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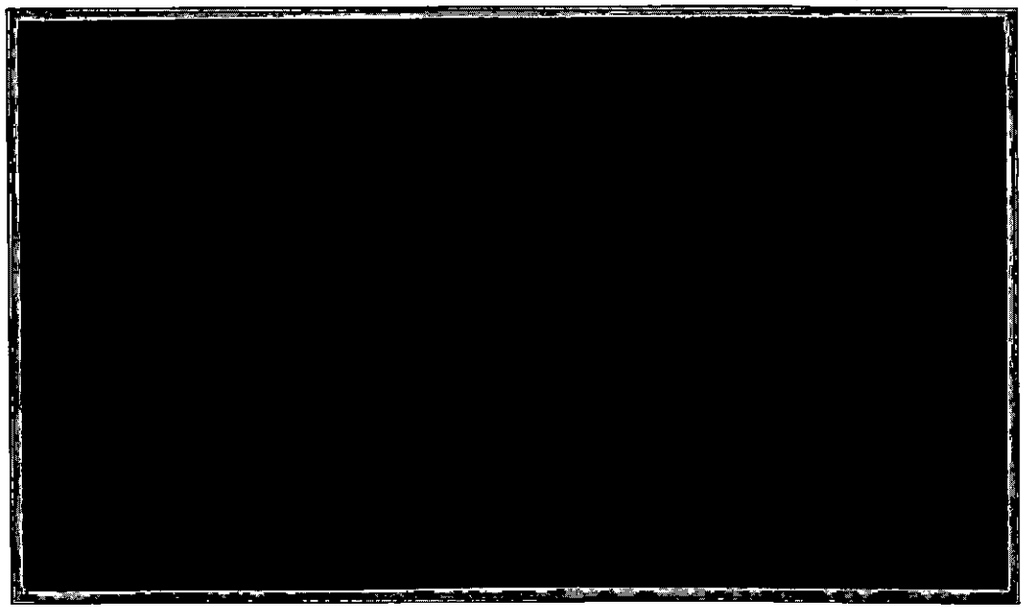

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Factors affecting the recruitment of river fish

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CONTENTS

INTRODUCTION

Problems of recruitment to natural fish populations in flowing water are of general concern. Although there are some fairly consistent correlations between summer temperatures and the occurrence of successful year classes, there is almost no understanding of the mechanisms which underlie such correlations. It is widely believed that "shelter" (from high flows and predation) is essential to the survival of the young of many fish species. No studies have yet been made on whether the critical transition phases in the first year of life are capable of resisting major environmental perturbations. Methods are to be developed for the study of seasonal changes in the distributions of the early stages of rheophilous fish species and subsequently to define the main conditions which favour their initial survival and which govern their distributions and year class success. The present study is the preliminary stage of work to establish methods which might be appropriate to obtain the information required to understand the processes involved.

OBJECTIVES

1. To compile and evaluate the relevant literature relating to fry distribution and survival in rivers.

2. The evaluation of polyacrylamide gel electrophoresis as a method of fingerprinting a number of fish species. This will be used to enable precise identification of individual fish at very early stages in their development.

3. A range of species will be examined using isoelectric focussing techniques and a catalogue of results prepared.

4. To describe the distributions of dace fry in the river Avon.

5. Examination of temporal and spatial distributions of the fry of some species of fish in natural river situations. Measurements will be made at intervals to establish the nature of changes in size and/or species distribution. This should enable the essential features of the habitats in use to be determined Construction and preliminary evaluation of artificially created habitats for fry in relation to current velocity requirements will be carried out.

SUMMARY OF CONCLUSIONS

1. The recruitment process in British freshwater fish is complex and the controlling factors differ from species to species. Temperature, macrophyte cover, substratum type, water depth, velocity variations, indirect influences mediated by food availability and the pressure of inter- and intra-specific predation have all been implicated in different cases.

2. High resolution Iso-Electric Focussing provides reproducible patterns of major protein bands for routine identification of fish.

3. IEF is of particular value for rapid determination of species and notably of small fry and hybrid forms which would otherwise be difficult or even impossible to distinguish on the grounds of standard morphological characteristics alone.

4. Significant differences in the growth of dace fry from the River Avon were related to the distance downstream. The cause of these differences seems to be due to a number of factors and to differ with the age of the fish concerned. In the first two years of life a temperature gradient along the middle reaches of the river seems to be implicated whilst some other factor, possibly food supply, seems to be implicated in the older fish.

5. The fry of several species of coarse fish observed in the River Frome at East Stoke all seemed to favour similar conditions and were found to broadly coexist in these habitats. It is probable that each species is associated with slightly different microhabitat conditions. Very small fry tend to occur near the surface and in association with dense plant cover but as they grow there is a tendency to disperse into deeper faster flowing areas.

1. LITERATURE RELATING TO FRY DISTRIBUTION, SURVIVAL AND YEAR CLASS STRENGTH

The ecology of coarse and game fish has been widely studied in recent years with a great deal of emphasis placed on habitat requirements and on the young stages of Salmonidae (see full reference list, paragraph 1.1). In British rivers work on coarse fishes has concentrated mainly on a few riverine species, for example the ecology of dace has been examined by Williams (1967); Cragg-Hine and Jones (1969); Hellawell (1974); Mann (1974); Mills (1982); Weatherly (1987) and Cowx (1988). Such studies have tended to concentrate on the ecology of the species at an isolated location. Cowx (1988) found that there were significant differences in growth between different reaches of the Exe catchment (Devon) and concluded that biotic factors and abiotic factors other than temperature may have strong influences on the growth of these fish.

Mann and Mills (1985) review the literature on common freshwater fishes including the main riverine cyprinids and the pike . They assess the natural density-independent fluctuations in the strength of year classes. The conclusion is that strong year classes are scarce in both salmonids and coarse fish and that correlations with temperature suggest that strong year classes generally occur following years when temperatures are high. A significant relationship was demonstrated between growth of 0 group dace and subsequent strength of the year class. It is suggested that management of the habitats for larval fish could provide the most practical approach to improving recruitment.

Koonce et al. (1977) review the factors influencing the year class strength of percid fishes. As with cyprinids there are significant correlations with water temperature in many water bodies but it appears that this may not be due to the direct influenc of temperature on survival of the "young of the year." it seems improbable that high temperatures would favour recruitment in all species, notably those which are existing near to the southern limits if their geographical range (in the northern hemisphere), and it is clear that our understanding of these interactions is still scant.

Habitat characteristics other than temperature have also been shown to influence survival of a number of freshwater species. Green (1975) found that the highest population densities of bullheads occurred at sites with a high cover of macrophytes and with coarse gravel or loose stones. Areas of exposed, compact gravel or of silt were less populated. Bullheads fed entirely on benthic invertebrates. The fry ate ostracods, cladocerans and chironomid larvae while larger fish fed mainly on *Gammarus* but also took larval stages of various aquatic insects. The observations are similar to those from other chalk streams (Mann and Orr, 1969; Abel, 1973) and the differences that occur are probably due to the local abundance of particular invertebrates.

Young trout fed mainly on *Gammarus* and the larval stages of aquatic insects but older trout relied less on these and took substantial quantities of surface food. Studies for *Salmo trutta* showed that waterdepth was a major factor affecting distribution, first-year trout being found chiefly in shallow water (mean depth <20 cm) and older fish in deeper water (Egglishaw and Shackley, 1982; Kennedy and Strange, 1982). That work confirms that older trout migrate into deeper water.

Grimm studied the regulation of biomass of the pike *Esox lucius* in relation to aquatic plants and concluded that the association of northern pike with aquatic vegetation is extensively described in literature (see Raat, 1988, for review). Grimm (1981; 1983) described this relationship in quantitative terms. The biomass of pike >54 cm is indirectly related to the vegetation, "because

it recruits" from this habitat. With the development of submerged plants in spring, pike habitat becomes available. This influences the recruitment of "young of the year" pike. When the lake bottom is overgrown with submerged vegetation a maximal 0+ pike biomass (75 $kg.ha^{-1}$) is produced (Grimm, 1983; Raat, 1988). The number of the young of the year pike can be reduced thereafter due to cannibalism (Bungenburg de Jong, 1968). In autumn, when most species of submerged macrophytes disappear, YOY pike abundance is also reduced (Grimm, 1981). Survival of 0+ pike in their first winter is determined by the amount of permanent cover, e.g. remnants of emersed plants. When permanent cover is both sparse and occupied by large pike, the recruitment of yearling pike is very low. This may result in a rather constant but low total pike biomass.

Eighty to ninety percent of the total numerical consumption of a 0+ pike class is realised in June and July. Therefore, the cutting of aquatic vegetation should not take place in the summer period. By a permanent reduction of the surface overgrown area the number and biomass of YOY pike decrease and hence their predation on the recruitment of cyprinid larvae. We do not know to what extent this reduction can take place without resulting in uncontrolled recruitment of cyprinids.

As Chapman and Mackay (1984) pointed out pike are to be found near the interface of vegetation and open water. The amount of vegetation appears to be an important factor enhancing pike biomass, if the distribution pattern and the spacing between plants allow for a habitat characterised by extensive interfaces between open water and vegetation. The management of aquatic vegetation with the aim of enhancing pike populations and predation pressure on cyprinids should, therefore, be directed at maximising the interface areas.

The fish communities associated with a gradient of vegetation change from pike-tench-rudd to bream-pike-perch associations. Intermediate species are roach and silver bream, perch and eel. The absence of bream in or near overgrown areas is related to the inefficiency of its foraging behaviour in this habitat. Perch and roach outcompete bream foraging in aquatic vegetation under clear water conditions, compared with bream which feeds most efficiently in open turbid water (Diehl, 1988). Besides, if the bottom is rich in plant remains the branchial sieves are blocked and the feeding activity is hindered. Also the body form hinders bream in its movements within overgrown areas, which may be a handicap in its avoidance reactions to predators.

Clearly recruitment of freshwater fishes is a complex process in which the controlling factors differ from species to species and from one situation to another. The examples quoted are only a small part of the available information which must be obtained and analysed if reasoned management of communities is to be undertaken.

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2. POLYACRYLAMIDE GEL ELECTROPHORESIS

2.1 Introduction

Phastsystem, manufactured by Pharmacia, is a modern integrated system for horizontal electrophoresis and isoelectric focusing on small gels, and includes automated staining and destaining.

The aim of the present study was to produce an electrophoretic "library" of British freshwater fish by staining for general proteins.

2.2 Background information, genotypic data and the process of electrophoresis

Five of the 20 common amino acids which make up proteins are charged; lysine, arginine and histidine have positive charges, while those of aspartic acid and glutamic acid are negative. Thus different proteins tend to have different net electrical charges. Electrophoresis uses this physical property of proteins to separate mixtures of proteins on the basis of charge. If allelic differences occur at a protein coding locus, the net charge of the protein often changes. Gel electrophoresis makes it possible to identify such allelic differences.

The basic procedures of gel electrophoresis can be outlined as follows:

a) Mixtures of proteins are extracted with water, or buffered aqueous solvents, from tissues such as muscle or liver.
b) The extract from each sample is introduced individually to the gel (usually either starch, agarose, cellulose acetate, or polyacrylamide).
c) When a direct electric current is applied, different forms of a particular protein often move different distances from the point of application because they do not have identical electrical charges.
d) These forms are readily identified by using stains specific to each protein type. The result is a pattern of bands, which indicate the locations of various forms of a single type of protein on a gel. This banding pattern contains information on the individual's genotype with respect to the locus, or loci, coding for that particular protein.

Most proteins that are studied by electrophoresis are enzymes because it is easy to develop histochemical staining procedures to visualise activities of specific enzymes.

2.3 Strengths and limitations of electrophoretic data for studying protein loci

In spite of the unquestionable power of electrophoresis to readily generate large volumes of reliable genotypic data, it must be kept in mind that an electrophoretic sample of 100 loci still represents substantially less than 1% of the total number of genes of a particular diploid organism (Crow, 1976). It must also be remembered that only about a third of the amino acid substitutions are detected by electrophoresis (Lewontin, 1974). Thus while electrophoretically detected differences among individuals are positive indicators of genetic differences, *the absence of differences does not necessarily mean genetic identity at the DNA level.*

2.3.1 Non-genetic factors

All electrophoretic expressions described so far have had a genetic basis. However, electrophoretic phenotypes of proteins can be strongly modified by length and conditions of storage. A common reflection of such variables is

extra bands expressed anodal or cathodal to the primary band of a particular protein. These artifacts are known as "shadow bands" and are more of a problem for some proteins than for others.

Two points are worth noting which may aid interpretation. Firstly, if one allele has a shadow band, a different allele of the same locus is likely to have an equivalent shadow band. Secondly, additional shadow bands may occur beyond the initial shadow band, with the same direction and spacing. They usually occur in progressively reduced intensities forming a serial pattern. Knowing the subunit structure of the protein, and therefore the expected number of bands and their relative intensities, helps in accurate gel interpretation.

2.4 The value of electrophoretic data

The differences detected by electrophoresis of proteins encoded by different alleles at the same locus appear to have very little or no effect on the fitness of the individual (see Kimura, 1968; Nei, 1983). This situation is a disappointment to those who had envisioned electrophoretically detected alleles as "useful genes" for breeding programs and had assumed that many such genes could be directly related to fitness (see Robertson, 1972). However, the general absence of phenotypic effects on fitness of most allelic proteins enhances the value of these variations as more or less neutral genetic markers. The main value of such markers is for inferring the distribution and magnitude of genetic variation resulting from evolutionary processes at the vast remainder of the genome that has not been sampled electrophoretically.

3. COMPARATIVE ISOELECTRIC FOCUSING OF SARCOPLASMIC PROTEINS FROM FRESHWATER FISHES

3.1 Summary

The sarcoplasmic proteins of 27 species and 1 hybrid form of British freshwater fish have been studied by thin layer polyacrylamide gel isoelectric focusing. Species-specific protein patterns were found. It is suggested that this method may be of value for the identification of fish fry and hybrids, which can be impossible to distinguish using morphological characteristics.

3.2 Introduction

Isoelectric focusing (IEF) is an electrophoretic technique using large-pore polyacrylamide gel in which is incorporated a mixture of synthetic polyamino polycarboxylic acids ("carrier ampholytes") with a range of isoelectric points. When an electric potential is applied to the gel, the ampholytes form a stable pH gradient from one end of the gel to the other.

When a mixture of proteins is introduced into this pH gradient, the various proteins will move electrophoretically until they reach the point on the gel where the pH is equal to their isoelectric point (pI). At this point the protein is electrically neutral. Should it diffuse from this point, it will develop charge and move back to the pI point to become concentrated, or focused, into a very narrow zone on the gel. This method gives very high resolution and excellent reproducibility.

IEF has long been recognised as a valuable tool for the reliable identification of fish species. For example, Lundstrom (1977) reported that 11 species of marine fish could be clearly distinguished through the IEF banding patterns of their sarcoplasmic proteins.

The object of this study was to produce the characteristic banding patterns, or "fingerprints", for a number of species of British freshwater fish. Such a collection of fingerprints would allow the routine identification of these species.

3.3 Materials and methods

At least two specimens of each fish species (see Table 1) were obtained from various parts of southern England. They were either frozen immediately to -20 deg.c and used within two weeks, or kept alive until required.

Approximately 0.25g of white skeletal muscle was removed from each fish. (If fish fry were being analysed the caudal region was used.) A crude homogenate was prepared by crushing the tissue in an equal volume of ice-cold extracting buffer (0.01M Tris-HCl pH 7.4). The homogenate was then centrifuged at 1000g for 5 minutes and the resulting supernatant used for IEF. The Pharmacia Broad Range pI Calibration Kit was also applied to each gel to ensure the accurate measurement of pI values.

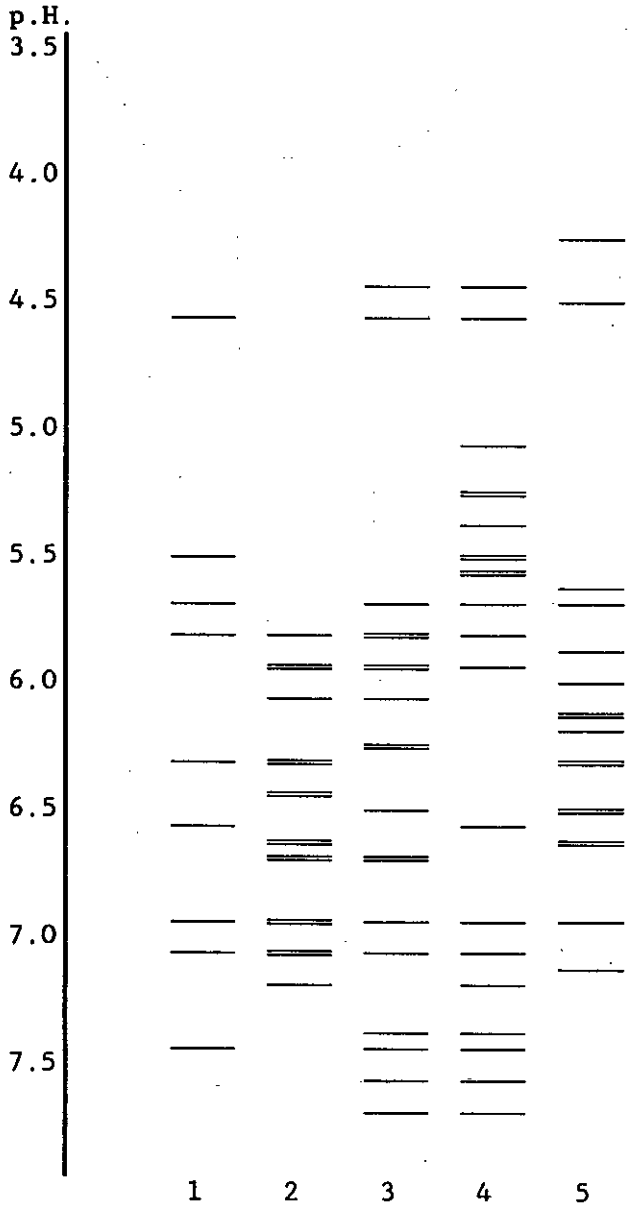
Table 1

Species studied	
Grayling	*Thymallus thymallus*
Rainbow trout	*Oncorhynchus mykiss*
Brown trout	*Salmo trutta*
Salmon	*Salmo salar*
Dace	*Leuciscus (Leuciscus) leuciscus*
Chub	*Leuciscus (Squalius) cephalus*
Golden orfe	*Leuciscus (Idus) idus*
Minnow	*Phoxinus phoxinus*
Gudgeon	*Gobio gobio*
Barbel	*Barbus barbus*
Bleak	*Alburnus alburnus*
Common carp	*Cyprinus carpio*
Goldfish	*Carassius auratus*
Bitterling	*Rhodeus sericeus amarus*
Roach	*Rutilus rutilus*
Rudd	*Scardinius erythrophthalmus*
Common bream	*Abramis brama*
Silver bream	*Blicca bjoerkna*
Brook lamprey	*Lampetra planeri*
Bullhead	*Cottus gobio*
Eel	*Anguilla anguilla*
Flounder	*Platichthys flesus*
Perch	*Perca fluvatilis*
Pike	*Esox lucius*
Ruffe	*Gymnocephalus cernua*
Stone loach	*Cobitis taenia*
Three-spined stickleback	*Gasterosteus aculeatus*
Hybrid studied	
Roach-Rudd	*Rutilus rutilus-Scardinius erythrophthalmus*

3.4 Results and discussion

Figure 1 shows the sarcoplasmic protein patterns from the five salmonid species that were analysed, as well as the Pharmacia Broad pI Calibration Kit. The pattern for each species appears to be unique and demonstrates much greater resolution than convential electrophoresis. Warping of bands which occurred towards the application point of brown trout and salmon is a well known phenomenon indicating that the ionic concentration of the samples was too high.

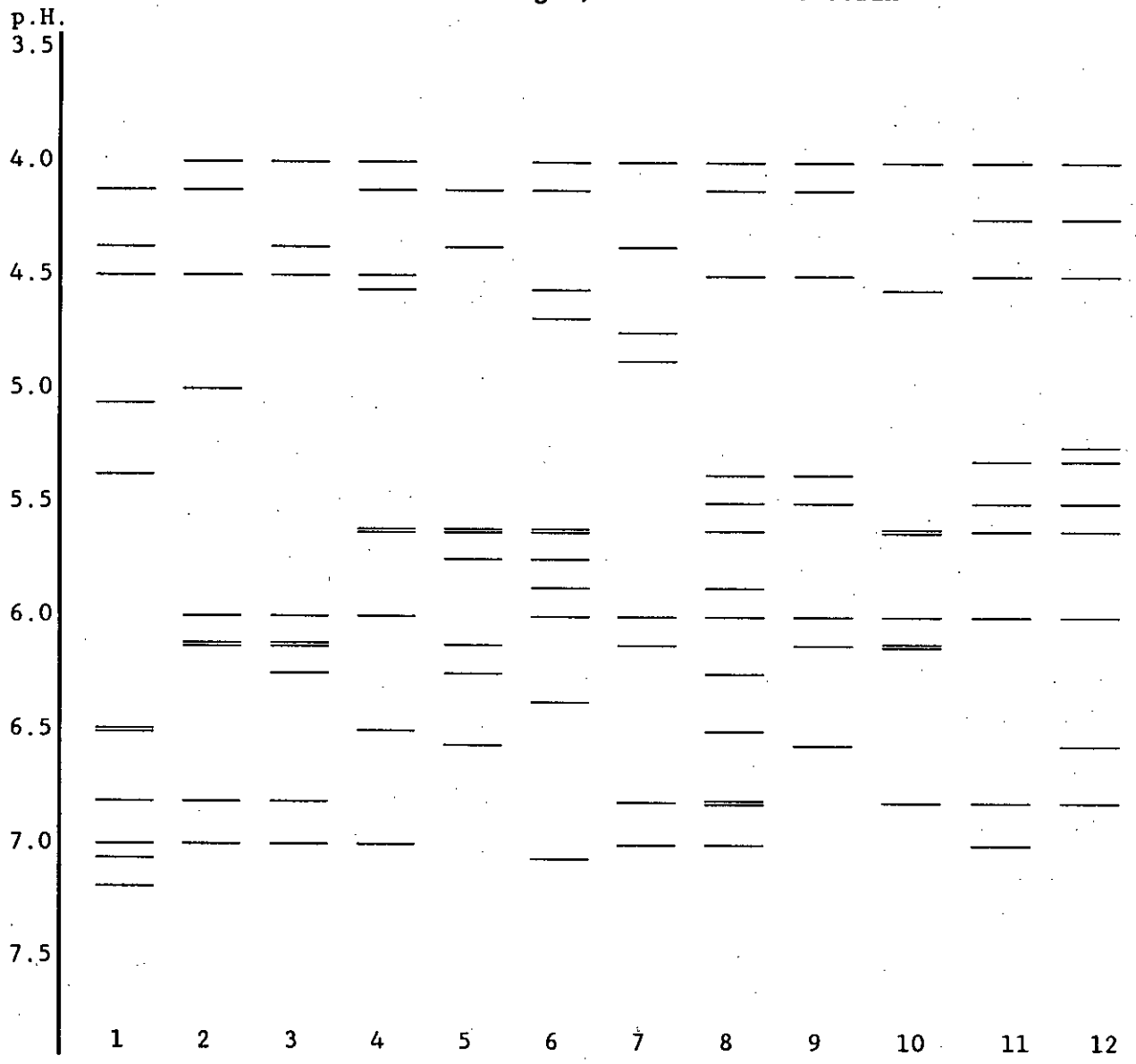
Figure 1 Major sarcoplasmic protein bands from five salmonid species obtained from IEF 3-9 Phastgel, Coomassie Blue stain



1 = Grayling *(Thymallus thymallus)*
2 = Rainbow trout *(Oncorhynchus mykiss)*
3 = Brown trout *(Salmo trutta)*
4 = Salmon *(Salmo salar)*
5 = Charr *(Salvelinus alpinus)*

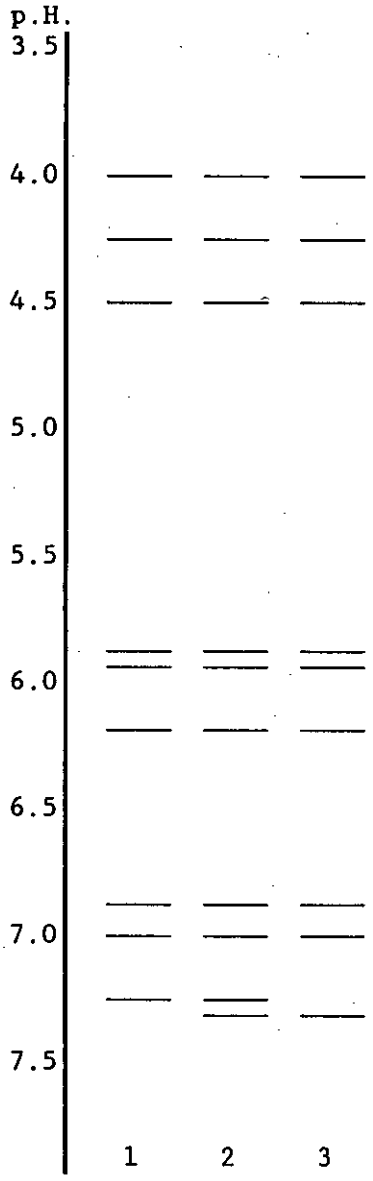
Figure 2 shows the fingerprints of 12 cyprinid species and Figure 3 of two cyprinids and their hybrid. Despite some intra-specific variation,which appears to be genetic, the banding patterns seem to be sufficiently different between species to allow a confident identification. Even roach, roach-rudd hybrids and rudd are distinguishable.

Figure 2 Major sarcoplasmic protein bands from twelve cyprinid species obtained from IEF 3-9 Phastgel, Coomassie Blue stain



1 = Dace *(Leuciscus leuciscus)*
2 = Chub *(Leuciscus cephalus)*
3 = Golden orfe *(Leuciscus idus)*
4 = Minnow *(Phoxinus phoxinus)*
5 = Gudgeon *(Gobio gobio)*
6 = Barbel *(Barbus barbus)*
7 = Bleak *(Alburnus alburnus)*
8 = Common carp *(Cyprinus carpio)*
9 = Goldfish *(Carassius auratus)*
10 = Bitterling *(Rhodeus sericeus amarus)*
11 = Common bream *(Abramis brama)*
12 = Silver bream *(Blicca bjoerkna)*

Figure 3 Major sarcoplasmic protein bands from two cyprinid species and their hybrid obtained from IEF 3-9 Phastgel, Coomassie blue stain

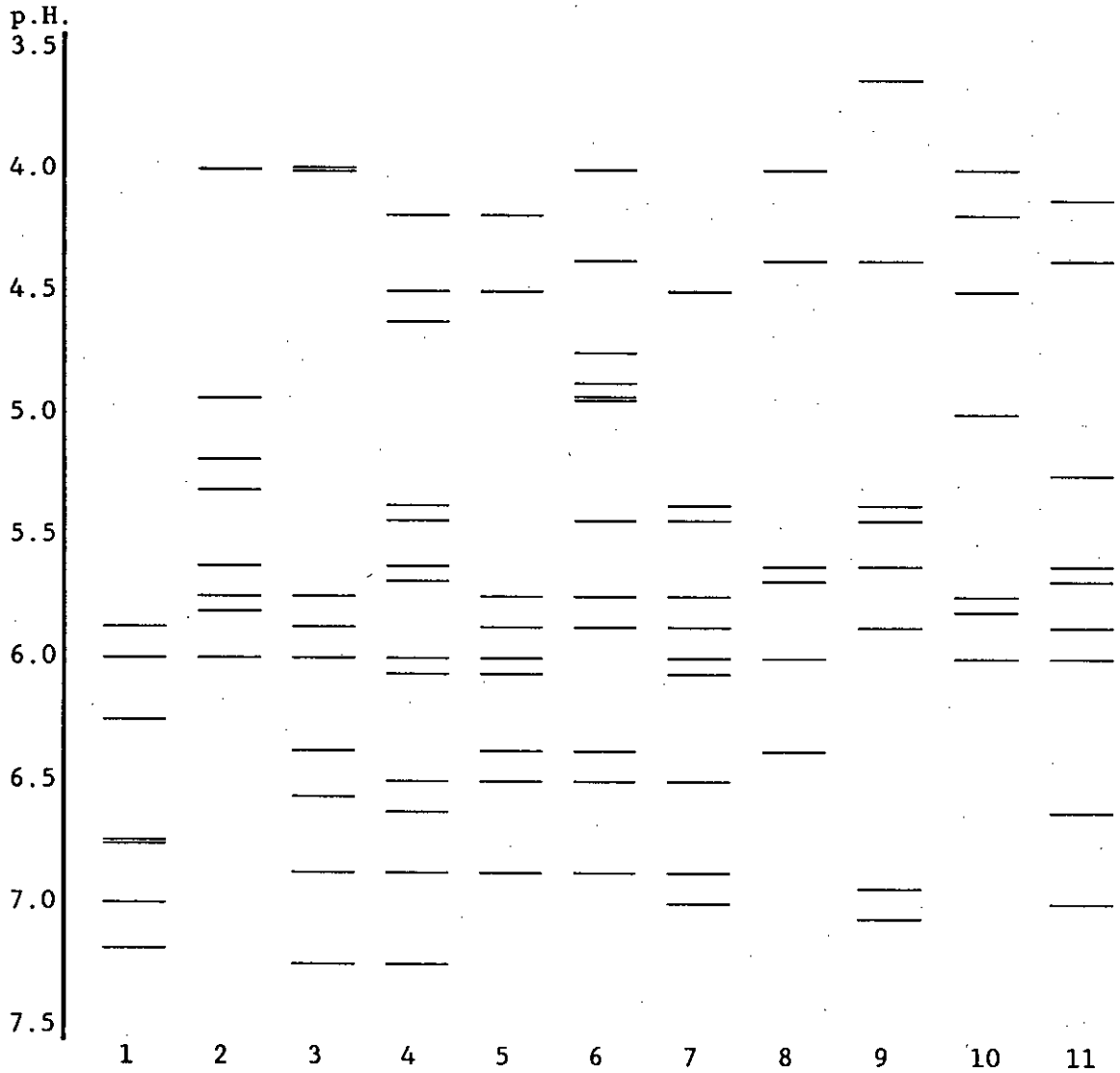


1 = Roach *(Rutilus rutilus)*
2 = Roach\Rudd hybrid *(Rutilus rutilus\Scardinus erythrophthalmus)*
3 = Rudd *(Scardinus erythrophthalmus)*

The banding patterns of five cyprinid fry corresponded with those of the adult fish. This implies that IEF would be a very useful tool for identifying the fry of certain cyprinids: for example, those which can be very difficult to identify using morphological characteristics only.

Figure 4 shows the sarcoplasmic protein patterns of the remaining 11 fish species. Again the banding patterns appear to be unique.

Figure 4 Major Sarcoplasmic protein bands from eleven, non cyprinid, fish species obtained from IEF 3-9 Phastgel, Coomassie Blue stain

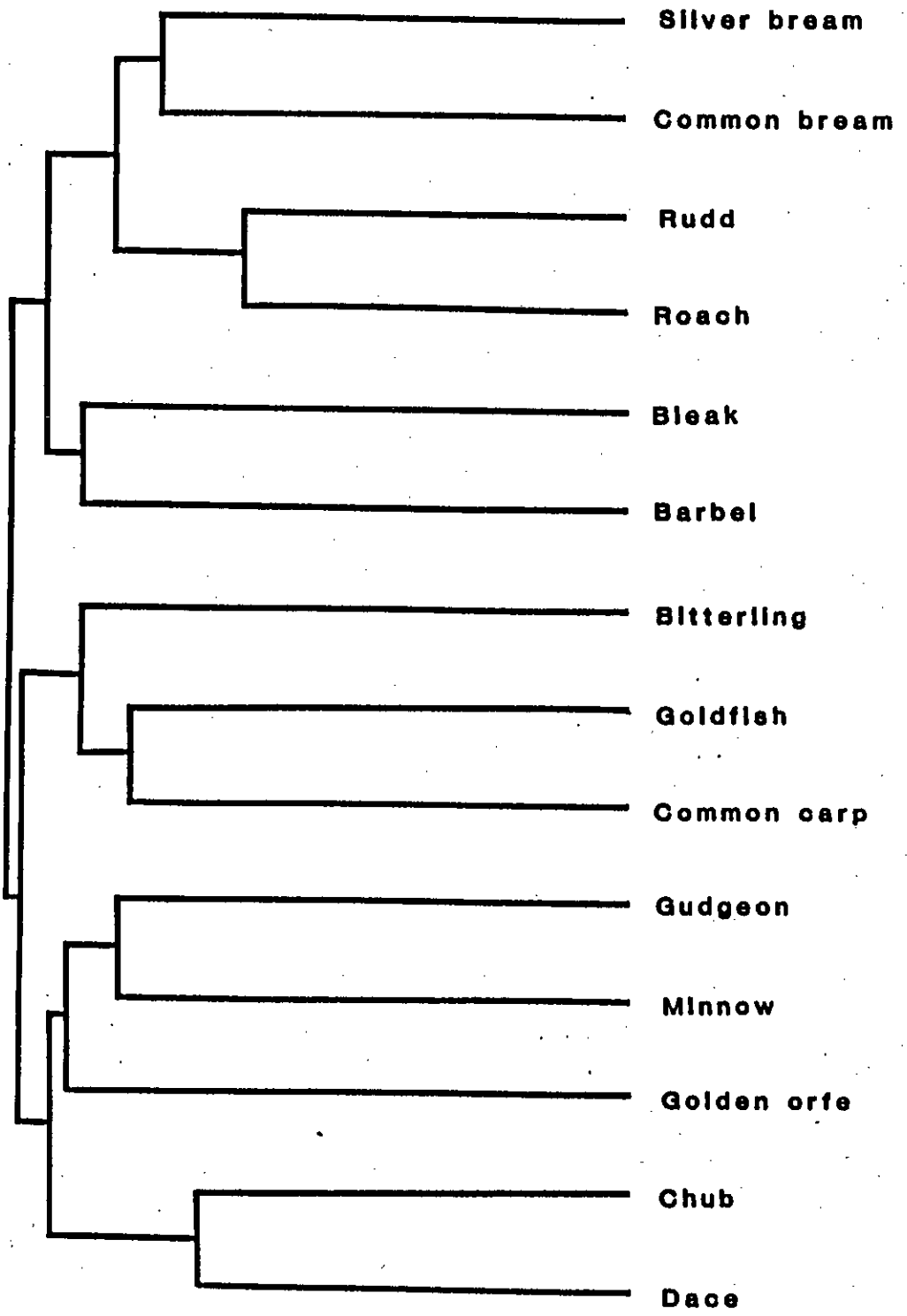


1 = Brook lamprey *(Lampetra planeri)*7 = Ruffe *(Gymnocephalus cernua)*
2 = Bullhead *(Coppus gobio)* 8 = Stone loach *(Noemacheilus barbatulus)*
3 = Eel *(Anguilla anguilla)* 9 = Spined loach *(Cobitis taenia)*
4 = Flounder *(Platichthys flesus)*
5 = Perch *(Perca fluviatilis)*
6 = Pike *(Esox lucius)*
10 = Three-spined stickleback *(Gasterosteus aculeatus)*
11 = Pumpkinseed *(Lepomis gibbosus)*

It is interesting that the salmonid banding patterns had relatively more bands than those of the other fish species. This is probably due to their tetraploid ancestry which has resulted in the salmonids having 50% more loci expressed than teleosts of diploid ancestry (see Allendorf and Thorgaard 1984).

An attempt was made to quantitatively assess the similarities and differences between the cyprinid species. A band counting method described by Ferguson (1980) was used to calculate coefficients of similarity between each species and then a dendrogram was drawn (see Figure 5). This method has serious

Figure 5 Dendrogram generated by UPGMA cluster analysis showing the relationships found among the cyprinids



limitations as far as inferring phylogenetic relationships is concerned. For example, electrophoretically coincident protein bands in two species may represent different proteins. In spite of these problems, a few tentative conclusions can be drawn from the dendrogram. The pair of fish deemed to have the most similar banding patterns are the roach and the rudd, which are from different genera. The dace and chub, of the same genus, are second in the order of similarity. This surprising result does correspond with what is known about hybridisation within the cyprinids. Wheeler (1969) states that roach-rudd hybrids are common and often fertile, while there is no mention of the existence of dace-chub hybrids. Both lines of evidence suggest that despite the way in which these species have been classified, roach and rudd are more similar at the genetic level than are dace and chub.

The major bands present for each fish species between the pH range 3.50-7.85 are represented above. A dotted line could be used to indicate a band that was not present in all individuals of a species. Since the isoelectric point of each of the bands is known, such data could easily be used to positively identify fish species without having to maintain a supply of known species as standards. This is one of the great values of IEF.

Returning to the earlier argument on the subject of phylogenetic relationships, the similarity between roach and rudd is emphasised in Figure 3. The common bream also appears to be similar in major protein bands to the roach and rudd. This again ties in with hybridisation data: roach-common bream hybrids are common and often fertile, while rudd-common bream hybrids also exist in certain waters. It would be interesting to know how these species, which are members of different genera, would have been classified had the early taxonomists had access to current biochemical techniques such as IEF.

3.5 Conclusions

Due to the high resolution and excellent reproducibility of IEF, this technique is an invaluable tool allowing the routine identification of fish species without the need to maintain a stock of known species as standards. It is recommended that IEF will be of particular use for identifying certain fish fry and hybrids, which are often impossible to distinguish on the grounds of morphological characteristics alone.

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4. STUDIES ON JUVENILE DACE IN THE HAMPSHIRE AVON

The Hampshire Avon downstream of Salisbury has long been regarded as one of Britain's best coarse fisheries. In recent years there has been widespread concern that the numbers of coarse fish, in particular dace, *Leuciscus leuciscus* (L.) and roach, *Rutilus rutilus* (L.) have declined dramatically. Such complaints are very subjective and in the autumn of 1987 a survey of the river, funded by the Department of the Environment, was carried out by the Freshwater Biological Association (FBA). Twelve sites between Salisbury and Christchurch were electrofished in order to determine the structure of the coarse fish populations and to provide a data bank against which to monitor any future changes (Mann et al., 1987).

There is very little factual information against which to compare the results of the FBA survey. The survey did, however, indicate that the total fish biomass in the River Avon is comparable with two nearby rivers, the Frome and

Stour. Anecdotal evidence from anglers and river keepers suggests that in the past the fish populations in the River Avon were considerably higher than both of these rivers.

The FBA survey revealed a number of trends in the growth of Avon dace. In particular 0, 1 and 3 group dace increased in size with increasing distance downstream, but this trend was absent from older fish. These observations were based only on actual lengths (no back-calculated lengths were calculated) the sample size was small. Mann et al. (1987) suggest that extensive movements of dace within the river is the reason that lengths at age 4 and older showed no significant trend of increasing growth at downstream sites. There are a number of large weirs on the River Avon; thus the validity of this hypothesis is open to question, particularly with respect to upstream movement.

The aims of the present study were to observe distribution and to gain a fuller knowledge of factors affecting the growth of Avon dace by looking in more detail at the scales of dace fry samples from this year. This included looking to see if there were any trends in growth that were not apparent from actual lengths (Mann et al., 1987) but which could be detected by examining growth rates in previous years obtained from back-calculated lengths.

4.1 Methods

4.1.1 Scale reading

A number of authors have demonstrated the annual occurrence of the annuli on dace scales (Hartley, 1947; Cragg-Hine and Jones, 1969; Matthews and Williams, 1972; Mann, 1974). Annuli were clearly visible on the dace scales without the need for cleaning and mounting. The scales were viewed on a 'Projectina' micro-macro projection microscope at x20 magnification.

False checks were rare and could easily be distinguished from true annuli because they did not form a complete ring around the scale. Confirmation of ages was made by comparison with the results obtained by the FBA staff.

4.1.2 Back-calculation

A single readable scale was selected at random and a strip of paper placed along the anterior radius of the projected image of the scale. The position of the scale centre, each annulus and the edge of the scale were then marked on the strip (Bagenal and Tesch, 1978). The relative length of each annulus was calculated and a nomograph (Le Cren, 1947; Mann, 1973) was used to correct for allometric growth. The nomograph used was one constructed from data obtained over a number of years from dace in the River Frome, Dorset, which is a similar habitat to the River Avon. Any scale over which there was doubt about the position of one or more annuli was excluded from the data.

Back-calculated lengths for a given age group of fish are frequently smaller the older the fish from which they are calculated. This is known as Rosa Lee's phenomenon (after Lee, 1920). The only way this can be detected is by comparison of back-calculated lengths with the actual lengths of fish of the same year-class measured in previous years. No such data exist for the River Avon. Therefore it is not possible to determine whether Lee's phenomenon has occurred in the present study.

Dace are known to grow very little over winter months (Kennedy and Fitzmaurice, 1969; Mann, 1974). As the present survey was carried out in the autumn it has been assumed that growth had finished for the year. For this reason any "+ growth" has been regarded as representing the total growth for the year 1987-1988. To avoid confusion ages are given as the age at the next birthday.

4.1.3 Growth rates

The instantaneous growth rates of 0 group dace were calculated from $G = \ln (Wt + i/Wt)$ where Wt = weight on hatching = 0.003 g (in Mann, 1974), and Wt + i was calculated from $\log W (g) = \log a + b \log L (mm)$. Instantaneous growth rates of dace older than 0 group were calculated directly from lengths using $G = b \frac{(\ln Li - \ln Lo)}{ti - to}$

The values of log *a* and *b* were taken from data for the River Frome (Mann, 1974) (Table 2).

Table 2 Values of log *a* and *b*

Category	*b*	log *a*
0 group ♀ ♂	2.762	-4.586
>0 group ♀ ♂*	3.198	-5.305

(*The sex of fish caught in the Avon was not recorded so a mean of the values for >0 group ♀ ♀ and >0 group ♂ ♂ was used.)

4.1.4 Temperature data for past years

Daily water temperatures recorded from the River Avon at Christchurch were obtained for the years 1982-1987 from the West Hampshire Water Company. The River Avon is known to exhibit significant temperature variation over 24 hours during the summer months (R. Foot, personal communication) and for this reason mean monthly temperatures from April to October were calculated using only those recordings taken within one hour of 11.00 a.m. so as to reduce variation caused by the time of recording.

Though temperature recordings made this year indicate significant variation along the river these data were used as an index of relative temperature between years. Temperature-growth correlations for each year-class at each site were made using cumulative degree days from April to July, August, September and October.

4.1.5 Fry collection

Dace fry were collected at two-weekly intervals from 17 May 1988 through to 9 August 1988 at six sites along the River Avon between Stratford-sub-Castle and Sopley. The location of these sites is given in Table 3. The fry were located visually and collected with a fine-meshed pond net and a long-handled micromesh "landing net" of the type used by anglers. The fry were killed and preserved in 90% alcohol. Any alteration in length caused by the method of preservation was assumed to be constant.

Table 3 Fry collection sites

Site	NGR	Relative distance downstream (km)
Stratford-sub-Castle	SU 179239	1
Charlton-all-Saints	SU 129330	9.5
Fordingbridge	SU 148140	27
Ibsley	SU 149097	32
Ringwood	SU 142048	38
Sopley/Avon Causeway	SZ 150978	50

Initially dace were found only in very slow-flowing and still water habitats, particularly behind stands of emergent vegetation such as *Glyceria* sp. As the fry grew they were found in a greater diversity of habitats, in particular behind large beds of *Ranunculus* sp. across the width of the river and on gravel shallows in sunny weather.

Fry were collected at a number of locations at each site whenever possible but this became increasingly difficult later in the year when the dace formed into large shoals, often with minnows and 1+ chub. These shoals were frequently found at the same location on successive visits. Initially 10 fry were collected from each site but this was increased to 30 from collection 5 onwards when the variation in size became larger.

Roach fry were also collected at each site and the lengths measured. However, from the end of June onwards they became very difficult to locate and with the available methods it was not possible to collect more than 3 or 4 from each site.

4.1.6 Fry identification and measurement

Larval dace were identified using the key prepared by Bracken and Kennedy (1967). Once the scales and fins had become fully formed they were identified using "The Key to Fishes of Northern Europe" (Wheeler, 1978).

When the dace had reached approximately 30 mm they could generally be distinguished from other species such as 0+ and 1+ chub in the field. Identification was confirmed in the laboratory at the time of measuring.

The fork length of the fry was measured by laying them along a ruler and viewing under a low powered stereo microscope. Measurements were taken to the nearest 0.5 mm.

4.1.7 Growth rates of dace fry

Instantaneous growth rates over the whole sampling period and for each fortnightly period were calculated directly from lengths using the same equation as for the back-calculated data. The value of *b* for 0 group dace is shown in Table 2.

4.1.8 Temperature data for 1988

Temperatures were recorded on "Squirrel" loggers at Salisbury and Ringwood. The mean daily temperature was calculated from hourly recordings and cumulative degree days calculated from daily means.

The River Avon is predominantly spring fed and hence an increase in water temperature downstream from the source is expected in the summer due to increasing exposure to warmer air temperatures. This is supported by the data from Salisbury and Ringwood. In all cases the mean daily temperature measured over two-week periods was higher at Ringwood (Table 4).

A linear relationship between temperature and distance downstream was assumed and the temperature gradient between Ringwood and Salisbury was calculated for each two week period. The temperature for each site was then calculated using the distance upstream or downstream of the nearest temperature recorder (Table 3).

Table 4 Temperature data from River Avon over summer 1988

Date	Collection period	Temperature degree days		Difference over 14 days	Mean daily difference
		Ringwood	Salisbury		
17.5.88-31.5.88	1	203.08	190.69	+12.39	0.89
1.6.88-14.6.88	2	209.49	197.74	11.75	0.89
15.6.88-28.6.88	3	253.72	234.66	19.06	1.36
29.6.88-12.7.88	4	232.12	215.87	16.25	1.16
13.7.88-26.7.88	5	237.46	218.46	19.00	1.36
27.7.88- 9.8.88	6	238.23	216.96	21.17	1.52

4.2 Results

4.2.1 Back-calculated data

Lengths at the end of each year's growth were calculated for all dace aged 7 and under (Appendix 2). The number of fish older than 7 was very small and for simplicity they were excluded from any statistical analysis. Instantaneous growth rates are shown in Appendix 3.

Analysis of variance shows that there is a significant difference in the size of fish of the same age between both sites and years. The sites surveyed were chosen so that they were equally spaced so for simplicity site numbers from 1-12 were used as an index of distance downstream. Allowing for variation between sites there is a highly significant difference ($p<0.01$) in lengths between years for all ages up to age 5 (Table 5). Similarly allowing for differences between years there is a significant difference in length between sites (Table 5). Using pooled data for all year-classes there is a highly significant ($p<0.01$) linear trend of increasing length with distance downstream at all ages up to age 3. At age 4 the significance of this trend is reduced ($p<0.05$), and at ages 5 and 6 there is no significant trend of increasing length at downstream sites (Table 5).

Table 5 Analysis of variance on back calculated lengths

Source of variation	Age of dace					
	1	2	3	4	5	6
Sites/year % total SS	19.5%	32.0%	34.5%	39.0%	44.9%	51.9%
Year/sites % total SS	3.8%	19.3%	22.0%	13.6%	4.5%	0.9%
$F_{YEAR}\frac{(Y/S\ MS)}{(Error\ MS\ (Y+S))}$	8.17** (5,837)	59.04** (5,837)	76.35** (4,663)	55.56** (3,589)	13.93** (2,314)	3.32 (1,182)
$F_{SITE}\frac{(Y/S\ MS)}{(Error\ MS\ (Y+S))}$	18.87** (11,837)	44.48** (11,837)	43.65** (11,663)	43.36** (11,589)	25.26** (11,314)	18.0** (11,182)
$F_{LINEAR/YEAR}$	10.58** (1,10)	37.48** (1,10)	23.09** (1,10)	9.94* (1,10)	2.25 (1,10)	0.96 (1,10)

** $p \geq 0.01$
* $p \geq 0.05$

Growth rates for each year of every year-class were regressed against distance downstream (Table 6). There is a tendency for 0 and 1 group fish to grow faster with increasing distance downstream. In 2 group and older fish this trend is reversed, with growth being faster at upstream sites.

Table 6 t-ratios of regression of instantaneous growth rate in each year against distance downstream (site number)

	Year class					
Age	1987	1986	1985	1984	1983	1982
0+	-	2.30*	-1.85	1.61	4.3**	1.31
1+	3.47**	2.21	-2.65*	2.44*	3.61**	-
2+	2.00	0.66	-2.31*	-2.28*	-	-
3+	-3.82**	-5.03**	-5.35**	-	-	-
4+	-3.88*	-2.28*	-	-	-	-
5+	-2.00	-	-	-	-	-

** $p<0.01$
* $p<0.05$

Although the growth rates from the 1982 and 1984 year-classes show no significant correlation with distance downstream it can be see that there are trends of increasing growth downstream in these years if sites 1-7 and 8-12 are treated separately (Figures 6-11). In both years this trend is not significant for sites 1-7 but it is significant ($p<0.05$) for sites 8-12.

Growth rates at each age were correlated against total degree days from the beginning of April to the end of July, August, September and October. This was done separately for each site because of the large variation in growth at different sites, even allowing for the differences between years (Table 5). No significant correlation was found, however. In nearly all cases the highest correlation was found with total degree days to the end of September.

4.2.2 Fry collection

The mean lengths of fry collected over the summer of 1988 are shown in Table 7. Due to the small variation in lengths at collection 1 growth was initially measured as length increase from this time. The regression of total length increase over the sampling period against distance downstream accounts for 69.2% of the variation in length increase. Thus assuming that there is a linear relationship between temperature and the distance downstream this length increase is significantly correlated ($p<0.05$) with temperature.

Table 7 Mean lengths (mm) of fry collected at the six sites

	Collection No.						
Site	1	2	3	4	5	6	7
1	13.05	14.70	19.25	21.65	26.82	35.03	40.70
2	14.05	15.25	19.45	24.55	28.17	35.00	39.90
3	14.25	16.80	20.60	21.70	29.53	36.37	44.07
4	15.10	18.10	20.65	22.65	32.93	39.83	45.12
5	14.40	17.80	20.30	22.85	36.67	38.95	43.63
6	14.75	17.70	20.50	27.30	35.77	44.57	46.28

Figure 6 Instantaneous growth rates of 0 group dace from 1982 year-class

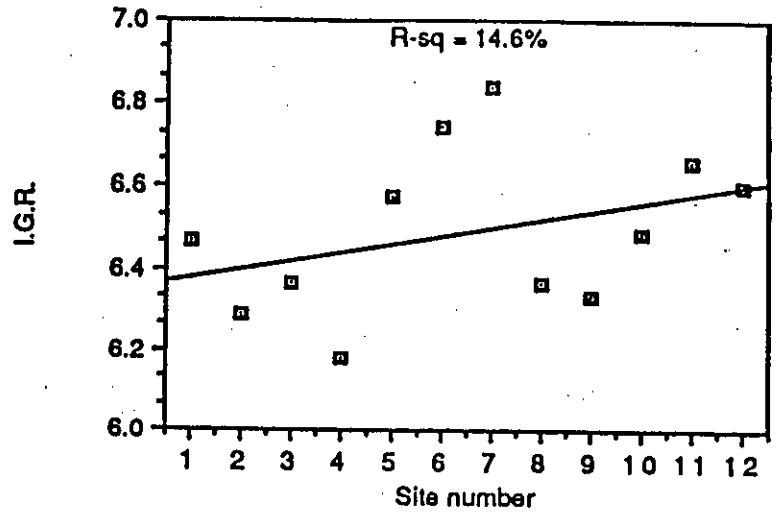


Figure 7 Instantaneous growth rates of 0 group dace from 1982 year-class

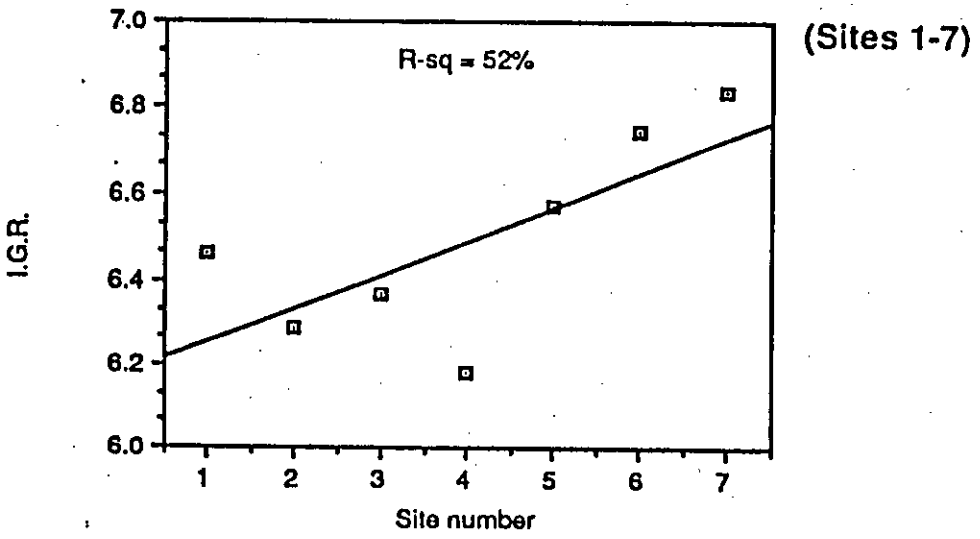


Figure 8 Instantaneous growth rates of 0 group dace from 1982 year class

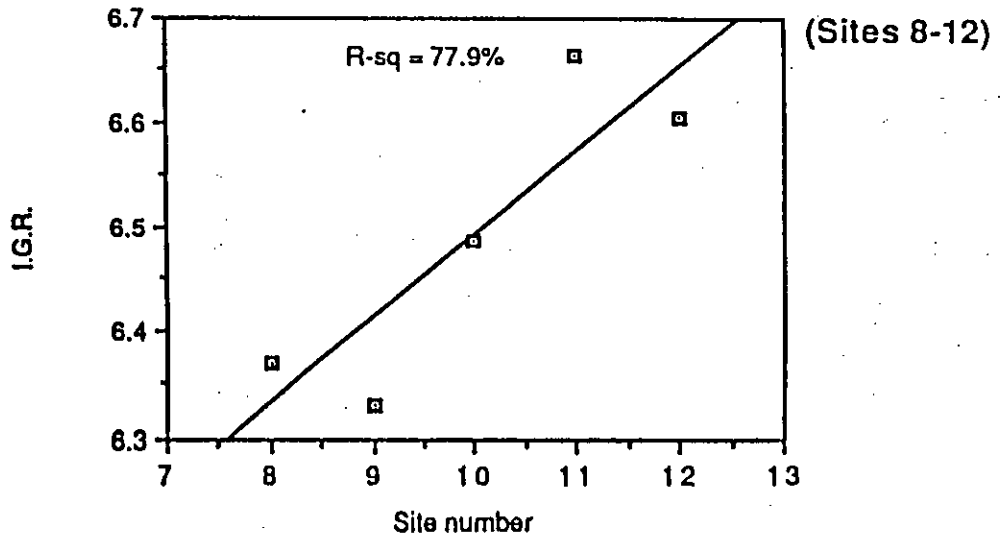


Figure 9 Instantaneous growth rates of 0 group dace from 1984 year class

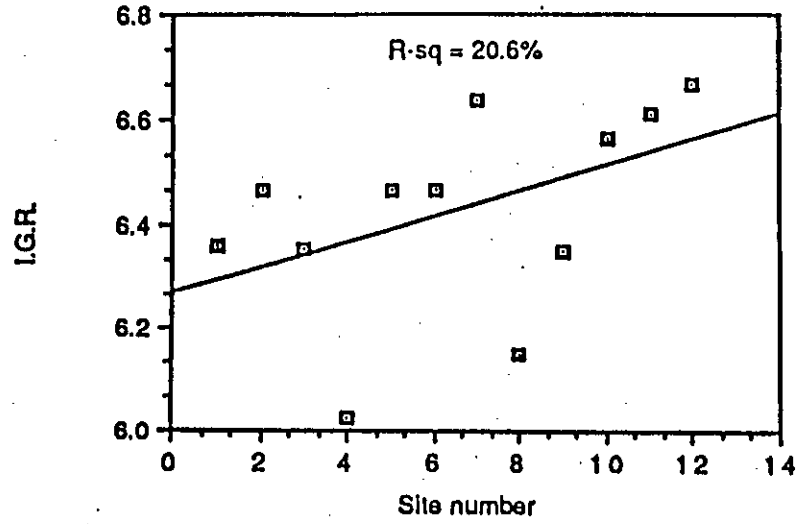


Figure 10 Instantaneous growth rates of 0 group dace from 1984 year class

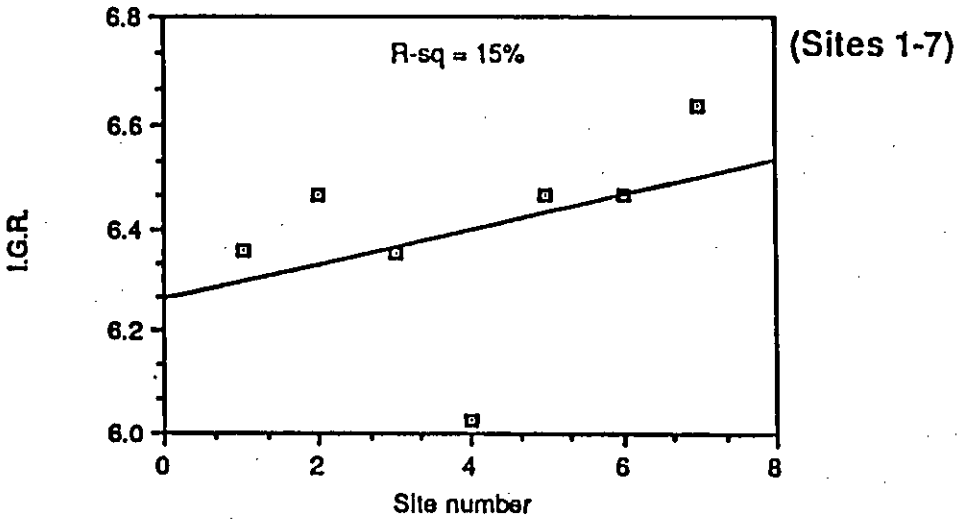
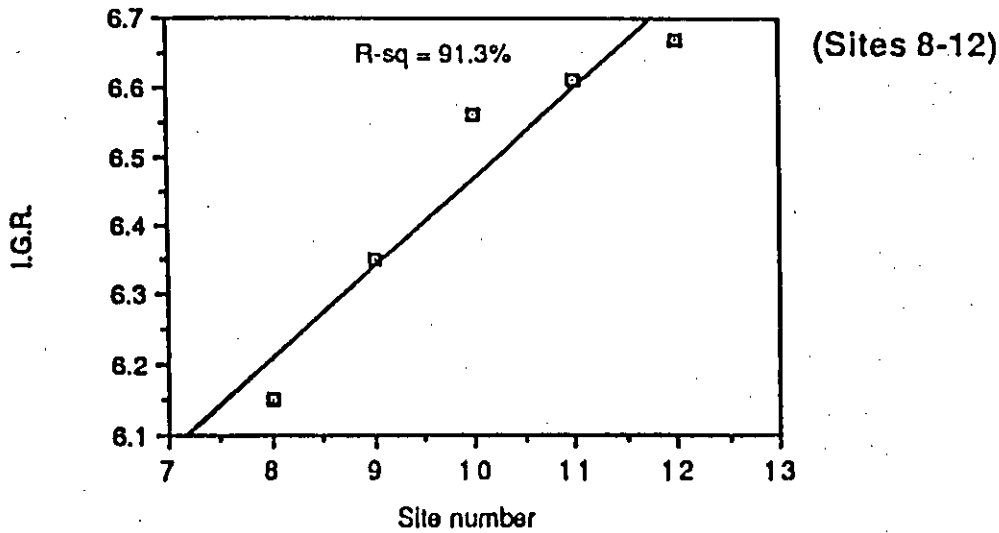


Figure 11 Instantaneous growth rates of 0 group dace from 1984 year class



The mean weights of dace at each collection were calculated (Table 8). The regression of weight against distance downstream shows a significant correlation ($p<0.05$) for weights at the first and last collections (Figures 12 and 13). The regression of instantaneous growth rates measured over the whole sampling period against distance downstream is not significant (Figure 14). Instantaneous growth rates measured between successive collections show no significant correlation with distance downstream.

Table 8 Mean weight (g) of dace fry at each collection

	Site number					
Collection	1	2	3	4	5	6
1	0.032	0.038	0.041	0.047	0.041	0.044
2	0.044	0.048	0.063	0.078	0.074	0.073
3	0.092	0.095	0.111	0.112	0.108	0.110
4	0.128	0.184	0.131	0.145	0.148	0.250
5	0.234	0.266	0.306	0.412	0.549	0.525
6	0.487	0.488	0.542	0.699	0.650	0.946
7	0.740	0.691	0.917	0.973	0.884	1.047

Instantaneous growth rates at each site were correlated against three sets of temperature data. These were:

i) data from the two weeks in which growth was measured (0 week lag);
ii) data set one week back (1 week lag);
iii) data set two weeks back (2 week lag).

This was to see if temperature had a delayed effect on growth. No significant correlation was found (Appendix 6).

4.3 Discussion

In common with most studies of freshwater fish growth in Britain the present study demonstrates significant differences in growth between years. Mills and Mann (1985) studying dace growth in the River Frome found a significant correlation between growth rate and temperature measured as degree days over 12°C. No correlation was found between temperature and growth rate in this study, but this may have been due to the temperature data available. Mean monthly temperatures were calculated from only approximately twenty readings each month, and the lack of suitable data meant no correlation with degree days over 12°C was possible.

Instantaneous growth rates of dace of each year-class show that growth of 0 and 1 group dace increases with distance downstream. This trend is reversed in 2 group and older dace, which grow quicker with increasing distance upstream. From this it is apparent that the observed trend of increasing size at downstream sites is caused by faster growth in the first two years only. At ages 5 and 6 there is no significant trend of increased or decreased growth with increasing distance downstream. Unfortunately very few dace aged 6+ and over were caught in the survey so it is not clear whether older fish continue to grow more quickly at upstream sites and become larger than those at downstream sites or whether they simply catch up with those at downstream sites.

The fact that back-calculated lengths of fish over the age of 3 show a trend of increasing growth in the first two years indicates that dace in the River Avon do not move large distances within the river. This conflicts with evidence from the River Frome where dace were found to move large distances

Table 12 Weight of 1988 0 group dace at collection 1

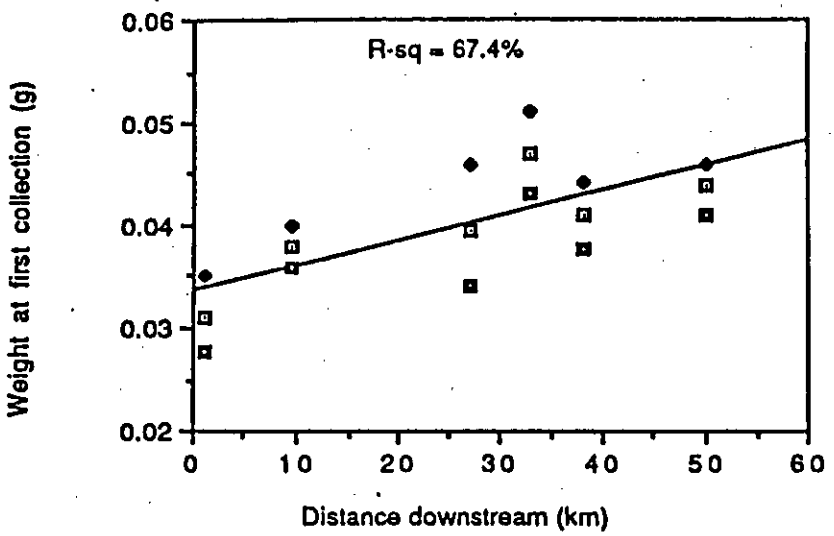


Figure 13 Weights of 1988 0 group dace at collection 7

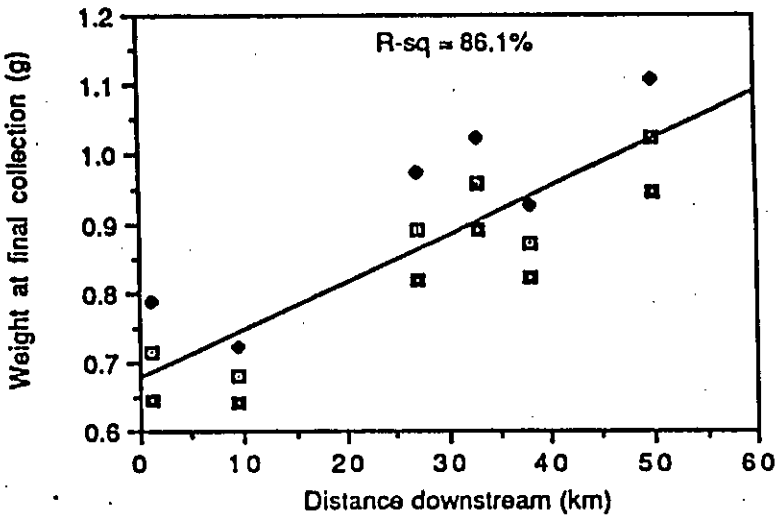
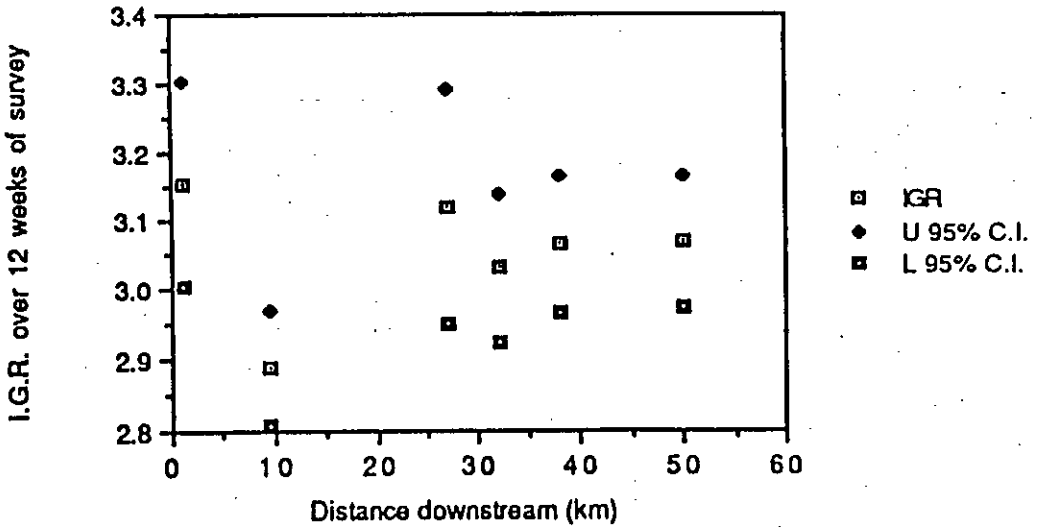


Figure 14 Instantaneous growth rates of 0 group dace over sampling period



(W. Beaumont, personal communication). The reason for the difference is probably that the weirs on the Hampshire Avon constitute a major barrier to the movement of small fish such as dace.

The observed trends in growth cannot be explained by variations in size-selective mortality between sites since the growth rates at each age were back-calculated from the same group of fish rather than from samples caught in successive years.

The two major factors known to affect growth are temperature and food supply (Kennedy and Fitzmaurice, 1969). Dace growth has also been shown to vary with the sex of the fish. Cragg-Hine and Jones (1969) and Hellawell (1974) found that male dace grew more quickly than female dace, particularly after the onset of maturity. However, Mann (1974) and Cowx (1988) found very little variation in growth between males and females. The sex of fish caught in the FBA survey was not recorded. However, even if males grow more quickly in the River Avon a higher ratio of males:females at upstream sites would not explain the reversal in the trend of increasing growth at downstream sites at age 2.

Temperatures recorded at Ringwood and Salisbury from May 1988 to August 1988 indicate that the downstream site, Ringwood, has a mean daily temperature of 16.4°C which is 1.2°C higher than the mean daily temperature at Salisbury. Mills and Mann (1985) found that in the River Frome, a similar chalk stream to the River Avon, the growth of 0 group dace was very significantly correlated with the annual temperature measured as degree days over 12°C. If this is the case in the River Avon a difference of 1.2°C between Ringwood and Salisbury represents approximately a 30% increase in the temperatures above which growth can occur. From this it is clear that if the same temperature regime is found in the River Avon in all years, as was the case for the Exe catchment (Webb and Walling, 1986) temperature differences may account for a significant amount of the increased growth of 0 group and possibly 1 group dace at downstream sites. The reversal of this trend in 2 group and older dace indicates that temperature does not have a limiting influence on the growth of dace aged 2 and over in the River Avon.

A growth curve for dace in the River Avon pooling data for all sites (Mann et al., 1987) indicates that growth does not slow up until fish attain a length about 180 mm. Other dace growth curves (e.g. Williams, 1967; Cragg Hine and Jones, 1969; Hine, 1970; Philippart, 1971; Hellawell, 1974; Mann, 1974) are all similar up to this length with the exception of Williams who studied a stunted population in the River Thames.

Given that the maximum mean length of dace in the River Avon at age 3 is less than 180 mm the reversal in the trend of increased growth at age 2 cannot be explained by dace at downstream sites reaching a levelling off point on the growth curve.

Cowx (1988) found that in the Exe catchment growth was higher at all ages for dace in stretches which were most similar to the "preferred" habitat characteristics of the species described by Wheeler (1969). In the case of dace this is clear, fast-flowing, well-oxygenated water. Subjective site assessments made during the survey do not indicate that depth, flow and weed cover show any trend along the river (Welton et al., 1988) though a more detailed survey would be necessary to confirm these findings. Cowx suggested that competition with roach was the major cause of changes in growth between sites with different physical characteristics and he cited a decline in the growth rate of one or other species, whenever the two species coexisted, as evidence for this. In the River Avon the two most abundant species which may compete for food with dace are roach and chub. Data from the FBA survey (Mann

et al., 1987) indicate that there is no relationship between the relative biomass of dace and that of i) roach, ii) chub and iii) roach and chub combined (Table 9). This suggests that competition is unlikely to be the cause of the observed trends in growth rate.

The diet of dace has been shown to change with age (Hartley, 1947; Cragg-Hine and Jones, 1969; Hellawell, 1974). In general it has been found that the consumption of algae and detritus declines with age, while the consumption of larger items such as molluscs and Trichoptera increases with age. The observed trends in growth strongly indicate that the growth of 2 group and older dace may be limited by food supply, whilst the growth of 0 and 1 group dace might not be limited by food and thus temperature may be the factor most likely to be limiting growth.

Table 9 Relative biomass of 2 group and older dace to biomass of 2 group and older roach, chub and roach and chub combined

Site	Dace:Roach	Dace:Chub	Dace:Roach + Chub
1	9.2	0.7	0.7
2	0.6	1.1	0.4
3	0.2	0.3	0.1
4	0.1	0.1	0.1
5	3.1	1.4	1.0
6	0.6	0.1	0.1
7	2.6	0.3	0.3
8	0.2	0.1	0.1
9	-	0.6	0.6
10	11.6	0.4	0.4
11	6.2	1.2	1.0
12	3.9	0.7	0.7

Three large trout farms are located on the main River Avon downstream of Salisbury. Trout farms are known to cause a number of changes in water quality, in particular raising organic matter and ammonia levels, and in certain circumstances reducing oxygen levels. Any one of these factors may reduce the population levels of invertebrates upon which older dace feed.

Weatherley (1987) found that 0 group dace consumed large amounts of detritus. Any organic loading caused by trout farms (e.g. uneaten trout pellets and trout faeces) may therefore serve as a food source for 0 group, and possibly 1 group, dace. This supports the theory that temperature is the factor limiting growth in these age classes.

It appears unlikely that trout farming activities have a direct effect on the growth of dace given that the observed trends occur in the same year. In 1982 and 1984 0-group dace do not show a trend of increasing growth with distance downstream (Figures 6 and 9). However, such a trend is apparent if sites 1-7 and 8-12 are treated separately (Figures 7, 8, 10 and 11). Bickton trout farm is situated at site 7 and it is possible that growth at sites downstream of here was suppressed by factors emanating from the farm in these years.

Dace are most likely to be vulnerable to changes in water quality early in their development which may explain why only 0 group fish were so affected. If the trout farm was the cause of the changing growth pattern it is unclear what the factor responsible was, and why only these years were affected in this way. It may be due to different management practices or different climatic conditions in these years. The fact that the trout farms at Britford and

Trafalgar had, apparently, no similar effect in these years suggests it may have been management practices at Bickton in 1982 and 1984, possibly accentuated by climatic conditions, that were the cause.

Data from 0 group dace collected in 1988 indicate that growth, measured as length increase over the sampling period, is significantly correlated with distance downstream. However, instantaneous growth rates measured over the same period are not. The reason for this is that by measuring growth as length increase the small differences in length at the start of the sampling period (<1.7 mm) are masked by the relatively large differences (>6 mm) at the end of the sampling period. In the calculation of instantaneous growth rates an equal weighting is given to differences at the start and end of the sampling period. This shows that the differences in size at the end of the sampling period are simply caused by the differences at the start, the percentage difference remaining very similar.

The larger size at downstream sites is thus mainly a result of some factor causing the fry at downstream sites to grow more quickly before sampling started in May. Mills and Mann (1985) found that the growth rate of 0 group dace in the River Frome was most strongly correlated with initial larval length and this may have been the cause of differences in length at the start of the study period. No data are available on size at hatching in 1988 so it is not possible to determine if this is the cause of the observed differences. Mills (1981) found that the incubation time of dace eggs was inversely related to water temperature. Given the observed increase in temperature with increasing distance downstream, dace larvae are likely to hatch earlier at downstream sites and as a result will have a longer growing season. It should be noted that the 95% confidence intervals for instantaneous growth rates are large (Figure 14) and intra-site variation may be masking a trend of higher growth rates at downstream sites during the study period.

To conclude, the results indicate that significant differences in growth occur along the River Avon but the cause of these differences appears to be a complex interaction of a number of factors which vary with age. The results show that in the first two years growth may be strongly affected by temperature, whilst some other factor, possibly food supply, limits growth in subsequent years. For this to be the case there must be a considerable change in the diet around age 2. Further work is needed into changes in diet with age, linked to a survey of the distribution of diet items along the river. The effect of on-river trout farms on the growth of dace is unclear. The results suggest that the farm at Bickton may have caused depression of fry growth in 1982 and 1984 and clearly further work into the possible effects such farms can have is necessary, particularly with respect to the growth and survival of 0 group dace.

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5. FRY DISTRIBUTION

5.1 Observations in 1980

Observations on fry distribution were first carried out in 1988 in the R. Frome and mill stream at East Stoke (Figure 15). Subjective assessments of flow and cover were made on the habitats where fry were found.

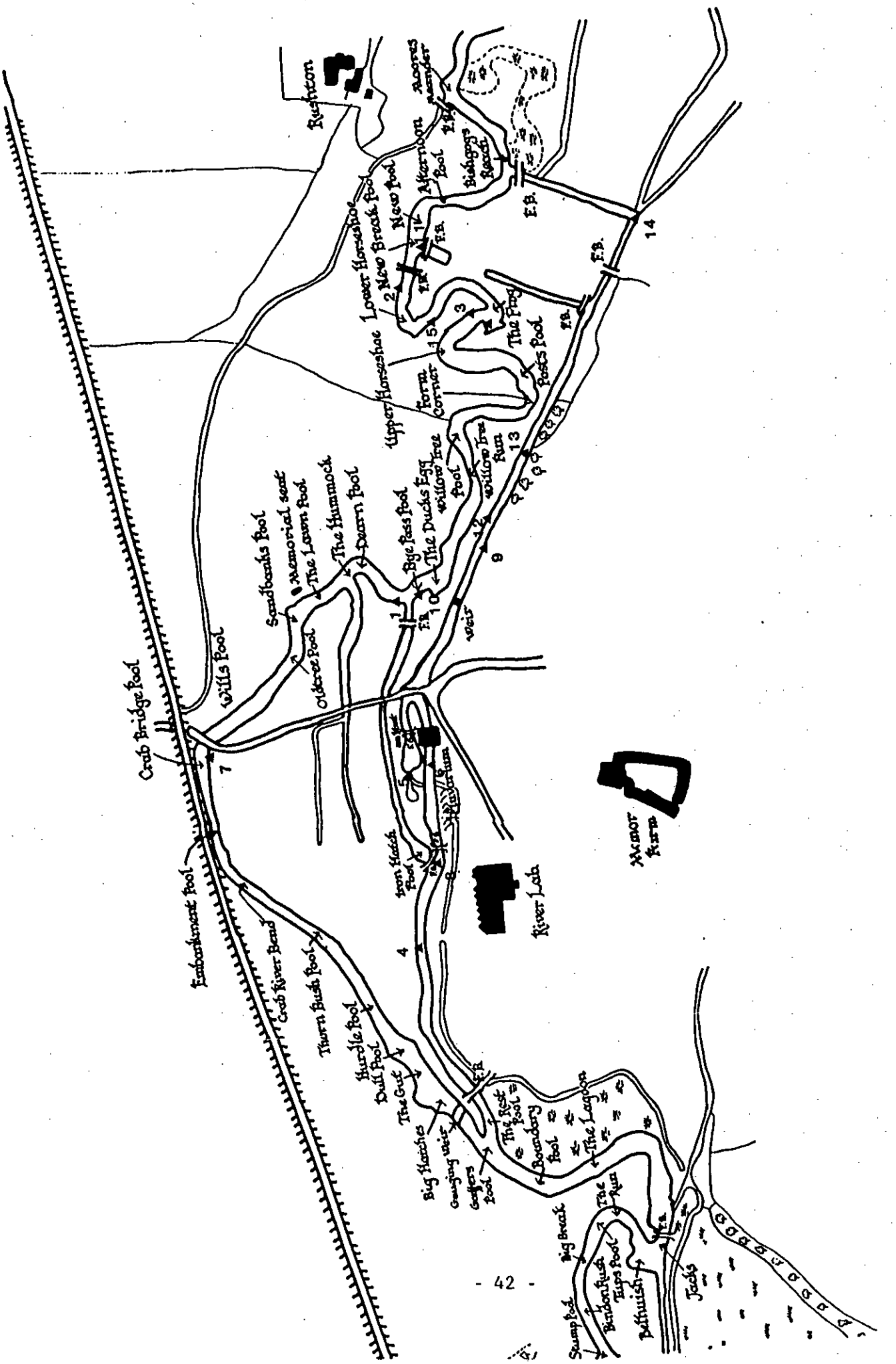
Dace fry were found in large numbers at only two sites on the main river, (1) upstream of the flood relief channel and (2) downstream of the Lower Horseshoe. Both sites were slacks in the margins and each had floating weed. Fry were near the surface associated with the floating weed. Estimated shoal size varied between sites and over time. At the first site a few hundred were seen on 6 May but by 10 May numbers had built up to between one and two thousand. One week later, however, the majority had dispersed and a few were seen in midwater further out from the bank. This dispersion also occurred at the second site. Many hundreds were seen on 10 May but only one was found on 17 May. The size of fry at this time was *c*.15 mm.

Around 700 fry were moved from the large shoal at site 1 on 11 May and put in the millhead. After 9 days they had redistributed into several smaller shoals of 10-25 individuals situated in slack eddies behind weed beds. Fry seen during June were still associated with the margins but were in faster flowing areas devoid of weed. Similar shoals were found in the runoff from the experimental channels.

Roach fry hatch slightly later than dace. Eggs were found on *Fontinalis* on the pilings in the Upper Horseshoe. A sample taken hatched in the fluvarium on 16 May when kept in river water at ambient temperature. A search of the *Fontinalis* on 17 May was made and no eggs were found. Roach fry appeared downstream at site 2 where dace fry were found. Several 7 mm larvae were associated with the surface scum. Small numbers were found at this site on 24 May having a mean size of 8.45 mm. Subsequently, none was found at this site.

Minnow fry first appeared on 27 May in small numbers along the margins of the river. They have an extended spawning season with larger individuals spawning earlier. Thus sizes of fry in shoals tend to have a greater variation than dace and roach and a progressive increase in size cannot necessarily be detected by sampling fry from several sites on the river. Four main areas of fry concentration were found and all sites had previously or still contained fry of the other species.

Figure 15. Fry sampling and observation sites 1-15 at East Stoke



Thus it seems that all species favour similar environmental conditions and do coexist in these habitats. Cover is important for very young fry and at this time they are near the surface. As they grow and are consequently able to move quicker and swim faster they disperse into deeper, faster flowing areas and are not necessarily associated with weed cover.

5.2 Observations in 1989

Dace fry were found at more sites than in the previous year although all sites had similar physical characteristics in terms of very low or zero flow situated in the margins. Depth of site was variable from very shallow cattle drinks which had little cover to deep slack marginal areas of the main river with floating weed. The position of the fish relative to the surface was similar, however, and cover appeared to be more important in the deeper areas. There was a greater size range of fry in this year on any one date. This is possibly due to the wider range of areas in which fry were found in 1989 which may have had differing temperature regimes or varying food availability. Dispersion from these marginal areas occurred at the end of May which was later than the previous year. The size of dace fry at this time was *c*.21 mm which was larger than the fish at the dispersion phase previously.

Growth rate was faster in 1989 than 1988 as a result of increased water temperatures. Roach and minnows were more abundant in 1989 and were found in several places in the main river. The distribution of roach was related to the limited areas suitable for spawning with fry found downstream of pilings covered with *Fontinalis*.

5.3 Distribution of dace, roach and minnow fry in the River Frome at East Stoke

1988

(1)	6.5.88	R. Frome margin just upstream of flood relief exit. Several hundred fry observed along a section of a few metres of margin in slack water among floating weed. Lengths of dace fry - mean 11.25 mm. 150 fish caught in single sweep of pond net.
(2)	10.5.88	R. Frome margin downstream of Lower Horseshoe pool. Two metres downstream of hawthorn bush amongst floating vegetation and scum. Large numbers of dace fry observed and *c*.100 retained in tank in fluvarium. Mean length 12.70 mm.
(1)	10.5.88	R. Frome margin just upstream of flood relief exit still with many hundreds of fry (*c*.1500 in 1 m shoal).
(1)	11.5.88	R. Frome margin just upstream of flood relief exit. 732 dace fry removed from and placed in millhead *c*.10 m downstream of junction with main river for observation.
(3)	13.5.88	Roach eggs present in *Fontinalis* growing on surface of pilings of Upper Horseshoe pool. Sample removed and placed in tank in fluvarium. Fry were swimming on 16.5.88 having hatched out overnight.
(1)	17.5.88	R. Frome margin just upstream of flood relief exit. A few larger dace fry still present but further from the margin and in mid-water.
(3)	17.5.88	No roach eggs found in search of Upper Horseshoe pilings.
(2)	17.5.88	Margin downstream of Lower Horseshoe. Sweep through surface scum with bucket, caught 1 dace fry length 15 mm and several newly hatched fry probably roach all 7 mm in length.
(2)	18.5.88	R. Frome margin downstream of Lower Horseshoe *c*.10 roach fry caught in pond net from surface scum. Overcast so no fry visible. Lots of rotifers in sample of scum and mud surface. Fry retained in container in laboratory.

(4)	20.5.88	East Stoke millstream millhead. c.10 quite large grayling fry in eddy in quite deep area of water near upstream end of millstream. Further downstream were shoals of dace larvae (10-25 in each shoal) mostly in eddies where flow is in an upstream direction behind weed clumps.
	23.5.88	Some of "wild" dace fry in fluvarium tank had died. Lengths of 3 dead specimens were 12.5, 13.5, 12.0 mm.
	24.5.88	Eight more dead fry, mean length 12.41 mm.
(2)	24.5.88	R. Frome downstream of Lower Horseshoe 1 larger fry (probably dace), 1 pike fry 35 mm and c.10 roach fry mean length 8.45 mm.
(5)	27.5.88	Outlet from Botany Pond, East Stoke mill stream little or no flow, large shoal of dace fry mean length 17.8 mm.
(1)	27.5.88	Margin of main R. Frome small fry (smaller than dace) present in margins including some very small minnows with yolk sac still present and fins undifferentiated. Others were probably roach?
(1)	7.6.88	Margins of main R. Frome upstream of flood relief exit, numerous minnow fry present in original dace fry area. Wide range of sizes not measured but many preserved.
(5)	10.6.88	End of main channel from Botany Pond, East Stoke mill stream and up into runoff from experimental channel system where flow is faster. Eight dace fry caught mean length 22.31 mm.
(5)	10.6.88	Small minnow and possibly gudgeon fry in runoff from Botany Pond.
(6)	13.6.88	Margins of East Stoke millstream upstream of fluvarium over stonework banks. Large numbers of dace and minnow fry. Mean length of dace 22.15 mm.
(7)	16.6.88	Main R. Frome just upstream of East Stoke road bridge in muddy cattle drink. Small shoal of dace, only two caught 23.5 and 24.5 mm.A few minnows also caught, size range 9.5-14 mm and one stone loach fry.
(8)	16.6.88	Large shoal of fry in East Stoke mill stream in millhead in inlet to Iron Hatch pool (closed). Shoal of more variable size and may have included roach? Mean length of dace 22.76 mm.
(6)	20.6.88	East Stoke mill stream upstream of fluvarium over stone banks (north bank). Dace, mean length 24.71 mm, minnow (2 only) 21, 17 mm.
(5)	20.6.88	Runoff from experimental channels. Many dace present but only 4 caught, 28.5, 29.5, 29.5, 32.5 mm.
(5)	21.6.88	East Stoke mill stream runoff from experimental channels. Mean length of 9 dace, 29.96 mm.
1989		
(9)	18.5.89	East Stoke mill stream cattle drink. Dace fry present, 12.5, 9.0, 9.5, 11.5, 10.0, 12.5, 8.5 mm.
(1)	18.5.89	R. Frome upstream of flood relief channel. Dace 16.25, 15.75, 17.25 mm.
(1)	18.5.89	R. Frome in flood relief channel. Dace 16.5 mm.
(10)	18.5.89	R. Frome downstream of flood relief channel. Dace 15.25, 16.00, 13.25, 15.26, 16.25, 15.25, 15.75.
(2)	18.5.89	Downstream of Lower Horseshoe. Dace 15.50 mm, roach/minnow 9.75, 8.25, 9.25, 8.75, 7.75, 8.50 mm.
(10)	23.5.89	R. Frome downstream of flood relief channel. Dace 19.0, 17.5, 18.5, 17.5, 17.5, 19.5, 18.0, 19.0, 18.0, 17.5 mm. Roach/minnows 9.5, 9.0 mm.
(2) (4)	23.5.89	Downstream of Lower Horseshoe. Minnows 9.75, 8.50, 10.50mm. Roach 7.50, 7.50 mm.
(11)	23.5.89	R. Frome East Stoke, Old East Stoke mill stream exit. Dace 17.0, 18.5 mm. Roach 18.00, 18.25 mm. Minnow 11.0, 9.0, 10.25, 11.00, 11.00, 10.5 mm.

(9) 23.5.89 East Stoke mill stream cattle drink. Dace 15.5, 15.0, 18.25, 18.0, 17.5 mm. Minnow 9.0, 9.5, 9.75, 10.25, 11.0 mm.

(12) 23.5.89 East Stoke mill stream Site 2 (10-15 m downstream of cattle drink, north bank). Dace 15.0 mm.

(13) 23.5.89 East Stoke mill stream Site 5 (Lily Pool). Dace 15.5, 15.5 mm.

(14) 23.5.89 East Stoke mill stream Site 8 (right angle bend). Dace 15.25, 15.25, 15.00, 15.00, 14.5, 15.50, 16.00 mm. Minnow 11.0 mm.

(15) 23.5.89 R. Frome East Stoke upstream of Lower Horseshoe. Dace 17.0, 19.0 mm. Minnow 19.5, 8.0 mm.

(10) 26.5.89 R. Frome downstream of flood relief channel 2.50 pm, surface temperature 19.5°C. Large shoal of fry ?minnows/roach, dace 19.75, 19.25 mm, roach 15.0 mm (lots of smaller minnows/roach).

(9) 26.5.89 East Stoke mill stream cattle drink 2.55 pm, temperature 19°C. Dace fry 16.0, 18.0 mm.

(5) 26.5.89 East Stoke mill stream above top hatch of botany channel. Dace 19.25, 20.00, 20.00, 18.00, 18.00, 18.00, 18.75, 18.50, 19.50, 19.50, 19.50 mm. Minnows 10.50, 12.00, 12.00, 11.50, 11.50, 10.00, 11.50, 11.50, 10.00, 11.50, 11.50, 11.50, 11.50, 11.00, 11.25 mm.

(10) 20.5.89 R. Frome East Stoke downstream of flood relief channel 2.40 pm, temperature 17°C. Large shoal of roach fry, NO minnows, beneath surface (not measured.

(9) 20.5.89 East Stoke mill stream cattle drink 2.45 pm, temperature 17°C (maximum 20, minimum 14). 10 dace, 2 caught 20.0, 21.0 mm.

(12) 20.5.89 East Stoke mill stream site 2 (10-15 m downstream of cattle drink), 3.00 pm, temperature 17°C (maximum 21, minimum 14). 12 minnow fry caught, not measured.

(14) 20.5.89 East Stoke mill stream site 8 (right angle bend), temperature 17°C (maximum 22, minimum 14), 1 dace caught.

(11) 20.5.89 Main R. Frome, old mill stream exit, temperature 16°C. Minnow 12 mm, roach fry 14.0, 15.0, 15.5, 12.0, 15.0 mm.

(2) 20.5.89 Main R. Frome downstream of Lower Horseshoe, temperature 16°C. Minnow and roach fry.

(15) 20.5.89 Main R. Frome upstream of Lower Horseshoe, temperature 16°C (maximum 18, minimum 15), no fish seen or caught.

(7) 20.5.89 R. Frome cattle drink upstream of road bridge, East Stoke. Lots of 1+ minnows and fry minnows, roach and dace. Roach 11.5, 13.25, 11.5, 11.75, 12.0, 12.75, 12.0, 12.0, 12.0, 12.0 mm. Minnows 13.25, 12.0, 12.0, 14.0, 13.0, 13.0, 13.5, 10.5, 9.5, 12.0, 13.25 mm. Dace 19.0, 23.0 mm.

(4) 20.5.89 East Stoke mill stream millhead upstream of top hatch. Dace 21.5 mm, minnow 9.5, 11.5, 13.0, 13.0, 13.5, 12.0, 12.0, 12.5, 14.0, 11.5 mm.

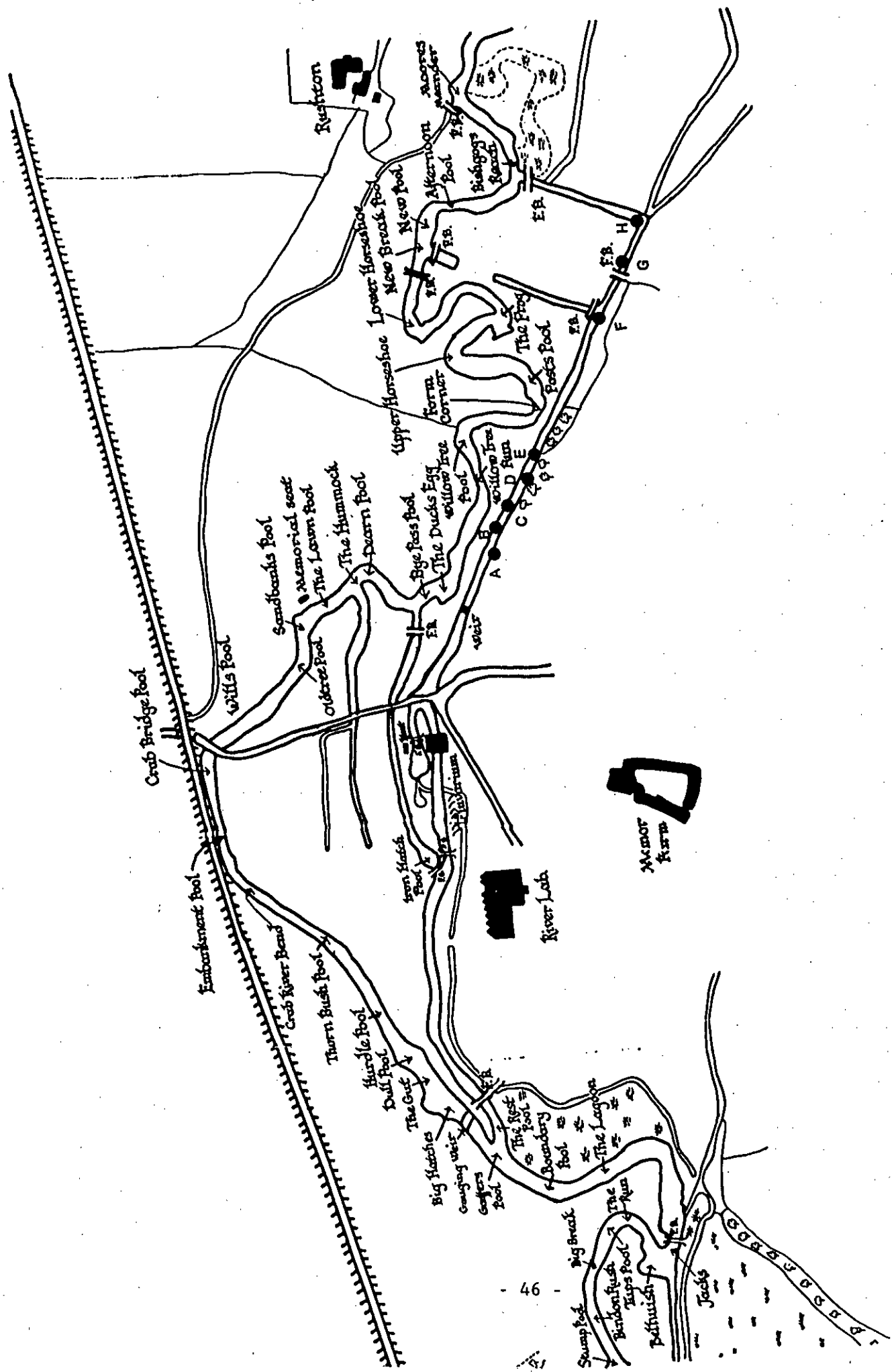
(10) 2.6.89 R. Frome downstream of flood relief channel, 2.10 pm, temperature 15°C, minnow and roach? fry caught. Minnows 9.0, 7.5, 8.5, 9.25, 11.25, 10.0, 10.5, 10.25, 14.0, 11.0, 11.5, 12.0, 11.5, 11.0, 12.0, 11.5, 10.0, 12.5, 13.0, 11.5 mm.

(9) 2.6.89 East Stoke mill stream site 1 cattle drink, 14°C (maximum 21, minimum 12), no fish visible.

5.4 Distribution of fry in East Stoke mill stream

In 1988 surveys of marginal areas of the R. Frome and the adjacent mill stream (Figure 16) (NGR 57865867) were carried out with the object of identifying areas in which small fish congregate. A number of such situations were recognised and mapped and the physical characteristics of each site and the abundance and identity of small fish present was noted. The occurrence of small fish at each point was recorded on a number of occasions with the objective of characterising the conditions which were favoured.

Figure 16 Fry sampling and observation sites A-H East Stoke mill stream



Site A (cattle drink, mill stream)

This marginal embayment was one created by drinking cattle which had trodden down the riparian vegetation and marginal soil to such a degree that on 25 May, when observations were first made, depths of 55 to 280 mm occurred within the small embayment. The substratum consisted of an uneven layer of silt and fine detritus and velocities were low, ranging from 0 to 40 mm s^{-1}.

26.5.89 Unidentified fry present in 140 mm of water at the upstream corner of the embayment (point 1) in velocities of 27-41 mm s^{-1}.

30.5.89 Temperature 17°C about 10 dace (*Leuciscus leuciscus* (L.)) fry were present at roughly the same position in 60 mm of water at velocities of 34-43 mm s^{-1}).

19.6.89 Only 5 mm of water were present at the upstream corner and 2 dace fry were present just streamward of the usual position in velocities of 15-19 mm s^{-1}.

9.6.89 Temperature 16°C, depth at 1.50 mm, velocity at A 15-19 mm s^{-1}, 2 fry at A.

26.6.89 Temperature 19°C, depth 60 mm, velocity 9 mm s^{-1}. Depth from usual place 200 mm, 2 small fry present, velocity 15-18 mm s^{-1} where large fry were present in 100 mm water at X.

4.7.89 Temperature 18°C, depth 210 mm, velocity 3.55 mm s^{-1}, 1 loach fry at L, 3 fry at M, velocity at usual place 0 mm s^{-1}.

11.7.89 Temperature 20°C, depth less than above, velocity 0, no fry.

Site B (10-15 m downstream of cattle drink - north bank)

26.5.89 This position was "created" by a large section of infallen bank which formed the shallower (130-310 mm depth) upstream region and just downstream was a deeper hole up to 720 mm in depth. A variety of submerged and emergent macrophytes were present. Velocities ranged from 0 to 100 mm s^{-1}.

20.5.89 Temperature 17°C. Macrophytes joined up to bank, deepest point now 670 mm, very small fish, probably minnows, near bottom, velocity 13-24 mm s^{-1}.

9.6.89 Temperature 15°C, 20 minnow fry at A facing bank, velocity at A 19-33 mm s^{-1}, depth 480 mm.

16.6.89 Temperature 13-27°C, 10-11 minnow fry at F1 only a few at A, depth 480 mm, velocity at A 22-31, at F1 19-28 mm s^{-1}. More fry than last time.

26.6.89 Temperature 20°C, depth 330 mm, velocity 12 mm s^{-1}, fry 100 mm from bed. Some fry further out between submerged reeds and mud below *Veronica* sp. facing upstream 13-15 mm.

Site C (Iron Loop Slack opposite Barry's Hole)

Lots of *Ranunculus* - clean flowing water on outer edge of slack. Some slack water and surface covered in diatoms. Plenty of cover, no fish seen. Bottom of silt.

26.5.89 Temperature 16-21°C, velocities 11-22 mm s^{-1} above *Ranunculus*, depth 270 mm.
30.5.89 Temperature 17°C, depth 200 mm, no fish seen.
9.6.89 Completely covered in *Ranunculus*. One fry seen near bank, temperature 14°C, depth 130 mm, velocity 22-33 mm.
16.6.89 Two fry near bank in only patch clear of *Ranunculus*, temperature 20°C, depth 110 mm, velocity 21-30 mm s^{-1}.
26.6.89 No fish seen, temperature 19°C, velocity 12-16 mm s^{-1}.
4.7.89 No fish seen, temperature 21°C, velocity 5-16 mm s^{-1}.
11.7.89 No open water, no fish seen, temperature 20°C, velocity 29-33 mm s^{-1}, depth now 7 mm.

Site D (opposite Alder tree)

26.5.89 Shallow marginal area with mat of diatoms on surface and growths of *Glyceria*, silty bed, temperature 15-19°C, velocity 3-24 mm s^{-1}.
30.5.89 Temperature 17°C, velocity at 1 23-29 mm s^{-1}, depth 230 mm, no fish.
9.6.89 Temperature 14°C, velocity at 1 11-18 mm s^{-1}, level very low, no fish.
16.6.89 Temperature 19°C, depth 110 mm, velocity 11-13 mm s^{-1}, no fish.
26.6.89 Temperature 19°C, depth 50 mm, velocity 0, no fish.
4.7.89 Temperature 21°C, as above.

Site E (Lily Pool)

26.5.89 Stream side slack with *Glyceria* and diatoms at surface. Plenty of cover, depth 730 mm, shoal of dace in mid water and a few at surface.

Site F (old mill stream channel)

26.5.89 Completely overgrown with *Glyceria* 180-240 mm deep, substratum silt/detritus, water velocity 0, only small amount of diatom growth, no fry.

Site G (bottom bridge pool)

26.5.89 Temperature 12-21°C, depth 3-198 mm s^{-1}, no cover, fast flow (*c*.500) over gravel. Plenty of medium sized minnows, no fry.
30.5.89 Temperature 17°C, depth 220 mm at *Veronica* 75 mm at edge of cress, velocity 138-156 mm s^{-1}, 100 mm from bed at 1.
9.6.89 Depth at 1, 200 mm, velocity 111-146 mm s^{-1}, velocity at minnow fry 10-30 mm s^{-1}. Loach fry in tussock region.
16.6.89 Main flow now bypassing cattle drink, bed covered with fine silt, temperature at 1 16°C, large and small fry off *Veronica* plus loach and gudgeon fry, velocity at 1 18-28 mm s^{-1}, velocity at fish 30-34 mm s^{-1}, odd fry all over.
4.7.89 Temperature 14°C, 1 loach fry in stagnant pool, velocity 128-132 mm s^{-1}.

Site H (Corner Pool)

26.5.89 Some leaves at surface bed of sand/silt, fair flow with *Simulium* on leaves, no surface diatoms, temperature 18°C, velocity 129-137 mm s^{-1}. Dace and minnows at F.

30.5.89 Temperature 17°C, depth 355 mm, velocity 142-146 mm s^{-1}, or 18-22 mm s^{-1} 10 cm from ed at point F where fry were still present.

9.6.89 Temperature 14.5°C, depth at 1, 390 mm, velocity 76-82 5 mm s^{-1}, no fish.

16.6.89 Temperature 20°C, depth 350 mm, velocity 89-108 mm s^{-1}, fry (+ large minnows) at surface at tail of *Ranunculus* just above corner pool.

26.6.89 Temperature 20°C, depth 280 mm, velocity 104-106 mm s^{-1}. Loach fry present at velocity point.

4.7.89 Temperature 20.5°C, depth 250 mm, velocity 68-81 mm s^{-1}. *Ranunculus* and *Elodea* matted in edge, 1 loach fry at velocity point.

11.7.89 Temperature 20.5°C, depth 240 mm, velocity 69 mm s^{-1}, nothing.

APPENDIX 1 Protocol for fast coomassie staining of IEF media

N.B. *Sample preparation*: See "Enzyme Analysis" protocol for crushing buffer recipe and for details on fish muscle sample preparation. For samples with a relatively high concentration of non-protein material, such as whole invertebrates, a longer centrifugation time may be beneficial. The centrifuge tube holders can be packed with ice to keep the sample cool.

Running of IEF media: See "Enzyme Analysis" protocol.

1. Stain preparation

Wear disposable gloves and safety spectacles.

1.1 FIX: 20% trichloroacetic acid
Make up and store in a large container at room temperature.
Recipe (to make 1l): Dissolve 200 g trichloroacetic acid in distilled water and make up to 1l

1.2 WASH/DESTAIN: 30% methanol and 10% acetic acid in distilled water.
Make up and store in a large container at room temperature.
Recipe (to make 1l): 300 ml methanol
100 ml acetic acid
600 ml distilled water

1.3 STAIN: 0.02% PhastGel Blue R solution in approximately 30% methanol and 10% acetic acid in distilled water and 0.1% (w/v) $CuSO_4$.

Recipe:
Stock solution: Dissolve 1 PhastGel Blue R tablet (from dessicator in fridge) in 80 ml distilled water by stirring for 5-10 mins. Add 120 ml and stir for 2 mins. Store in plastic bottle in fridge.
Final solution: Mix 1 part filtered stock solution with 9 parts, wash/destain solution.
Add $CuSO_4$ to 0.1% w/v.

i.e. to make 90 ml:
Dissolve 0.141 g $CuSO_4.5H_2O$ in 81 ml wash/destain solution by warming with a bunsen. Then add 9 ml filtered (through filter paper) stock solution.

N.B. The fix solution and the wash/destain solution can be recycled 3-4 times by passing through a column of activated charcoal.

2. Running the development method

Wear disposable gloves.

2.1 Prepare the development unit
- 2.1.1 Remove the caps on the Cap Set from the ports 0, 1, 2, 3 and 5.
- 2.1.2 Connect port 0 to waste with PVC tubing.
- 2.1.3 Connect port 1 to a 100 ml beaker containing approximately 90 ml, fix solution.
- 2.1.4 Connect port 2 to a 250 ml beaker containing approximately 180 ml wash/destain solution.
- 2.1.5 Connect port 3 to a 100 ml beaker containing 90 ml stain solution.
- 2.1.6 Connect port 5 to an empty 250 ml beaker.
- 2.1.7 Ensure that the tubing is securely submered in the solutions. All beakers and tubing should be labelled with their corresponding port number.
- 2.1.8 Open the lid of the development chamber by pressing on the right end of the red bar.

2.2 Insert the gels

2.2.1 Remove one gel from the separation bed with a pair of forceps.

2.2.2 Slide the gel, gel surface down, into the upper position of the gel holder. Use gloved fingers or forceps, whichever is the easier.

2.2.3 Remove the other gel and slide it, gel surface up, into the lower position of the gel holder. If only one gel is being developed, slide it into the lower position, gel surface up.

2.2.4 Close the lid and lock it by simultaneously pressing down on the top of the lid and pushing in the red bar.

2.3 Start the run

2.3.1 Press "DEV start/stop"

2.3.2 Enter "1" for the Coomassie-IEF development method.

2.3.3 Press "do" to confirm.

The method is carried out as follows:

Step no.	Solution	IN-port	OUT-port	Time	Temperature
1	Fix	1	1	5 mins	20°C
2	Wash/destain	2	0	2 mins	20°C
3	Stain	3	0	10 mins	50°C
4	Wash/destain	2	5	10 mins	50°C

2.4 Remove the gels

2.4.1 The method finishes automatically.

2.4.2 Remove the gels with gloved fingers or forceps.

2.4.3 Place the gels on filter paper to dry.

APPENDIX 2 Mean lengths at age of each year-class of Avon dace

	Year class					
Age	1986	1985	1984	1983	1982	1981
Site 1						
1	50.000	60.667	55.800	57.636	58.000	54.000
2	89.000	113.000	99.330	108.000	112.220	109.000
3	-	146.000	136.930	154.550	167.670	149.000
4	-	-	167.600	187.550	202.440	178.000
5	-	-	-	205.820	222.110	192.000
6	-	-	-	-	235.670	195.000
7	-	-	-	-	-	202.000
Site 2						
1	51.667	-	58.100	54.643	54.381	56.750
2	85.000	-	104.300	106.860	113.050	120.250
3	-	-	148.900	150.930	163.640	176.000
4	-	-	183.350	185.210	192.760	212.250
5	-	-	-	203.070	208.210	228.000
6	-	-	-	-	218.450	235.500
7	-	-	-	-	-	241.000
Site 3						
1	52.333	59.000	53.167	53.500	56.000	51.000
2	88.250	85.000	91.400	110.300	114.080	109.000
3	-	113.000	134.370	160.100	176.330	174.000
4	-	-	169.430	190.700	212.500	217.000
5	-	-	-	207.900	228.670	240.000
6	-	-	-	-	237.420	247.000
7	-	-	-	-	-	252.000
Site 4						
1	48.308	-	49.500	55.000	52.333	-
2	91.310	-	99.000	111.500	116.670	-
3	-	-	142.600	153.500	160.330	-
4	-	-	179.200	186.000	191.000	-
5	-	-	-	200.000	206.330	-
6	-	-	-	-	219.000	-
7	-	-	-	-	-	-
Site 5						
1	63.125	59.222	61.800	59.111	60.372	58.000
2	96.620	112.670	121.500	132.830	132.670	106.000
3	-	162.110	167.450	185.940	194.770	167.000
4	-	-	207.350	215.670	222.330	211.000
5	-	-	-	232.220	236.910	227.000
6	-	-	-	-	247.330	236.000
7	-	-	-	-	-	247.000
Site 6						
1	47.000	51.000	58.056	56.167	64.222	-
2	89.000	87.000	116.780-	126.500	134.440	-
3	-	145.000	163.440	188.500	194.330	-
4	-	-	199.390	210.170	221.220	-
5	-	-	-	228.170	235.330	-
6	-	-	-	-	245.780	-
7	-	-	-	-	-	-

APPENDIX 2 (continued)

Age	Year class					
	1986	1985	1984	1983	1982	1981
Site 7						
1	50.500	59.500	61.750	59.417	66.400	58.000
2	107.000	113.000	120.690	127.330	141.500	146.000
3	-	167.000	171.560	177.670	200.200	234.000
4	-	-	210.870	210.250	226.500	259.000
5	-	-	-	230.250	242.700	264.000
6	-	-	-	-	254.400	272.000
7	-	-	-	-	-	275.000
Site 8						
1	54.615	50.750	51.750	57.600	56.000	-
2	109.380	107.000	113.620	130.200	127.670	-
3	-	164.750	154.120	186.800	180.330	-
4	-	-	183.870	206.800	201.330	-
5	-	-	-	218.600	212.670	-
6	-	-	-	-	224.330	-
7	-	-	-	-	-	-
Site 9						
1	60.000	55.125	55.636	57.095	55.273	52.000
2	110.330	112.500	119.910	126.710	121.360	91.000
3	-	159.620	163.360	175.050	179.000	152.000
4	-	-	192.910	198.050	198.450	180.000
5	-	-	-	212.480	212.360	196.000
6	-	-	-	-	222.550	204.000
7	-	-	-	-	-	212.000
Site 10						
1	57.905	52.000	60.083	63.500	58.500	56.500
2	118.290	105.200	115.830	151.000	144.000	111.000
3	-	166.000	166.750	194.000	193.000	169.500
4	-	-	199.330	212.000	204.500	222.000
5	-	-	-	226.500	218.500	239.000
6	-	-	-	-	229.000	246.500
7	-	-	-	-	-	252.000
Site 11						
1	58.290	54.071	61.182	64.278	62.320	62.667
2	118.970	110.790	123.890	141.470	141.440	130.000
3	-	174.070	171.640	191.930	200.040	184.330
4	-	-	205.000	212.870	221.040	221.330
5	-	-	-	226.670	229.760	238.000
6	-	-	-	-	237.480	244.330
7	-	-	-	-	-	249.330
Site 12						
1	59.770	56.179	62.459	65.824	61.000	75.000
2	121.000	110.360	123.020	139.760	135.090	139.000
3	-	172.000	174.440	186.760	189.090	190.000
4	-	-	205.310	209.410	212.450	220.000
5	-	-	-	223.180	224.090	238.000
6	-	-	-	-	232.180	252.000
7	-	-	-	-	-	262.000

APPENDIX 3 Instantaneous growth rates of Avon dace from each year-class

	Year class					
Age	1986	1985	1984	1983	1982	1981
Site 1						
1	6.054	6.588	6.358	6.447	6.456	6.267
2	1.844	2.005	1.841	1.992	2.111	2.246
3	-	0.815	1.034	1.142	1.286	1.000
4	-	-	0.651	0.632	0.611	0.569
5	-	-	-	0.304	0.304	0.242
6	-	-	-	-	0.193	0.050
7	-	-	-	-	-	0.113
Site 2						
1	6.145	-	6.469	6.300	6.287	-
2	1.562	-	1.860	2.151	2.334	-
3	-	-	1.155	1.115	1.196	-
4	-	-	0.653	0.660	0.530	-
5	-	-	-	2.958	0.248	-
6	-	-	-	-	0.154	-
7	-	-	-	-	-	-
Site 3						
1	6.181	6.512	6.356	6.242	6.368	6.109
2	1.680	1.168	1.727	2.346	2.283	2.429
3	-	0.910	1.233	1.189	1.404	1.496
4	-	-	0.748	0.564	0.604	0.706
5	-	-	-	0.278	0.238	0.322
6	-	-	-	-	0.121	0.092
7	-	-	-	-	-	0.064
Site 4						
1	5.959	-	6.027	6.318	6.181	-
2	2.021	-	2.193	2.260	2.553	-
3	-	-	1.166	1.015	1.020	-
4	-	-	0.753	0.610	0.566	-
5	-	-	-	0.237	0.248	-
6	-	-	-	-	0.192	-
7	-	-	-	-	-	-
Site 5						
1	6.698	6.522	6.640	6.517	6.575	6.465
2	1.388	2.068	2.163	2.594	2.520	1.928
3	-	1.165	1.031	1.084	1.233	1.454
4	-	-	0.689	0.479	0.427	0.748
5	-	-	-	0.237	0.204	0.233
6	-	-	-	-	0.138	0.124
7	-	-	-	-	-	0.146
Site 6						
1	5.883	6.109	6.467	6.376	6.746	-
2	2.042	1.708	2.225	2.594	2.359	-
3	-	1.634	1.085	1.277	1.180	-
4	-	-	0.650	0.351	0.417	-
5	-	-	-	0.263	0.201	-
6	-	-	-	-	0.140	-
7	-	-	-	-	-	-

APPENDIX 4 Mean monthly temperatures of R. Avon from April to October for years 1982-1987

	Year					
Month	1982	1983	1984	1985	1986	1987
April	9.9	9.1	10.2	10.5	8.3	10.6
May	13.3	12.2	14.1	13.6	12.4	12.8
June	17.0	16.4	18.2	16.0	16.7	15.8
July	17.4	22.0	21.2	18.5	18.4	18.5
August	16.9	19.9	19.7	18.0	16.0	18.4
September	14.7	19.7	15.6	16.3	13.1	17.0
October	10.8	12.0	12.8	12.4	12.4	11.5

APPENDIX 5 Degree days (above °C) from 1 April in years 1982-1987 in R. Avon

	Year					
Month	1982	1983	1984	1985	1986	1987
July	1758	1823	1946	1769	1705	1759
August	2281	2441	2556	2349	2199	2330
September	2723	3033	3023	2837	2592	2838
October	3057	3403	3420	3222	2976	3194

APPENDIX 6 Correlation coefficient of instantaneous growth rate of Avon dace at each collection with temperature

Site	0 week lag	1 week lag	2 week lag
1	-0.162	-0.181	0.147
2	0.412	-0.133	-0.375
3	0.320	-0.238	0.700
4	-0.108	0.494	0.742
5	-0.149	0.440	0.561
6	0.265	0.254	0.003

None significant

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