Hunter (1964, 1978) argued that it would be of a selective (adaptive) advantage for freshwater snails in general, and pulmonates in particular, to conserve high flexibility in their physiology and life-history tactics. Significantly, Brown (1985a) discovered that the differences in age and size at reproduction, and growth rate between two populations of a freshwater pulmonate, *L. elodes,* disappeared when reared for two generations in the same habitat, and suggested that the phenotypic plasticity in life-history patterns was an adaptation to life in an unpredictable environment. Similarly, phenotypic plasticity was found to be an

important component of the life history of *L. palustris* (Hunter 1975a). In general, the results of the present study support the above hypothesis; most of the interpopulation variations of *L. peregra* observed in the field (chapter 4.1 and 4.2) can be ascribed to proximate, ecological factors, and only one or two out of the 15 traits exhibited significant genetic variance in each population. This study, however, demonstrates that the action of a strong selection pressure can result in local adaptations involving genetic divergence, for example in the reproductive timing of the Don snails, in freshwater environments.

6. Testing predictions of the Sibly and Calow model

The differences in growth rates and survival regimes among the three populations of *L. peregra* reported in chapter 4.1 are identified by the Sibly & Calow (1985) model as key selection pressures in habitat classification (section 2.5). The model predicts that poor conditions for growth (i.e. low G) should favour higher reproductive investment per individual offspring (RI_i) i.e. larger young (Prediction 1), and that higher juvenile relative to adult survivorship (i.e. high S) should favour higher total reproductive investment (RI_t) (Prediction 2). I tested the above predictions based on the life-history data of the three *L. peregra* populations documented in preceding chapters.

6.1 Test of Prediction 1

In chapter 4.1, it was reported that the Don juveniles had lowest growth rates (i.e. lowest G) while the Sheaf snails had the highest G in the field. The Sibly & Calow (1985) model predicts that under low G, the snails should produce a small number of bigger propagules as larger initial size usually means shorter development time. In this context, larger propagules could be bigger eggs if they have a shorter hatching time, and/or larger hatchlings if they take a shorter time than smaller ones to reach a size at which they can themselves reproduce. Indeed, the existence of a trade-off between reproductive timing and hatchling size in the Don population does suggest that hatchling size is an important component determining the age at first breeding. Since I have no data on the relationship between egg size and hatching time for *L. peregra* and, moreover, Spight (1975) discovered for prosobranch gastropods that larger eggs had longer pre-hatching developmental time, I limit the test of Prediction 1 to a comparison of hatchling size among the three

populations.

Unfortunately, it is practically very difficult to measure size at hatching sufficiently accurately from field samples. The main problem is that it is impossible to assess precisely the age of the hatchlings in a field sample, and that any substantial variations in the age of the hatchlings among samples would be likely to render any interpopulation comparisons inappropriate for two main reasons. First, juvenile growth rates were found to vary markedly among sites (chapter 4.1). Secondly, the snails showed fastest (exponential) growth during the initial stages of their life span. Therefore, considerable amount of between-site differences in snail size could have accumulated within a relatively short period after hatching.

It is, however, reasonable to assume that egg size may be a 'good enough' predictor of hatchling size. This assumption, if valid, would be very useful as egg size does not change appreciably during the course of egg development, and hence can be accurately determined from field samples. This would, then, allow an indirect test of Prediction 1. I examined the above assumption by performing correlation analyses between egg size (v_e) and hatchling size (SL_0) based on means from individual breeding pairs within each population (data from experiment 5.2.1). The results of the above tests revealed no significant relationship between the two parameters (Table 6.1).

Table 6.1 Correlation analyses between egg size and hatchling size based on means from individual breeding pairs. *r* and *n* are the correlation coefficient and sample size respectively. NS = Not significant.

generation	population	r^2	*n*
F_1	Sheaf	0.0049 NS	29
	Rivelin	0.19 NS	13
	Don	0.0055 NS	29
F_2	Sheaf	0.0040 NS	18
	Rivelin	0.083 NS	13
	Don	0.000025 NS	32

The above results suggest that egg size and hatchling size may be unrelated. In that case, Prediction 1 will not be amenable to testing without direct information on size at hatching in the field. Alternatively, it is possible that the lack of significant correlation between v_e and SL_0 may be an artefact resulting from low within-population variabilities of either one or both parameters. I differentiated between the above two possibilities by re-examining the relationship between v_e and SL_0 based on population averages from a wide range of habitats:

Adult snails ($n \geq 10$) were collected from 14 sites in Britain: 4 in Sheffield; 7 in the Peak District, Derbyshire; 1 in North Yorkshire; 2 in Aberdeen, Scotland. These sites included 6 lotic (river/stream) and 8 lentic (pond) habitats. Samples were taken in April 1985 after the onset of the breeding season (i.e. egg capsules were observed in the field). Snails from each site were maintained

separately at 15 ($\pm$1)^{o}C in 40 litre glass tanks containing 20 litres of reconstituted water (RW, Appendix 2). Tanks were inspected every day for egg capsules which were then isolated (n > 40 for each population). The volume of 10 of the typically ellipsoidal eggs was calculated for each capsule using the formula: $v_e = 4/3(\pi)(a/2)(b/2)^2$ (a = longer axis, b = shorter axis). The dimensions of the eggs were measured with a graticuled eyepiece on a stereomicroscope. Ten egg capsules were isolated from each of the 14 populations, and each capsule was incubated individually at 15 ($\pm$1)^{o}C in plastic pots containing 25 ml of RW. Egg capsules were inspected every day and the shell length of 5 hatchlings (SL_0) from each capsule measured within 24 hours. Correlation analyses revealed that mean v_e was significantly positively related to mean SL_0 (r = 0.72, n = 14, P < 0.01), suggesting that it is probably legitimate to use v_e as an indicator of SL_0 for interpopulation comparisons. It is noted that although SL_0 was positively correlated with v_e, the increase in SL_0 reduced progressively with increase in v_e (Fig. 6.1). This deviation from a simple linear relationship suggests that v_e might not be a 'good enough' predictor of SL_0 when v_e exceeds a critical value of $c.$ 0.46 mm^3. As mean sizes of eggs laid in the field were all less than 0.45 mm^3, I assume that variations in egg sizes reflect similar differences in hatchling sizes.

Prediction 1 was in general supported by the data from the three snail populations. The Don snails with lowest G consistently produced the largest eggs while the Sheaf snails with highest G laid the smallest eggs (Table 6.2), although the difference was significant at the 5% level only in 1986.

Fig. 6.1 Relationship between hatchling size (mm) and egg size (mm^3). Points are population averages. Vertical and horizontal bars represent standard errors. The curve was fitted by eye.

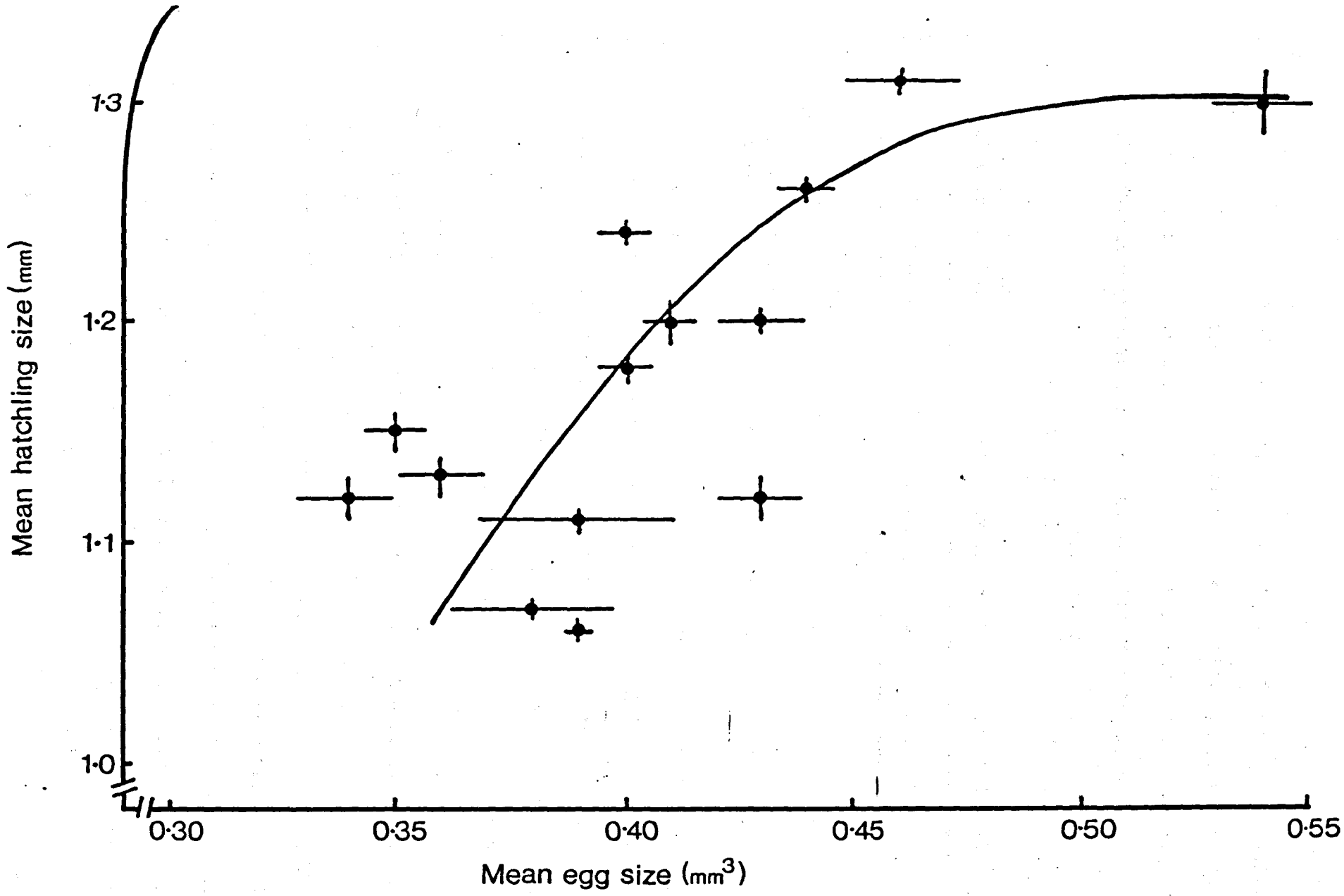

Mean hatchling size (mm)
1·3
1·2
1·1
1·0
0·30
0·35
0·40
0·45
0·50
0·55
Mean egg size (mm³)

Table 6.2 A test of Prediction 1 of the Sibly & Calow model involving G and RI_i. G, index of juvenile growth rate; RI_i, reproductive investment per individual offspring; v_e, egg volume (mm^3). › = significantly greater than; - = no significant difference.

		populations arranged in descending order according to population means	references
Observations on G in 1985 and 1986	:	Sheaf › Rivelin - Don	Table 4.1.10
Predictions on RI_i	:	Don - Rivelin › Sheaf	section 2.5
Field data on v_e in 1985	:	Don - Rivelin - Sheaf	Table 4.1.2
Field data on v_e in 1986	:	Don - Rivelin › Sheaf	Table 4.1.2

There is no evidence to show that egg size has a genetic component in any of the three populations studied (chapter 5). The absence of significant genetic variances in egg size could be a result of intense selection, reducing genetic variability of the trait to a level too low to be detected by the method employed. Alternatively, egg size might be phenotypically plastic, and any observed differences between the populations in the field could have been due to direct environmental effects on the phenotype. By contrast, there is significant genetic variation in the hatchling size of the Rivelin and the Don populations (chapter 5). The high heritabilities of hatchling size in the Rivelin and Don populations might have been maintained through antagonistic pleiotropy i.e. genetic trade-offs (Charlesworth 1984). These results do not contradict the hypothesis that natural selection could have acted upon the gene(s) controlling hatchling size, modifying this trait according to different growth conditions. Indeed, one of the

underlying assumptions of the Sibly & Calow (1985) model is that there is a genetic trade-off between hatchling size and age at the onset of breeding. The existence of such a trade-off has been demonstrated in the Don population (chapter 5), indicating that the above assumption is a biological reality in at least one population.

6.2 Test of Prediction 2

A test of prediction 2 requires an estimate of the index of age-specific survivorship (S) for each population. Unfortunately, the computation of S [expressed as the juvenile relative to adult survival probabilities due to extrinsic factors (*sensu* Sibly & Calow 1986)] is not straightforward. The reason is that, while the juvenile survivorship (S_j) can be derived directly from the appropriate section (birth to first breeding) of the life table, the survivorship of a reproducing adult is affected by both extrinsic and intrinsic (due to reproduction) mortality factors (Parry 1982, also see review by Sibly & Calow 1986). This means that the overall adult survivorship will have to be partitioned into the extrinsic (S_{ex}) and intrinsic (S_{in}) components before S (defined as S_j/S_{ex}) can be quantified. To date there is still no standard procedure for an independent assessment of S_{ex} and S_{in} from field data.

I followed the method employed by Maltby & Calow (1986b) which uses the survivorship of animals that are of reproductive size, but before the breeding season begins, to approximate the S_{ex} of breeding adults. Clearly, the application of the above technique is restricted to situations where the costs of reproduction are incurred only after breeding has begun [referred to as absorption costing by Sibly & Calow (1984)]. The assumption that *L. peregra* is absorption costing can be justified by the same reasons outlined by Maltby & Calow (1986b), namely the absence of any behavioural and morphological

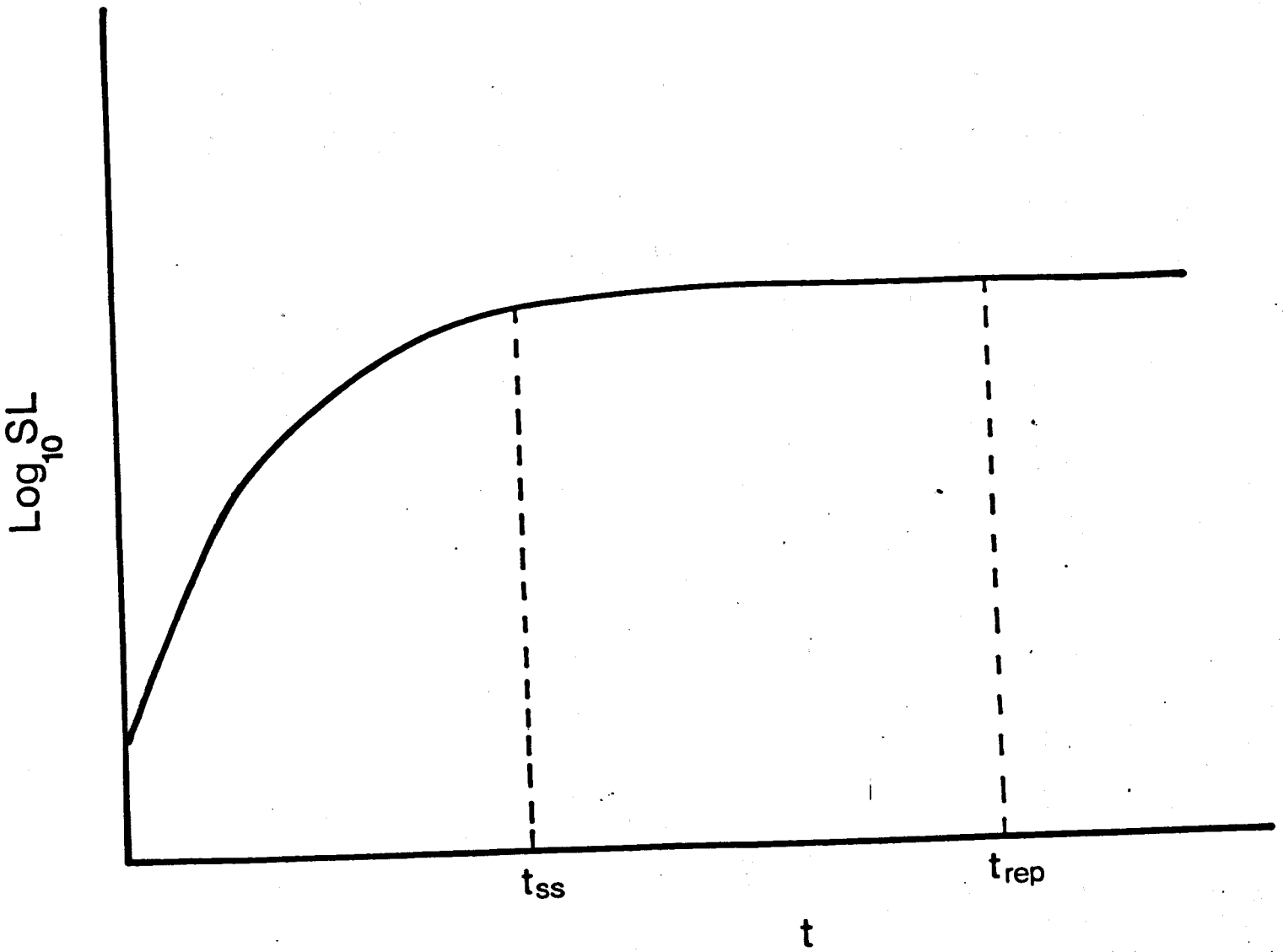
changes prior to breeding that may reduce the survival chances of the parent, and the observation of a sudden increase in mortality only after the onset of breeding (*cf.* Fig. 4.1.10). In the present study, S_{ex} was estimated as the survival probability of snails during the period from when the animals first attained reproductive size (t_{ss}) to the onset of breeding (t_{rep}). In practice, t_{ss} was taken as the time when the growth curve started to level off (Fig. 6.2 and *cf.* Fig. 4.1.11). Estimates of S for the Sheaf, Rivelin and Don populations are summarized in Table 6.3.

Table 6.3 Estimation of S (= S_j/S_{ex}) based on the life tables of the respective populations (Tables 4.1.3, 4.1.4 and 4.1.5). All estimates are based on the 1985 cohorts.

	Sheaf	Rivelin	Don
number of snails (out of 1000 originally born) surviving to reach reproductive size l_{ss}	7.4	0.3	44.7
number of snails (out of 1000 originally born) surviving to first breeding l_{rep}	0.02	0.006	42.3
survival probability of juveniles from birth to first breeding $S_j \times 100$	0.002	0.0006	4.23
survival probability of snails from first attaining reproductive size to first breeding S_{ex} (= l_{rep}/l_{ss}) x 100	0.27	2.00	94.63
index of age-specific survivorship $S = S_j/S_{ex}$	0.0074	0.0003	0.045

Data on the reproductive efforts [measured by an indirect index of reproductive effort (IEI) of Calow (1978), described in section

Fig. 6.2 A generalized growth curve of a cohort of *L. peregra* describing the increase in mean shell length ($Log_{10}SL$) with age (t). t_{ss} is the time when the snails first attain reproductive size, t_{rep} is the time when the breeding season begins.


Log10 SL
tss
trep
t

4.1.3.5] of the Sheaf and the Rivelin populations were consistent with Prediction 2, but those of the Don snails were not (Table 6.4).

Table 6.4 A test of Prediction 2 of the Sibly & Calow model involving S and RI_t. S, index of age-specific survivorship; RI_t, total reproductive investment; IEI, indirect index of reproductive effort.

		populations	references
Observations on S based on 1985 cohort	:	Don > Sheaf > Rivelin	Table 6.3
Predictions on RI_t	:	Don > Sheaf > Rivelin	section 2.5
Field data on IEI in 1985	:	Sheaf > Rivelin > Don	Table 4.1.13
Field data on IEI in 1986	:	Sheaf > Rivelin > Don	Table 4.1.13

Assuming that the above estimates of juvenile survivorship (S) and reproductive efforts (IEI) were legitimate, the results in Table 6.4 suggest the possibility that some of the assumptions on which the model is based may not be valid. For example, the assumption that different levels of reproductive effort exhibited by the three populations have been *genetically* tuned by selection pressures of varying S is questionable. Indeed, contrary to the pattern in the field, experimental observations revealed that, under the same controlled laboratory conditions, the Don snails showed higher reproductive efforts (IEI) than the other two populations (Table 6.5).

Table 6.5 Summary of the indirect indices of reproductive effort (IEI) of laboratory-cultured *L. peregra*. n, total number of eggs produced per individual; v_e, egg volume (mm^3); SL, shell length (mm); SW, shell width (mm); v_a, parental volume (mm^3).

	F_1 generation					
	n	v_e	SL	SW	v_a	IEI[1]
Sheaf	192.7	0.4257	10.42	6.36	441.38	0.19
Rivelin	168.0	0.4384	10.20	6.23	414.58	0.18
Don	316.9	0.4677	11.30	6.55	507.68	0.29
	F_2 generation					
	n	v_e	SL	SW	v_a	IEI
Sheaf	131.4	0.4242	10.54	6.53	470.65	0.12
Rivelin	132.8	0.4332	9.82	6.01	371.44	0.15
Don	160.9	0.4402	10.09	5.98	377.85	0.19
	Mass cultures					
	n	v_e	SL	SW	v_a	IEI
Sheaf	202.9	0.4537	10.21	6.28	421.67	0.22
Rivelin	254.8	0.4992	9.99	6.10	389.27	0.33
Don	292.7	0.4378	7.46	4.40	151.24	0.85

[1] $IEI = n(v_e)/v_a$ (*cf.* equation 4.20)

It is well documented that limitations in resource availability could result in reduced snail fecundities (Brown 1982, 1985a, 1985b, McMahon 1983). It is possible that the lower availability of food at the Don than the other two sites (chapter 4.1) could have contributed to the decreased fecundities of *L. peregra* at the former. This is

supported by the fact that the Don snails did not show lower reproductive efforts than the Sheaf and the Rivelin individuals in laboratory cultures (Table 6.5) where food was present in excess.

Furthermore, I proposed in chapter 4.1 that the shortage of colonizable surfaces, and consequently the strong intraspecific and interspecific competition for oviposition sites at the Don might have limited snail fecundity to a level below its physiological optimum. Indeed, when the above external constraint was relaxed in the laboratory cultures (egg capsules deposited on the walls of the containers were removed every three days), the Don snails exhibited the highest reproductive efforts among the three populations (Table 6.5).

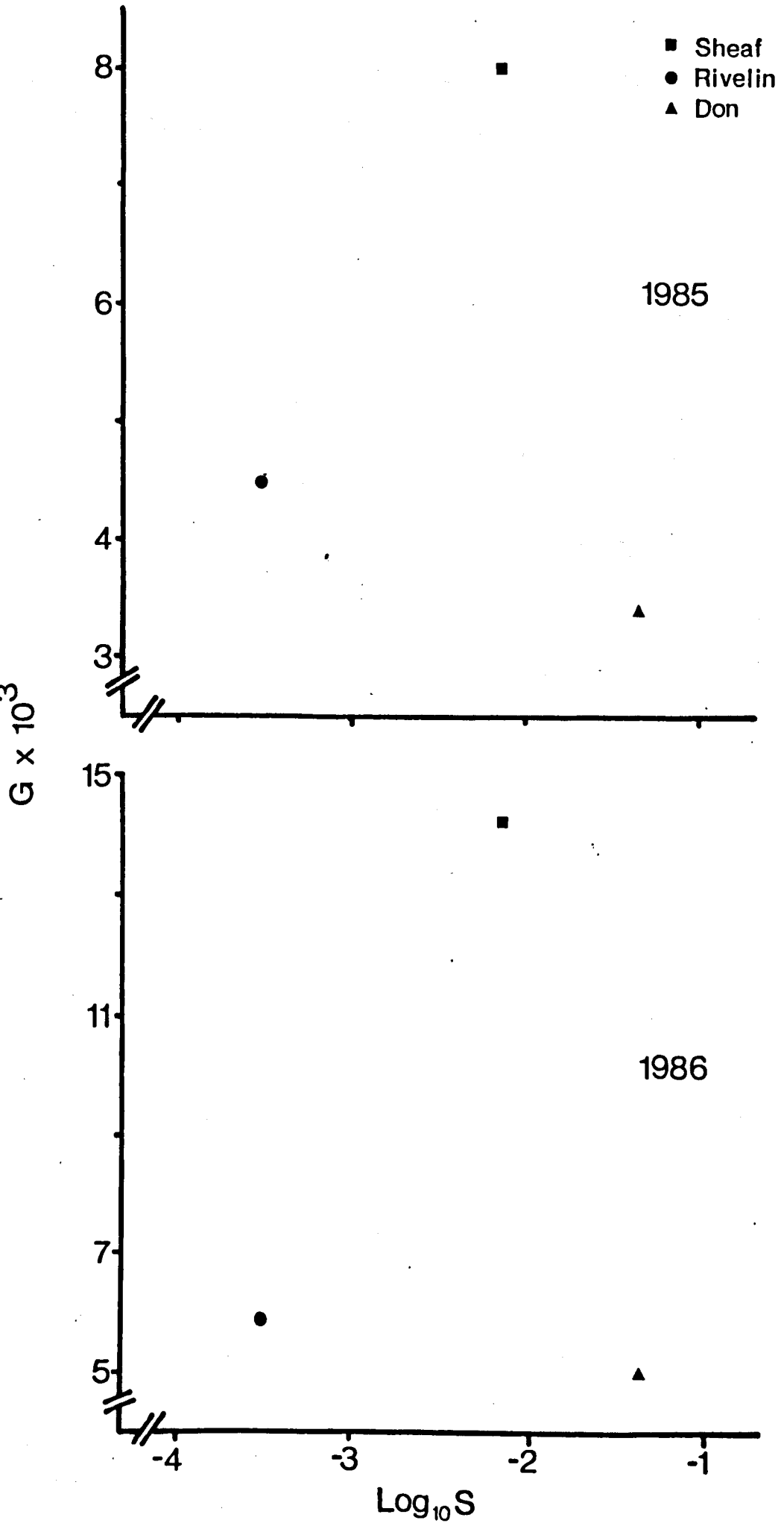
Although the IEIs of the Don snails appeared to be higher than those of the other two populations in the first laboratory generation (i.e. the F_1 snails and the mass cultures), the three populations showed similar levels of reproductive effort in the F_2 generations (Table 6.5). All the above findings point to the conclusion that the low total reproductive investment exhibited by the Don snails in the field was a result of environmental constraints upon reproductive output (i.e. phenotypic), rather than any specific reproductive tactics that might be of an evolutionary origin (i.e. genotypic).

It is generally accepted that freshwater pulmonates arose relatively recently in the Devonian period from a primitive, nearly-terrestrial ancestral stock (Morton 1955, McMahon 1983). The fact that freshwater pulmonates still retain many of the traits of the more terrestrial members of the group, ranging from the absence of ctenidia in lymnaeids to the lack of surface ciliation on the secondary, neomorphic gills of the relatively advanced freshwater limpets and some planorbids (Russell-Hunter 1964, 1978), suggests

that the invasion of aquatic habitats by the freshwater basommatophorans is still incomplete (McMahon 1983). Very often, freshwater habitats are considered to be transient in nature [estimated existence of 10^2 to 10^4 years (Russell-Hunter 1964, 1978)], with conditions generally not conducive to long term evolutionary changes (Hubendick 1952, 1954, 1960, Russell-Hunter 1978, McMahon 1983). It is, therefore, conceivable that the life-history tactics of *L. peregra* may represent a combination of traits that evolved specifically to deal with particular challenges in the current environment and the remnants of those of their ancestors. This underlines the possibility that some of the life-history traits observed in this study may not be optimal in the present habitat (*cf.* Stearns & Sage 1980, also discussed in McMahon 1983).

Another complication is that the differences in the juvenile relative to adult survivorship among the three populations of *L. peregra* (Table 6.3) might be too 'subtle' to invoke any appreciable evolutionary shift in the levels of total reproductive investment. The fact that all three populations are semelparous suggests that the variance in S may be insufficient to cause the evolution of iteroparity. In the context of the Sibly & Calow model, this would mean that the interpopulation variations in S were relatively small, while the differences in G were large (Fig. 6.3). It should also be noted that the life-history data reported in this study were based on only one (in the case of S) or two (in the case of G) cohorts, and hence the pattern observed might not have been consistent over time. Nevertheless, the evidence suggests that the Sibly & Calow model is probably useful in predicting reproductive investment per individual offspring under different growth conditions [Fig. 2.3(b)], but possibly less powerful, at least within the range of S reported in

Fig. 6.3. The proposed classification of selection pressures (S and G) at the Sheaf, Rivelin and Don sites, according to the Sibly and Calow (1985) model. S is the index of age-specific survivorship, G is the index of juvenile growth rate.


Sheaf
Rivelin
Don
1985
1986
8
6
4
3
15
11
7
5
G x 10^3
-4
-3
-2
-1
Log10S

the present study, in predicting total reproductive investment based on juvenile relative to adult survivorship [Fig. 2.3(a)].

7. Overall conclusion

The present study revealed that the environmental conditions prevailing at the three study sites had important influences on the life histories of the three populations of *Lymnaea peregra*. For example, annual variations in water temperature were found to have a direct effect on the timing of the breeding seasons in different years. Although temperature may be expected to have an influence on growth rates, the inter-site differences in water temperature did not account for the divergence in growth rates among the three populations. Instead, food availability or habitat productivity was evidently the key factor affecting snail growth in the field, which in turn determined the number of breeding bouts per year. The Sheaf population, characterized by a high growth rate, had two recruitments per year (i.e. bivoltine) while the more slow-growing Rivelin and Don snails were typically univoltine.

Although all the snail populations suffered high mortalities immediately after hatching, the three populations differed in their survival regimes during the subsequent stages of their life history. The Don snails had lower winter mortalities than the Sheaf and the Rivelin individuals, and this was attributed at least partly to the higher minimum temperatures prevailing at the Don site than the other two during the winter, as a consequence of the warming effects of the submerged pipes. There is circumstantial evidence to show that the high current speeds at the Sheaf and the Rivelin sites might also constitute an important size-specific mortality factor; larger snails appeared to be more prone to fast currents. Indeed, snails collected from lotic habitats (including the Sheaf and the Rivelin sites) generally possess proportionally larger apertures than their lentic counterparts (e.g. the Don site), probably as an adaptation to

withstand fast-flowing conditions. Although there is some suggestion that shell shape in *L. peregra* might partly be genetically determined in some lotic populations (e.g. the Sheaf and, to a lesser extent, the Rivelin snails consistently produced larger apertures than the Don individuals), the observed variations clearly contained a large environmental component.

The Don snails showed significantly lower reproductive efforts than both the Sheaf and the Rivelin populations in the field. The life-history theory in general, and the Sibly & Calow (1985) model in particular, predict that a reduced fecundity should only evolve in situations where adults have relatively higher survival probabilities than juveniles. Contrary to the above expectation, the Don snails exhibited lower juvenile relative to adult mortalities than the other two populations, and there was no indication of the existence of any other potential selection pressure at the Don that might select for the lowered fecundity. Indeed, data from the field study highlighted the possibility that the lower reproductive efforts exhibited by the Don snails, as compared with the Sheaf and the Rivelin individuals, could be a direct result of a number of environmental influences such as low food availability, high population density, and shortage of oviposition sites. The above claim gains support from the observation that the Don snails had reproductive outputs that were at least as high as the other two populations in laboratory cultures when limitations of food and space had been relaxed.

The Don snails generally commenced breeding earlier than the other two populations both in the field and in the laboratory. The fact that age at the onset of breeding was found to be heritable ~~and, to a certain extent, genetically fixed~~ in the Don population suggests that the early-breeding trait is more than just a phenotypic

response to higher environmental temperatures, and could be an adaptation to specific selection pressures at the Don. The existence of high additive genetic variance in the trait controlling age at first reproduction (lower limit of $h^2_N > 0.47$) is not inconsistent with the above suggestion as reproductive timing is negatively genetically correlated with hatchling size in the Don population. By contrast, reproductive timing of both the Sheaf and the Rivelin snails was environmentally initiated, most probably by water temperatures. Since oviposition can only occur above a critical temperature, and low temperature stress can cause high juvenile mortality, the higher water temperatures at the Don than the other two sites might have the important effect of removing the physical (temperature) barrier, and allowing the evolution of the early-breeding trait.

The early breeding season, coupled with the relatively low growth rates at the Don meant that the snails were of smaller size at breeding. Although a smaller reproductive size is often associated with reduced absolute fecundity, this potential cost is likely to be less important in this instance as the *in situ* reproductive outputs of the Don snails were evidently well below their physiological limit. Thus, it would be of a selective advantage for the Don snails to start breeding earlier, though at a smaller size, to produce in real terms a higher total number of eggs by exploiting a longer breeding season. There is, therefore, evidence to indicate that the early reproduction at the Don is an evolutionary response (adaptation) to the particular ecological circumstances (external constraints on egg production).

Furthermore, the genetic trade-off that exists between reproductive timing and hatchling size at the Don indicates that the

early-breeders probably have relatively larger size at hatching. Importantly, the Sibly & Calow (1985) model, on the basis of lower juvenile growth rates, also predicts bigger hatchling size at the Don. The Don snails did appear to produce larger eggs, and probably bigger hatchlings, than the Sheaf individuals in the field. However, the fact that hatchling size is negatively correlated with reproductive timing in the Don population means that natural selection is likely to have acted upon the above two traits to produce an optimal genotype.

The Sibly and Calow model predicts that the Rivelin snails should invest more per individual offspring than the Sheaf snails as an adaptation to the poor conditions for growth. The finding that hatchling size of the Rivelin snails is heritable means that the trait may potentially be amenable to natural selection, and hence does not contradict the above prediction. However, in this instance, the possible mechanism by which the high additive genetic variance in hatchling size is maintained is unknown. It is important to note that, unlike the Don snails, there was no evidence of any genetic trade-off between hatchling size and any of the other traits in the Rivelin population. With the absence of such a genetic constraint, selection would be expected to drive hatchling size to its physiological maximum. This is supported by the fact that the Rivelin snails produced the largest hatchlings under laboratory conditions.

Quantitative genetical analysis revealed that the juvenile growth rate of the Sheaf snails had significant genetic variance. However, the possible adaptive significance of this trait is unclear. It is worth noting that a heritable trait is not necessarily adaptive as the presence of genetic variance can be a result of random processes such as founder effects and/or genetic drift.

On a phenotypic level, post-reproductive survivorship was found to be positively related to total number of eggs produced, but negatively associated with rate of egg production. These results suggest that rate of egg production might be a better measure of reproductive investment than total number of eggs produced. However, data of the present study revealed no evidence of any genetic correlation between the above fitness components, and hence the results cannot be taken as a convincing support for the hypothesis of reproductive cost.

Overall, the findings of the present study support the general hypothesis that freshwater pulmonates are primarily phenotypically plastic. This underlines the danger of presuming any specific adaptation involving genetic divergence, and the necessity to demonstrate, in each case, the presence of genetic variance of the trait concerned before invoking evolutionary inferences. This work shows that, in the presence of an appropriate selection pressure, freshwater snails can evolve local adaptations within a relatively short period of evolutionary time.

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Appendix 1. A worked example of the calculation of the density of *Lymnaea peregra* based on field data collected by the stratified sampling technique of Wrona *et al.* (1986).

(a) Stone profile of the Sheaf site.

		Number of stones of each size class				
	Size class	1	2	3	4	5
Quadrat number	1	12	15	2	1	1
	2	18	12	6	4	0
	3	37	8	2	1	0
	4	28	17	6	0	1
	5	15	21	3	1	0
	6	32	23	2	0	0
	7	36	12	3	0	2
	8	9	14	3	1	0
	9	39	14	3	2	0
	10	40	22	5	0	0
	11	5	3	0	0	0
	12	24	19	3	3	1
	13	32	19	5	0	0
	14	14	20	1	1	2
	15	11	3	0	2	1
X_i		23.47	14.80	2.93	1.07	0.53
S.D.		12.10	6.36	1.91	1.22	0.74
$(S.D.)^2$		146.41	40.46	3.64	1.50	0.55
V_{Xi}		9.76	2.70	0.24	0.10	0.04

(Note: $V_{Xi} = (S.D.)^2/n$, where n is the sample size)

(b) Number of snails per stone for each size category collected on July 29 1985 at the Sheaf site.

Sample number	size class	Number of snails per stone of each size class 1	2	3	4	5
1		2	10	83	63	120
2		2	26	60	92	160
3		2	26	39	9	78
4		0	2	37	89	30
5		2	5	30	20	16
6		0	10	45	69	120
7		0	9	44	10	85
8		3	7	14	13	109
9		3	7	20	35	80
10		1	5	18	33	50
11		0	26	30	68	75
12		0	19	12	74	40
13		0	14	35	30	74
14		0	1	13	38	75
15		1	4	19	37	45
16		2	6	26	76	62
17		0	9	18	30	50
18		2	17	14	13	77
19		0	9	7	31	15
20		3	2	7	49	44
Y_i		1.15	10.70	28.55	43.95	70.25
S.D.		1.18	8.07	19.13	26.72	36.80
$(S.D.)^2$		1.40	65.06	365.84	714.05	1354.20
V_{Yi}		0.07	3.25	18.29	35.70	67.71

Calculation of weighted density:

$$D = (23.47 \times 1.15) + (14.8 \times 10.7) + (2.93 \times 28.55) + (1.07 \times 43.95) + (0.53 \times 70.25)$$

$$= 353.26$$

Calculation of weighted variance, and standard error:

$$V_D = [9.76 + (23.47)^2][0.07 + (1.15)^2] - (23.47)^2(1.15)^2 +$$

$$[2.70 + (14.80)^2][3.25 + (10.70)^2] - (14.8)^2(10.7)^2 +$$

$$[0.24 + (2.93)^2][18.29 + (28.55)^2] - (2.93)^2(28.55)^2 +$$

$$[0.099 + (1.07)^2][35.70 + (43.95)^2] - (1.07)^2(43.95)^2 +$$

$$[0.037 + (0.53)^2][67.71 + (70.25)^2] - (0.53)^2(70.25)^2$$

$$= 52.15 + 1029.78 + 357.03 + 235.64 + 204.12$$

$$= 1878.72$$

$$S.E. = (1878.72)^{1/2}$$

$$= 43.34$$

Therefore, the density of *L. peregra* (0.25 m^{-2}) at the Sheaf site on July 29 1985 was 353.26 $\pm$ 43.34.

Appendix 2. The procedure for the preparation of reconstituted water, which is equivalent to the ISO medium (International organization for standardization ref no. ISO 6341-1982 (E)).

(a) Prepare the following solutions:

(1) Calcium chloride solution

Dissolve 11.76 g of calcium chloride dihydrate ($CaCl_2.2H_2O$) in distilled water and make up to 1 litre with distilled water.

(2) Magnesium sulphate solution

Dissolve 4.93 g of magnesium sulphate heptahydrate ($MgSO_4.7H_2O$) in distilled water and make up to 1 litre with distilled water.

(3) Sodium bicarbonate solution

Dissolve 2.59 g of sodium bicarbonate ($NaHCO_3$) in distilled water and make up to 1 litre with distilled water.

(4) Potassium chloride solution

Dissolve 0.23 g of potassium chloride (KCl) in distilled water and make up to 1 litre with distilled water.

(b) Mix 25 ml of each of the four solutions (1) to (4) and bring the total volume to 1 litre with distilled water.

Appendix 3. A worked example of the log-rank test for comparing the life tables of the Sheaf and the Don cohorts. S, D and T represent Sheaf, Don and total respectively.

Age	Standardized density$_{-2}$ ($0.25\ m^{-2}$) at the start of x			Observed deaths between x and $x+1$			Expected deaths between x and $x+1$		
(x)	S	D	T	S	D	T	S	D	T
0	1000	1000	2000	973.4	935.3	1908.7	954.35	954.35	1908.7
			stage 1:	973.4 (O_1)	935.3 (O_2)		954.35 (E_1)	954.3 (E_2)	
1	26.6	46.7	73.3	8.3	0.5	8.8	3.193	5.607	8.80
2	18.3	46.2	64.5	2.1	0.5	2.6	0.738	1.862	2.60
3	16.2	45.7	61.9	6.7	0.5	7.2	1.884	5.316	7.20
4	9.5	45.2	54.7	2.1	0.5	2.6	0.452	2.148	2.60
5	7.4	44.7	52.1	4.3	0.5	4.8	0.682	4.118	4.80
6	3.1	44.3	47.4	2.5	0.5	3.0	0.196	2.804	3.00
7	0.6	43.8	44.4	0.4	0.5	0.9	0.012	0.888	0.90
8	0.2	43.3	43.5	0.1	0.5	0.6	0.003	0.597	0.60
9	0.09	42.8	42.89	0.04	0.5	0.54	0.001	0.539	0.54
10	0.05	42.3	42.35	0.03	0.5	0.53	0.001	0.529	0.53
			stage 2:	26.57 (O_1)	5.0 (O_2)		7.162 (E_1)	24.41 (E_2)	

11	0.02	41.8	41.82	0.012	0.5	0.51	0.000	0.512	0.51
12	0.008	41.3	41.31	0.005	28.4	28.40	0.005	28.393	28.40
13	0.003	12.9	12.90	0.003	8.5	8.50	0.002	8.501	8.50
			stage 3:	0.02	37.4		0.08	37.41	
				(O_1)	(O_2)		(E_1)	(E_2)	

Illustration: $x = 5$, $4.8 \times (7.4/52.1) = 0.682$

$4.8 \times (44.7/52.1) = 4.118$.

Test of significance for stage 2:

$$x^2 = [(O_1 - E_1)^2]/E_1 + [(O_2 - E_2)^2]/E_2$$

$$x^2 = [(26.57 - 7.162)^2]/7.162 + [(5 - 24.41)^2]/24.41$$

$$= 68.03$$

Appendix 4. An example of the estimation of the heritability of age at the onset of breeding of the F_2 Don snails using one-way analysis of variance with unequal number of hatchlings per egg capsule (unbalanced design) (Becker 1975).

Egg capsule	number of snails surviving to breeding
A	2
B	3
C	2
D	3
E	2
F	2
G	9
H	2
I	3
J	3

Analysis of Variance Table

Source of variation	*d.f.*	SS	MS	EMS
between-capsule	9	83664	9296	$\sigma^2_w + r\sigma^2_b$
within-capsule	21	54027	2573	σ^2_w
total	30	137691		

The coefficient (r) is calculated as follows:

Total number of egg capsules (s) = 10

Total number of snails (n) = 2+3+2+3+2+2+9+2+3+3 = 31

$r = [1/(10 - 1)][31 - (2^2+3^2+2^2+3^2+2^2+2^2+9^2+2^2+3^2+3^2)/31]$

$= 2.95$

$\sigma^2_b = (9296 - 2573)/2.95$

$= 2278.98$

$t = 2278.98/(2278.98 + 2573)$

$= 0.47$

The standard error (S.E.) of t is given by:

$$S.E. = \{2(n - 1)(1 - t)^2[1 + (r - 1)t]^2/r^2(n - s)(s - 1)\}^{1/2}$$

$$= \{2(30)(1 - 0.47)^2[1 + (1.95)0.47]^2/(2.95)^2(21)(9)\}^{1/2}$$

$$= 0.19$$

Therefore, the lower and upper limits of the heritability of age at the onset of breeding of the F_2 Don snails is 0.47 ($\pm$0.19) and 0.94 ($\pm$0.38) respectively.