SOME INVESTIGATIONS INTO THE RELATIONSHIP OF GRAYLING (<u>THYMALLUS THYMALLUS</u>)
AND THE BROWN TROUT (<u>SALMO TRUTTA</u>) IN THE EDEN WATER, ROXBURGHSHIRE

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In partial fulfilment of the requirements for the Degree of Master of

Philosophy.

UNIVERSITY OF EDINBURGH

DEPARTMENT OF FORESTRY AND NATURAL RESOURCES

1975

I hereby declare that this thesis has been composed by myself and the research presented in it is my own.

ABSTRACT

The inter-relationship of sympatric populations - and the question of their competition with each other - has been a much debated subject in both academic and angling circles, but one which has frequently lacked quantitative investigation. Nilsson (1963) introduced the notion of interactive segregation to describe the process whereby co-habitating fish species become more specialised (as regards the limiting resource) through the operation of certain inherent differences in their behaviour, morphology, and physiology. This concept was applied in this research and it was hypothesised that the sympatric grayling and trout populations in the Eden do interactively segregate and therefore exhibit some degree of niche specialisation, in this instance relating to diet; it was also proposed that the major mechanisms effecting their specialised feeding behaviour are morphological and physiological.

Part I of this research was concerned with the demonstration of interactive segregation in the grayling and trout populations in the Eden. Investigations into the feeding habits of these species indicated that although there is some overlap in the type of prey consumed by grayling and trout, particularly for the immature fish in the summer, they take the major portion of their diet from different parts of the invertebrate fauna and at opposite times of the year and therefore exhibit some specialisation in their feeding. The indexes of growth and condition were used to assess fish well-being as an indirect means of detecting any low-grade competition. The growth rate of the grayling is nearly double that of the trout in the first year of life, however, both species grow at a similar rate after that time which was shown to

be maximal for that environment. Various indexes of condition - the coefficient of condition, the percentage of dry matter, and the amount of fat - demonstrated that both species achieve a similar (numerical) peak in condition which occurs at different times of the year.

Part II examined some of the mechanisms which probably effected interactive segregation between grayling and trout in the Eden. Morphological differences in feeding-related structures - fin and body form, size and orientation of mouth, dentition, etc. - were measured and indicated significant differences between these species which would tend to channel them into slightly different feeding niches. Physiological differences in metabolism which could largely regulate the seasonal periodocity shown in feeding, growth and condition, were investigated in food consumption and growth studies at three different temperatures; results suggested that there are intrinsic differences in the levels of grayling and trout metabolism which are probably related to the species' origins.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

PART II: SOME OF THE MECHANISMS EFFECTING INTERACTIVE SEGREGATION

LIST OF TABLES

LIST OF FIGURES

No.	Title	Page

LIST OF PLATES

"Whilst yielding to its sisterspecies the Trout
in the qualitites of dash and obstinate courage,
the Grayling is yet a sturdy and mettlesome fish-
a foeman worthy of our steel; and if the former
is the handsomer, the latter must I think be
admitted to be the prettier species of the two.
The trout has so to speak a Herculean cast of
beauty; the Grayling rather that of an Apollo -
light, delicate, and gracefully symmetrical."

(The Angler Naturalist, Pennel 1863)

CHAPTER I

GENERAL INTRODUCTION

The grayling (<u>Thymallus</u> <u>thymallus</u>) and the brown trout (<u>Salmo</u> <u>trutta</u>) are both members of the family Salmonidae which live in well oxygenated freshwater streams, rivers, and lakes. In Europe, both species are found roughly from the Alps to the Arctic circle, that is from latitude 42 35 N to almost 70 N and from longitude 65 E to 5 W, as illustrated in Figures 1a, 1b (Jankovic 1964, Frost and Brown 1967). The distribution of the grayling is somewhat more restricted than that of the trout: In Britain the northern-most populations of grayling are found in the Tay watershed, whereas the brown trout are native to most waters of Scotland. Grayling are most abundant in the Limestone country of the Pennine Chain and in the chalk-streams of the south of England and in the Triassic sands of the Central Districts (Pennel 1863).

The diverse distribution of the grayling is attributed to the effects of the Ice Ages and to the various introductions of the species for food and sport. Before the Ice Ages the British Isles were joined to the continent of Europe and much of the North Sea is believed to have been land, and the Thames, a tributary of the Rhine. The ice sheet penetrated furthest south during the second glacial period and covered the whole of Britain north of the line of the Thames valley, forcing fish as far south as the Mediterranean Sea, and almost all of the remaining fish fauna are thought to have been destroyed (Varley 1967). As the ice began to retreat, probably around 15,000 B.C., salmonid fish are believed to have recolonised more northern rivers by swimming along the coasts. Grayling and trout most probably exhibited certain differences in their

Fig. 1b   Distribution of grayling
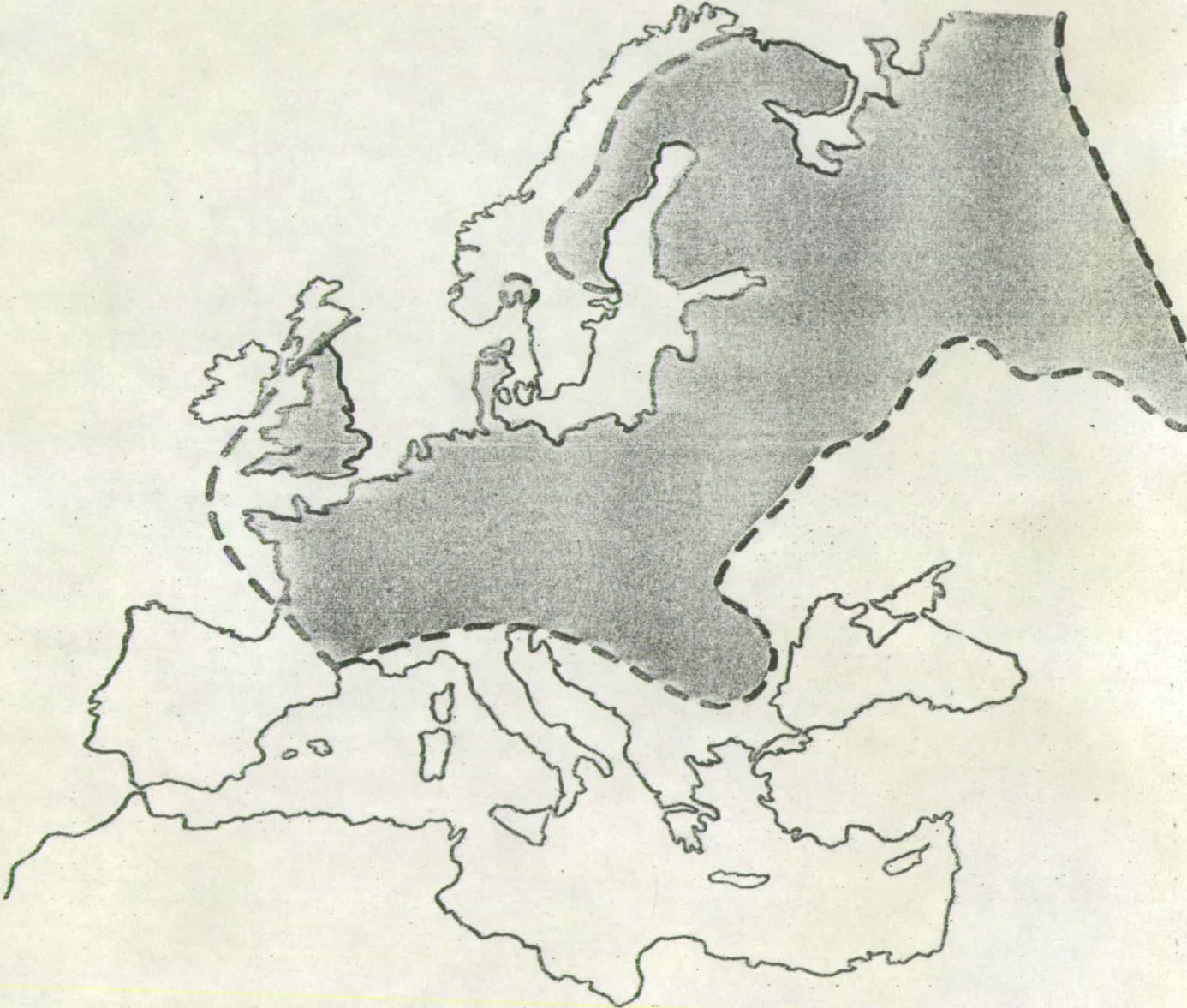
tolerance to salinity which could have affected their rate of success in recolonising various freshwater systems in Britain. (The sea trout, _Salmo trutta_, which is an anadromous salmonid, that has survived to the present millenium and is testimony to the high level of salinity tolerated by the brown trout.)

The grayling is thought to have been introduced into Scotland (Jankovic 1964) and Pennel (1863) relates the suggestion that monks introduced the species into the Tweed watershed for winter food. The gastronomics of the species were clearly appreciated by the clericals; Father Sanctus called it the "Queen of Delight" (Pennel, 1863). Ritchie (1920) attributes the introduction of grayling into the River Tweed to some fish which escaped from a pond at Monteviot House, Roxburghshire, in 1885.

The grayling typically inhabits the middle reaches of cool, not very swift rivers with gravelly or sandy bottoms (Huet 1959, Jankovic 1964) and the trout occurs in more torrential reaches with a substrate composed of solid rock, stones, boulders, and perhaps some gravel (Huet 1959, Frost and Brown 1967). Because these zones are rarely well-differentiated physically, a high degree of overlap of fish species may occur naturally or artificially which raises the question of interspecific relationships, and of possible competition in particular.

Several definitions of competition have been composed: (1) Interspecific competition may occur between sympatric[1] species when the growth or survival of one is deleteriously affected by another which may arise as a result of similar requirements for limited resources,

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1. Sympatric species are those which occur together, that is with areas of distribution which overlap or coincide. Allopatric species are conversely those which do not occur together (Odum 1971).

such as nutrients, space and light (Gause 1934a); (2) Clements and
Shelford (1939) wrote that competition is the active demand by two or
more individuals of the same species population (intra-specific) or
members of two or more species at the same trophic level (inter-specific)
for a common source or requirement that is actually or potentially
limiting; (3) Andrewartha and Birch (1954) suggest that competition
occurs when a valuable or necessary resource is sought together by a
number of animals when that resource is in short supply or if the
resource is not in short supply, competition occurs when the animals
seeking that resource nevertheless harm one another in the process;
and (4) Milne (1961) wrote that competition is the endeavor of two or
more animals to gain the same particular thing when that supply is not
sufficient for all.

The subject of competition remains a topical and dynamic ecological
issue and therefore many ecologists are still discussing the appropriate
application of the term (Miller 1967). For the purpose of this research,
the following definition of competition was adopted: Inter-specific
competition may occur between sympatric species when the growth or
survival of one is deleteriously affected by another which may arise as
a result of similar requirements for limited resources.

There are a number of ways in which these 'deleterious' inter-actions
may occur, such as the use of the same spawning area, the consumption of
the same food and of the eggs or young of another species or the total
displacement of a species by inter-specific aggression. It is extremely
difficult to discern the causal relationships of competition because
the natural inter-actions between populations and their environment are
so complex; in such an analysis, it would be necessary to inter-relate
all the productive factors such as the biotic potential, size, and
predation within the given (although fluctuating) environmental context

(Crombie 1947). Furthermore, in any consideration of competition some appreciation must be given to the geographical distributions and rates of evolution and the faunal association of the species concerned (Larkin 1956, Nikolskii 1963).

Various researchers in Britain have attempted to analyse competition between fish over food (Synnerton and Worthington 1939, Frost 1946, Hartley 1948, Thomas 1962, Mills 1964, Maitland 1965, Mann and Orr 1969). Hartley examined the food of 11 freshwater species in the River Cam, Shepreth Brook, and Bulbeck Dyke, Cambridgeshire, from 1938 to 1941, giving particular reference to the problem of inter-specific competition. The gut contents of fish samples were identified and then the fish were divided into four rather arbitrary groups on the basis of differences in general feeding habits. Competition was assessed by the degree of similarity of diet in terms of the percentage of frequency of occurrence of various food items in each food type.

Hartley admits that the major failing of such an analysis of competition is that no account was taken of the relative abundances of food organisms: "In the absence of data upon the density of food organisms and the proportion removed by the fish in the course of the year, competition here means nothing more than taking an appreciable proportion of a common food without any implication that the food was a limiting factor (p. 4)".

A similar study was carried out by Maitland in 1965 on the feeding relationships of brown trout, salmon (Salmo salar), minnows (Phoxinus phoxinus), stone loach (Nemacheilus barbatula), three-spined stickleback (Gasterosteus aculeatus) in the River Endrick in Scotland. The analysis centred mainly on the feeding relationships of the fish, although the research did include an assessment of the relative abundance of food in the nursery areas. Samples were taken of each

species mid-monthly during 1961, along with samples of the bottom fauna to compare specific feeding patterns in relation to food availability - termed the 'availability factor'. The availability factor was first used by Allen (1942) and is derived from the ratio of the proportion of the organisms in the stomachs to the proportion of the same prey species in the fauna. However, in practice there are certain limitations to the use of the availability ratio which are related to inaccuracies of sampling and enumeration (Hynes 1950) and to the qualitative nature of this measure; the availability factor incorporates such characteristics of the prey as size, appearance, habits, and taste because they affect the extent to which they are selected by the predator species and various size groups in particular (Allen 1942, Ivlev 1961). Elliott (1967) concluded that availability factors can only be used to show general tendencies in the feeding patterns of fish.

The problem of the inter-relationship of grayling and trout with special reference to their feeding has been the subject of many angling debates and surveys in the last century in Britain, where the brown trout is highly respected as a game fish and the grayling is regarded as a pest.

"Grayling are very rapid breeders and are extremely greedy.
If they are not kept down, the trout larder is sure to suffer."

(Bell 1966, p.141)

Sawyer (1946) examined the stomachs of grayling and trout taken from the Avon and discovered that the grayling were full of fly species and that the trout contained benthic invertebrates such as snails and shrimps; he deduced that the grayling which is quicker to the surface than the trout had harassed the trout to such an extent that they had relinquished the mid-water to the grayling and fed off the bottom and suffered accordingly. Carrington (1947) made a study of the feeding

habits of the grayling of the Wye and reported that following the introduction of rainbow trout, grayling changed from surface risers to bottom feeders and that both species were well maintained.

Gerrish (1938, 1939) examined the feeding relationship between grayling and trout in the River Avon and noted that sometimes the trout suffered and sometimes it was the grayling which suffered. Furthermore, he reported a definite relationship between the abundant food type and the dominant fish species:

> "The best food for trout is snails, which the grayling do not like and therefore where snails are abundant, the latter will not be a serious competitor....when amphipods were the dominant food (available) the two species competed."
>
> (Gerrish 1938, p.57)

Gerrish then demonstrated this relationship experimentally by transferring trout and grayling from waters where grayling and shrimp dominated to snail waters where he found that the trout improved and the grayling declined.

Gerrish's paper is inconclusive as it fails to take into account the range of differences in habitats which might be expected between shrimp and snail dominated waters, such as the relative alkalinity levels and consequent productivity. However, he does put forward an interesting and most progressive outlook on grayling and trout interrelations in his conclusions:

> "In any given season, trout and grayling rarely choose the same thing as their basic food...I have little doubt in my own mind that trout and grayling do exercise a definite selection among the many sorts of food animals available and also that nature has so arranged matters that trout and grayling generally select a different basic food in any given

season. This selection is clearly influenced by weather
and water conditions, but it is impossible to be more
specific at this stage." (Gerrish 1938, p.58)

In effect, Gerrish suggests that grayling and trout exhibit niche
specialisation (see following paragraphs for explanation), as regards
feeding. This view agrees with that stated by Muller (1954b) following
a comprehensive examination of grayling and trout stomach contents
taken from sympatric populations in Sweden – "Trout and grayling do
not compete because they fill different ecological niches in the
stream" (p. 182).

The concept of niche and of niche specialisation is axiomatic
the theory of evolution. The niche was first used by Grinnel (1917,
1928) to stand for the concept of "the ultimate distributional unit
within which each species is held by its structural and instinctive
limitations.... no two species in the same general territory can occupy
for long identically the same ecological niche" (in Odum 1971, p 234-235).
Grinnel therefore viewed the niche as a spatial concept similar to micro-
habitat. Elton (1927) used the term niche in the sense of the functional
state of the organism in its community and emphasised the energy relations
so that the meaning of niche was expanded from Grinnel's rather rigid
concept of a micro-habitat to a more dynamic notion of an animal's role
in the biological community.

In 1965, Hutchinson suggested that the niche could be better visu-
alised as a multi-dimensional space or hyper-volume within which the
environment permits an individual or species to survive indefinitely
and it is this extremely elastic view of niche which will be adopted
for this research. Hutchinson also made the distinction between the
'fundamental niche', the maximum abstractly inhabited hyper-volume,

occupied when the species is not constrained by competition, and the 'realised niche', a smaller hyper-volume utilised when under biotic constraints.

It is in fact the evolution of the 'realised niche' which forms the basis of the first published work on niche specialisation. Darwin (1859) examined the occurrence of niche specialisation in a finch (Geospisidae) community in the Galapagos Islands. These finch-like birds had developed certain adaptations which enabled them to exploit very different food sources on the islands; the types of birds which evolved included 6 species of ground feeders, several of which developed heavy beaks to crush seeds and 8 species which are adapted to perching on trees and to feeding on insects, largely by removing the bark with their beaks. Darwin theorised that these closely-related finches continued to co-exist in one community by avoiding competition through niche specialisation.

When the question of the inter-relationship of grayling and trout is examined in the light of evolutionary theory and the adaptive notion of niche specialisation in particular, it would be expected that the numerous sympatric populations of grayling and trout which do occur exhibit some degree of niche specialisation. Such ecological plasticity has been termed 'interactive segregation' by Nilsson (1963) and it relies on the operation of certain inherent differences between species in their behaviour, morphology, and (or) physiology which in a competitive situation can be selected upon to afford some advantage in feeding, habitat, spawning, and predation and thus could ultimately enable a species to become adapted to a slightly different niche(Nilsson 1967, Odum 1971, Schutz and Northcote 1972).

Some of the characteristics of interactive segregation have been summarised by Nilsson (1967): (1) Interactive segregation can occur between taxonomically distant species, but may be most severe between

closely related species if competition is occurring; (2) total or partial co-existence may be apparent, but in each case, the species will be segregated into different food niches or habitats. (I should like to add that these nuances of niche are typically undetectable to the human observer.); (3) segregation is governed by behaviour, that is to say the fish respond to attraction or avoidance stimuli such as food or habitat preferences, conditioning, threats or other interference. Slight differences in,for instance, the inherent preferences or the ability to threaten in different situations maintain segregation when resources are limited"(p. 307). (I should like to qualify this third characteristic: Behaviour provides a more flexible mechanism which operates to optimise a fish's survival in a changing environment within the limitations of a species' morphological and physiological characteristics.)

Ideally, interactive segregation may be demonstrated in situations where comparisons can be made: Wherever changes in the environment have been monitored, there exists a tremendous opportunity for studying species' inter-actions. One of the most dramatic examples of a sudden change in resources (and its effect on species) is the great fluctuations in the water levels of impoundments; in the sub-arctic impounded lakes of Scandanavia, a very high proportion of large benthic crustaceans (Gammarus lacustris) and insect larvae are eliminated when the water level fluctuates. Ultimately, the bottom fauna is reduced to a few species and in response to this, brown trout and char become more sharply segregated into different feeding niches than before, the brown trout feeding primarily on terrestrial insects and the char chiefly on planktonic crustacea (Nilsson 1960, 1964).

Larkin and Smith (1954), Crossman (1959), and Johannes and Larkin (1961) examined the complex web of trophic side-effects resulting from

the introduction of the red-side shiner (<u>Richardsonius balteatus</u>) into Paul Lake, British Columbia. They discovered that: (1) Competition for food existed between the shiner and its predecessor, the rainbow trout; (2) predation ensued, the red-side shiner preyed on trout fry and the adult trout fed on the adult shiners. After 15 years, it was reported that some form of interactive segregation had occurred as regards feeding because following the introduction, both the species were feeding on amphipos whereas fifteen years later, the shiners were feeding mainly on planktonic crustacea and the rainbow trout (over 30 cm) were preying on the shiners.

Another approach to the demonstration of interactive segregation has been to compare the ecology and the behaviour of allopatric and sympatric stocks of the species in question. Svardson (1949c) and Nilsson (1955, 1960, 1963, 1965) have studied char and trout as regards feeding and spawning when they live separately and when they occur together. They found that when these species live allopatrically, the species have similar food habits and when they live sympatrically, they segregate into separate food niches. Similarly, they noted that trout which live in the absence of char regularly spawn in running water. Lindroth (1955) demonstrated habitat segregation between interacting populations of juvenile Atlantic salmon and seas trout in a Baltic river and found that when living allopatrically the preference of the salmon changed to a habitat corresponding to the trout habitat in the Baltic river.

In this research into the inter-relationship of grayling and trout in the Eden, it is expected that some degree of niche specialisation has been attained related to feeding and therefore it is hypothesised that some form of interactive segregation has occurred between these

populations. Furthermore, it is suggested that the behaviour effecting this niche separation is related to certain inherent differences between these species as regards their morphologies and physiologies.

Part I of this research is conerned with the demonstration of some degree of interactive segregation in the Eden. In situations where allopatric comparisons are not possible, as when limited to investigations in one stream, the occurrence of interactive segregation amongst closely related species may be demonstrated directly by quantitatively assessing the similarity in species' feeding and indirectly by estimating fish growth and condition. These latter indexes are especially useful when evaluating a sympatric situation where a finer appraisal of fish well-being is required to detect the occurrence of any low-grade competition. The sympatric analysis of interactive segregation does confer the advantage that it is more likely the environmental and genetic factors are the same for both populations which cannot be assumed in the allopatric comparison.

Part II of this research explores some of the probable inherent differences between grayling and trout in the Eden, namely in their morphology and physiology, which have tended to separate these closely related salmonids, especially as regards feeding - i.e. the type and size of prey and the seasonal periodocity of feeding. Feeding behaviour regulates such characteristics as food preference (Ivlev 1961) and even the mode of feeding - shoaling or solitary - through such mechanisms as aggression, territoriality, etc. (Nilsson 1967). These behavioural patterns, however, must operate within the various morphological constraints of the species, such as the type of mouth, fin, swim bladder, body type, and eye, all of which will affect the type of prey selected and therefore ultimately, the quality of the fish diet (Keast and Webb 1966). The amount of food consumed, both daily and seasonal, is

governed primarily by metabolism and the neuro-endocrine system which relate to the environment.

Part II will therefore include some morphological examinations which consist of quantitative anatomical comparisons to detect possible differences in the shapes and (or) orientation of the feeding-related structures and physiological investigations into the food consumption, growth, appetite, and metabolism of grayling and trout at different times of the year.

THE STUDY AREAS

1. Tweed Basin.

The River Tweed rises among the upland moors of the Lowther Hills at an altitude of 520 m , approximately 8km north of Moffat, Dumfriesshire. From Moffat, it flows in a northerly direction for 32 km and then follows an eastern course from near Peebles which it continues for the length of the Tweed Valley, between the Cheviot Hills to the south and the Moorfoot and Lammermuir Hills to the north, until it flows into the North Sea at Berwick-upon-Tweed, a total of 160 km.

The Tweed area is composed of high-folded rocks which have been weathered to masses of broad-topped but steep-sided hills (Craig 1965). Much of the rock consists of mud-stones and greywackes which have been heavily eroded forming gentle rounded uplands composed of siliceous soils which are acidic and infertile and steeply entrenched valleys with deposits of heavy glacial till which is slow-draining. Broad bands of Ordovician and Silurian rocks underlie most of the region. Towards the east, however, the valley is covered with red or reddish-brown till which is usually sandy with free drainage, and sometimes with a clay loam which originated from the Old Red Sandstones.

The climate of the lowlands lying to the east is dry and sunny, and the weather of the upper Tweed is relatively cloudy and wet. At Berwick-upon-Tweed, 12.5 km. east of Norham, the mean air temperature is 8.8°C and the mean annual rainfall is 56.6 cm. (All rainfall and air temperature data are taken from Met. Office reports and Tweed Purification Reports.) At Kelso, the average annual temperature is 8.3°C and the annual rainfall is 63.5 cm. At West Linton, the average annual rainfall may be as high as 178 cm and the mean annual temperature is 7.1°C. The mean annual rainfall at Glentress is 110 cm and the average annual temperature is 7.6°C.

Fig. 2   The Tweed Basin and location of study area
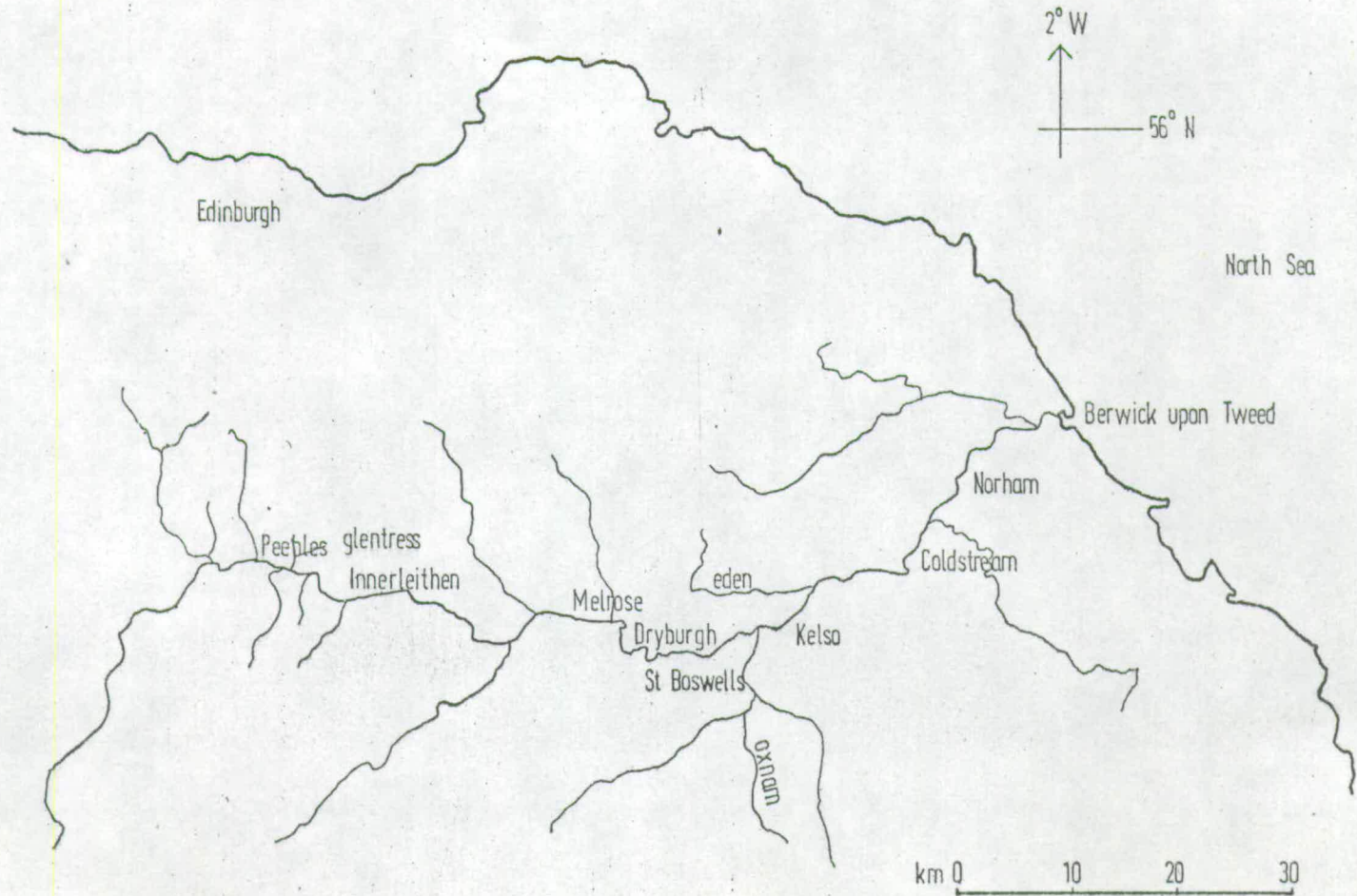
2. The Study Streams.

i. The Eden Water. The Eden is a minor tributary of the Tweed, entering from the northside, one mile downstream of Kelso. For the most part it meanders through agricultural land on the fluvio-glacial material of the Tweed valley. Most sampling took place at the Ednam station, altitude 120 ft., Grid Reference NT 739537, immediately downstream of the road-bridge. The most important sub-station was a large pool flanked on the left side by a high bank with trees and some houses recessed from the bank of the stream and on the right side by a flat field which is generally used for rough grazing (see photograph - Plt. 1a). When more fish were required than could be fished from this sub-station, it was then necessary to make up the desired numbers by sampling further along the Eden at Grid Reference NT 740537, where it flows between flat pastoral grasslands.

The nature of the stream bed at these sub-stations is similar: In the faster water, the substrate is comprised of large gravel and stones with fine gravel in the shallower parts; in the pools and in slower reaches, the bottom is covered by silt with many large stones; some sub-merged plants are present in the glides and the emergents are found around the edges of the pools. This information has been quantified by Mills (1973) in terms of a stream profile which incorporates regular measurements of stream characteristics by transect sampling (Table 1).

ii. Glentress Burn. Glentress Burn flows through the Glentress Forest on the north side of the Tweed Valley and joins the Eshils Burn 1.6 km upstream from where it flows into the Tweed. The surrounding head-waters are predominately heather-moorlands which continue through a young coniferous mixed plantation of Douglas fir (Pseudotsuga taxifolia), Norway spruce (Picea abies), and Sitka spruce (Picea sitchensis), and then through the mature forest of Douglas fir,

SAMPLING AREAS

Plate 1a. The Eden Water

Plate 1b. Norham Boat House on the River Tweed

Table 1. Summary of Characteristics of Three Study Streams

(Mills 1973 and unpublished)

	Eden Water	Glentress Burn	River Tweed
Gradient (%)	0.6	6.5	-
Average depth (cm)	26.9	10.0	-
Average width (m)	8.0	1.1	-
Silt (%)	20.0	16.3	-
Unstable banks (%)	3.5	60.0	-
Mean flow (m^3/sec)	1.12	0.06	34.07
Forest (%)	0.0	56.1	-
Brush (%)	48.8	3.8	-
Open (%)	51.2	40.1	-
Fish species	11.0	1.0	
Land Use	AF	F	G&P
pH	7.9 -8.5	7.8	9.5
Alkalinity (ppm)	182 -206	58.0	88.2

(AF=arable farmland; F=forest; G=grazing; P=parklands.)

Norway and Sitka spruce, Scots pine (<u>Pinus</u> <u>sylvestris</u>), and both the European and Japanese larch (<u>Larix</u> <u>decidua</u> and <u>Larix</u> <u>kaempferi</u>, respectively).

The sampling sub-station for this research is located at Grid Reference 288402. The general characteristics of the stream are again summarised in Table 1.

iii. <u>The</u> <u>River</u> <u>Tweed</u> <u>at</u> <u>Norham</u> <u>Boat</u> <u>House</u>. The Tweed flows through intensive agricultural land around Norham, including sheep and cattle farming. The sampling sub-station is located at Grid Reference NT 893464. The river bed is composed mainly of bed-rock and large stones which are embedded in gravel and reaches a depth of 6 m. Some of the stream characteristics are also recorded in Table 1 and these indicate that the stream's productivity (in terms of the number of fish species and chemical composition) is intermediate to the Eden Water and Glentress Burn.

GENERAL FIELD METHODS.

1. Sampling.

i. <u>Electro-fishing</u>. All the fish samples were obtained by electro-fishing except for the fish which were required for conversion efficiency experiments. This method involves creating an electric field in the water by passing a current between two submerged electrodes. The electro-fishing gear which was used in this work incorporated pulsed D.C. One of the machines which was used was a model manufactured by Marine Electronics of Donegal, Ireland; this machine consists of a 12 volt car battery and power converter which were placed on the stream bank and the electrodes were connected to the coverter by 100 m of flex. This apparatus produces a pulsed D.C. current of about 500 volts which induces 'galvano-taxis' and attracts fish towards the annode where they can be easily

removed by means of a hand-net. A D.C. machine is particularly useful in turbid conditions (Lagler 1970) such as often occurred following a spate. The other shocking machine which was used was a portable model manufactured by the same company and which used smaller batteries (two 6 volt); the complete apparatus is held in a pack frame which together weigh 13 kg. This electro-fisher is preferrable because it is much less cumbersome to operate and also requires fewer people working in the field to catch the fish.

The use of electricity for capturing fish for studies of wild populations dates from the 1920's (Schiemen and Schonfelder 1927). It is accepted as one of the least selective of all active fishing methods (Boccardy and Cooper 1963, Libovarsky and Lelek 1965, in Ricker 1968), although a certain amount of selectivity for larger fish was noted by this author: The electro-fisher was always more efficient in stunning larger fish and this may be related to the greater potential difference between the head and tail of the bigger fish and therefore a corresponding increase in the efficiency of the stunner.

There are some hazards attached to the use of this sampling method. A very strong electric current or prolonged exposure or direct contact with the electrodes can damage or kill fish (Allison 1973 unpublished). Electro-fishing is also potentially dangerous to the operator and to any other warm-blooded animal within the electrical field generated by the apparatus.

ii. Seining. The active form of seine netting was used to obtain samples of the young of the year grayling from the River Tweed at Norham Boat House. The river seine consists of nylon mesh (stretched mesh diameter of 35mm in the wings and 10mm in the bag end) with a total length of 61m and a centre depth of 8m, tapering to 1.6m in the wings. The buoyancy of the net is obtained by cork floats fixed intermittently

along the length of the seine and a lead line is located on the bottom of the net to ensure that it fishes the river bed as it is drawn in. Most shots were made by slowly and carefully letting out the net from the end of a row boat which was completing a loop from the shore (where the net was anchored by at least two people) out into the main body of water and back to a second group who(together with the first group) began to pull in the net to the shore. The only problems experienced with the seine net was that occasionally, such as when the river was full of weed (Rannunculus fluvitans), the net tended to roll up, and in so doing, to become ineffective.

2. Measurements.

i. Length. The lengths of fish were read off measuring boards to the nearest tenth of a cm. Care was always taken to place the anterior extremity of the fish against the stop of the measuring scale with the fish lying on its side with its jaws closed before taking the readings. In all instances (unless other-wise stipulated),the fork length of the fish was measured which takes into account the distance between the most anterior extremity of the fish and the tip of the median rays of the tail. Fork length is regarded as a useful standard of fish measurement because it avoids the problem of how much of the tail to include as in a measure of total length (Lagler 1956 and 1970).

There are certain inherent and experimental inconsistencies related to the measurement of fish. Live fish tend to tense and to contract muscles and thereby to reduce their length, whereas dead fish are comparatively relaxed and their true length is therefore more easily assessed. The efficiency of the experimenter in handling and measuring will affect the accuracy of measurements, particularly in the instance of live fish; care should always be taken to spread the tail fin and to apply consistent pressure to all specimens to assure more precision in measuring fish.

ii. Weight. For most of this research, the weights of live or freshly killed fish were taken by means of spring balances which measure to the nearest 0.1 of a gram. Fish which weighed between 300-500g were weighed on small balances by slipping a hook into the corner of the mouth around the gill arch. Lighter fish were attached to the larger balances by means of a clip to the tail fin. The only instance where spring balances were not used was when measuring the changes in weight for the live fish in conversion efficiency experiments which demanded a higher degree of accuracy as well as a method of weighing which involved the minimum of handling stress and abrasion. (This weighing method will be discussed in the appropriate chapter - VI.)

Fish which were measured alive were not anesthetised because the grayling in particular did not always recover from the anesthetic. The fact that fish could not be reliably anaesthetised does introduce some variation into the fish weights related to persistent wriggling. However, this variation was on the order of only 1-2% of body weight and was expected to be similar for all fish weighed in this manner, and therefore slight differences in weight were not considered to be problematic. Furthermore, the true weight of a fish may vary with the amount of food in the gut or in the amount of water swallowed at the time of capture (Lagler 1970). Finally, it is recommended that the tail clip attachment for the balance is not so satisfactory as the hook, in that it was seen to abrade the tail fin which would probably increase the susceptibility of the fish to disease as well as to retard growth.

3. General Statistical Methods.

All means recorded in this research are listed with standard errors. The following statistical tests were applied to results - student's t test, regression analysis, and analysis of variance (according to Bailey, 1959). Biometrika statistical tables were consulted one-tailed F Variance ratios.

PRACTICAL CONSIDERATIONS

This research has been carried out in the hope of providing some objective insight into the controversial inter-relationship of grayling and trout, both for the improved academic understanding of inter-specific relationships and for the obvious implications that this sort of investigation has for fisheries management.

It should be mentioned, however, that there are many limitations to the interpretation and application of this research. Most of the investigations in the Eden were of short duration and in biological research, where dramatic and unpredictable fluctuations in the environment are expected, short-term analysis will not necessarily reflect or encompass the representative biological situation. This is especially true where only seasonal values are available which may tend to mask monthly or even daily fluctuations. However, for the sake of discussion, the results of this research will be considered as representative of the ecological situation examined in the Eden. Also for the purpose of discussion, because of the limited amount of (published) research on the inter-relations of grayling and trout in Britain, many comparisons have been made with continental work, although the validity of same cannot be necessarily assumed.

Many of the practical limitations of sampling should be considered. Firstly, the size and frequency of samples was largely dictated by whether or not a particular examination required dead specimens. Those fish which were taken for growth data, such as scales, lengths, and weights, were returned to the stream and therefore these samples tended to be large. Those fish which were destined to be slaughtered for stomach analyses, histological examinations and calorific determinations formed small samples, usually 10 of each species, which is the minimum level for statistical assessment (Carpenter 1940). Carpenter showed

that a sample of 10 salmon parr was adequate to give valid results for estimating the food of a population of that species.

There are problems associated with obtaining a representative sample of a population which relate to the distribution and the sampling methods employed and to the sampling time. Elliott (1967) states that because of the 'contagious' (clumped) dispersion of many animals, a large amount of variation may be encountered when sampling natural populations and small samples may therefore be statistically inaccurate. Different species have their particular environmental requirements and behavioural pattern and therefore their dispersion follows a characteristic pattern which is seldom random. Because of the small sample sizes used in some of this research, there are certain restrictions as far as attaching some level of significance to the results and this must be borne in mind in any discussion of these results.

The time of sampling must receive careful attention in the planning and the discussion of investigations. Where examinations necessitated the use of dead fish, the two sampling times which were chosen represented the most extreme temperature conditions experienced during the average annual cycle(Varley 1967) and should therefore tend to accentuate any seasonal differences between grayling and trout. February and August were selected as sampling times because these months typically mark the transition from decreasing to increasing temperature, that is the change from winter to spring and from summer to autumn, respectively. This choice of sampling time assumes that temperature is the critical environmental factor regulating pokilotherms (Fry 1957). The actual time of day for sampling was largely determined by the day-light hours as well as by the working hours of field volunteers, rather than by the circadian rhythms of the fish(Frost and Brown 1967), but the time of sampling was always consistent - midday to late afternoon.

PART   I

THE DEMONSTRATION OF INTERACTIVE SEGREGATION IN THE EDEN

FEEDING

INTRODUCTION

The methods for the determination of the similarity in the diets of
two or more species have already been criticised – the problem of the
interpretation of stomach analyses without quantitative data on the
fauna (Hartley 1948) and the questionability surrounding the use of
the availability factor to relate the numbers of prey species in fish
stomachs to the densities in the fauna (Hynes 1950, Elliott 1967).

In this research, any competition between grayling and trout in
the Eden over food was expected to have been ameliorated through the
processes of interactive segregation and to be reflected by the degree
of specialisation in the diet. It was therefore decided to approach
the problem of dietary similarity by : (1) Discovering whether or not
there was any seasonal periodocity in feeding (both the time of year
and intensity) and if so to evaluate to what extent these overlapped;
(2) learning whether or not these salmonids consume the same prey
species, again with special reference to temporal overlap; and (3)
assessing how specialised are the feeding habits of these species.

METHODS

1. The Sample.

Twelve grayling and eight trout comprised the February samples and ten
of each species, the August samples (Table 2 for fish lengths). Equal
numbers of adult and juvenile fish were obtained whenever possible.
All fish were caught early in the afternoon and were transferred to
a holding box placed in the stream until the station had been 'fished
out'. It is important to note that in a study of competition, where

Table 2.

Lengths (cm) of Grayling and Trout used for Stomach Analyses

February 1973 Sample		August 1973 Sample	
Grayling	Trout	Grayling	Trout
12.0	22.0	24.7	19.7
26.0	24.7	24.5	20.0
36.0	24.0	28.8	16.2
26.0	21.0	19.0	18.3
12.7	14.6	25.0	17.0
25.3	15.0	27.0	13.2
24.6	14.2	19.1	14.5
10.5	9.9	18.6	14.8
10.9		16.5	13.7
13.0		18.7	13.4
10.6			
23.6			

comparisons are made between the diets of two or more species, it is extremely important that the fish samples are taken as closely as possible in both time and space (Maitland 1965).

2. Examination.

Fish lengths and weights were measured and recorded and the scales were removed for later age determinations. The fish were killed by a sharp blow to the skull and then stomachs were removed immediately and preserved in 10% formalin in individually marked vials. Although sometimes the stomachs were not preserved for up to two hours after initial capture, while the sampling was completed, this extra time which the fish spent in the holding boxes did not seem to affect the state of digestion of the gut contents adversely as regards identification.

Each stomach was then treated as a single unit in the laboratory examination which followed. Food items which were found in the oesophagus within one-half inch of the stomach were included with the stomach contents, and the intestinal contents were excluded because they were generally indistinguishable (Windell 1968). The contents of the individual stomachs were then sorted under a low power binocular microscope and all the food items were identified to species whenever possible using the keys of Macan (1959) and Mellanby (1963), and various keys published by the Freshwater Biological Association. In some cases, the identification was more difficult because the animal was macerated, although it was still possible to identify the major group (family) on the basis of certain hard parts, particularly the head capsule and the wings.

3. Quantification.

i. Stomach Fullness. An arbitrary estimation of the degree of fullness of each stomach was determined visually according to Ball (1961) and

this grading system is reproduced in Table 3 . As fish size was not taken into account in this estimation, the values were assessed collectively as a mean and were compared statistically by a student's t-test. (As mentioned in practical considerations, the diurnal feeding rhythms of the fish were assumed to be similar for this analysis.)

Visual Estimate	Points
Distended	5
Full	4
3/4 Full	3
1/2 Full	2
1/4 Full	1
Empty	0

Table 3 : Indexes for grading stomach fullness (after Ball 1961).

ii. Composition. There are many methods available for enumerating the stomach contents of fish, as reviewed by Hynes (1950), Graham and Jones (1962), and Windell (1968); although the approach of these methods differs considerably, they do provide similar comparative results (Windell 1968).

The numerical method was used in this research to provide an actual account of the numbers of different prey species consumed by each fish. The number of individuals of each food type in each stomach is counted to give totals for each kind of food item in the whole sample and then a grand total of all items: The quotient of these provides a percentage representation by number of each food item. However, it is apparent that this method will tend to distort the importance of different food items because of the large differences in the sizes of individual prey

species. There is also the possibility that counting may be distorted by the state of maceration of various prey: One animal may be counted more than once because of fragmentation; soft-bodied animals are digested more rapidly than hard-bodied prey (Reimers 1957, Hess and Rainwater 1939).

The frequency of occurrence method was used as a second means of food enumeration. The number of stomachs in which each food item occurs is recorded and expressed as a percentage of the total number of stomachs examined. This method indicates what organisms are being eaten but does not include information on numbers and it tends to bias the smaller prey species.

iii. Similarity of Diet. An assessment of the similarity in the diets of grayling and trout was carried out in two different ways.

The overlap coefficient is calculated from a formula developed by Morista (1959) and modified by Horn (1966) to provide a numerical expression for the degree of food overlap between species. Proportional food values were estimated for individual stomachs and a mean taken for that species (Zaret and Rand 1971). The formula for calculating the coefficient for overlap is:

$$C = \frac{2 \sum X_i \; Y_i}{\sum X_i^2 + \sum Y_i^2} \qquad \text{(Zaret and Rand 1971)}$$

X_i = that proportion of the total diet of species X taken from a given food category (i); Y_i = that proportion of the total diet of species Y taken from a given category of food (i).

The overlap coefficient varies from 0 when the samples are completely distinct to 1 when the samples are identical (with respect to the proportional food category composition). It must be remembered that the overlap formula tacitly makes great assumptions about the predators and prey species, in so far as the overlap estimated by the probability

that an item of food was taken randomly by the fish- that the prey were equally available to both predator species and that the distributions of both were random. Most species have been found to exhibit a contagious distribution (Elliott 1967) and inter-specific differences in acquiring food related to morphological and physiological constraints which will make some prey species more or less available to different predator species (Nikolskii 1963).

The trophic diversity estimate is defined as the measure of the diversity of prey species taken by each predator species. This estimate provides a quantitative comparison of predator specialisation and consequently it is expected to afford some insight into the feeding relations of these sympatric salmonids.

The diets of grayling and trout were analysed to give estimates of trophic diversity for each species according to Pielou (1965a) as modified by Hurtubia (1973). The measurement of dietary diversity of a predatory population is calculated by treating each stomach as a unit, thereby enabling the calculation of trophic diversity for the whole population. The appropriate function is given by the formula:

$$H = (1/N) \ (\log_2 N! - \log_2 N_i!)$$

(Hurtubia 1973)

H = the diversity per individual predator; N = the total number of individuals; N_i = the total number of individuals in the i_{th} species. This function was adopted because of the previously mentioned problems of obtaining random samples in a patchy environment. This function estimates the diversity of a finite population where larger samples for an infinite population are not possible and therefore a mean for trophic diversity can be ascertained for a series of stomachs of each predator species.

The mean trophic diversity (H) is not, however, representative of the trophic diversity of the predator population as a whole because each stomach will contain only some of the possible prey species used by the population. To resolve this difficulty, a method devised by Pielou (1966a) was applied to estimate the diversity of a patchy population from a series of randomly placed quadrats. Each predator's stomach is treated as an equivalent to a quadrat and its trophic diversity is estimated separately according to the above formula. The counts for individual stomachs were then pooled one at a time for k=1....z stomachs: As the stomach contents are pooled one at a time, at random, the accumulated trophic diversity (H_k) resulting from the greater number of prey individuals and species increases until it reaches stability at point t. The stability of H_k is assessed by plotting curves of H_k coordinates against k, the pooled number of stomachs for the species sample.

To reiterate, the final value for H_k obtained from the pooled sample of prey individuals from all the stomachs should not be assumed to represent an estimate of the trophic diversity of the population (Hurtubia 1973). It is not a random sample of the prey population, as the contents of each stomach do not represent a random sample of the prey species(Pielou 1966a).

RESULTS.

1. Stomach Fullness.

Results for stomach fullness analyses are recorded in full in Table 4. These results suggest that the grayling are feeding more than the trout in the winter and that in the summer, both species are feeding at an intermediate level. The trout is generally thought to exhibit reduced food consumption in the winter and this occurrence is commonly

Table 4.

Seasonal Change in the Stomach Fullness Indexes of Grayling and Trout

(Stomach Fullness Index Scale: 0-empty; 1- $\frac{1}{4}$ full; 2- $\frac{1}{2}$ full; 3- $\frac{3}{4}$ full; 4- full; 5- distended.)

	Summer		Winter	
	Grayling	Trout	Grayling	Trout
	1.0	3.0	5.0	1.0
	0.0	2.0	3.0	4.0
	2.0	2.0	5.0	1.0
	3.0	3.0	5.0	1.0
	1.0	2.0	4.0	2.0
	3.0	1.0	4.0	1.0
	4.0	1.0	4.0	2.0
	4.0	1.0	4.0	1.0
	1.0	1.0	4.0	
	1.0	2.0	5.0	
	S.F.= 2.0	S.F.= 1.8	S.F.= 4.4	S.F.= 1.6

termed 'over-wintering'[2].

2. Composition of the Diets.

A record of the contents of grayling and trout stomachs are listed in Tables 5 and 6 both numerically and by frequency of occurrence for the February and August samples, respectively. This data is also presented as histograms in Figs. 3 and 4.

In the winter samples, the dominant food for both species was chironomids, forming 70% of the grayling diet numerically and occurring in 100% of the fish sampled and composing 67% of the trout diet in 25% of the sample. Grayling also fed commonly on Simulium (92%), on Trichoptera (92%), on Ephemeropter (50%), and Amphipoda (34%). Trout contained little other than chironomids with a mean of 6 food items as against 91 in the grayling.

In the summer samples, _Lymnaea peregra_ was the dominant food on a numerical basis, comprising 50% of the grayling diet and occurring in 60% of the fish examined. Aerial insects also contributed to the grayling diet, amounting to 40% numerically and 60% by frequency of occurrence. The trout diet in the summer was dominated by aerial fauna which comprised 63% of the samples numerically and occurred in 70% of the fish.

3. Similarity of Diets.

i. Coefficient of Overlap. The figures for the combined overlap between the diets of grayling and trout are presented in Table 7.

2. Over-wintering. Fish in northern climes typically undergo over-wintering which involves reduced activity, the cessation of feeding and the use of fat reserves to supply the energy required during the winter. This physiological deceleration is assumed to be related to a reduction in the metabolism of the organism because chemical reactions are depressed by low temperatures and therefore all natural body functions such as feeding and digestion will require more time than at higher temperatures. (Pennel 1863, Nikolskii 1963, Frost and Brown 1967, Varley 1967).

Table 5.

Stomach Analyses Results for Grayling and Trout   February Sample

Method of Enumeration:	Numerical				Frequency of Occurrence			
Fish Species:	Grayling		Trout		Grayling		Trout	
Prey Categories	no.	%	no.	%	no.	%	no.	%
1. Ephemeroptera	4	0.4			4	33.0		
2. Baetis rhodani	4	0.4			2	17.0		
3. Plecoptera	1	0.9			1	8.5		
4. Trichoptera	4	0.4	3	6.5	1	8.5	2	25.0
5. Rhyacophila spp.	24	2.2			10	85.0		
6. Hydropsyche spp.	23	2.1			1	8.5		
7. Hemiptera								
8. Corixa sp.	2	0.2			2	17.0		
9. Diptera								
10. Simulium spp.	184	16.6			11	92.0		
11. Chironomidae	785	70.1	31	67.0	12	100.0	2	25.0
12. Pericoma sp.	3	0.3	4	8.8	2	17.0	2	25.0
13. Tipulidae			1	2.2			1	12.5
14. Ceratopogon sp.	25	2.3			3	25.0		
15. Dicranota sp.								
16. Aerial								
17. Hirudinea								
18. Mollusca								
19. Lymnea peregera	3	0.3			1	8.5		
20. Planorbis sp.								
21. Potomapyrgus jenk.								
22. Crustacea								
23. Asellus aquaticus			5	11.0			2	25.0
24. Gammarus pulex	16	1.4			4	34.0		
25. Coleoptera	3	0.3	2	4.5	2	17.0	1	12.0
26. Elmis aenea	4	0.4			1	8.5		
27. Hydroperus sp.								
28. Stones, debris	13				7	59.5		

Table 6.

Stomach Analyses Results for Grayling and Trout   August Sample

| Method of Enumeration: | Numerical | | | | Frequency of Occurrence | | | |
| Fish Species: | Grayling | | Trout | | Grayling | | Trout | |
Prey Categories	no.	%	no.	%	no.	%	no.	%
1. Ephemeroptera								
2. Baetis rhodani	7	3.0	1	2.1	3	30.0	1	10.0
3. Plecoptera								
4. Trichoptera	3	1.0	4	8.4	2	20.0	2	20.0
5. Ryacophila spp.								
6. Hydropsyche spp.								
7. Hemiptera								
8. Corixa sp.	9	4.0	2	4.2	4	40.0	2	20.0
9. Diptera								
10. Simulium spp.								
11. Chironomidae	4	2.0			1	10.0		
12. Pericoma sp.								
13. Tipulidae								
14. Ceratopogon sp.								
15. Dicranota sp.								
16. Aerial	91	40.0	30	63.0	6	60.0	7	70.0
17. Hirudinea								
18. Mollusca								
19. Lymnea peregera	127	50.0	2	2.1	6	60.0	2	20.0
20. Planorbis sp.	2	1.0			2	20.0		
21. Potomapyrgus jenk.	1	0.4	4	8.4	1	10.0	1	10.0
22. Crustacea								
23. Asellus aquaticus								
24. Gammarus pulex			3	6.3			2	20.0
25. Coleoptera								
26. Elmis aenea								
27. Hydroperus sp.			1	2.1			1	10.0
28. Stones, debris								

Fig. 4 Overlap in diet of grayling and trout taken in February and August

February: grayling

August: grayling

Prey categories (numbers correspond to those of preceding Tables - Nos. 5 & 6)
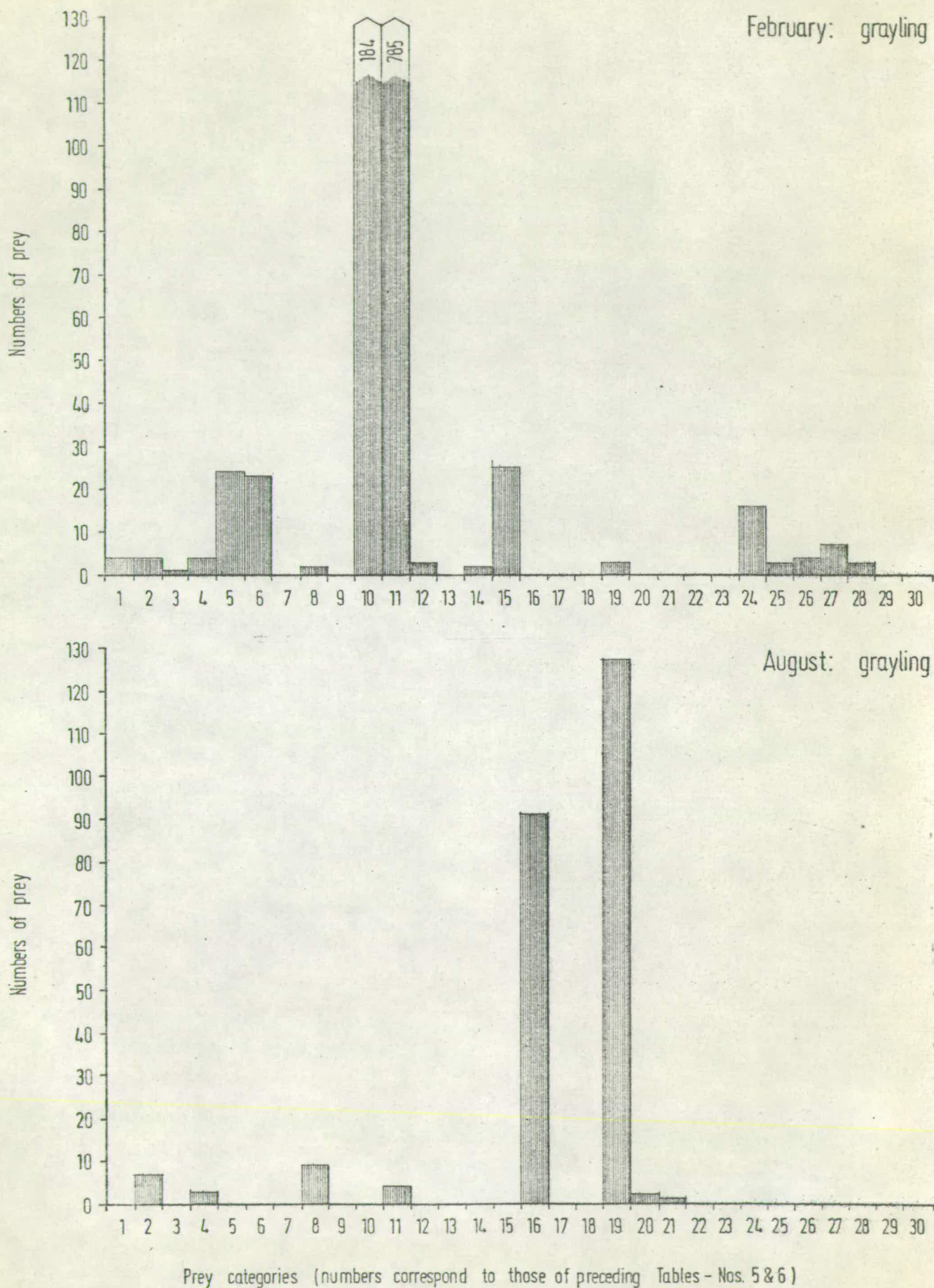
In both samples, the combined coefficients are below 0.60 which is the level of significant overlap according to Zaret and Rand (1971).

Time of Sample	Overlap Coef. for Imm. Fish	Overlap Coef. for Mat. Fish	Overlap Coef. for All Fish
February	0.02	0.39	0.38
August	0.84	0.18	0.54

Table 7 : Coefficients for overlap in the diets of grayling and trout in the Eden.

ii. Trophic Diversity Estimates. Trophic diversity estimates were calculated from the stomach contents of 10 grayling and 10 trout in the August sample and 12 grayling and 8 trout in the February sample and these results are presented in Table 8 along with figures for the accumulated trophic diversity. These results are also presented graphically for better comprehension in Figs 5ab, 6ab, 7ab, and 8ab. Grayling exhibit a higher H_k value than trout in the winter, and conversely trout show a greater H_k value in the summer

Species	$\bar{\bar{H}}$	February Sample range	H_k	$\bar{\bar{H}}$	August Sample range	H_k
Grayling	1.06	0.20-1.45	1.04	0.58	0.0-1.39	1.56
Trout	0.64	0.00-1.15	1.74	0.34	0.0-1.15	1.70

Table 8 : Mean trophic diversity estimates and mean accumulated trophic diversity estimates for grayling and trout in the Eden.

DISCUSSION

1. Stomach Fullness.

The stomach fullness index has been used by other workers as a quantitative means of assessing feeding intensity (Ball 1961, Graham and Jones

Fig. 5a   Trophic diversity estimates (H) from grayling in February
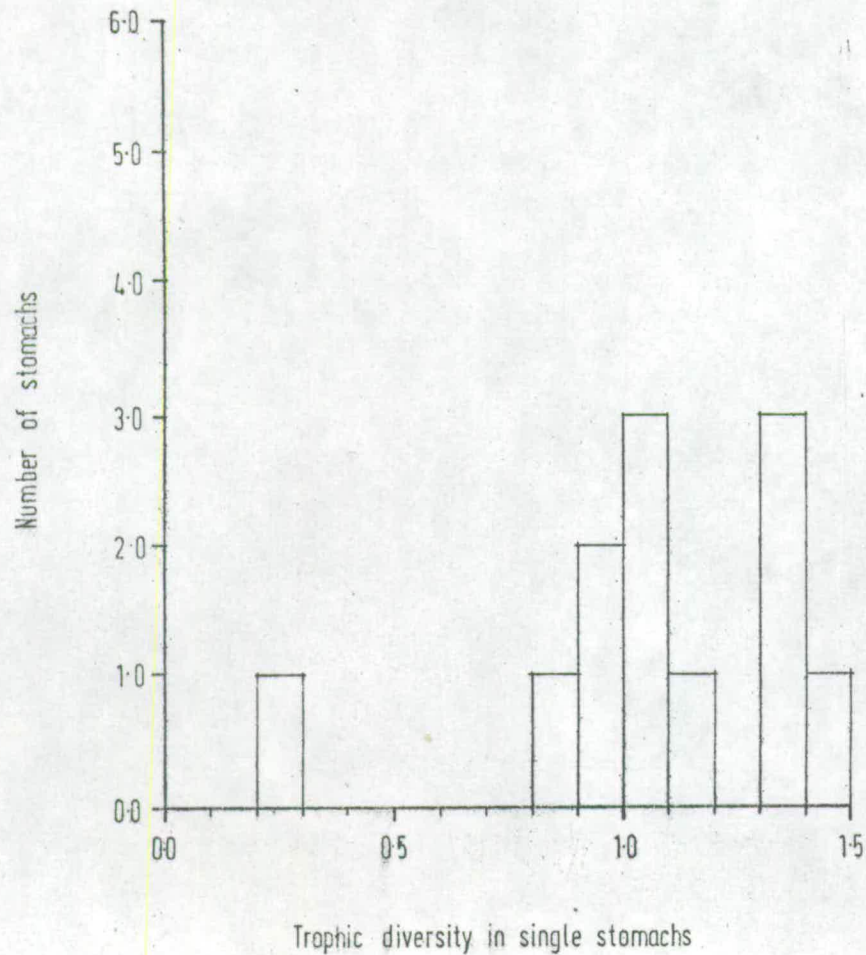
Fig. 5b   Graph of accumulated trophic diversity (H_k) for grayling in February
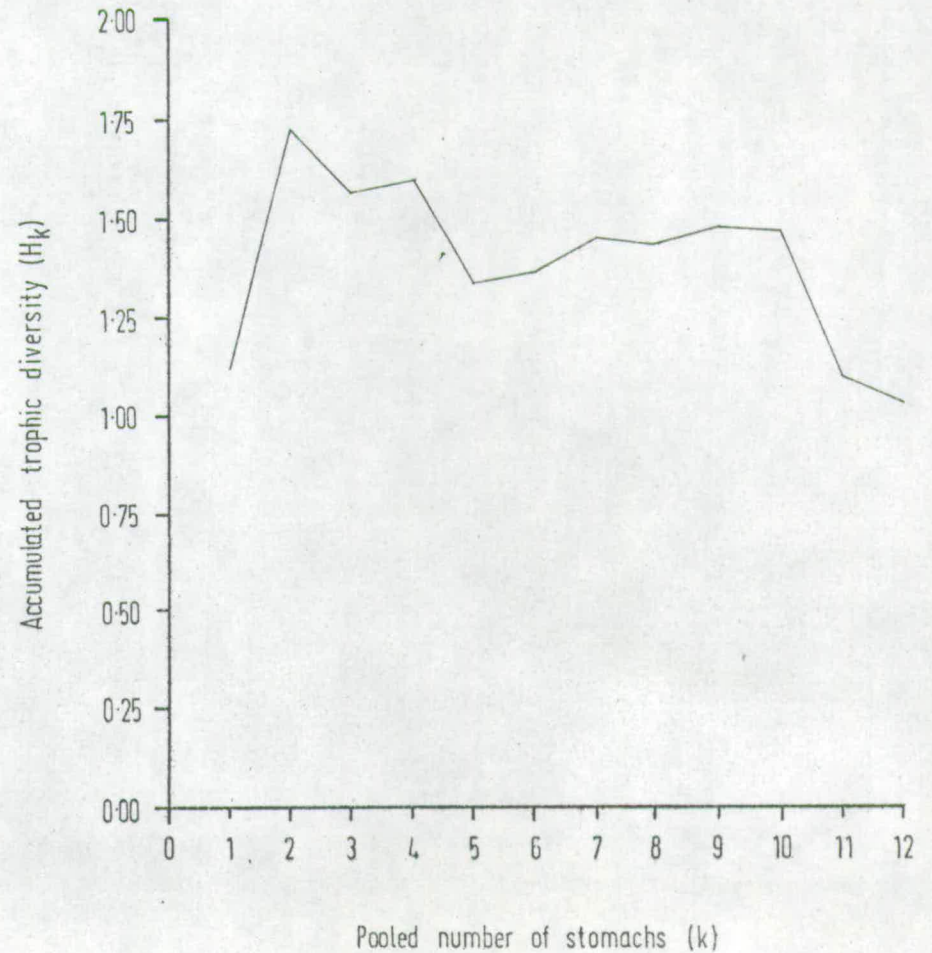
Fig. 6a Trophic diversity estimates (H) for trout in February
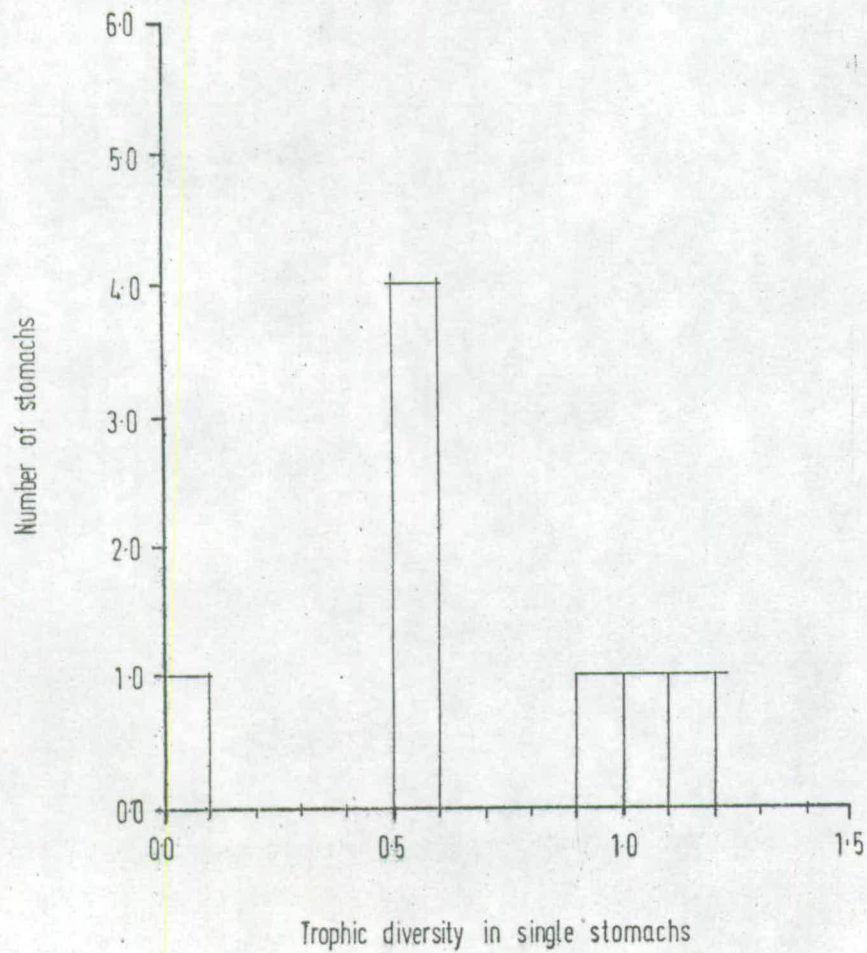
Fig. 6b Graph of accumulated trophic diversity (H_k) for trout in February

Fig. 6a axes: Number of stomachs (y-axis, 0·0 to 6·0) vs Trophic diversity in single stomachs (x-axis, 0·0 to 1·5)

Fig. 6b axes: Accumulated trophic diversity (H_k) (y-axis, 0·00 to 2·00) vs Pooled number of stomachs (H_k) (x-axis, 0 to 12)
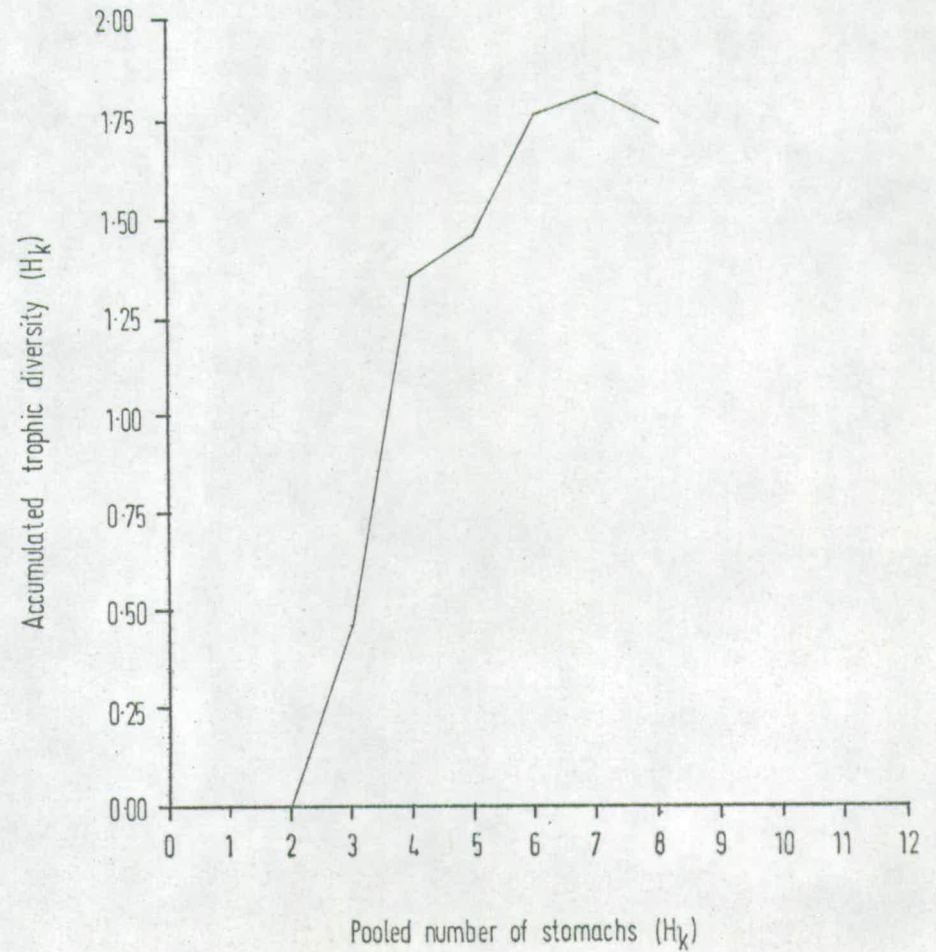
Fig. 7a Trophic diversity estimates (H) from grayling in August
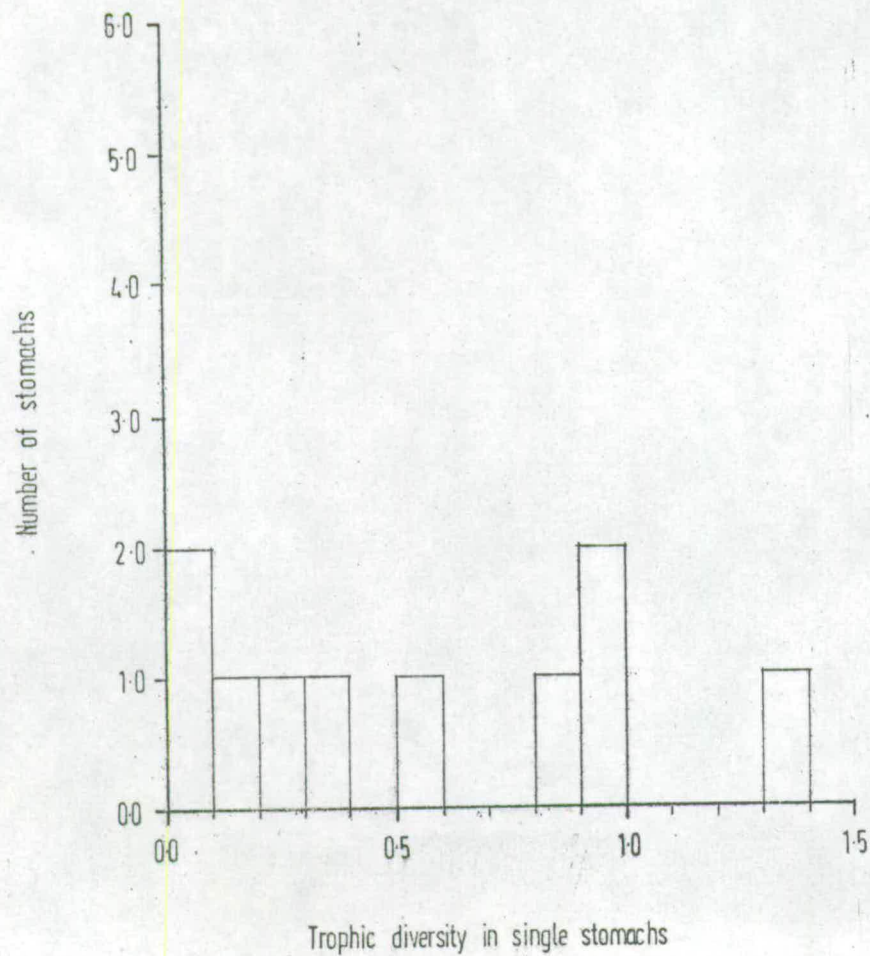
Fig. 7b Graph of accumulated trophic diversity (H_k) for grayling in August

Number of stomachs

Trophic diversity in single stomachs

Accumulated trophic diversity (H_k)

Pooled number of stomachs (H_k)
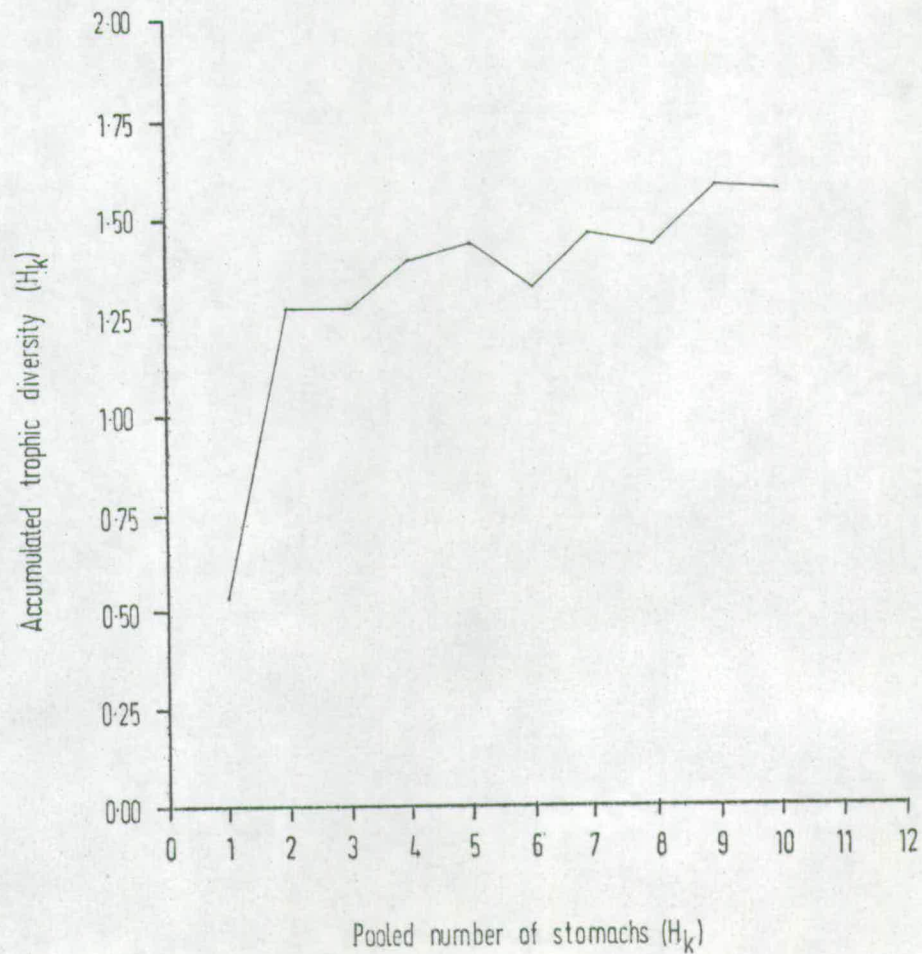
Fig. 8a   Trophic diversity estimates (H) for trout
in August
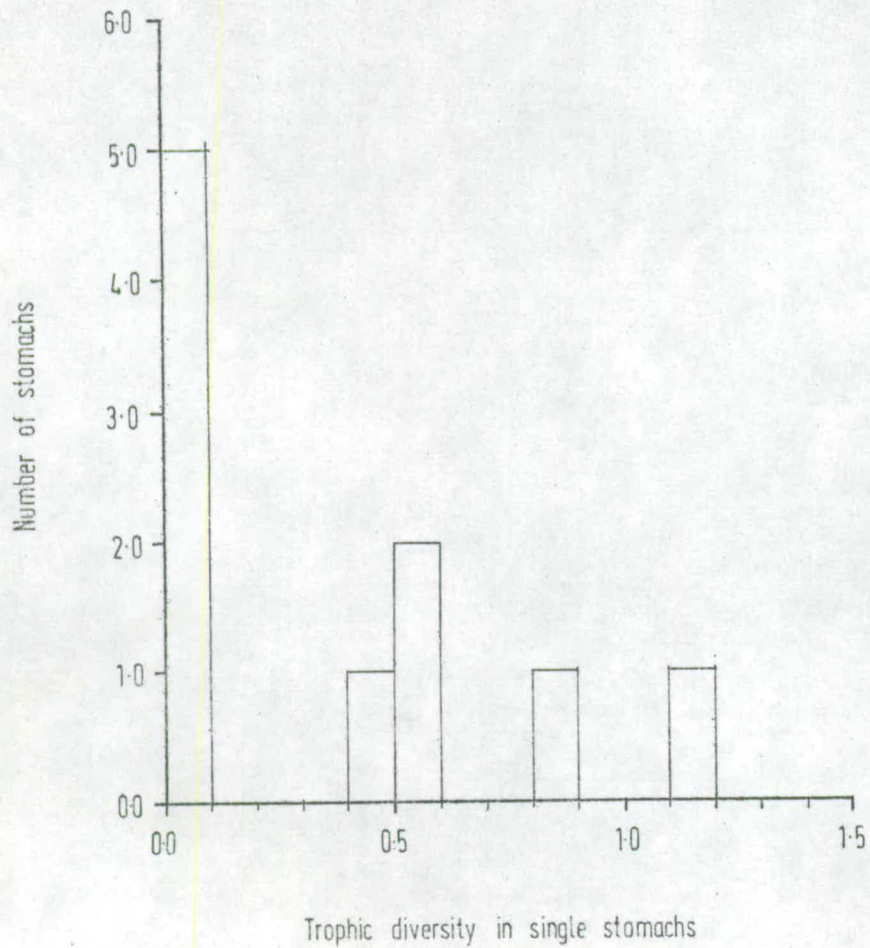
Fig. 8 b   Graph of accumulated trophic diversity (H_k) for trout
in August

Number of stomachs

Trophic diversity in single stomachs

Accumulated trophic diversity (H_k)

Pooled number of stomachs (H_k)
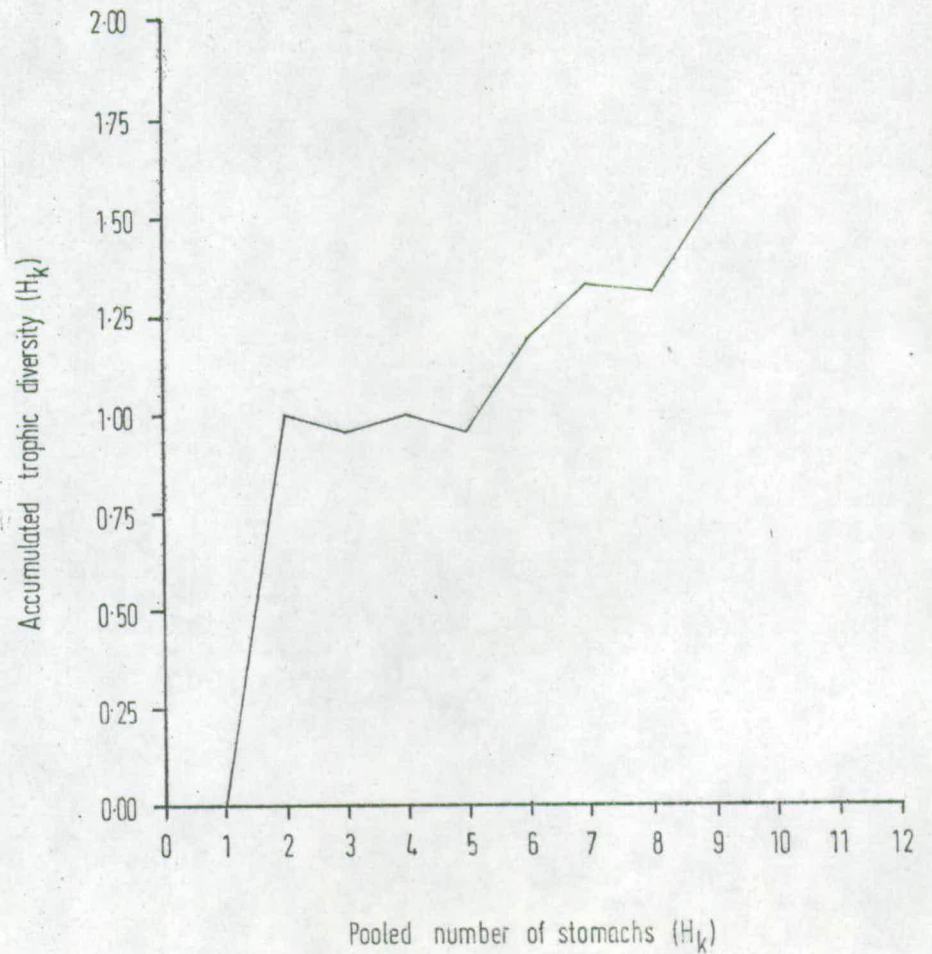
1962) and it therefore may provide some insight into those times of
year when species might be competing because of intensive feeding.
In these papers, high stomach fullness values have been equated with
increased feeding and conversely low values or empty stomachs have been
interpreted as reduced feeding. Accordingly, Dahl (1962) deduced from
a high incidence of full stomachs that grayling were feeding actively.

It has already been mentioned that the gastric digestion rates and
food intake of fish are greatly affected by temperature (see the def-
inition of 'over-wintering') and this has been shown experimentally
by Fry (1947), Nikolskii(1963), and by Elliott (1973); in view of bio-
chemical aspects of digestion (enzyme kinetics as regards temperature)
it is possible that a high stomach fullness index may indicate an accum-
ulation of food at low temperatures (depressed digestive activity)
rather than the direct interpretation of intensive feeding.

Because of the contradictory natures of these two interpretations
of stomach fullness results, a discussion of meaning of stomach fullness
indexes and of changes in these values in the summer and winter will
be taken up in chapters IV and VI of this manuscript when information
on seasonal changes in fish condition and metabolism and appetite are
presented.

2. Composition of Diets.

The interpretation of stomach analyses is not straightforward. It is
obvious that there is a great amount of variation in feeding between
different days and the nature of this variation depends on both the
fauna on which the fish are feeding and on the physiological condition
of the fish themselves, all of which relate to the environment. Further-
more, the extent of the variation possible between stomachs in any one
sample and exactly what this sample represents in terms of the whole
diet must be carefully assessed. Elliott (1973) examined the

complicated question of diurnal feeding rhythms of rainbow trout in relation to prey abundance and rhythms and found that the amount of food consumed by trout was directly related to the activity cycles of both predator and prey which are greatly influenced by temperature.

The abundance of individual food items in grayling stomachs was noted by Radforth (1940) and by Dahl (1962). Although the grayling has been termed a voracious and unfastidious feeder (Dahl 1962), it has a general preference for the bottom fauna (Seeley 1886, Day 1887, Muller 1954a,b, Jankovic 1964, Siddiqui 1967, and Peterson 1968), and for Gastropoda, Amphipoda, Ephemeroptera, and Trichoptera larvae, Simuliidae and Tendipedinae larvae and pupae. Grayling are known to eat aerial insects during the late summer and early autumn (Seeley 1886, Day 1887, Muller 1954a,b, Jankovic 1964, Hellawell 1971), although Dahl considers the aerial fauna to represent an emergency food source for the grayling.

Gammarus was not so important to the Eden grayling as might have been expected from the results of other studies (Day 1887, Gerrish 1939, Dahl 1962, and Hellawell 1971). However, Radforth, who also worked on grayling from the Tweed watershed, found only a few gammarus in the grayling stomachs she examined and reported that the fish consumed mainly chironomids, simulids, ephemeropterans, and Elmis larvae, most of which were found in the stomachs of grayling taken from the Eden.

Molluscs were extremely important in the grayling diet in the August sample, occurring in 50% of the sample, however these did not appear in the February sample. Hellawell (1971) found that molluscs did not contribute much to the diet of the Lugg grayling, however, the virtual absence of molluscs in the grayling diet in February is surprising in view of the reports of Dr. Mills and some under-graduates in this

department: They found molluscs in the stomachs of Eden grayling which had been sampled in December and in February. It is therefore considered that these results serve to emphasise the point made earlier that due to the size of the samples and to natural biological fluctuations these results cannot be regarded as truely representative of the ecological situation being examined.

Fish were never recovered from the stomachs of Eden grayling. Other workers have also noted that fish are not a characteristic component of the grayling diet and therefore play a minor role in the grayling diet (Radforth 1940, Dahl 1962, Hellawell 1971). There have been occasional reports of piscivorous fish: Grayling have been found to consume stickle-backs (Gasterosteus aculeatus), minnows (Phoxinus phoxinus) and dace (Leuciscus leuciscus) (Dahl 1962, Radforth 1940, and Muller 1954a,b, respectively). Trybom (1908) recovered young whitefish (Coregonus spp.) perch (Perca fluviatilis), roach (Rutilus rutilus), and bullheads (Cottus gobio) from grayling stomachs.

Reports of grayling predation on fish ova are more common (Day 1887, Trybom 1908, McIntyre 1968, Peterson 1968, and Hellawell 1971), although these eggs do not seem to form a major component of the grayling diet. In Hellawell's paper, he noted that salmonid ova composed only 1-2% of the diet by frequency of occurrence. Siddiqui (1967) reported that 25% of the Lynn Tegid grayling contained correogonid ova.

Numerous studies have been conducted on the food of the brown trout and these indicate that trout consume both a greater number and a broader range of prey during the summer months than in the winter (Nilsson 1955, Ball 1961, Graham and Jones 1962). The February sample of trout (although rather sparse) contains most of the prey species recorded in Elliott's trout captured in February (1967): He noted that Baetis sp., chironomids, and plecopterans were the major food types and these

resemble Eden results with the exception of the plecopterans. In the August sample, Elliott (1967) found that the surface and terrestrial insects comprised most of the trout food, which again is similar to the Eden trout, along with ephemeropterans, plecopterans, tricopterans, Baetis sp., chironomids, and Elmis sp.

Siddiqui (1967) conducted an investigation into the feeding habits of brown trout in Lynn Tegid, an out-flow of the Bala Catchment Scheme; however, because of the large numbers of grayling caught when seining for trout, the investigation was enlarged to include analyses of the stomach contents of both trout and grayling to compare their feeding habits. In February, Assellus sp., ephemeropterans, and correogonid ova were the chief type of grayling food (approximately 25% by frequency of occurrence) whereas trout consumed trichopterans predominately (25% by frequency of occurrence) and Asellus sp. and some fish (each 10% by frequency of occurrence). In August, the trout diet was chiefly comprised of aerial fauna (80% by volume in 45% of the sample) and also of coleopteran adults (40% by frequency of occurrence); the chief prey species taken by grayling was Chironomidae (both pupae and larvae formed 35% by volume in 40% of the sample) and only 5% of the grayling consumed aerial fauna.

The feeding habits of grayling and trout in Lynn Tegid appear to be quite different to those of the Eden populations where in the winter the dominant food of both species was clearly Chironomidae, and the summer samples indicated that both species fed extensively off aerial fauna - the trout nearly exclusively - and that the grayling consumed a large proportion of molluscs. However, it should be remembered when comparing the results of stomach analyses of grayling and trout taken from the Eden with other research on these species, that any discrepancy between investigations over either the types and the importance of

various food species consumed by these salmonids may be attributed to the differences in the habitats in which these populations live (Mann and Orr 1969). Lynn Tegid is an out-flow lake and constitutes a 'lentic' environment, a relatively self-contained, still body of water, and the Eden is a 'lotic' environment , that is characterised by flowing water; therefore, the types and abundances of flora and fauna which will survive in these freshwater habitats may be expected to differ considerably.

3. Similarity of Diets.

The measurement of the overlap or the degree of specialisation between the diets of two species is complex because two individuals in the same environment may find different foods by foraging in different ways or alternatively, they may obtain the same foods but in slightly different environments. Therefore any interpretation of the similarity in diets greatly depends on a broad general knowledge of the fish and the prey species concerned and the biology and distribution of both.

Quantitative evaluations of the similarity of grayling and trout diets have been lacking in previous studies of these species. A rough comparason of their food was made by Sawyer (1946) who deduced from the fact that both species responded readily to the fly that the grayling overlapped seriously with the trout as regards food. A more objective study was carried out by Muller (1954b) who counted and classified prey species taken from grayling and trout stomachs by means of pie-shaped diagrams; he concluded that during the summer both species fed off aerial fauna, however the grayling also took bottom fauna.

i. Coefficient of Overlap. A superficial summary of the various categories where grayling and trout diets overlap in the Eden indicates that there is a greater similarity in their diets in the summer than in the winter - in the summer they overlapped in 6 out of 10 food

categories, whereas in the winter they overlapped in 4 out of 18 prey species which were taken. The coefficients for overlap also suggest this seasonal difference in dietary overlap, although the figures were not considered to demonstrate significant overlap. However, when the coefficients are assessed by age groups, a different feeding relationship is revealed (Table 7): The contents of the immature fishes' stomachs show a negligible overlap in the winter (0.02), but in the summer they exhibit a significant overlap in diets (0.84); the adult fish do not overlap significantly in either sample. The marked overlap in the diet of immature fish in the summer may be explained by the fact that the grayling were consuming only 8 different prey categ ories and the trout only 5, and the species overlapped in four of these prey categories, 3 of which were the main constituents of both predators' diets. The coefficients for overlap tend to suggest that the only time that the food of grayling and trout overlap significantly is in the summer and therefore that this is the time of year when competition is likely to occur.

ii. Trophic Diversity Estimates. The results of the composition of grayling and trout diets indicate that each species eats a wide variety of organisms, and that their diets have certain characteristic features. Grayling and trout both fed off the bottom fauna during the winter - both species took high percentages of chironomids, although grayling consumed more of this prey category in sheer numbers than did the trout, as well as large amount of Simulium sp., trichopterans, and ephemeropterans. In the summer, there was a shift to surface and aerial prey species produced: Trout fed nearly exclusively on aerial fauna, whereas grayling divided their feeding between aerial and bottom fauna, with slightly more emphasis to the latter (40% and 50% numerically).

The superficial summary of dietary overlap in the Eden as presented

in the preceding section also delimited the feeding spectra for each species at the two sampling times, and the breadth of these feeding patterns have been compared by means of trophic diversity estimates. The H_k figures for grayling and trout suggest that there are specific differences in the extent of their feeding specialisation. In the summer, the grayling H_k is only 1.40 against 1.70 of the trout. These figures may be interpreted to mean that a greater number of prey species are available to the trout or in other words that the grayling is a more specialised feeder than the trout, possibly related to its morphological propensities or constraints. Grayling fed from 8 food categories, but 90% of their food was taken from only 2 prey types; trout consumed 60% of their food from 1 prey category (aerial) and the remaining food was taken from 3 other prey categories.

In the winter there was a marginal increase in the H_k for trout (1.74). This result is surprising because the higher accumulated trophic diversity for the trout sample occurred at a time when trout intake is expected to be low (or reduced) because of 'over-wintering'. This may be explained by the fact that although the species on the whole were consuming a great range of prey types, the individual fish typically consumed only a very few number of prey which tended to represent different species than those taken by the next trout, resulting in an unexpectedly high value for accumulated trophic diversity. Grayling H_k showed a definite decline from the summer value of 1.40 to a winter figure of 1.06. Again the trophic diversity estimate may be somewhat surprising because most grayling consumed a large number of food organisms, but these prey represented a similar range of prey species; the grayling therefore consistently exhibited more specialised feeding than the brown trout.

Other researchers who have examined the feeding habits of grayling

and trout have speculated on their feeding specialisation. Gerrish
(1939) wrote that grayling and trout were both specialised to the ex-
tent that in any given year, they rarely chose the same invertebrate
as their basic food: In one year, the grayling took caddis and the
trout fed off gammarus, and in the next year, the reverse was true.
Muller (1954b) in his comprehensive examination of the invertebrate
fauna and the feeding habits of grayling and trout in various Swedish
streams, concluded that the species were both highly specialised in
that they removed different proportions of the same prey categories.
Siddiqui (1967) demonstrated that grayling and trout exhibit a certain
degree of feeding specialisation: His results "...support Ball's
suggestion that trout are more superficial feeders than grayling,
eating the more obvious and readily accessible larvae." (p. 42).

SUMMARY AND CONCLUSIONS

The problems of interactive segregation as regards feeding was invest-
igated in the grayling and trout populations living in the Eden. The
similarity in their diets was assessed from stomach analyses made on two
samples taken at two extreme times of the year - February and August.
The stomach contents were quantified for stomach fullness and composi-
tion before evaluating the dietary similarity in terms of the coefficient
for overlap and the trophic diversity estimate.

1. A direct interpretation of stomach fullness analysis indicates that
grayling feed all year round, but most intensively in the winter, and
that trout feed heavily in the summer, but exhibit a reduced intake of
food in the winter. This interpretation however would appear to be
complicated by the known bio-chemical relationships of metabolism, tem-
perature, and digestion (enzymes), and therefore this apparent seasonal
periodocity of feeding will be attested by the following investigations
into seasonal changes in fish condition and into conversion efficiency
of grayling and trout.

2. In the winter, the dominant food for both species was Chironomus sp.
but the grayling also commonly fed on Simulium sp., trichopteran larvae,
ephemeropteran and Amphipoda. In the summer, Lymnaea peregra was the
dominant food in the grayling, although aerial fauna were nearly as
important. The trout fed almost exclusively on aerial fauna.

3. Results from the calculations for overlap coefficients and trophic
diversity estimates suggest that there is a similarity in the diets of
grayling and trout, particularly in the summer, but that there are some
important differences in the intensity of consumption of their common
invertebrate stock. These differences are thought to be related to the
fact that some degree of feeding specialisation does occur in these
salmonid populations which lend support to the concept of interactive
segregation in the Eden.

CHAPTER III

GROWTH

INTRODUCTION

Any appraisal of growth begins with an analysis of the age structure
of the population. Typically, the age of a fish is determined by
counting the annular configurations in various hard parts of the body
which are generally accepted as representative of seasonal irregular-
ities in fish growth (Le Cren 1947, Lagler 1956). Seasonal variation
in tissue activity results in deposits of different extracellular
material in the internal skeletal parts during the winter and summer
in temperate climes, where usually a very rapid spring growth follows
a relatively depressed winter growth, and thus well-defined alternating
annual bands are formed (Van Utrecht and Schenkan 1972).

The calcareous structures which are commonly used for age deter-
minations are otoliths, fin-rays, and scales,and the latter are used
almost exclusively for freshwater research because they can be collected
easily, with a minimum of damage to the fish. A great amount of work
has been done with scales and a comprehensive tabulation of age studies
which have to a large extent relied on scale-reading was compiled by
Graham (1929b) and by Carlander (1950).

Although the use of seasonal variations in the growth of the cal-
careous tissue has been accepted as a standard technique for age deter-
minations of teleosts, the validity of this method must be confirmed for
each species (Lagler, 1956). The prerequisites for obtaining reliable
age and growth determinations from scales, fin-rays, and or otoliths,
have been outlined by Graham (1929a), Van Oosten (1929), and De Bont
(1967), and may be summarised by the following: (1) A correspondence
between the growth of rings of a specimen of a known age and the

interpreted age; (2) a constancy of scale number throughout life; (3) Growth rings formed yearly and at the same time of year; (4) a regular increase in body size correlated with an increased number of growth rings; and (5) a constant ratio between the annual increment in body length and the radius of the scale.

To my knowledge the published work on the age and growth of the grayling has relied on the use of scales for age determinations, but has failed to include any verification of the above criteria (Hutton 1923, Platts 1936, Gerrish 1938, 1939, Jones 1953, Hellawell 1969, and Mackay 1970 have examined the grayling in Britain; Dyk 1939, 1959, Gustafson 1949, Dahl 1962, Peterson 1968, and Jankovic 1964 have studied the grayling on the continent). In view of this assumption made by fellow researchers, it was therefore considered necessary to attempt to satisfy the De Bont criteria as a part of this research. However, as the laboratory facilities proved to be unsuitable for the maintenance of salmonids over an extended period of time, i.e. one year (see appendix I), it was impossible to assess the first and second requirements. Therefore the verification of the use of scales for age and growth determinations of the grayling was limited to the latter three criteria.

In the instance of the brown trout, the verification of the use of scales for aging brown trout was clearly recorded as early as 1910 by Dahl in Norway, so it was only necessary to attest the validity of an 'isometric' relationship between body and scales for growth deter-minations (De Bont criteria 5).

Scales not only provide information on the age of the fish, but may also be utilised for estimating the previous growth of the fish. The length of a fish at the end of each year of its life may be computed from a series of measurements of a scale when the length of

the fish at the time of capture is known. These estimations which are termed back-calculations of growth are based on the assumption that the body to scale relationship is 'isometric', that is the scales size increases in proportion with body length.

The principle of back-calculation was first applied to salmonids by Dahl (1910), who assumed that the brown trout exhibited an isometric body-scale relationship throughout life. This assumption was queried by Kipling (1962) and she discovered that the body-scale relationship changed from allometric when fish were less than 10cm at the end of the first year to isometric after the fish attained that size. If the fish were consistently rapid growers, they attained isometric growth by the end of the first year (at 10 cm) and the body-scale relationship was accordingly always proportional. When, however, allometry persisted through the lifetime of the fish, the back-calculations underestimated previous growth and a specific correction factor was applied.

METHODS.

Age Determination.

1. Terminology and Ideology of Age Determination.

A consistent system for designating the age of fish is required for comparative purposes regardless of the actual method utilised to ascertain the age of the fish. 'Circuli' are concentric ridges, rings or bands which are laid down as the fish grows and form the basis of age determination in fish. Dahl (1910) discovered and confirmed that the age of a fish could be calculated by counting the alternating bands of summer and winter growth which together form each 'check' or 'annulus'. Dahl examined the scales of brown trout caught at different seasons of the year and studied those of artificially reared trout of a known age and found that in the summer, the circuli were widely spaced and that

in the winter, the circuli were closely spaced. "It is therefore quite clear that the summer-zones and the winter bands on the scales of trout are formed in the corresponding seasons, which affect their growth, and that these zones indicate annual epochs in the life of the fish. If therefore, we count the number of these zones, we can ascertain the age of the fish." (in Frost and Brown 1967, p. 90).

To assure accuracy and consistency in age determination, the precise location of the check must be defined: An annulus has formed only when the termination of winter growth is delineated by the resumption of spring growth. Therefore, a fish is termed 0+ during the first year of life until it has formed an annulus at which point is called 1+ because of the presence of at least one band of spring growth which is appropriately termed 'plus growth' (Tesch 1970).

2. Collection and Preparation of Fish Material.

Scales were removed with a scalpel just anteriorly to the dorsal fin between it and the lateral line and placed in a scale packet on which the specimen's length and weight were recorded. Many of the scales examined in this research had been collected by Dr. Mills in the 4 years preceding the commencement of my research and therefore scale samples extended from 1968 to 1973, inclusively.

| | Grayling | | | Trout | |
Month	Year	Number of Fish	Month	Year	Number of Fish
May	1968	12	July	1969	11
June	1968	9	October	1969	10
October	1968	4	November	1970	8
December	1968	10	December	1970	7
June	1969	10	January	1971	8
October	1969	19	February	1971	15

Month	Year	Number of Fish	Month	Year	Number of Fish
February	1969	10	March	1971	8
February	1970	16	May	1971	13
April	1970	23	June	1971	25
May	1970	28	August	1971	4
March	1972	20	February	1973	8
February	1973	82	August	1973	10
August	1973	59			

Total number of fish= 302   Total number of fish= 127

Before the scale was examined, it was cleansed and a permanent impression was made by placing a few scales between two strips of cellulose acetate (approximately 2 cm x 5 cm x 1 mm) and passing these through a hand roller (Nesbit 1934, Smith 1954).

3. Scale Reading.

i. The scale impressions were magnified on a projectina by a 10X objective lens which facilitated counting the annuli as well as measuring the scale's radii for later work in back-calculation of growth. The checks were read off from the focus (scale centre) to the edge of the scale, taking care that it was always along the same anterior axis (Frost and Brown 1967).

ii. Sources of Error. The accuracy of scale reading as a means of determining the age of the fish depends to a great extent on the experience and objectivity of the operator in so far as the interpretation of the specimen's age is complicated by various irregularities and ambiguities in the formation of the scale itself (Frost and Brown 1967). Quite often, the scale marks are not necessarily present on all of the scales of a fish and the actual circuli may vary on the same specimen. The extreme examples of these inconsistencies are the occurrences of false annuli, secondary growth rings (Cragg-Hine and ____ 1965), the

complete failure of the annulus to form in a particular year, the erosion of the outer margins of the scale possibly related to spawning and (or) a regenerated scale, all of which may considerably influenced by food supply, temperature, and stress (Brown 1943). There is also the question of the selection of a 'normal' or 'key' scale, that is one of the first scales to develop and therefore, the scale which will most accurately represent the age and the growth of the fish (Tesch 1970).

4. Verification of the Use of Scales for Age Determination of Grayling.

i. The determination of regular annulus formation was made from a careful inspection of circuli evolution during a given year and was documented by photographs which were taken by means of a Polaroid camera which attaches to the projectina screen.

ii. In order to assess whether or not there was a consistent increase in body size which could be correlated with an increase in the number of circuli, the mean number of rings which comprised the first year of growth was calculated for all age classes (252 fish) and this was compared with the actual mean length of grayling at the end of the first year of life. The calculations for the actual mean length were based on the 158 scale samples which were taken around the time of assumed check formation:12 in May 1968, 5 in March 1969, 16 in February 1970, 23 in April 1970, 20 in March 1972, and 82 in February 1973. An appraisal of the relationship between circuli and body size during the first year of life was considered to provide sufficient evidence for the verification of this relationship and tended to minimise any possible differences between fish as regards spawning and its effect on scales (Lagler 1956, Frost and Brown 1967).

5. The Accuracy of Age Determinations.

The accuracy of age determinations can be substantiated by the following means (Van Oosten 1929, Graham 1929a): (1) A length frequency curve

where each age group is expected to be represented by a distinct mode; (2) differences in the relative abundance of the year classes may make a particular year class recognisable(as unusually scarce or abundant) and may by its annual appearance in increasingly older age classes act as a check on age determinations made from annuli on scales; (3) if the rings on the scales are annual, a close examination of their growing edge throughout the year should reveal any annual cycle of growth; (4) fish of a known age (fry) may be marked and released and recaptured at a later time and the number of annuli on the scales may be compared with their known age; (5) fish reared in ponds or aquaria are typically of a known age and this information may be used to confirm annuli present on their scales.

In this research the first method - length frequency histogram- was chosen to confirm the accuracy of age determinations of grayling because: method (2) was inappropriate to the size of the study area and samples and the period of research, method (3) has been examined in the section dealing with the verification of the use of scales for aging grayling, method (4) was not feasible because the tagging program begun by Dr.Mills was small and there were no recaptures, and method (5) was impossible to execute in this department because of previously mentioned difficulties experienced in maintaining salmonids.

Petersen (1891) demonstrated that the multi-modal length distribution of a population permits a statistical classification of the individuals into different age groups. A length frequency histogram was constructed with class intervals of 0.5 cm which was based on 84 grayling caught in February, 1973. For such a histogram to be meaningful, it should be based on data collected(nearly as possible)at the same time (Le Cren 1947), and this was the largest sample of grayling. The agreement between the age of the fish sample was determined by successive

comparisons of modes of the length frequency distribution with annuli on the scales. The percentage agreement between the two methods was calculated by dividing the number of fish in a particular year class as determined by scale-reading by the number of fish in the same year class as determined by the length frequency modes.

Determination of Past Growth.

1. Back-calculations.

i. Method. The yearly increases in the length of each fish were determined by marking off the individual distances from the focus to each successive annulus to the edge of the scale, onto a cardboard strip held against the projected image of the scale. The length of each fish when each annulus is laid down can then be calculated from the following proportion:

$$\text{length at the end of year Y} = \text{length at capture} \times \frac{\text{distance from focus to annulus Y}}{\text{distance from the focus to the edge}}$$

(Frost and Brown 1967)

This method is often known as the Dahl-Lea method, named after its principal originators (1910). Alternatively, the corresponding back-calculated lengths can be determined graphically by direct proportion as illustrated in the following diagram, and this latter method was chosen in this research (Le Cren 1947). Until the proportionality of growth could be tested, it was assumed for working purposes that either there was an isometric relationship between scale and body growth or that these back-calculations could be corrected later if an allometric relationship was shown to occur.

ii. The Accuracy of Back-calculations. According to Le Cren (1947), the accuracy of back-calculations may be tested in two ways: (1) The mean lengths of samples of fish caught in one year ('actual') can be compared with the means of back-calculated lengths from samples caught

Fig. 9a Dahlea's method for back-calculation of previous growth by direct proportion

Fish length (cm)

Fig. 9b Method for correcting back-calculations
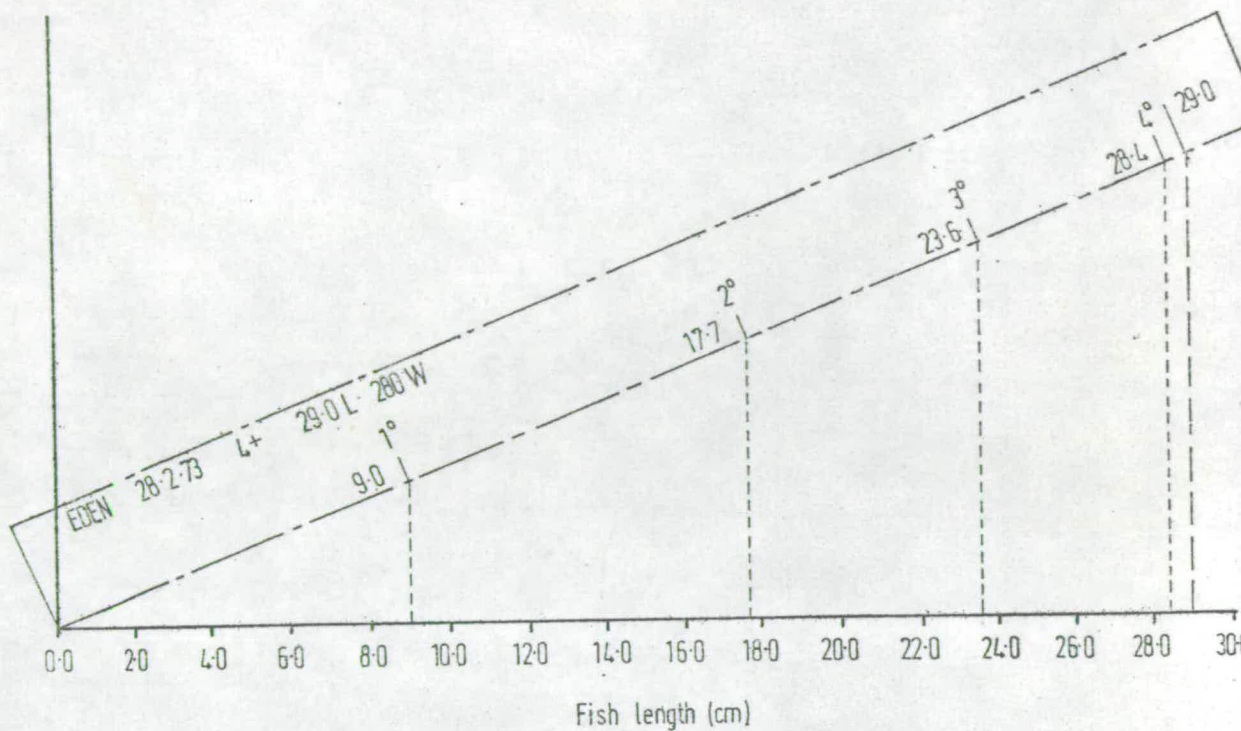
Scale length (arbitrary units)
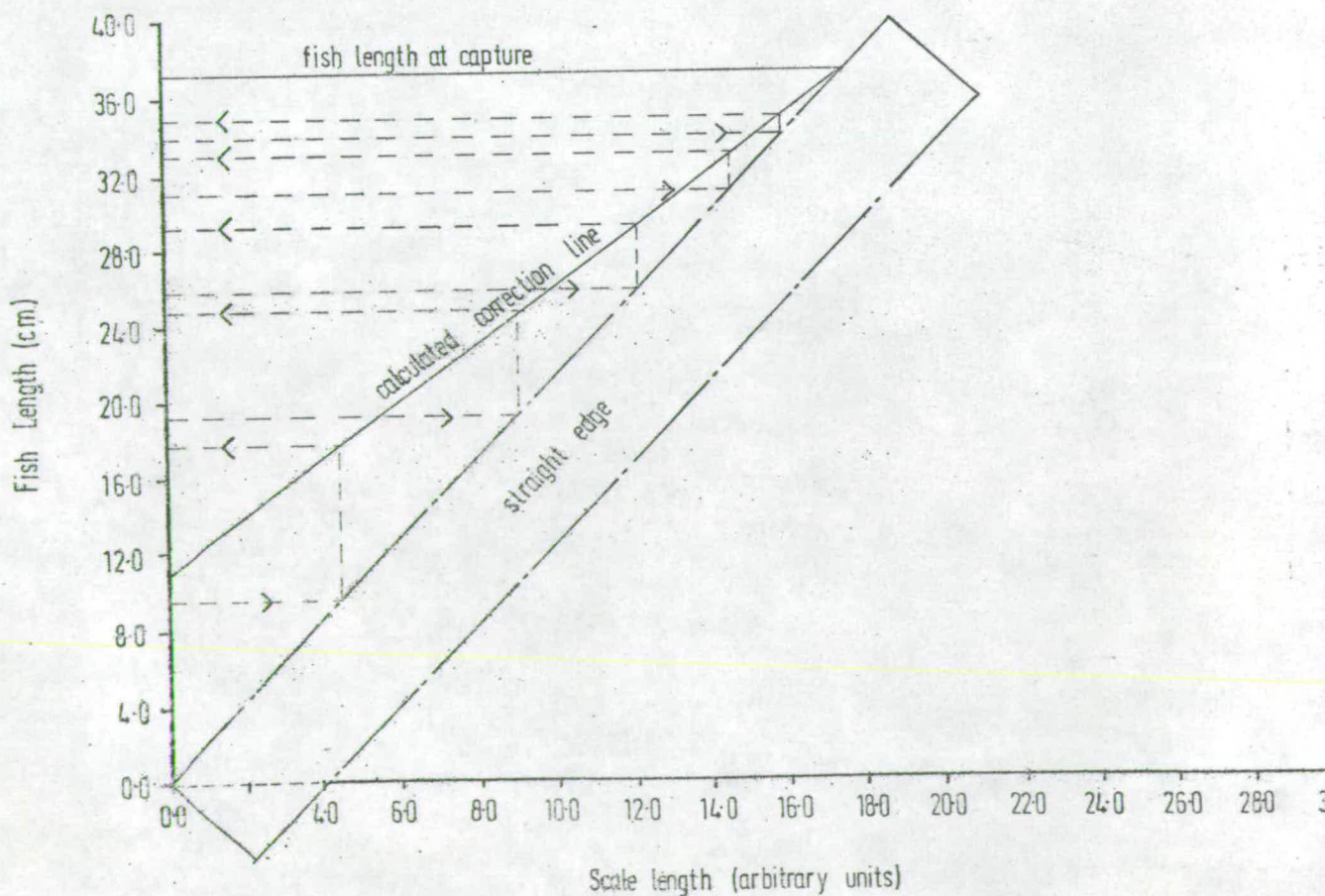
at a later time; (2) records made at different times of the lengths of fish which are individually marked and which are maintained in an aquarium. The former method was chosen to confirm the accuracy of back-calculations for grayling and trout because of the aforementioned maintenance problems in the laboratory. Back-calculated lengths were tested statistically by intra-specific comparisons with the actual means for the same year. The actual mean lengths (and standard error) for trout were calculated from a length frequency histogram based on the following fish samples which were taken in late autumn to early winter (around the time of assumed check formation): 6 in October 1969, 40 in November 1969, 36 in January 1970.

2. Body and Scale Growth Relationship.

i. In order to discover whether the relationship between body and scale growth was isometric or allometric, measurements were made on one scale per fish and the distance from the focus to the edge of the scale was recorded in arbitrary units. These measurements were then paired with the length of the same fish at the time of capture and the data was punched onto cards for an SPSS program to calculate the exact regression coefficients in logarithmic form (Le Cren 1947, Whitney and Carlander 1956). The resulting coefficients were then applied to various values of the log scale length (arbitrary units) and the values of log body length (cm) were then calculated and the paired figures plotted on graph paper and transformed back into arithmetic numbers. If this fitted regression line formed a straight line, then no correction factor was required, but if the calculated co-ordinates fell along a curved line, a correction for allometry was required.

ii. Corrections for Allometric Growth. An efficient way of correcting back-calculated lengths obtained by the above method of direct proportion was described by Le Cren (1947) and is best comprehended

by means of a diagram (Fig 9b). Two lines, one illustrating the empirical relationship between fish length and the length of the structure with a positive intercept on the Y-axis (fish length) and the other as an imaginary line passing through the origin which is used as a basis for a proportion and these lines will approach each other as the values of the variable increase. The necessary correction will, therefore, become smaller, the more recent the annuli have formed. In practice a straight edge is laid from the origin to the point where the fitted line cuts the final length of the fish. The correct reading for any back-calculated length will be the ordinate of the point on the fitted line which is vertically above the point at which the straight edge cuts the horizontal line which represents the back-calculated length arrived at by direct proportion. The corrected-back-calculated length therefore differs from the length obtained by direct proportion by the vertical distance between the straight edge and the calculated line.

iii. The Accuracy of Corrected Back-calculated Lengths. The accuracy of the corrections made for allometric growth evaluated statistically by student's t-test (Bailey 1959), that is comparisons between the mean corrected back-calculated lengths and the mean actual lengths of the same year. The significance of the magnitude of correction was assessed by comparisons of the mean corrected and uncorrected back-calculated lengths for the same year (student's t-test in Bailey 1969).

iv. Lee's Phenomenon. Back-calculated lengths show a tendancy for computed lengths at a given length to be progressively smaller, the older the fish from which they are computed. This is known as the Rosa Lee Phenomenon (Lee 1920) which has been the subject of many studies (Van Oosten 1929, Jones 1958, Bryuzgin 1961),but which is not always present in a back-calculated series. Statistical comparisons were made between mean corrected and uncorrected back-calculated lengths for the

first year, beginning with paired values from the most extreme age classes - i.e. 1+/5+, 2+/5+, 3+/5+ and 4+/5+.

3. Growth.

There are several ways in which the data on the past growth of grayling and trout may be used to compare their growth:

i. Growth Graph. A simple growth graph may be constructed by plotting the mean fish length at each year of life. Alternatively, the annual increments, i.e. the amounts by which the lengths of the fish increase during each year, can be calculated and graphed. The former method is more commonly used in fisheries research and therefore growth graphs were plotted for both species which were based on actual, back-calculated and corrected back-calculated mean lengths attained at the end of each year of life. Statistical comparisons of the mean actual lengths of grayling and trout for each year were made by the usual student's t-test (Bailey 1959).

ii. Growth Rates. Growth made during each year can be expressed as a percentage of the size of the fish at the beginning of the year, however, this is not considered to be an accurate assessment of annual growth because it does not incorporate the growth of the newly formed tissue but rather the growth present at the start of the year. A formula which does take into account the initial and final sizes of the fish is called specific growth rate and is calculated as follows:

$$G = \frac{\log_e Y_T - \log_e Y_t}{T - t}$$

(Frost and Brown 1967)

Y_T = the final size of the fish at time T

Y_t = the initial size of the fish at time t

G = the growth rate

RESULTS.

Age Determination.

1. Verification.

i. Photographs of scales which have been taken from a 22.8cm grayling in April and from a 25.3cm fish taken in June (Plts. 3a,b) illustrate that the annulus of the grayling scale is formed in the spring and that typically it occurred in April. The regularity of the time of check formation as seen in the 305 fish examined supports the use of scales for age determination of grayling.

ii. The relationship between the number of circuli elaborated in a given year, here the first year of life, and the increase in actual length during the same year showed a 0.91 correlation coefficient (r) in a regression based on 252 grayling. The significance of this correlation was tested with a student's t-test and was highly significant (p = 99.9%).

2. Accuracy of Age Determinations-Length Frequency Histogram.

The relative agreement between the two methods for aging fish, that is from scale reading and from a length frequency histogram is presented in Table 10. According to Petersen (1891), the first two modes should correspond very well to the youngest age classes of the population and the young grayling do form two distinct modes: 0+ fish ranged in length from 11.0cm to 16.0cm and the 1+ fish extended from 22.5cm to 27.0 cm. The correspondence between the two methods was high, as there was 96% agreement between them over the classification of the 0+ fish and 94% concurrence over the 1+ grayling. Again the agreement between scale reading and the length frequency histograms is very good for the 2+ fish- 94% -but decreases for the older age classes -60% over the 3+ and 86% for the 4+ fish (see Table 9).

THE TIME OF ANNULUS FORMATION IN GRAYLING SCALES
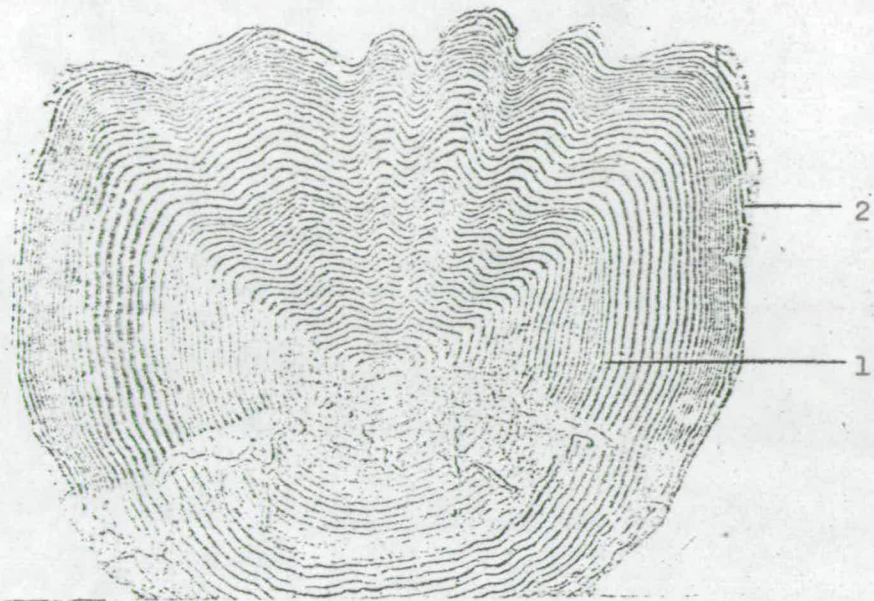
Plate 2a. Scale of a 22.8 cm grayling taken in April

Plate 2b. Scale of a 25.3 cm grayling taken in June

Fig. 10   Petersen length frequency histogram based on grayling sample taken from Eden Water on 28th February, 1973.
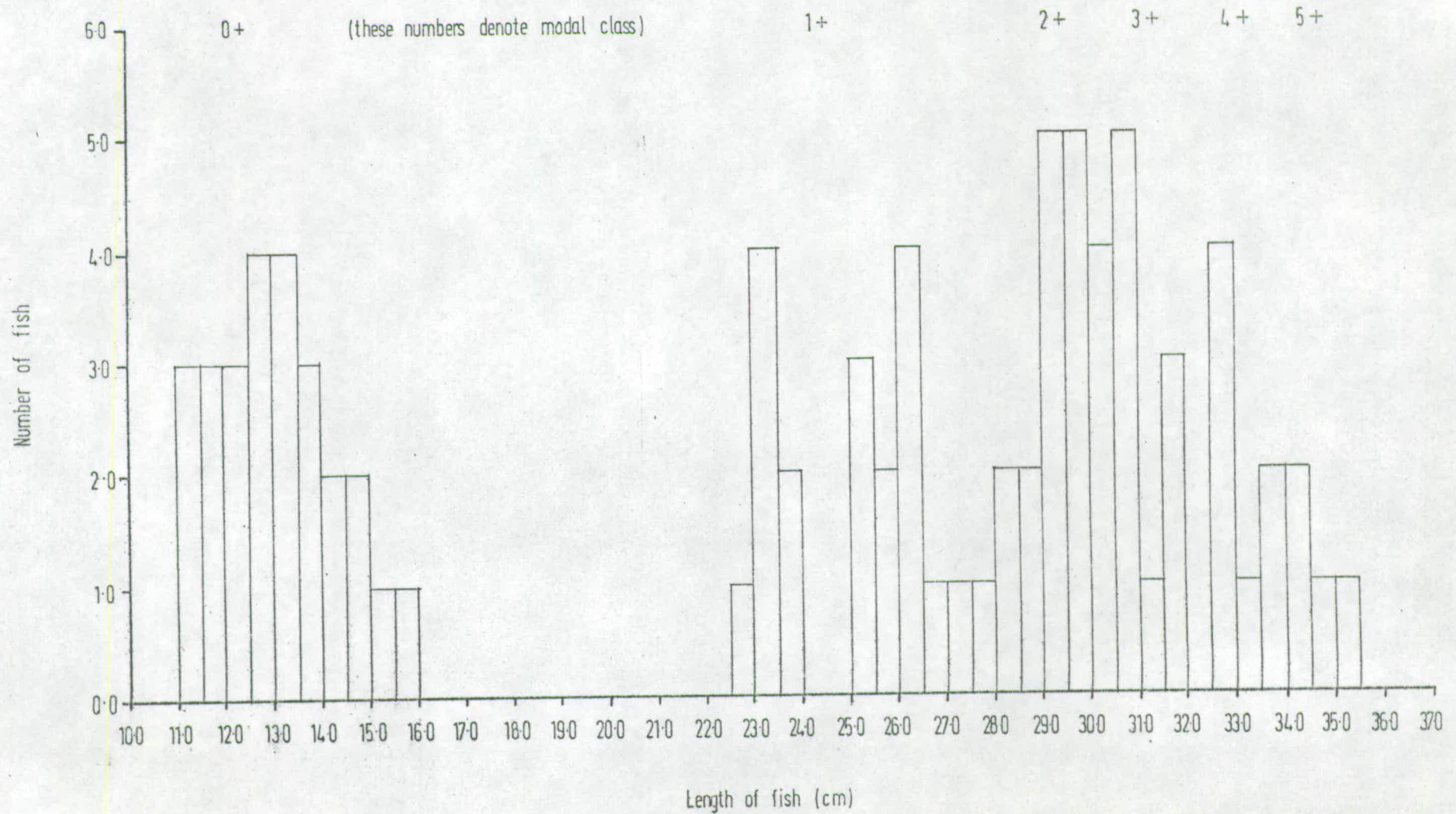
Table 9 : Confirmation of the use of scale-reading for aging grayling - a comparison of the ages and lengths of a grayling sample determined by scale-reading and by a Petersen length frequency histogram.

Age Class	Range of Modes from Length Frequency Histogram	Length of Modal Class (class interval of 1 cm) and number per age class	Range of Lengths from Scale-reading	Length of each Age Class and number per age class	$\dfrac{\text{number fish age}_x \text{ (scale)}}{\text{number fish age}_x \text{ (len.freq)}}$
0+	11.0 - 13.5	12.5 - 13.5 (25 fish)	10.5 - 16.9	13.5 (24 fish)	96%
1+	22.5 - 26.5	23.2 - 25.2 (17 fish)	12.0 - 29.8	24.2 (18 fish)	94%
2+	27.0 - 30.0	29.0 - 30.0 (16 fish)	26.3 - 32.9	29.6 (17 fish)	94%
3+	30.5 - 31.0	30.5 - 31.0 (10 fish)	27.6 - 34.1	30.4 (14 fish)	71%
4+	31.5 - 33.0	32.5 - 33.0 (7 fish)	30.9 - 35.0	33.1 (8 fish)	86%
5+	33.5 - 35.0	33.5 - 34.5 (7 fish)	33.9	33.9 (1 fish)	14%

Determination of Past Growth.

1. Back-calculations.

i. Back-calculated lengths were determined for 232 grayling ranging in age from 1+ to 5+ (0+ fish were excluded) and for 109 brown trout ranging in age from 1+ to 5+. The mean back-calculated lengths of each year class and standard error are summarised in Tables 10 and 11 with similar data on the mean actual lengths for both species.

ii. The Accuracy of Back-calculations. Results of student's t-tests to compare actual and back-calculated means for each year of life are recorded in Table12 for grayling and in Table13 for trout. Both sets of results indicate that there is a significant difference between the back-calculated and actual mean lengths attained at the end of the first year(p =99.9%). This difference persists in the older age classes of grayling, that is in the 2+, 3+, 4+, and 5+ fish, but not in the older age classes of trout.

2. Body and Scale Growth Relationship.

The relationship between scale length and body length for both species indicated that growth was allometric and therefore that the back-calculations would require a correction factor. The regression coefficients along with the standard errors are recorded in Table14 in logarithmic form. The relationship was linear and intercepted the Y-axis at a positive length (Fig.11).

Species	m	c	S.E.	no. fish
Grayling	0.416	1.026	0.0441	252
Trout	0.567	0.778	0.0662	111

Table 14: Regression coefficients for the relationship between scale and body growth in logarithmic form.

Table 10: Grayling   A Summary of Mean Actual, Back-calculated, and Corrected Back-calculated Lengths for Each Year.

Age Class	No. Fish Sample for Actual L.	No. Fish Sample for B-cal. L.	Year 1 Actual Length (cm)	Year 1 B-cal. Length (cm)	Year 1 Cor. B-cal. Length (cm)	Year 2 Actual Length (cm)	Year 2 B-cal. Length (cm)	Year 2 Cor. B-cal. Length (cm)
1+	46	92	14.20±0.24	11.74±0.23	15.89±0.22			
2+	42	83	14.20±0.24	9.73±0.21	16.27±0.23	23.12±0.40	21.30±0.20	23.30±0.20
3+	29	26	14.20±0.24	8.42±0.28	16.32±0.22	23.12±0.40	17.78±0.70	22.17±0.50
4+	24	25	14.20±0.24	8.46±0.27	16.77±0.24	23.12±0.40	19.17±0.73	23.95±0.56
5+	17	6	14.20±0.24	7.73±1.05	16.35±0.89	23.12±0.40	18.70±0.46	23.72±0.50

Age Class	Actual Length (cm)	B-cal. Length (cm)	Cor. B-cal. Length (cm)	Actual Length (cm)	B-cal. Length (cm)	Cor. B-cal. Length (cm)	Actual Length (cm)	B-cal. Length (cm)	Cor. B-cal. Length (cm)
3+	27.69±0.38	25.22±0.63	26.93±0.55						
4+	27.69±0.38	25.40±0.77	28.22±0.60	30.46±0.47	30.17±0.58	31.47±0.48			
5+	27.69±0.38	24.40±0.76	27.45±0.68	30.46±0.47	28.53±0.91	30.18±0.78	34.28±0.29	31.68±0.97	32.33±0.88

Table 11: Trout: A Summary of Mean Actual, Back-calculated and Corrected Back-calculated Lengths for Each Year.

Year				1			2	
Age Class	No. Fish Sample for Actual L.	No. Fish Sample for B-cal. L.	Actual Length (cm)	B-cal. L Length (cm)	Cor. B-cal. Length (cm)	Actual Length (cm)	B-cal Length (cm)	Cor. B-cal. Length (cm)
1+	11	14	9.46±0.43	7.16±0.46	9.27±0.69			
2+	50	34	9.46±0.43	6.97±0.65	10.88±0.27	15.55±0.24	15.84±0.46	16.53±0.45
3+	11	38	9.46±0.43	6.60±0.08	10.83±0.20	15.55±0.24	14.40±0.53	17.30±0.39
4+	7	18	9.46±0.43	5.51±0.43	10.59±0.33	15.55±0.24	12.16±0.60	16.73±0.47
5+	3	5	9.46±0.43	4.68±0.65	10.28±0.33	15.55±0.24	12.41±0.85	15.10±1.02

Year	3			4			5		
Age Class	Actual Length (cm)	B-cal. Length (cm)	Cor. B-cal. Length (cm)	Actual Length (cm)	B-cal. Length (cm)	Cor. B-cal. Length (cm)	Actual Length (cm)	B-cal. Length (cm)	Cor. B-cal. Length (cm)
3+	27.69±0.38	25.22±0.63	26.93±0.55						
4+	27.69±0.38	25.42±0.77	28.22±0.60	30.46±0.47	30.17±0.58	31.47±0.48			
5+	27.69±0.38	24.41±0.76	27.45±0.68	30.46±0.47	28.53±0.91	30.18±0.78	34.28±0.29	31.68±0.97	32.33±0.88

-71-

Summary of Statistical Comparasons of Actual, Back-calculated, and Corrected Back-calculated Mean Lengths for Grayling.

Table12a: Comparasons of means for actual length with back-calculations.

Age Class	Number Fish in Sample	F Variance Ratio (= or ≠)	Degrees Freedom	Levels of Significance (t and p)	
1	46, 92	not applicable	-	d=7.37	p= 99%
2	42, 83	not applicable	-	d=4.05	p= 99%
3	26, 29		42	d=3.34	p= 99%
4	25, 24		47	t=0.39	p= 30%
5	17, 6		21	t=2.84	p= 99%

Table12b: Comparasons of means for actual lengths with corrected back-calculated lengths.

Age Class	Number Fish in Sample	F Variance Ratio (= or ≠)	Degrees Freedom	Levels of Significance (t and p)	
1	92, 46	not applicable	-	d=5.14	p= 99%
2	83, 42	not applicable	-	d=0.25	p= 20%
3	26, 29		53	t=1.16	p= 75%
4	25, 24		47	t=1.49	p= 85%
5	17, 6		21	t=2.20	p= 97%

Table12c: Comparasons of means for back-calculated and corrected back-calculated lengths.

Age Class	Number Fish in Sample	F Variance Ratio (= or ≠)	Degrees Freedom	Levels of Significance (t and p)	
1	92, 92	not applicable	-	d=13.1	p= 99%
2	83, 83	not applicable	-	d=7.10	p= 99%
3	26, 26	F=1.33 (=)	50	t=0.68	p= 45%
4	25, 25	F=1.44 (=)	48	t=0.65	p= 45%
5	6, 6	F=1.21 (=)	10	t=0.22	p= 15%

Summary of Statistical Comparisons of Actual, Back-calculated, and Corrected Back-calculated Mean Lengths for Trout.

Table 13a: Comparison of means for actual length with back-calculations.

Age Class	Number Fish in Sample	F Variance Ratio (= or ≠)	Degrees Freedom	Levels of Significance (t and p)	
1	14, 11	F=1.44 (=)	23	t=3.55	p= 95%
2	50, 34	not applicable	-	d=0.56	p= 45%
3	38, 11	F=7.09 (≠)	44	d=0.77	p= 63%
4	18, 7	F=2.51 (=)	23	t=0.91	p= 10%
5	3, 5	F=11.4 (≠)	2	d=1.56	p= 75%

Table 13b: Comparison of means for actual lengths with corrected back-calculated Lengths.

Age Class	Number Fish in Sample	F Variance Ratio (= or ≠)	Degrees Freedom	Levels of Significance (t and p)	
1	14, 11	F=3.26 (=)	23	t=0.22	p= 15%
2	50, 34	not applicable	-	d=1.94	p= 45%
3	38, 11	F=7.54 (≠)	45	d=1.62	p= 15%
4	7, 18	F=2.50 (=)	23	t=0.93	p= 65%
5	3, 5	F=3.75 (=)	6	t=0.25	p= 15%

Table 13c: Comparisons of means for back-calculated lengths with corrected back-calculated lengths.

Age Class	Number Fish in Sample	F Variance Ratio (= or ≠)	Degrees Freedom	Levels of Significance (t and p)	
1	14, 14	F=2.26 (=)	26	t=2.54	p= 98%
2	34, 34	not applicable	-	d=1.07	p= 75%
3	38, 38	not applicable	-	d=0.43	p= 35%
4	18, 18	F=1.00 (=)	34	t=3.00	p= 99%
5	5, 5	F=3.45 (=)	8	t=3.95	p= 99%

Fig. 11   Regression of scale length (log) against body length (log)
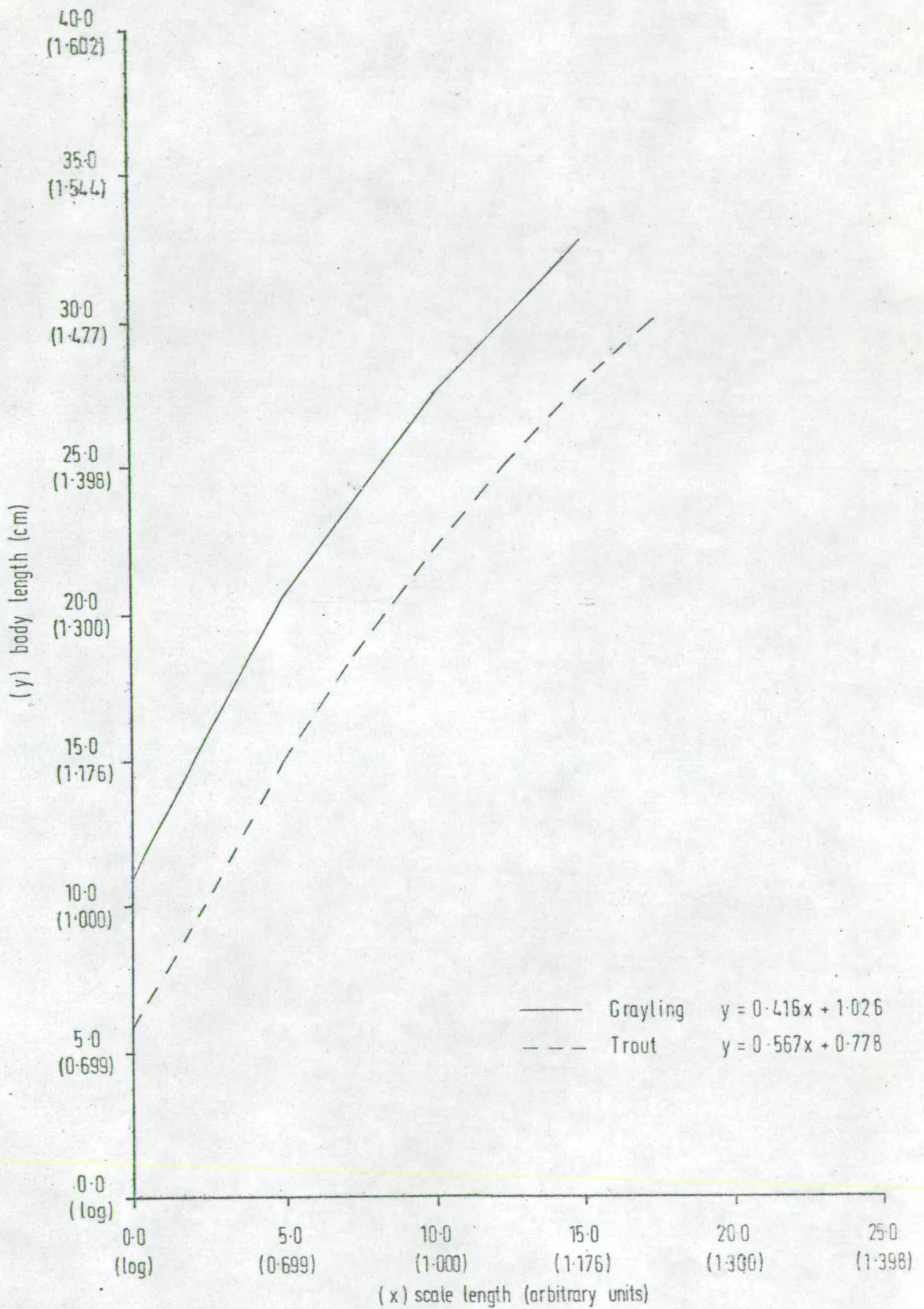
3. <u>Corrections for Allometric Growth.</u>

i. Corrections for allometric growth were determined for all grayling and trout and the mean corrected back-calculations for each year class are summarised for both species in Tables 10 and 11.

ii. <u>The Accuracy of Corrections for Back-calculated Lengths.</u> The results of statistical analyses to compare the corrected back-calculated and actual mean lengths for grayling and trout are recorded in Tables 12 and 13, respectively, and those for the corrected and uncorrected mean back-calculations are listed in Tables12 and 13, respectively. Significant differences were found in some of the age classes in both species and these differences will be examined in the following discussion.

4. <u>Lee's Phenomenon.</u>

Lee's phenomenon is detectable in the data for mean back-calculated lengths at the end of the first year of life for the first two year classes of grayling ($p = 93\%$) and for the first and second year of trout ($p = 99\%$). The magnitude of the difference between the mean back-calculated lengths was highest for the 1+ and 5+ year classes - 4.01 cm in the grayling and 2.48 cm in the trout. Lee's phenomenon was not detected in the corrected back-calculations.(See Table 15).

5. <u>Growth.</u>

i. <u>Growth Graphs</u>. Growth graphs based on mean lengths calculated from actual, back-calculated, and corrected back-calculated data have been plotted and are presented in Fig.13 for the grayling and in Fig. 12 for the trout. Grayling growth appears to be consistently better than that of the trout, particularly in the first three years of life.

ii. <u>Specific Growth Rates</u>. The calculations for specific growth rates are included in Table 16 and are presented graphically in Fig. 14. There is a marked difference between growth rates of grayling and trout in the first year - a difference of 129%.

Table 15a: Tests for the occurrence of Lee's Phenomenon in the corrected and un-corrected back-calculations for grayling.

| | corrected back-calculations | | | | un-corrected back-calculations | | | |
Age Class	Number Fish each Sample	Degrees Freedom	Level of Significance		Age Class	Number Fish each Sample	Degrees Freedom	Level of Significance
1+/5+	92, 6	96	$t=0.24$ $p=20\%$		1+/5+	92, 6	96	$t=1.84$ $p=93\%$
2+/5+	83, 6	87	$t=0.32$ $p=25\%$		2+/5+	83, 6	87	$t=1.87$ $p=93\%$
3+/5+	26, 6	7	$d=0.53$ $p=39\%$		3+/5+	26, 6	5	$d=0.78$ $p=50\%$
4+/5+	25, 6	6	$d=1.39$ $p=80\%$		4+/5+	25, 6	6	$d=1.51$ $p=82\%$

Table 15b: Tests for the occurrence of Lee's Phenomenon in the corrected and un-corrected back-calculations for trout.

| | corrected back-calculations | | | | un-corrected back-calculations | | | |
Age Class	Number Fish each Sample	Degrees Freedom	Level of Significance		Age Class	Number Fish each Sample	Degrees Freedom	Level of Significance
1+/5+	14, 5	17	$d=1.32$ $p=85\%$		1+/5+	14, 5	17	$d=4.53$ $p=99\%$
2+/5+	34, 5	37	$t=0.60$ $p=55\%$		2+/5+	34, 5	36	$d=3.21$ $p=99\%$
3+/5+	38, 5	41	$t=1.00$ $p=65\%$		3+/5+	38, 5	41	$t=7.61$ $p=99\%$
4+/5+	18, 5	21	$t=0.50$ $p=35\%$		4+/5+	18, 5	19	$d=1.60$ $p=85\%$

time of
hatching

c.l.	————	Actual Lengths
c.l.	– – – –	Back-calculated Lengths
(c.l.)	—x———x—	Corrected Back-calculated Lengths
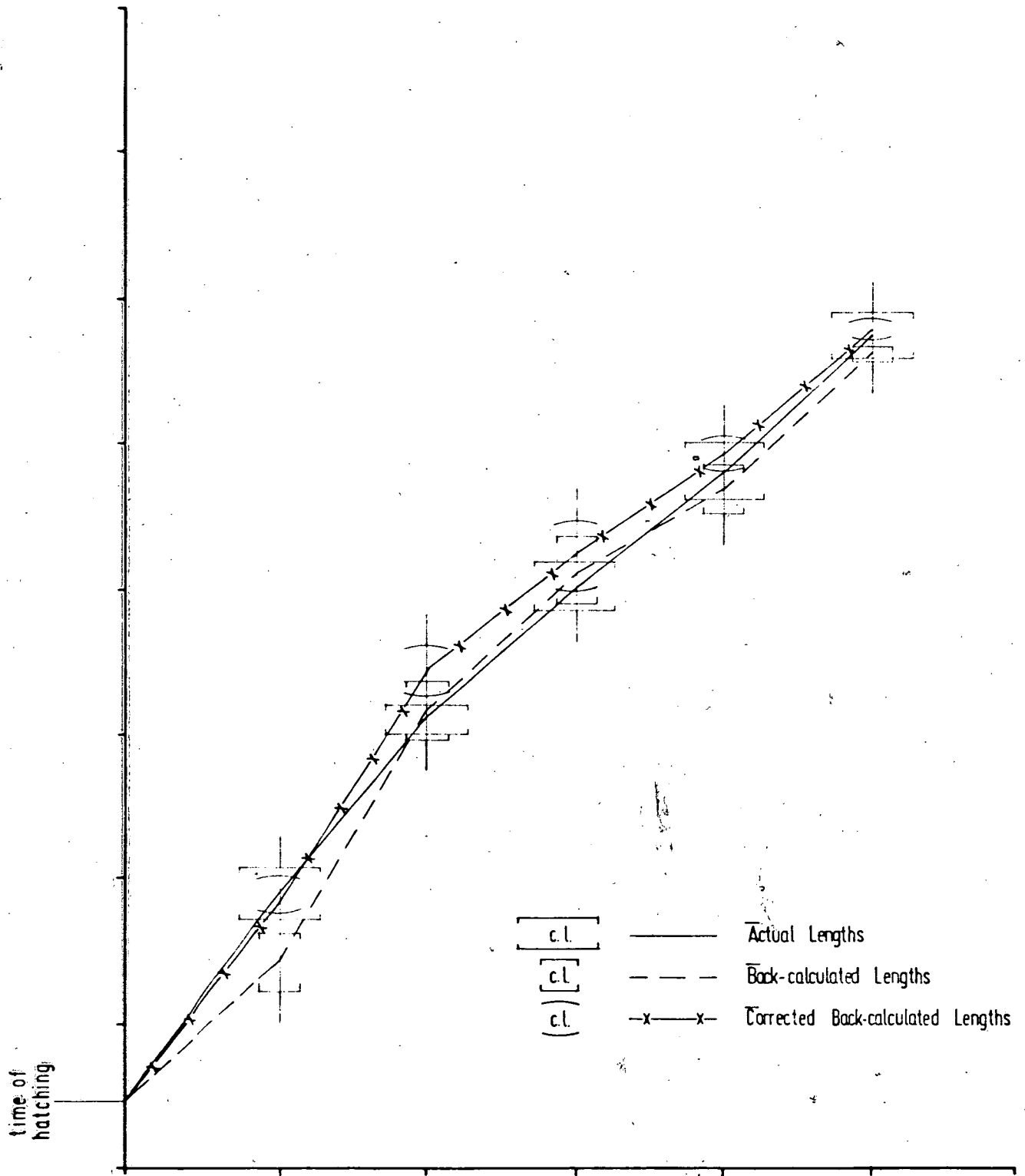
Fig. 13   Growth of grayling
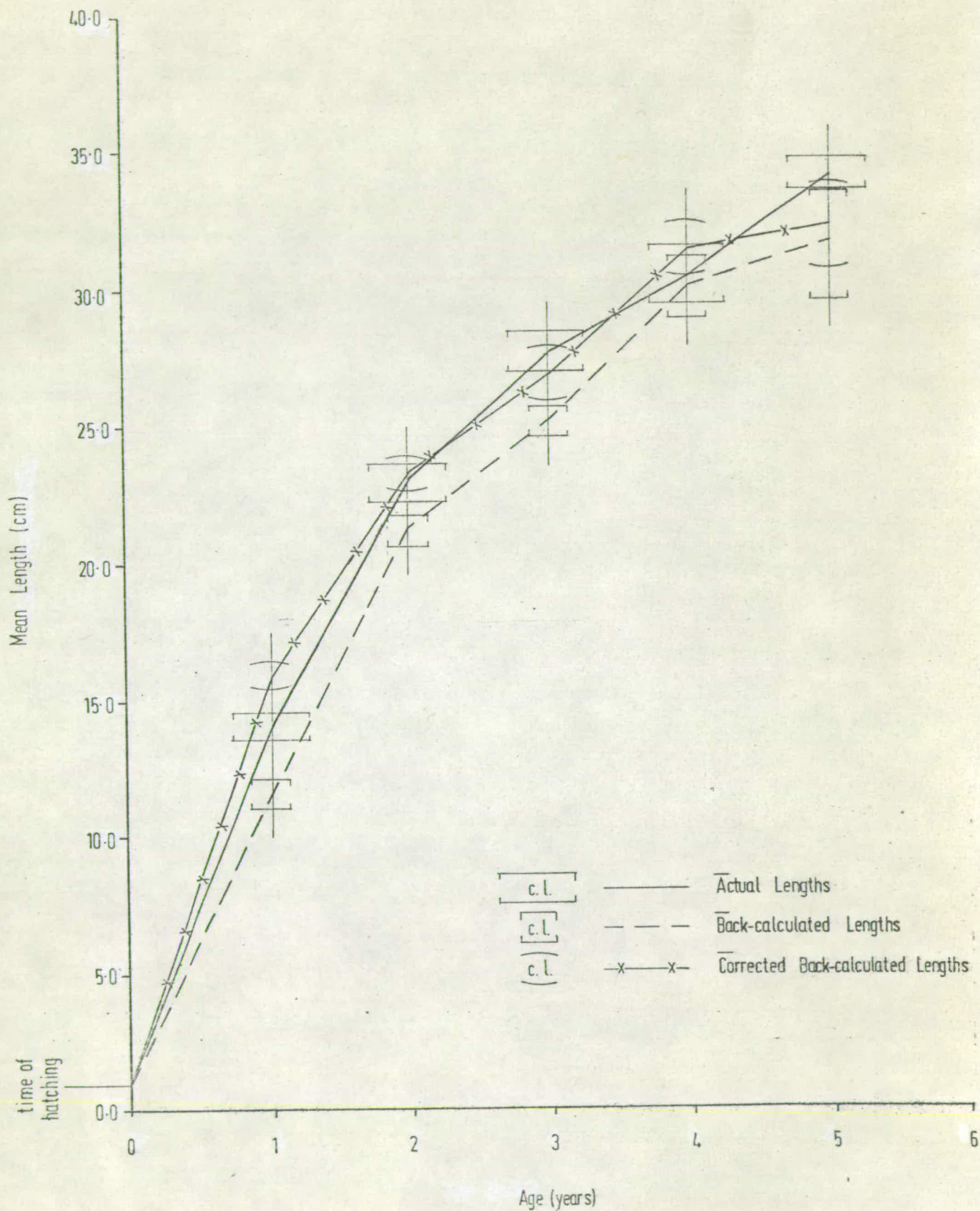
Table16a: <u>Summary of the calculations for specific growth rates of</u> <u>grayling, based on mean actual lengths.</u>

Age Class	Mean Length (cm)	Log Mean Length	Difference Mean Log Length	x 2.3026 x 100%
5	34.28	1.5350		
4	30.46	1.4838	0.0512	11.79
3	27.69	1.4423	0.0415	9.56
2	23.12	1.3640	0.0783	13.38
1	14.20	1.1523	0.2117	48.75
0	0.95	1.9777	1.1746	270.47

Table16b: <u>Summary of the calculations for specific growth rates of</u> <u>trout, based on mean actual lengths.</u>

Age Class	Mean Length (cm)	Log Mean Length	Difference Mean Log Length	x 2.3026 x 100%
5	28.81	1.4594		
4	24.08	1.3816	0.0778	17.91
3	20.07	1.3025	0.0791	18.21
2	14.55	1.1628	0.1397	32.17
1	9.46	0.9759	0.1869	43.04
0	2.32	0.3617	0.6142	141.43

Fig. 14   The relationship between specific growth rate and age from grayling and trout in the Eden
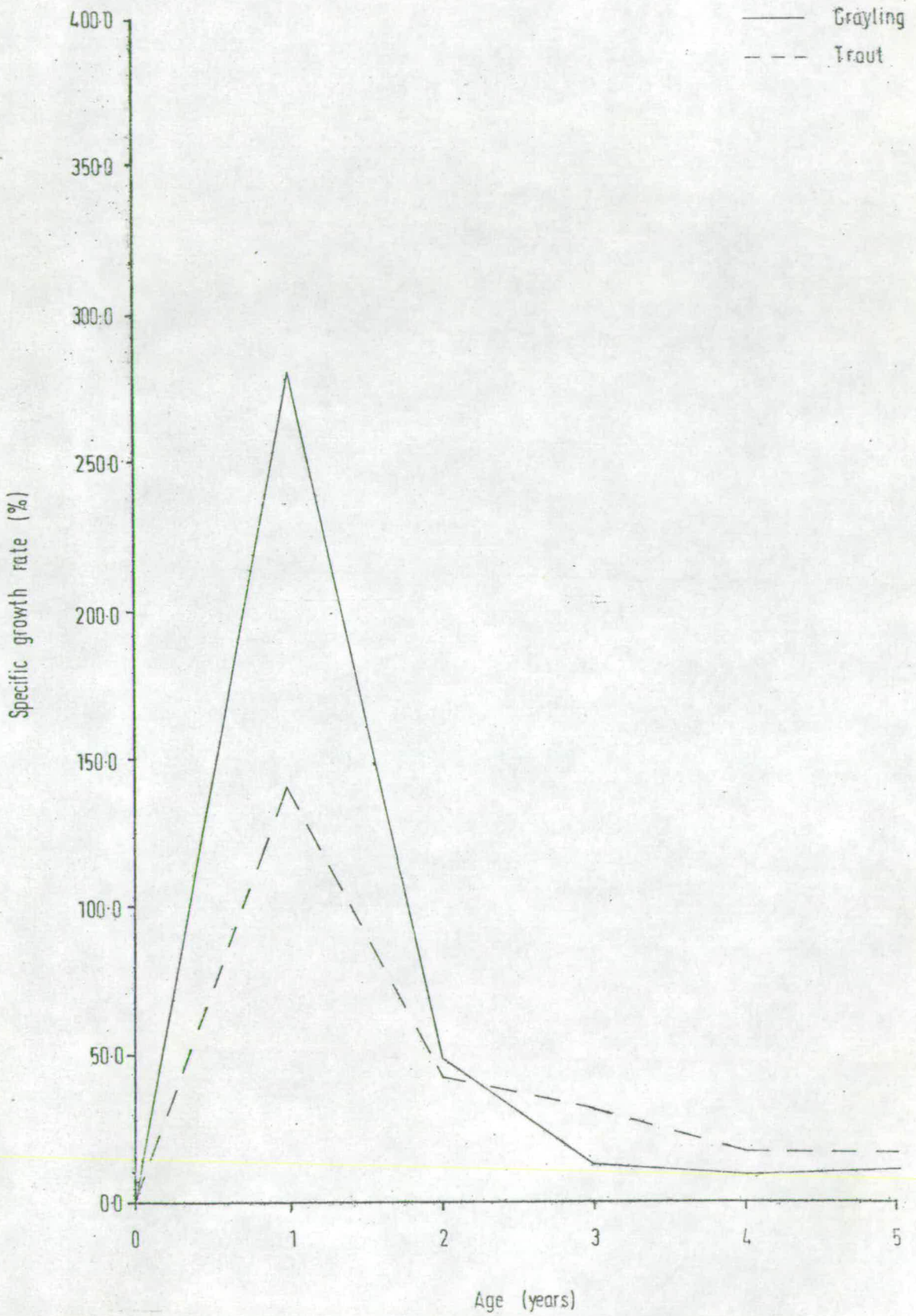
DISCUSSION

Age Determination.

1. Annulus Formation.

The work carried out on scales collected from grayling in the Eden
consistently indicated that the annual check was formed in middle to
late spring and usually in April. These results agree with those of
Peterson (1968), but do not concur with the findings of other workers.
In general the apparent disagreement with the results of other scien-
tists seems to stem from the inconsistent use of scale reading termin-
ology and the subsequent mis-interpretation of the scale configurations.
Hutton (1923) used scales for age determination but his paper did not
provide an account of how he deduced the pattern of circuli evolution.
Gerrish's work (1938) is confusing in that he discussed 'winter'bands
which were formed in July. Hellawell (1969) and Jones (1953) reported
that the River Lugg grayling showed a check in November and that the
Tegid grayling formed an annulus in August to September, respectively.

In Plt.3a taken from Hellawell's paper, Hellawell has labelled the
annuli of a fish caught in December to illustrate the recent formation
of an annulus which is "about three rings in from the edge of the scale".
However, in such an older fish, the few circuli which have formed beyond
the previous check cannot be immediately interpreted as plus growth
demarcating the annulus. In fact what is probably being exhibited by
these ridges is a fish which has only begun to recover (in terms of
circuli elaboration) from spawning: The somatic losses related to spawn-
ing are well documented and are on the order of 20% of the total fish
weight (Frost and Brown 1967) and therefore a great amount of time is
required to replace somatic losses associated with spawning before
fresh fish growth is manifested on the scale of the fish.
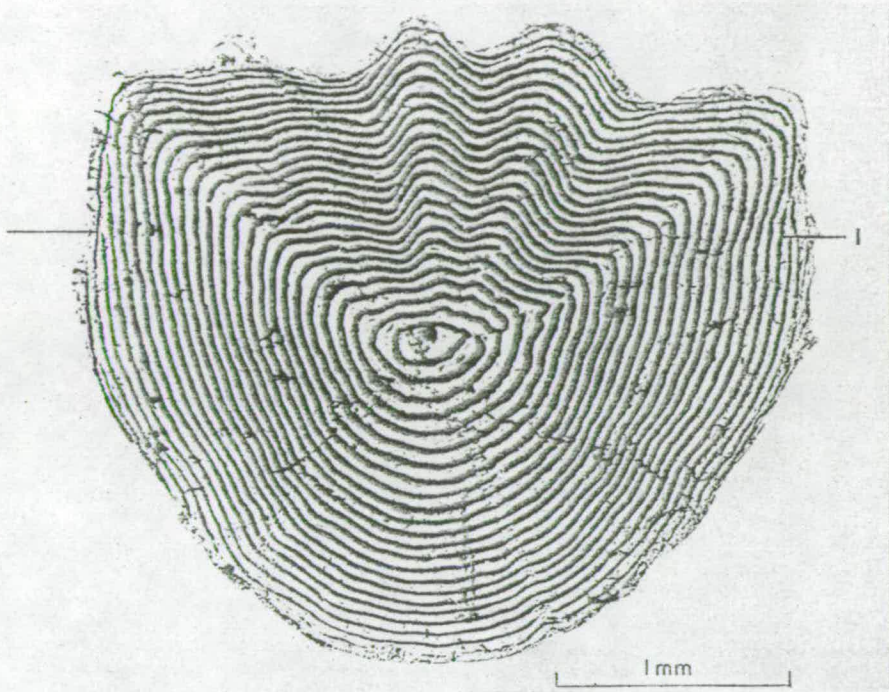
Plate 3b. Scale of 12.4 cm grayling taken in February (Hellawell)
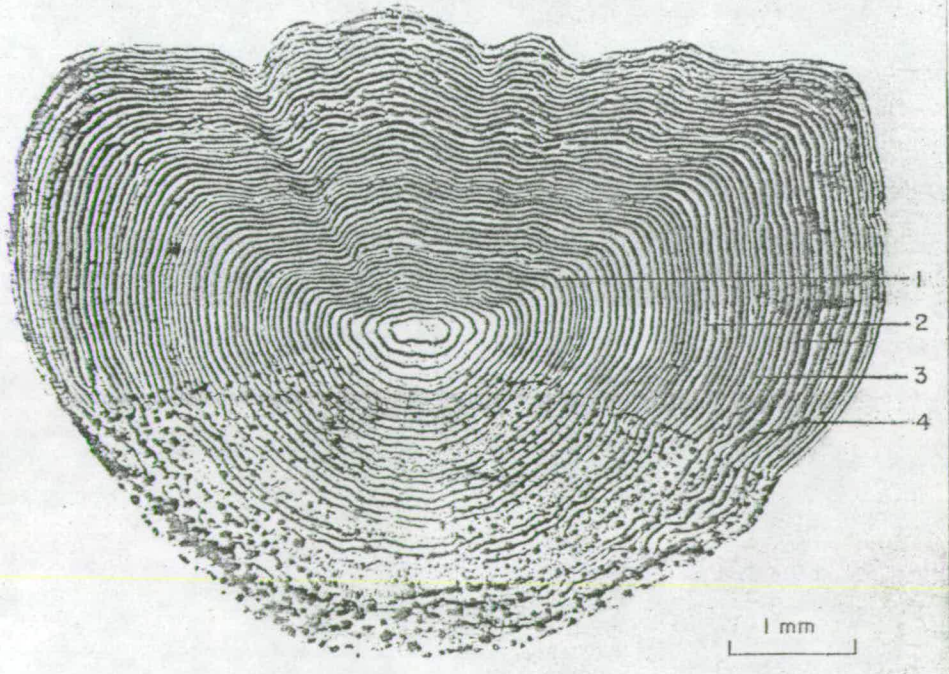
Plate 3a. Scale of 24.5 cm grayling taken in December (Hellawell)

In another plate (3b) Hellawell shows a 12.4 cm fish taken in February with a check visible at the edge of the scale. In fact, this is not a completed check, but rather the first narrowly spaced circuli typical of winter growth. This scale serves to illustrate that the grayling of the River Lugg does not form its check in November, but that annulus formation occurs sometime in the spring.

Jones (1953) did not provide photographs of circuli evolution in his paper which therefore precludes a similar sort of examination into his scale reading, interpretation, and terminology. Finally, MacKay (1970) analysed the age and growth of grayling in two tributaries of the Clyde, but no mention was made of the time of check formation.

Determination of Past Growth.

1. Back-calculation.

The accuracy of back-calculations is by definition closely related to the growth rates of the fish and to its scales, and when an allometric relationship does exist, all back-calculations will be under-estimated (Kipling 1962). In the instance of the grayling, statistical differences between actual and back-calculated mean lengths indicated an allometric relationship between fish and scale growth. Brown trout were also shown to exhibit an allometric body-scale relationship, however, the statistical comparisons of actual and back-calculated mean lengths were only significant in the first year (p = 99%).

According to Frost and Brown (1967), in fast-growing trout, isometry probably begins by the time the fish has reached 10 cm and there is no error in back-calculations for a fish which reaches or exceeds this length at the end of the first year. In this study, the statistical difference between trout actual and back-calculated mean lengths for the first year of life is probably related to the fact that brown trout in the Eden do not attain a mean size of 10 cm at the end of their first

year and consequently, the back-calculations for that year will be under-estimated. Furthermore, as the results for all older age classes show no significant differences between actual and back-calculated means, it would appear that the trout body-scale relationship is allometric during the first year and once fish attain approximately 10 cm, the relationship changes to isometric.

2. Corrections for Allometric Growth.

The statistical comparisons of the corrected back-calculated means with the actual mean lengths of grayling indicated significant differences in the first and fifth years with the differences between means being 1.69 cm and 1.95 cm respectively. In both years, these differences may be attributed simply to allometry, because an error of up to 2.54 cm can be expected due to allometry (Frost and Brown 1967) and in the fifth year, the error may be associated with the size of the samples used for age and growth determinations. On the whole, the differences between corrected back-calculated and actual lengths are small and tend to concur and bearing in mind the indication in the previous section that the grayling body-scale relationship is always allometric, the necessity of the use of a correction factor for allometric growth in the grayling has been demonstrated.

In all year classes, the magnitude of correction was highly signif-icant for the first check (p=99.9%). The amount of correction required for back-calculations in the first year increased progressively for the older fish, i.e. the correction for a 1+ fish amounted to 4.15 cm and the correction for a 5+ fish was 8.62 cm. The amount of difference in the correction is probably related to the known tendency of the back-calculated lengths taken from older fish to increasingly under-estimate the younger age classes and not to a real increase in allometry in older fish. Finally, the differences between the corrected back-calculations

and the back-calculated lengths for older age classes were smaller and were not statistically significant and this corresponding decrease in the amount of correction for increasingly older fish is an obvious and inherent feature of the nomographic method for correcting back-calculations (Le Cren 1947).

It has already been suggested that the trout shows an allometric relationship between body and scale growth during the first year of life and an isometric one thereafter. Any corrections for back-calculations would therefore be superfluous for all but the first year of life and statistical comparisons of corrected back-calculated and actual lengths tend to support this. At the end of the first year, there is no significant difference between these mean lengths ($p=15\%$), and in the second, third, fourth, and fifth years, the differences between means are not significant ($p=15\%$, 85%, 75% 15% respectively). Therefore the validity of the correction factor for back-calculations of brown trout in the Eden has been demonstrated for the first year only and corrections in the older age classes are not necessary.

A comparison of the corrected and uncorrected back-calculated lengths of the first year of life for all age classes indicates a highly signif-icant difference only in the first year ($p=98\%$). These results concur with those preceding comparisons and lend support to the conclusion that a correction is only justified for back-calculations to the end of the first year.

3. Growth.

A comparison of grayling and trout growth was undertaken as an indirect means of investigating the possible competitive side-effects of grayling and trout living sympatrically in the Eden. To reiterate, a specie's growth is considered to mirror its success in a given environment, incorporating such vital factors as spawning, habitat, and feeding

into a piscatorial standard of living index for that community. In this research, it has been shown that grayling achieve better growth than trout and also that they have a superior growth rate during the first year of life. In order to assess whether or not this fact points to the grayling's(successful)competition with the trout or simply to the grayling's inherently faster growth rate, the growth of Eden trout was compared with growth data of trout taken from similarly productive streams. In reality, such comparisons are enormously complicated by the ecological variation of each community and involve sweeping assumptions concerning the similarity of the habitat - such as the topography, water chemistry, fish and prey species composition and dynamics, etc. - and therefore it is very important to bear these limitations in mind in the course of the discussion.

Mills (1971) has examined the growth of brown trout in the Oxnam Burn, a tributary of the Tweed, and its stream characteristics are very similar to those of the Eden (Table 17). The mean back-calculated lengths for trout (after Mills 1971) are presented graphically in Fig. 8 and these may be compared with the mean corrected back-calculated lengths for Oxnam grayling (collected and analysed by this author) which are also included in Fig. 15 . In the first year the growth of grayling is more than twice that of the trout, which is similar to the results in the Eden, and grayling growth continues to surpass trout growth in all later years. Correspondingly, the specific growth rates of grayling are extremely high in the first year (273%) and these drop thereafter, falling below those of the trout by the end of the second year. These figures from the Oxnam tend to reinforce the picture presented of grayling and trout growth in the Eden, but they do not indicate whether or not the grayling is in some way depressing trout growth.(see Table 18 and Fig. 16).

Table 17: Summary of the stream characteristics of the Oxnam Burn.

	Oxnam	Eden
Gradient (%)	0.3	0.6
Average depth (cm)	19.0	26.9
Average width (m)	6.1	8.0
Silt (%)	10.7	20.0
Unstable banks (%)	23.0	3.5
Mean flow (m^3/sec)	1.07	1.12
Forest (%)	17.9	0.0
Brush (%)	0.0	48.8
Open (%)	82.1	51.2
Fish species	10.0	11.0
Land Use	AF	AF
pH	8.8	7.9-8.5
Alkalinity (ppm)	192.0	182-206

(AF = arable farmland)

Fig. 15   Growth of grayling and trout in the Oxnam
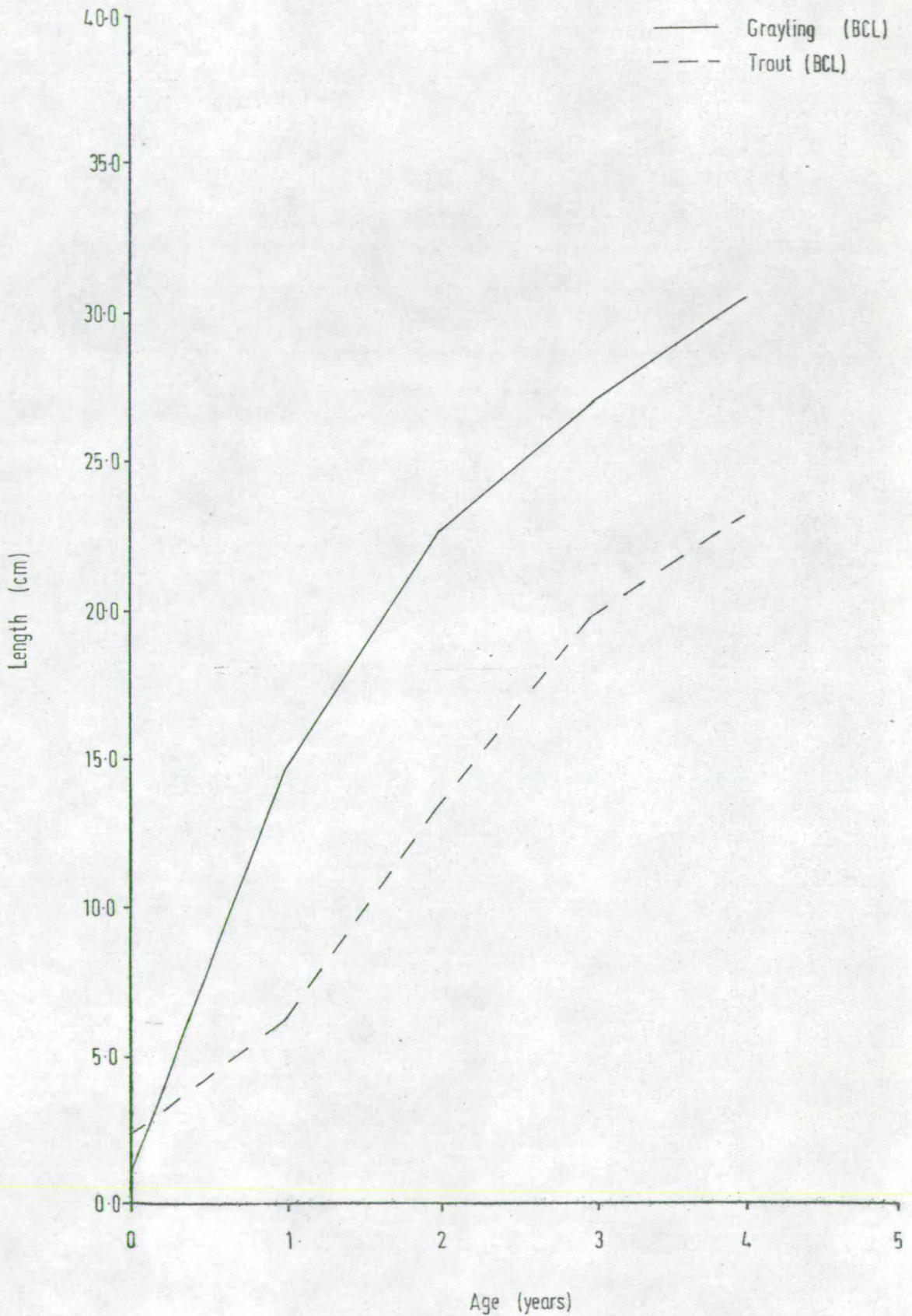
Table18a: Summary of the calculations for specific growth rates of grayling, based on corrected back-calculated lengths (Oxnam).

Age Class	Mean Length (cm)	Log Mean Length	Difference Mean Log Lengths	x 2.3026 x 100%
4	31.6	1.4997		
3	27.0	1.4314	0.0683	15.7
2	22.7	1.3560	0.0754	17.4
1	14.6	1.1644	0.1916	44.1
0	0.95	0.9777	1.1867	273.2

Table 18b: Summary of the calculations for specific growth rates of trout, based on back-calculated lengths (Oxnam Burn).

Age Class	Mean Length (cm)	Log Mean Length	Difference Mean Log Lengths	x 2.3026 x 100%
4	23.4	1.3692		
3	19.9	1.2989	0.0703	16.2
2	13.4	1.1271	0.1718	39.5
1	6.2	0.7924	0.3347	77.1
0	2.3	0.3617	0.4307	99.2

Fig. 16   The relationship between specific growth rate and age from grayling and trout in the Oxnam
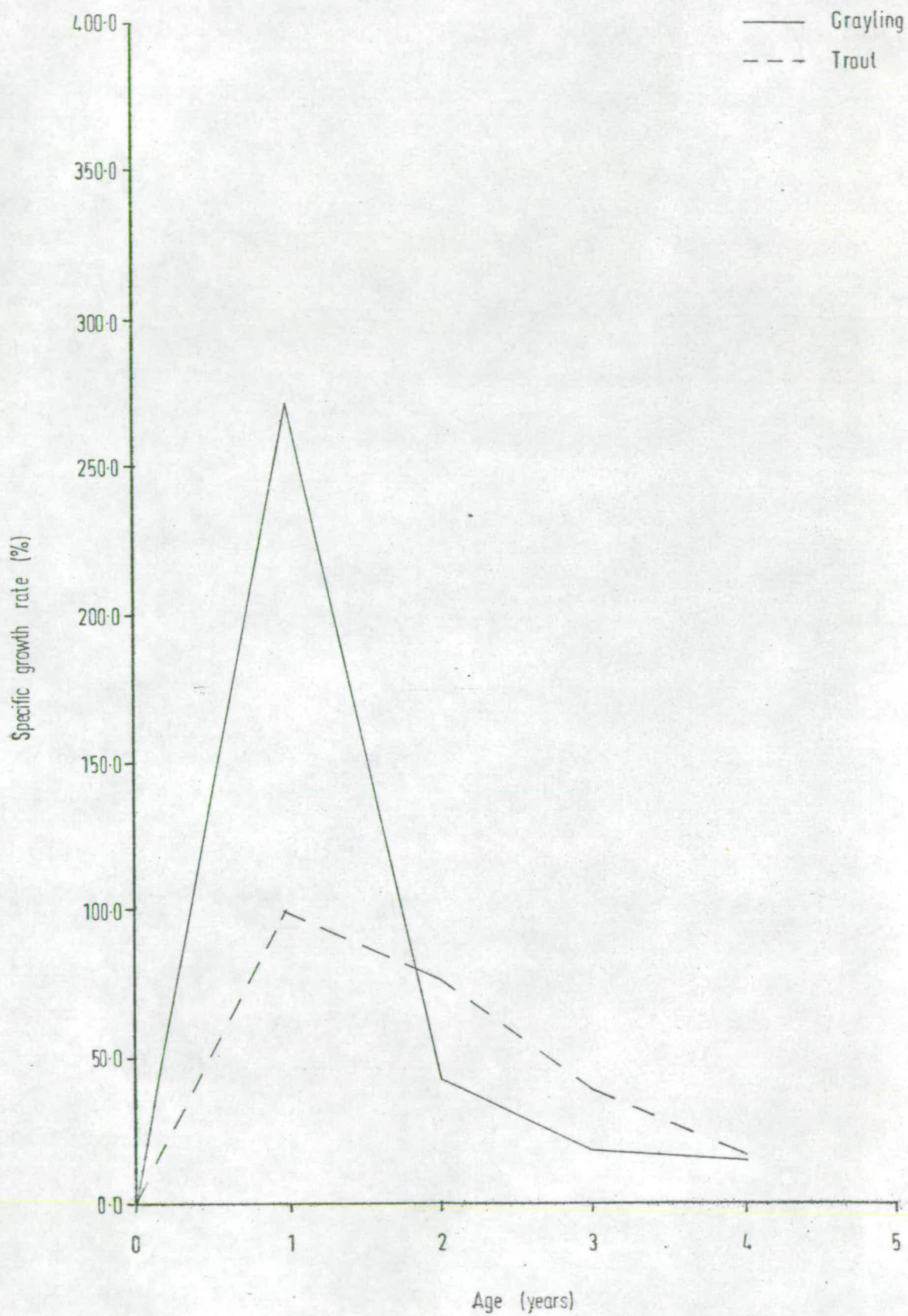
What is really required is a standard (here an average) figure for brown trout growth to provide a comparison with trout growth in the Eden. As trout and its growth in particular have been frequently studied in this country, a great amount of growth data has accrued and these have been compiled by Frost and Brown (1967); the average lengths of the various year classes have been listed in relation to water hardness ($CaCo_3$ppm). Water hardness was selected as an index of productivity of an aquatic environment because it has been shown to be naturally related to the geology, topography, and prey and fish species' diversities and abundances (Nikolskii 1963, Frost and Brown 1967).

According to the analysis made by Frost and Brown (1967), trout which are taken from waters with calcium carbonates from 150 to 249 ppm (as in the Eden) should exhibit the maximum growth of 20.0 to 24.7 cm by the end of the third year of life. Trout in the Eden achieve a mean back-calculated length of 20.6 cm (mean actual length of 20.1 cm) at the end of the third year. When consideration is given to the fact that the value for water hardness in the Eden is at the bottom of the Frost and Brown scale, is is therefore reasonable to conclude that trout growth there falls within the mean range of values for maximum growth expected in that quality of environment.

It therefore seems probable that the presence of grayling in the Eden does not depress trout growth. However, it may be argued that the stream could hold more trout in the absence of grayling because such a change in the density of one species when another species is removed has been demonstrated for trout and eels (Smith 1952). Ecologically this implies a reduction in the stream's carrying capacity and therefore productivity through the loss of a 'realised niche'.

Finally, the question remains as to whether or not the low specific growth rate of the trout is related to its intrinsically low growth rate.

Fish growth is considered to be a plastic mechanism, that is it adjusts to the constraints of the environment (Brown 1957), but it is likely that some indication of the inherent growth patterns of the species may be seen in the graph of the growth rates. Trout exhibit a more stable though less dramatic growth pattern than the grayling which display a most astonishing growth rate in the first year and plummet thereafter. In fact, the growth rates of the species are very similar in mature age classes and therefore it is extemely likely that this rapid growth rate of the grayling during the first year of life represents an inherent species' characteristic.

SUMMARY AND CONCLUSIONS

In this chapter, the problem of interactive segregation was investigated indirectly by an assessment of grayling and trout growth in the Eden to provide an index of specie's well-being. Actual age determinations were made by reading scales which had been collected over the preceding seven years, having taken care to verify the use of this method for the grayling. To enlarge the samples for growth analyses, back-calculations were made by the Dahl Lea method of direct proportion and corrections were applied where allometry existed. The growth of grayling and trout was then examined and compared by means of graphs based on mean lengths and on specific growth rates.

1. The verification of the use of scales for aging grayling was demonstrated by the regular evolution of circuli and formation of an annulus. Check formation was shown to occur in the spring (typically in April) for the grayling examined. The accuracy of age determination for grayling was demonstrated by the high concurrence between the two methods used - scale reading and length frequency histogram.

2. The relationship between scale and body growth in grayling was always allometric and therefore a correction factor was applied to all age classes. The relationship between body and scale growth in trout appears to be allometric only in the first year and this is thought to be related to the fact that it does not achieve 10 cm by the end of the first year, and accordingly a correction was applied only to the first year.

3. Graphs presenting the growth of grayling and trout in relation to age clearly indicate that grayling growth surpasses trout growth. However, when Eden trout growth is compared with a standard it is apparent that Eden trout attain the maximum length for that quality of

environment, and therefore the presence of grayling does not appear to retard trout growth by removing resources. The comparison of specific growth rates shows that grayling have nearly twice the growth rate of trout in the first year and it is therefore considered that the initial grayling growth rate represents an intrinsic difference between these species rather than competition.

CHAPTER IV

CONDITION

INTRODUCTION

Condition and the coefficient of condition (K) in particular have been used widely in fishery investigations to express the relative robustness of fishes (Lagler 1956, Nikolskii 1963). Fish condition has been used as an adjunct to age and growth studies to indicate the suitability of an environment for a species by comparing the condition value for a specific locality with those from other regions; they have been employed to measure the effects of environmental improvement, including stocking (Cooper and Benson 1951). In this research, fish condition was evaluated to provide an assessment of the well-being of sympatric grayling and trout in the Eden which would afford some indication of their inter-relationship.

The condition coefficient of factor (K) represents the principal approach to an examination of fish condition. It provides a numerical appraisal of the length-weight relationship of the fish, that is whether or not the fish is heavier or lighter than the average fish of that length. Another method for assessing fish condition involves the controlled dehydration of the specimens to detect the relative percentages of dry matter and moisture content; when fish are in poor condition, such as starved fish, the percentage water content of the body increases to replace eroded somatic stores (Love 1957). Finally, one of the most important indexes of fish condition is fat, which comprises the main energy store of the organism (Nikolskii 1963). The extent to which a fish stores fat around various organs of the body, such as the pancreas and the liver, can be evaluated qualitatively and the extent to which a fish stores fat in its somatic tissue can be assayed quantitatively

in terms of calories, as fat content is the major determinant of the calorific value of fish flesh (Lusk 1968).

METHODS

1. Calculation of the Condition Factor.

The condition factor is calculated from the following formula which is derived from the length-weight relationship:

$$K = \frac{W}{L^3}$$

(after Tesch 1970)

The data used for these calculations are the actual lengths and weights recorded at the time of capture. Care was taken to analyse fish which were mature separately from immature fish because of the known effect of spawning on fish condition: The months preceding spawning are typically characterised by a build-up of nutrients in the fish, particularly as sexual products, and consequently there is a definite increase in the condition of a fecund fish (Hoar 1955, Frost and Brown 1967). The amount of food present in the stomach may also affect the fish weight and therefore to obtain more accurate condition calculations, it would be necessary to pump the stomachs of the fish sample. Such a procedure was too time consuming and therefore impracticable, so the effect of stomach fullness on the condition factor was not evaluated.

The measurements from various fish samples were then pooled into four different seasons which represented quarterly divisions of the year based on Varley's summary of annual climatic cycles (temperate) and its effect on freshwater fish: Winter included December, January, and February; spring was taken from March through May; summer included June, July, and August; autumn was comprised of the remaining months - September, October, and November.

Statistical comparisons of the means of immature and mature fish were made for each season and the means were plotted on a graph. Where

significant differences existed between sexually mature and immature fish in a given season, these means were plotted separately and, conversely, where no significant differences were found in seasonal mean condition regarding sexual maturity, the values were combined for that season.

2. Determination of the Percentage Dry Matter.

The bodies which were used for the determination of the percentage of dry matter were the same fish that were examined for stomach contents. After returning from the field, the heads and gonads were removed from all of the fish before determining their wet weight (individually). Each body was then placed in a labelled piece of foil and was weighed again and then placed in an oven for 5 days at 60C to dehydrate (after Mackinnon 1972). The bodies were removed from the oven and the dry weights determined and were then returned to the oven for an additional 6 hours to discover if the drying process was complete. The usual statistical tests were carried out on the means.

3. Determination of the Fat Condition.

A. Qualitative Analysis: Histological Examination of Fat in the Omentum Surrounding the Pancreas.

i. Preparation of Materials. The omenta which were examined in this study were carefully removed from the fish samples at the time of the stomach analyses and were immediately preserved in 4% formalin in individually labelled bottles and stored. At a later time, the specimens were cut into small pieces (approximatedly $\frac{1}{2}$ cm) for the histological work.

ii. Dehydration. The specimens were dehydrated in the following series of concentrations of alcohol: 70% for 1 hour, 90% for 45 minutes, 90% for 45 minutes, 99% for 45 minutes and 99% for 45 minutes. Care was always taken to change the specimens into fresh solutions of alcohol.

In order to remove the alcohol, methyl benzoate was dribbled down the side of the containers until the specimens began to float. At this point, all the liquid was then poured off the specimens and fresh methyl benzoate was added and the specimens were allowed to remain in this for 12 hours.

iii. Embedding. The specimens were transferred to benzene for 30 minutes and then into 52° C melting point wax for 45 minutes. Specimens were transferred to fresh wax after 45 minutes and this was repeated for a third time. Using hot tweezers, the specimens were individually removed from the wax and were placed in a glycerine-coated watch-glass while fresh wax was poured simoltaneously into the glass. A hot needle was then swirled around the specimen in the wax to attract any air bubbles and a smooth finish was achieved by passing a hot spatula across the top of the wax and gently blowing until a skin was formed. The embedded specimen in the watch-glass was then plunged into cold water to solidify.

iv. Sectioning. The wax blocks were welded onto 1 inch cubes of wood and the five exposed wax facets were squared off to ensure a good ribboning effect. The micro-tome was set to cut sections at 5μ. The ribbons were cut into 3 or 4 frames before placing them in a 50° C water bath which served to remove any wrinkles from the wax and facilitated mounting the sections. Slides were cleansed in acid alcohol and dried and a drop of Haupts adhesive was rubbed on the slide. The slide was then dipped into the water bath and angled under-neath a series of wax frames and was then lifted up and allowed to dry.

v. Staining. Once the sections had been well dried, they were stained with Haemotoxylin and Eosin (Gurr 1973) according to the following method:

1. Xylol (5 minutes).

2. Absolute alcohol (2 minutes).

3. Methylated spirit (1 minute).

4. Rinse in tap water.

5. Haemotoxylin (8 minutes depending on the strength of solution).

6. Rinse in tap water (until stain stops running).

7. Differentiate in 1% acid alcohol (3 quick dips).

8. Blue in Scott's Tap Water.

9. Rinse in tap water.

10. Eosin (3 to 4 minutes).

11. Dehydrate, clear, and mount.

vi. Statistics. Because of the difficulties involved in the quantification of fat in each specimen, all the comparisons between samples are qualitative (McBride 1967). Fat accumulation was therefore assessed as absent, present, densely present, sparsely present, and the percentage of each category were calculated for the four samples.

B. Quantitative Analysis: Determination of the Calorific Value of the Fish Body.

i. Preparation of Materials. The sample of fish used for this evaluation were the same as in the gut analysis. The oven-dried fish bodies were stored in dessicators with silica gel to ensure an air-tight seal which prevented the absorption of moisture. A fish powder was then prepared by milling each fish separately in a Culatti grinder using a 1.0 mm sieve plate. The pulverised fish was then poured into an individually labelled vial and was returned to the dessicator until calorific determinations were made at a later time.

ii. Bomb Calorimetry. A Gallenkamp automatic adiabatic bomb calorimeter was used, model CB-110, for all calorific determinations. The apparatus measures the total amount of heat liberated when the sample

of dry matter is combusted; the measurement is made in terms of the
amount of temperature increase in the water blanket around the bomb
containing the sample. The steps for bombing the samples and for cal-
culating their calorific values are as follows:

1. Weigh accurately, to 3 decimal places, a 25 mm crucible and then
weigh out(approximately) 1 gram of dry fish powder in the crucible.

2. Stretch a piece of platinum firing wire between the electrodes
of the bomb cap and knot a 9 cm piece of cotton mid-way on the firing
wire. Lengths of both wire and cotton should be kept constant to
assure consistent calculations for combustion and heat loss. The
crucible is then placed in the ring of the bomb cap and the cotton
ends are carefully buried in the dry matter in the crucible.

3. Before assembling the bomb, 1 ml of water (distilled) is pipetted
into the bomb case and then the bomb cap is screwed onto the base.

4. The bomb is then filled with oxygen by connecting the oxygen
filling tube to the bomb and slowly filling the bomb to a pressure of
25 atmospheres. After removing the filling tube, fit the sealing cap
to the inlet tube.

5. The calorimeter is adjusted before the bombing by weighing out
3 kg of water which correspond to the total heat capacity of the appar-
atus of 2537 calories. The water temperature within the calorimeter
must be low enough to allow for the expected rise in temperature within
the water blanket. The bomb may then be lowered into the water and
aligned with the firing plug. The cover to the water jacket is then
lowered and the water flow through the cooling coil is reduced to approx-
imately 300 ml per minute at which point the bomb is ready for firing.

6. The apparatus should be run for about 5 minutes to allow the
temperature in the jacket and the calorimetry vessel to stabilise and
to assure that the initial reading is constant. The sample is ignited

by depressing the fire switch and the first reading is taken after 8 minutes. A second reading should be taken as a check about 5 minutes later.

7. To find the total heat release from the combustion of the sample, the temperature rise is multiplied by the heat capacity of the apparatus. This product of total heat release per given milligrams of dry matter can be easily converted to calories by dividing the number of milligrams in that particular sample and then multiplying by 1000(mg).

iii. _Statistics_. Two to five replicate sub-samples were combusted for each fish in the February sample; the number of replicates was determined by the amount of fish powder available for each fish body. Analysis of variance carried out on these results indicated that the variation in the means of two or three fish were not significant and therefore only three replicates were made on the August samples(to conserve time!). Analysis of variance was also carried out on August replicates to determine the within and between variation for calorific values of grayling and trout.

RESULTS

1. Coefficient of Condition (K).

The mean condition factors which were calculated from length-weight data taken from 173 grayling and 222 trout are presented in Table 19 according to the time of year and state of sexual maturity. Statistical comparisons between the mean K values for mature and immature fish in each season are also included in this table.

Various intra-specific comparisons indicate that there are significant differences in the mean condition factors between mature and immature fish for grayling only, and that these differences occur in the winter and spring samples (p= 99.9%). Inter-specific comparisons (see Table 20) indicate that there is a significant difference between mature

Table 19a: <u>Summary of intra-specific comparisons of changes in the mean condition (K) of mature and immature grayling during the year.</u>

Season	Mature Fish Number	Condition	Immature Fish Number	Condition	F Variance Ratio (assumed = or ≠)		Degrees of Freedom	Levels of Significance t (or d)	p (%)
Winter	55	1.28±0.02	20	1.07±0.03	1.13	(=)	73	t = 5.47	p = 99%
Spring	33	1.38±0.01	25	1.27±0.02	0.11	(=)	56	t = 4.93	p = 99%
Summer	16	1.34±0.05	3	1.43±0.00	0.05	(=)	17	t = 0.72	p = 55%
Autumn	11	1.20±0.02	10	1.18±0.03	0.12	(=)	19	t = 0.58	p = 45%

Table 19b: <u>Summary of intra-specific comparisons of changes in the mean condition (K) of mature and immature trout during the year.</u>

Season	Mature Fish Number	Condition	Immature Fish Number	Condition	F Variance Ratio (assumed = or ≠)		Degrees of Freedom	Levels of Significance t (or d)	p (%)
Winter	28	1.03±0.01	57	1.05±0.03	9.64	(≠)	75 (calc.)	d = 0.60	p = 45%
Spring	9	1.23±0.02	30	1.12±0.06	34.00	(≠)	32 (calc.)	d = 1.84	p = 93%
Summer	14	1.31±0.03	73	1.28±0.04	9.00	(≠)	52 (calc.)	d = 0.63	p = 45%
Autumn	14	1.18±0.02	42	1.15±0.01	2.01	(=)	54	t = 1.18	p = 75%

Table 20 : <u>Summary of inter-specific comparisons of seasonal changes in the mean condition of grayling and</u> <u>trout.</u>

Season	Species (X) State maturity Number	Species (Y) State maturity Number	F Variance Ratio (= or ≠)	Degrees of Freedom	Levels Significance t (or d) p
Winter	Mature grayling 555	Immature and mature Trout (combined) 85	not applicable	140	d = 8.13 p = 99%
Winter	Immature grayling 20	Trout (combined) 85	(=)	103	t = 0.72 p = 55%
Spring	Mature grayling 33	Trout (combined) 39	not applicable	72	d =14.37 p = 99%
Spring	Immature grayling 25	Trout (combined) 39	(=)	62	t = 7.57 p = 99%
Summer	Grayling (combined)19	Trout (combined) 87	(=)	104	t = 0.88 p = 65%
Autumn	Grayling (combined)21	Trout (combined) 56	(=)	75	t = 1.44 p = 85%

grayling and trout (combined) in the winter (p=99.9%) and between both mature and immature grayling with trout in the spring (p=99.9%) and these results may be better comprehended in a graph (Fig. 9).

Mature grayling show a peak in condition in the spring (1.38 $\pm$ 0.01) and immature fish rise to top condition in the summer (1.43+0.0) which is statistically similar to the condition of mature fish (1.35+0.01). Mature grayling fall to their lowest condition in the autumn (1.20+0.02) and immatures drop to their lowest condition in the winter (1.07+0.03). Both mature and immature trout attain their best condition in the summer (1.29+0.03) and drop to their lowest condition in the winter (1.05+0.02).

2. Determination of the Percentage Dry Matter.

The means with standard error for the percentage dry matter in grayling and trout bodies are included in Table 21 and Table 22 for the February and August samples, respectively. The results of various intra-specific statistical analyses are presented in Table 23 which indicate that very significant differences do occur in the dry matter content within species and between sexually mature and immature fish as well. Student's t-test shows a highly significant difference between grayling and trout at both times of sampling (see Table 24): The grayling has a higher percentage of dry matter in February and the trout shows a greater percentage of dry matter in its body in August (p=99%).

3. Determination of Fat Condition.

A. Results of histological investigations of the fat condition of grayling and trout are presented in the following photographs (Plts. 4ab, 5ab). In all photographs, the fat cells are 'white goblet cells'.[3] In Plt. 4a of the grayling in winter, the fat cells are so dense as to

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3. In the photographs one is actually looking at negative space of the fat cell(s) rather than seeing the goblet cell which has been dissolved by the dehydration and preserving procedures. This negative space is accepted as a valid indication of fat (pers. com. Dr. R. Roberts 1973).

Fig. 17   Seasonal change in the mean condition of grayling and trout in the Eden
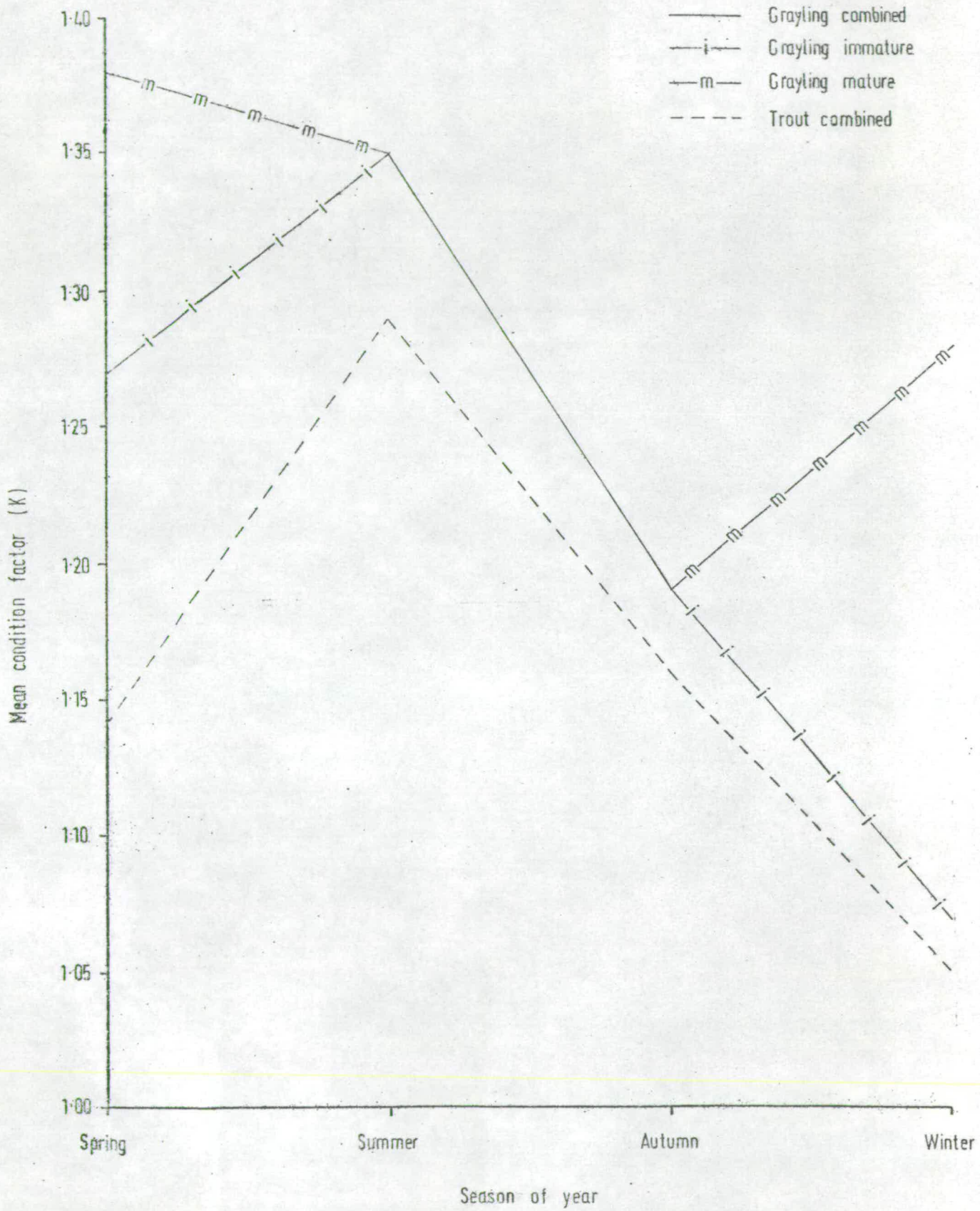
Table 21a : Summary of % dry matter and calorific value of individual grayling. (February sample.)

Wet weight (g)	Dry weight (g)	%dry weight	Number of Replicates	Mean number of calories ± S.E. per g dry weight
124.3	35.1	28.2	5	4868.4±23.6
123.2	33.9	27.5	5	4856.8±10.4
165.1	46.2	28.0	5	4897.0± 8.9
111.0	30.3	27.3	5	4809.2±15.4
145.4	40.1	27.6	5	4860.2±14.7
91.4	22.5	24.6	5	4830.8±12.9
12.7	2.8	21.9	2	4884.9± 5.9
14.9	3.3	22.3	3	4849.4±30.0
10.8	2.3	21.1	2	4992.4± 6.4
21.0	4.8	22.8	5	4750.9±18.2
10.9	2.4	21.6	2	4826.4±15.4
11.4	2.5	21.5	2	4881.2±19.7
		mean = 24.5±0.85		mean = 4849.7± 8.7

Table 21b : Summary of % dry matter and calorific value of individual trout.

Wet weight (g)	Dry weight (g)	%dry weight	Number of Replicates	Mean number of calories ± S.E. per g dry weight
87.1	17.1	19.6	5	5077.7±10.7
108.8	22.2	20.4	5	5106.3±15.7
110.0	21.2	19.3	5	5142.9±14.4
112.9	21.2	19.1	5	5034.6±48.2
117.2	22.4	19.4	5	5099.4± 4.7
61.2	14.2	23.2	2	5060.3±46.7
8.8	2.0	22.7	2	5048.4±46.8
7.8	1.6	20.5	2	5050.9±32.9
		mean = 20.3±0.70		= 5084.6± 9.9

Table 22a: Summary of % dry matter and calorific value for individual grayling. (August sample)

Wet weight (g)	Dry weight (g)	%dry weight	Number of Replicates	Mean number of calories ± S.E. per g. dry weight
127.8	29.5	23.1	3	4821.6±13.2
150.9	37.4	24.8	3	4840.1±26.3
230.0	60.5	26.3	3	4852.9±23.0
57.1	11.4	20.0	3	4893.6±23.0
137.7	31.7	23.0	3	4909.9±34.7
194.3	47.6	24.5	3	4861.5±23.2
65.4	12.9	19.8	3	4849.9±29.9
61.2	12.2	19.9	3	4793.8±31.2
47.4	9.1	19.2	3	4848.0±21.7
58.7	11.4	19.5	3	4795.7±25.3
		mean = 22.0±0.82		mean = 4846.4± 9.3

Table 22b : Summary of % dry matter and calorific value for individual trout.

Wet weight (g)	Dry weight (g)	%dry weight	Number of Replicates	Mean number of calories ± S.E. per g. dry weight
65.3	177.0	27.2	3	5111.0± 9.1
71.1	19.2	27.0	3	5075.9±14.7
39.7	9.9	25.0	3	5077.7±31.0
50.2	13.3	26.4	3	5097.3±14.0
59.2	15.7	26.5	3	5064.1±26.3
18.2	4.2	22.9	3	5103.4±19.9
27.0	6.6	24.3	3	5023.7±19.8
27.9	6.8	24.3	3	5159.5±24.4
18.6	4.2	22.6	3	5061.0±24.9
21.8	5.0	23.0	3	5117.4±12.1
		mean = 24.9±0.56		= 5089.1± 8.5

Table 23a: **Intra-specific comparisons of the mean percentage dry matter in mature and immature grayling.**

Time of sample	Number of fish	State of maturity	Time of sample	Number of fish	State of maturity	Degrees freedom	Level of significance
February	6	immature	February	6	mature	10	$t = 8.96$ $p = 99\%$
August	5	immature	August	5	mature	7	$d = 7.54$ $p = 99\%$
February	6	immature	August	5	immature	9	$t = 7.22$ $p = 99\%$
February	6	mature	August	5	mature	9	$t = 3.58$ $p = 99\%$

Table 23b: **Intra-specific comparisons of the mean percentage dry matter in mature and immature trout.**

Time of sample	Number of fish	State of maturity	Time of sample	Number of fish	State of maturity	Degrees freedom	Level of significance
February	4	immature	February	4	mature	6	$t = 2.35$ $p = 94\%$
August	5	immature	August	5	mature	8	$t = 2.43$ $p = 95\%$
February	4	immature	August	5	immature	7	$t = 2.48$ $p = 95\%$
February	4	mature	August	5	mature	7	$t = 6.36$ $p = 99\%$

Table 23c: Intra-specific comparisons of the mean percentage of dry matter in grayling and trout.

Fish species	February sample		August sample		Degrees freedom	Level of significance
	Number	% dry matter ± S.E.	Number	% dry matter ± S.E.		
Grayling	12	24.8 ± 0.89	10	22.0 ± 0.83	20	t = 2.26 p = 95%
Trout	8	20.3 ± 0.70	10	24.9 ± 0.56	16	t = 5.90 p = 99%

Table 24 : Inter-specific comparisons of the mean percentage of dry matter in grayling and trout.

Time of sample	Grayling sample		Trout sample		Degrees freedom	Level significance
	Number	% dry matter ± S.E.	Number	% dry matter ± S.E.		
February	12	24.8 ± 0.89	8	20.3 ± 0.70	18	t = 3.90 p = 99%
August	10	22.0 ± 0.83	10	24.9 ± 0.56	18	t = 2.89 p = 99%

HISTOLOGICAL EXAMINATIONS OF FAT STORED IN THE OMENTUM AROUND THE PANCREAS
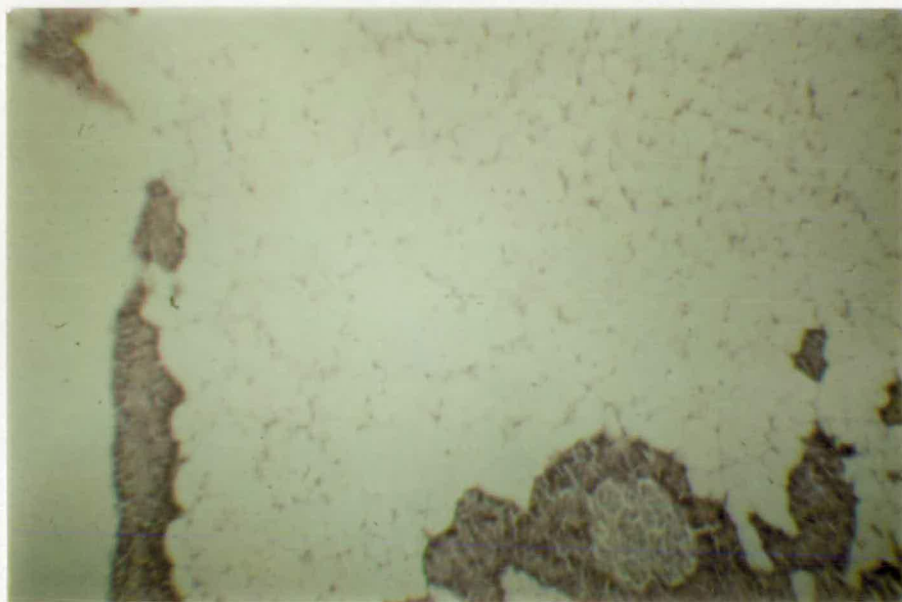
Plate 4a. Specimen of a grayling taken in February (x 100)
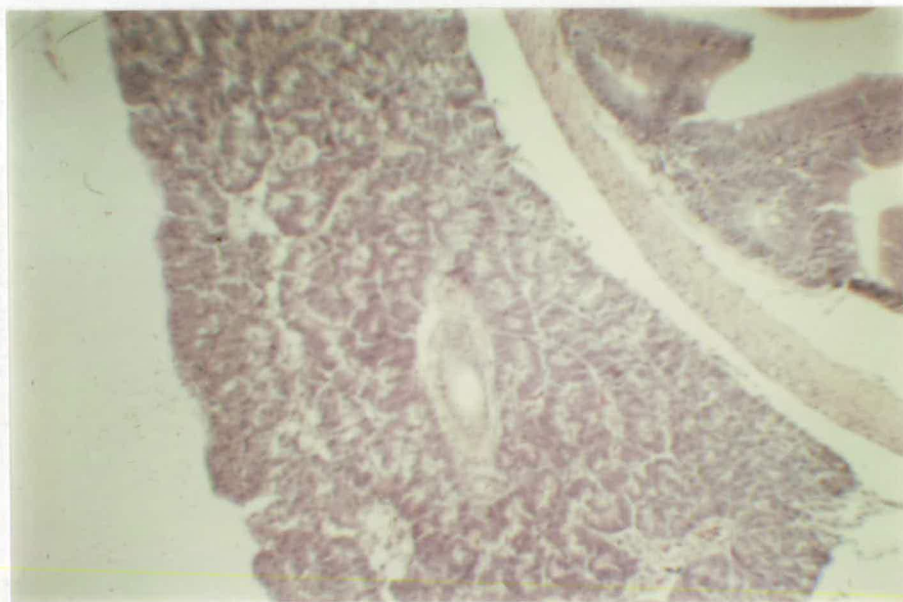
Plate 4b. Specimen of a trout taken in February (x 100)

HISTOLOGICAL EXAMINATIONS OF FAT STORED IN THE OMENTUM AROUND THE PANCREAS
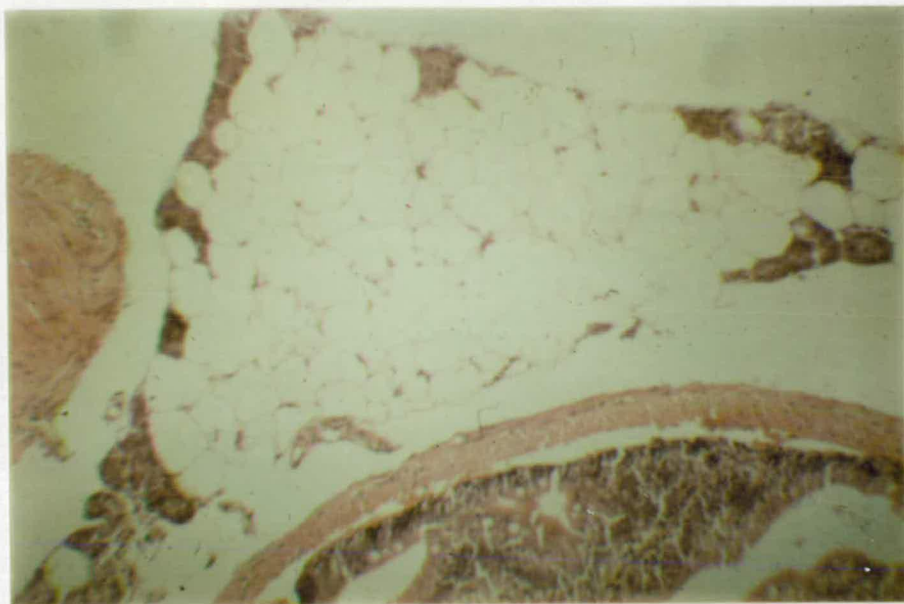
Plate 5a. Specimen of a grayling taken in August (x 100)
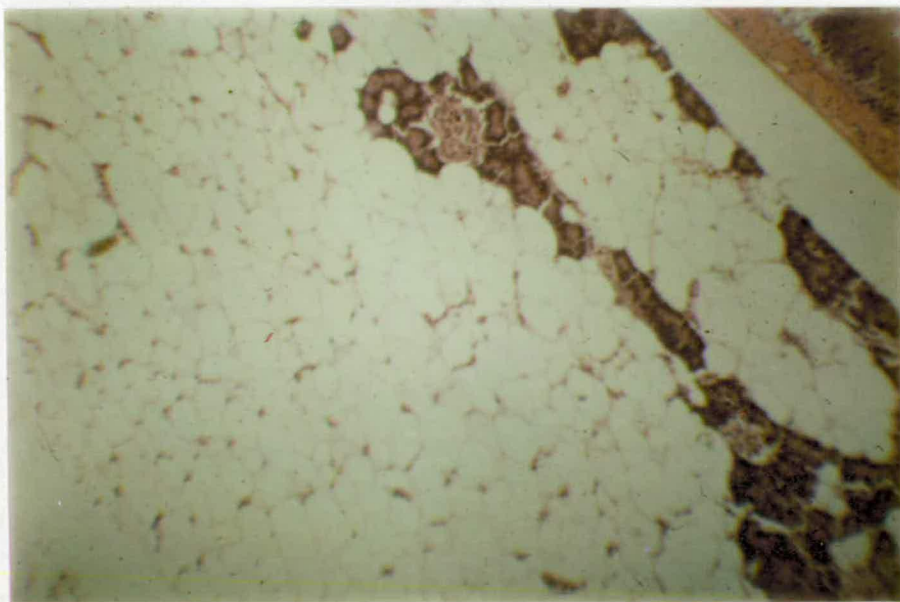
Plate 5b. Specimen of a trout taken in August (x 100)

obscure all but the margin (violet) of the pancreatic tissue beneath.
In the summer (Plt. 5a), the amount of fat exhibited in the grayling
omenta ranged from present in 90% of the sample to densely present in
the remaining fish.

Plt. 4b is a picture of a trout omentum in the winter in which
the pancreatic tissue is completley revealed due to the total absence
of fat cells in 87.5% of the sample, and the remaining fish exhibited
fat cells which were classified as sparsely present and also exposed
some of the compacted cellular structure of the under-lying pancreas.
Plt. 5b of the trout in the summer shows an omentum which resembles
that of the grayling in the winter and this high density of fat cells
was found in 80% of the sample; in the remaining 2 fish samples, the
the fat cells were classified as present.

B. The calorific values determined for replicate samples of each fish
body are included in Tables 21 and 22 for the February and August sam-
ples respectively: The mean calorific values with standard errors are
also recorded in these tables. Analysis of variance carried out on
these results is summarised in Table 25 and indicates that the exper-
imental error is on the order of 44.18 calories in the February sample
and 39.8 calories in the August sample. The intra-specific variation
in both sampling times and for both species did not exceed the tabu-
lated value in the one-tailed variance ratio table and therefore the
within variation was not significant. Inter-specific variation, how-
ever, was extremely significant for the calorific values of grayling
and trout in both the February and August samples, with the calculated
values of 500.83 and 557.2, respectively.

DISCUSSION

1. Coefficient of Condition.

Intra-specific differences in the mean condition factors based on the

Table 25a: Variance Analysis on the Calorific Values of Grayling and Trout Bodies Sampled in February.

Source of Variation	Degrees of Freedom (N-1)	Sum of Squares (SS)	Mean SS	F Value
Between Grayling	11	91,136.10	8,285.10	2.79
Between Trout	7	39,224.25	5,603.50	2.75
Between Species	1	1,022,213.32	1,022,213.32	500.83
Residual Error	57	116,339.97	2,041.05 =45.18	
Total	76	1,268,913.64		

Table 25b: Variance Analysis on the Calorific Values of Grayling and Trout Bodies Sampled in August.

Source of Variation	Degrees of Freedom (N-1)	Sum of Squares (SS)	Mean SS	F Value
Between Grayling	9	37,473.13	4,163.70	2.63
Between Trout	9	38,446.38	4,271.80	2.69
Between Species	1	883,682.84	883,682.84	557.21
Residual Error	40	63,438.03	1,585.95 =39.82	
Total	59	1,023,040.38		

state of sexual maturity were exhibited by grayling alone and these may be related to the significant differences already shown in the intrinsic growth rates of these species during the first year of life. The grayling samples were predominately composed of young of the year fish which grew in length from 0.95 cm to 14.20 cm (mean actual length) at the end of the first year - a specific growth rate of 270.5% - whereas the trout samples which were largely composed of 1+ fish grew from 2.4 cm to 9.46 cm at the end of the first year -a specific growth rate of 141.4%. It would therefore appear that immature grayling concentrate their nutrients into the elaboration of length and it is probably not until they reach 1+, the time when their intrinsic growth rate plummets, that nutrients are directed increasingly towards weight rather than to body length. Similarly, it is likely that trout which show a significantly lower specific growth rate in the first year of life are perhaps consequently better able to maintain higher condition because of a lower intrinsic growth rate and therefore a reduction in the proportion of nutrients utilised for increasing body length.

The mean condition values of the grayling and trout in all seasons are greater than 1.00 and therefore, although grayling condition is always higher than that of trout, both populations appear to sustain good condition all the year in the Eden. Furthermore, their over-all changes in condition exhibit similar sorts of extreme values within the annual cycle - the condition of grayling range from 1.07 to 1.35 (a difference of 0.28) and trout condition range from 1.05 to 1.29 (a difference of 0.24) - and follow a similar pattern in so far as both species attain their peak condition in the months preceding spawning and leading up to spawning and fall off thereafter. Other researchers have also noted a relationship between condition and spawning: Pennel (1863) mentions that grayling are in the height of condition in the

pre-spawning months and Frost and Brown (1967) noted the same for the trout.

The implications of the results of inter-specific comparisons must be discussed from two points of view - the meaning of significant and of insignificant differences. The significant difference between the mean condition of mature grayling and trout (combined) in the winter and in both the mature and immature grayling and trout (combined) in the spring suggest that mature grayling are feeding and assimilating in preparation for spawning and that immature grayling are probably growing during the winter in length until the spring, when their growth rate alters and they too begin to become heavier for their length. Mature trout appear to be slow to recover somatic losses associated with spawning in the winter, probably related to over-wintering and immature trout, although in slightly better condition than the mature fish, also show a drop in condition in the winter and all trout begin to increase in condition in the spring.

In the summer and autumn samples, however, there are no significant differences between the mean condition of grayling and trout: Both species are in extremely good condition in the summer which demonstrates that they both feed and assimilate intensively and in the autumn, the mean condition of both species drops which implies a reduction in feeding for all fish as well as somatic and gonadic losses in the mature trout. These differences and similarities in condition coefficients for grayling and trout in the Eden resemble the results of the stomach fullness examination which indicated that only the grayling fed intensively during the winter and suggested that both species were feeding intensively during the summer but were probably digesting food rapidly at higher temperatures. Furthermore, it would appear that the inter-specific differences in the changes of condition lend support to the

previous suggestion that it is during the summer that these salmonids are likely to compete over food. However, the fact that both grayling and trout do attain very good condition in the summer demonstrates that both species do in fact obtain (and assimilate) abundant food and therefore that competition over food does not occur to any measurably deleterious degree.

2. Percentage Dry Matter.

Intra-specific comparisons between mature and immature fish taken in the same sampling times demonstrate that mature fish have generally a significantly greater percentage of dry matter in their bodies than the immature fish which confirms the positive effect of maturity on the dry matter content of the body. This was not, however, found to be true for the February sample of trout where the percentage of dry matter of mature fish was lower than immature fish, although this was anticipated because of somatic loss associated with spawning (Chapman 1967).

Inter-specific comparisons of the changes in the mean percentage of dry matter in the bodies of grayling and trout suggest that there are significant differences in fish condition within the annual cycle. These results concur with those of Brett et al (1969) who found that a change in the percentage of dry matter in the body of the sockeye salmon (Oncorynchus nerka) and therefore reinforce the pattern of seasonal change already seen in the condition coefficients of grayling and trout. These differences may again be related to spawning and to the associated accumulation of nutrients. The mean over-all percentage of dry matter in grayling and trout in their pre-spawning seasons are virtually identical, 24.8 and 24.9% respectively, and similarly the difference between the over-all percentage of dry matter in their post-spawning samples is not significant (p = 90 %).

Changes in the percentage of dry matter within the year also afford insight into those months when fish are feeding intensively. Brett et al. (1969) found that the growth rate, fat and protein contents of the body are directly related whereas growth and body water are inversely related; the time of year when a species exhibits the greater dry matter content is therefore during and shortly after a period of intensive feeding, and decreases in the percentage of dry matter reflect a reduction in feeding and the concomitant increase in the water content of the fish body (Love 1957). Changes in the mean percentage dry matter indicate that grayling feed intensively in the winter and that feeding (and assimilation) fall significantly in the summer (p = 96%). Changes in the mean percentage of dry matter show that the trout feeds intensively in the summer and exhibits a drastic reduction in food intake (and assimilation) in the winter (p = 99.9%). These changes are similar to those seen in condition factors and in stomach fullness indexes and provide further support to the indications that grayling and trout feed most intensively at different times of year and would therefore be less likely to compete for food.

3. Fat Condition.

Histological evidence for densities of fat deposition clearly portray the changes in fat condition of grayling and trout which have occurred at the two extreme sampling periods in 1973. These results offer further confirmation to the specific patterns of annual fluctuations in condition as suggested by changes in the mean condition coefficients and percentages of dry matter. Moreover the photographs convey the intensity of these changes: In Plts. 5b and 4b are the trout omenta before and after spawning and the totally depleted pancreas with no fat cells present in the omentum in the latter photograph emphasises the severity of the loss of condition in the trout after spawning. In Plts. 4a and 5a of the

of the grayling omenta before and after spawning, fat condition
appears to be virtually the same in both samples. It is important to
note that there is no obvious difference between the fat conditions of
grayling and trout in their pre-spawning samples (Plts. 4a and 5b,
respectively) and this was also shown to occur for their condition
coefficients and percentages of dry matter.

Other authors have reported seasonal changes in the amount of fat
in fish. Mills (1953 unpublished) examined the changes in fat content
of the herring liver throughout the year. Ananichev (1959) reported
that the accumulation of fat reserves in the burbot (Lota lota) and the
pike perch (Lucioperca lucioperca) occurred during the periods of inten-
sive feeding. Lusk (1969) noted a variation in the fat content of the
brown trout liver which he attributed to the intensity of feeding.

When histological results of this research are interpreted accord-
ingly, the high level of fat deposition found in the omentum around the
pancreas indicates that graylingwere feeding intensively in both Feb-
ruary and August samples and that trout were feeding intensively in the
summer but showed reduced feeding in the winter sample. These results
again demonstrate the bi-annual periodocity in feeding for grayling and
trout and provide further support to the previous interpretation of
stomach fullness analyses and to the suggestion that both trout and
grayling obtain surplus food (i.e. energy in excess of maintenance re-
quirements and sufficient for growth, fat deposition, and sexual products)
during the summer and therefore do not appear to compete over basic re-
sources at a time of year when both species are feeding intensively.

Intra-specific variation in the calorific values of grayling and
trout are not significant and suggest that grayling and trout do not
vary in the amount of fat stored in somatic tissue within the annual
cycle. Furthermore, these results indicate that there are no real

differences between the calorific content of immature and mature fish bodies of the same species. Such results are initially surprising in light of the previous results which have demonstrated changes in condition during the year and have the following implications: (1) There is no significant or measurable change in the calorific content of the fish bodies as regards the state of maturity (and spawning in particular); (2) it is probable that the somatic chemical composition, i.e. the relative lipid and protein constituents of the tissue, is reasonably stable and this may serve as a safeguard to assure fish survival in stressful situations, such as when food is scarce. It is, however, important to note that although these constituent ratios appear to be stable, the quantity of tissue has been shown to change (the actual percentage of dry matter of the body) within the annual cycle and particularly in relation to spawning and sexual maturity. Because of the constancy of tissue composition, as seen in the consistent calorific values found in the February and August samples of both species, this index of condition is probably only useful in the most extreme situations - such as epidemics and starvation - and is therefore considered as inapplicable as a quantitative index of fish condition in normal circumstances.

SUMMARY AND CONCLUSIONS

In this chapter the demonstration of interactive segregation was investigated indirectly in terms of various condition indexes - the coefficient of condition, the percentage of dry matter in the fish body, and fat deposition in the omentum around the pancreas and in somatic tissue.

1. Condition coefficients indicated that both species always exhibited better than average condition and so it appears that both populations are well-sustained in the Eden. There is a definite change in condition with the seasons and this is most probably related to the effects of spawning. Intra-specific differences were found between the condition coefficients of mature and immature grayling only, and the lower condition of the latter is thought to reflect its remarkable intrinsic growth rate during the first year of life.

2. Intra-specific differences in the percentage dry matter show the positive effect of sexual maturity on body constituents. Inter-specific comparisons indicate significant bi-annual changes in the body composition of the grayling and trout which again relate to their spawning cycles.

3. Fat deposition in the omentum around the pancreas showed a marked bi-annual variation for the trout only - densely present in the summer and absent in the winter - whereas the grayling exhibited a high concentration of fat cells at both sampling times. Fat deposited in somatic tissues was assessed calorifically and did not vary bi-annually (which was considered to represent a survival safeguard) and therefore this was not found to be a suitalbe index for fish condition. Trout on the whole maintained a higher level of fat in its tissues than the grayling.

All the condition indexes have been shown to relate (and in some instances to depend) on feeding and assimilating - a high condition coefficient, percentage of dry matter, and fat deposition all presuppose both intensive feeding and the efficient utilisation of food. The condition indexes of both grayling and trout were typically high in the summer sample which imply that both species were feeding heavily in the months preceding the sampling. The condition indexes may therefore be used to provide insight into the appropriate interpretation of the results for stomach fullness analyses (Chapter II): The intermediate values for stomach fullness in grayling and trout in the summer should be interpreted to mean that both species are feeding intensively, but also digesting rapidly, probably due to the higher temperatures experienced at that time of year.

Condition indexes therefore also suggest that the summer is the time of year when grayling and trout are most likely to compete for food and this was also indicated by the results for stomach fullness, coefficient of overlap and trophic diversity estimates in Chapter II. However as all the condition indexes do concur in that both grayling and trout are in very good condition at this time of year, competition does not appear to occur in the Eden to any measurably deleterious degree.

PART I

FINAL DISCUSSION

Grayling and trout are both salmonids and are frequently found living sympatrically which indicates either great plasticity or specificity of their fundamental habitat requirements, such as spawning and feeding. According to Nilsson (1967), when closely related species overlap spatially they tend to exhibit some form of interactive segregation - niche specialisation - in an effort to maintain slightly different habitat requirements from one another. Therefore, in an examination of the spawning sequence for these species, it is expected that any great similarity in their spawning requirements would result in either competition over basic reproductive-associated resources or in some form of interactive segregation. Similarly, it is anticipated that any overlap in feeding habits would also result in competition or interactive segregation.

A species may limit the spawning success of another species in many ways: One species may physically interfere directly by disturbance, aggression, and (or) by predation on ova and fry of another species; a species may interfere indirectly by cropping a greater proportion of the nutrients of a common food supply and in so doing, possibly affecting the quality and viability of the ova (Nikolskii 1963) or by usurping better spawning grounds.

Grayling are reputed to dig up trout redds when (grayling) spawning and in so doing to reduce the hatching success of the trout. However, in practice grayling and trout do not appear to limit the survival of the other species by such potentially competitive acts. Both species deposit their eggs in clean, well-oxygenated gravel, but that used by the grayling is slightly finer than that used by the trout (5mm and 10 mm, respectively) and is generally located in a different part of

the riffle; both species appear to occasionally consume each other's egg and alevins (Hellawell 1971); both species spawn at widely separate times of the year, the grayling in the spring and the trout in the late autumn (Dahl 1962, Jankovic 1964, Frost and Brown 1967, Peterson 1968). Therefore, although the spawning of grayling and trout overlaps spatially to some extent, they do not overlap temporally and it is considered that they do not seriously interfere (or compete) with each other's reproductive success.

The question then arises of whether or not the differences in the spawning times of these species should be considered to represent the occurrence of interactive segregation - i.e. was the temporal separation of spawning effected by interactive segregation? In view of the information concerning grayling and trout reproduction published in various papers, it seems reasonalbe to assume that the temporal separation between the spawning periods of these species is a universal characteristic which occurs allopatrically as well as sympatrically, rather than an ecotypic variant. The time of spawning itself is thought to be a physiological adaptation of protective significance related to the particular environmental constraints, such as the amount of food produced and the presence of predators (Nikolskii 1963).

In the instance of the closely related salmonids, grayling and trout, the difference in their spawning periods may be related to a fundamental difference in their physiologies and in their metabolisms in particular, which are probably attributable to the origin of the grayling: The grayling is considered to be an arctic fish (Henderson 1951) and arctic fish generally spawn in the spring to summer (Norman and Greenwood 1963) when the water temperature is increasing and food is more abundant. The trout is considered to be a temperate species which regularly spawns in the autumn when predation is at a minimum and eggs are assured of

good aeration.

With regard to the similarity of grayling and trout feeding habits, an examination of their diets is expected to reveal the amount of overlap and specialisation in the types of food they consume and therefore to indicate whether some form of competition or interactive segregation occurs. Food is of fundamental importance to the organism (Lack 1954, Wynne-Edwards 1962, Nikolskii 1963) and it is therefore a resource which is extremely susceptible to over-exploitation in the form of competition. "Endeavours to analyse the possible forms of relations between animals convinces one that the fight for food is of overwhelming importance amongst them, and that it occupies a predominant position among all forms in the struggle for existence" (Ivlev 1961).

The results of bi-annual stomach analyses made on grayling and trout from the Eden Water indicate that there was no overlap in their diets in the winter and that there was some overlap in the summer. However, this was only significant for the first and second year classes. Hartley (1948) confirmed that finding different food in different species which suggested that there was little overlap in their diets was not sufficient evidence that some form of competition was (or was not) occurring. Therefore, in order to detect any persistent, low-grade competition in the Eden, the quantitative indexes of fish growth and condition were applied to assess the 'well-being' of each species. The growth rates of both species appeared to be at a maximum for the given environmental conditions (productivity), although grayling growth always exceeded trout growth. Both species always exhibited better than average condition - 1.0 - and they also attained a similar peak in condition (numerically) which was thought to be related to spawning and to increased feeding and assimilation. These indexes demonstrated that both species are well-maintained in the Eden and therefore that grayling and trout

did not seem to compete to any (measurably) limiting extent.

The fact that competition over food was not seen to occur in the Eden suggests that these closely related species have segregated their feeding habits to some degree. The results of the stomach analyses which were quantified in terms of trophic diversity estimates indicate that grayling and trout do exhibit feeding specialisation; during the winter, when the trout is 'over-wintering', the grayling feeds on a broad spectrum of prey species, whereas in the summer, at a time when both species have been shown to feed intensively, the grayling appears to restrict the types of prey it consumes. This dietary specialisation is probably reinforced by inherent differences in their seasonal feeding patterns which are most likely related to the specific relationships between their metabolisms and the environment (see Chapter VI).

Interactive segregation describes the occurrence of ecological differences between species through such mechanisms as food or habitat selection to develop slightly different niches. This phenomenon is believed to be typical of young fauna, from recently de-glaciated areas where ecosystems are still in a relatively rapid process of evolution (Dunbar 1960) and theoretically, the populations of the most northern temperate zone should provide examples of interactive segregation. (Nilsson 1967).

The results of this research provide partial fulfillment of the above premises of interactive segregation and therefore they are considered to contribute substantial evidence towards the demonstration of the occurrence of interactive segregation between the grayling and trout populations in the Eden.

PART II

SOME OF THE MECHANISMS EFFECTING INTERACTIVE SEGREGATION

CHAPTER V

COMPARATIVE MORPHOLOGY OF FEEDING RELATED STRUCTURES

INTRODUCTION

Fish are commonly classified according to the amount of variation in
the types of food they consume: (1) euryphagic fish feed on a great
variety of prey species; (2) stenophagic fish consume only a few dif-
ferent types of prey; (3) monophagic fish eat only a single type of
food (Nikolskii 1963, Odum 1971). Feeding diversity is a specie's
characteristic which is thought to have been formed during its evolu-
tion (Nikolskii 1963); the more stable the feeding conditions of the
species, the smaller the range of foods to which it is adapted, and
conversely, the more variable the food supply, the greater the range
of prey eaten by a species.

In the temperate latitudes, where both the quantity and the compo-
sition of the food supply is considered to vary at different times of
the year, most fish exhibit euryphagy, although many have developed
certain adaptations which permit some degree of specialisation as
regards feeding. The pike (Esox lucius) which is a predator which has
evolved sensory organs which enhance the efficiency of its method for
capturing prey; the pike generally seize their food by lying-in-wait
and then chasing their prey, orienting by means of the lateral line
and the eyes (Nikolskii 1963). The brown trout largely depends on its
eyes for hunting food (Frost and Brown 1967). The stone loach exhibits
special out-growths (barbels) in the area around the mouth which act
as tactile organs for feeding from the benthos.

Closely related to the variety of foods consumed by fishes is the
function of the organs which sieze, comminute, and swallow the food,
and these may be classified as follows (Nikolskii 1963): (1) grasping

mouth which is typical of predatory species is usually large with very sharp teeth on the jaw bones and often on the vomerine and palatine bones; (2) sucker mouth has no jaws and the teeth are in the form of horny tubercles; (3) imbibing mouth is in the form of a tube which is sometimes extensible and usually toothless; (4) crushing mouth sometimes is in the form of a beak with powerful teeth and other times is plate-like or spine-like, all of which serve to crush the hard exoskeleton of invertebrates such as molluscs, echinoderms, or corals; (5) plankto-phagic mouth is typically a medium to large size which is immobile with teeth small or absent and food is strained by long gill-rakers; (6) periphyton-eating mouth is located on the lower side of the mouth and is shaped like a transverse crack and the lower lip usually has a sharp cutting edge (horny) without teeth.

According to Keast and Webb (1966) "the significance of the mouth in leading to specialised feeding and to a (probable) reduction in inter-specific competition needs assessing (p.1846)". Icthyologists have given little attention the the influence of minor structural dif-ferences between the mouths of fish in effecting the segregation of co-habitating species of fresh-water fish as compared with other ani-mals: Extensive ornithological research has been conducted to invest-igate the minor differences in the bill shape, wing form, and tarsus length all of which have been correlated with differences in diet, feeding habits, and way of life (Darwin 1859, Lack 1954, Hamilton 1961, Osterhaus 1962).

Morphological investigations made on samples of grayling and trout from the Eden consisted of both qualitative and quantitative anatomical comparisons to detect any possible differences in the shapes, sizes and orientation of their mouths, eyes, fins and body which could affect their feeding. These results were then interpreted according to

accepted theory of functional design of fish structures and to field
and experimental observations of the use of these structures in
feeding, swimming, and caturing prey (Mitchell 1967, Greendale 1973,
unpublished).

METHODS

Morphometric measurements were made on fresh specimens of grayling and
trout which ranged in size from 13.4 cm to 33.0 cm and 8.9 cm to 34.2
cm, respectively. The size range included both   immature (2) and
mature (5) fish because certain body characteristics have been shown
to change with age (Lowe-McConnell 1966). Most measurements were made
by using Vernier steel calipers which permitted measurements to be made
as fine as 0.1mm. As the objective of this examination was to compare
the relative sizes and shapes of various body characters, highly accur-
ate measurements were demanded and therefore all measurements were
taken a second time and if any discrepancy occurred, a third measure-
ment was taken to confirm the correct distance.

1. Standard Length.

In systemic work this is typically taken as the distance from the
anterior part of the snout or upper lip (whichever extends farthest
forward) to the caudal peduncle base in a straight line.

2. Mouth Position.

The mouth position is classified according to its relation with the
body axis (according to Nikolskii 1963):(1) dorsal mouth lies above the
body axis; (2) terminal mouth is located along the line of the body axis;
(3) ventral mouth is underneath the body axis.

3. Width of Mouth.

The aperature of the mouth is measured by the distance across the inside
of the open mouth (Keast and Webb 1966).

4. Extensibility of the Lower Jaw.

This distance is measured from the anterior margin of the eye to the lower lip with the mouth closed and open, and the difference is expressed as a percentage.

5. Dentition.

The heads of the fish were simmered until the flesh came away and the boney parts were then dried, examined, and photographed.

6. Body Height.

This measurement is taken at the deepest part of the body excluding the fins (Keast and Webb 1966).

7. Body Width.

This is a measure of the maximum width which is characteristically in the region of the pectoral fins (Keast and Webb 1966).

8. Body Form.

The body form may be classified as follows: (1) rounded fusiform, (2) compressed fusiform, (3) foreshortened tubular, (4) elongate tubular, (5) sub-gibbose, (6) gibbose. (Keast and Webb 1966, see illustrations in Fig. 18).

9. Pectoral-Pelvis Distance.

This is a measurement of the horizontal distance between the mid-points of the bases of the paired fins expressed as a percentage of standard length.(Keast and Webb 1966)

10. Caudal Peduncle Length.

This measures the horizontal distance from the anus to the end of the spinal collumn expressed as a percentage of the standard length (Gray 1953).

11. Fin Form.

The fin is described as falcate, sub-falcate, or rectangular and these fin types are illustrated in Fig. 18 (Keast and Webb 1966).

Fig. 18   Illustrations of body and fin form classification according to Keast and Webb (1966)

Body form

Elongate tubular   Sub gibbose   Gibbose

Rounded fusiform   Compressed fusiform   Foreshortened tubular

Fin form

Falcate   Sub-falcate   Spatulate   Rectangular   Rounded
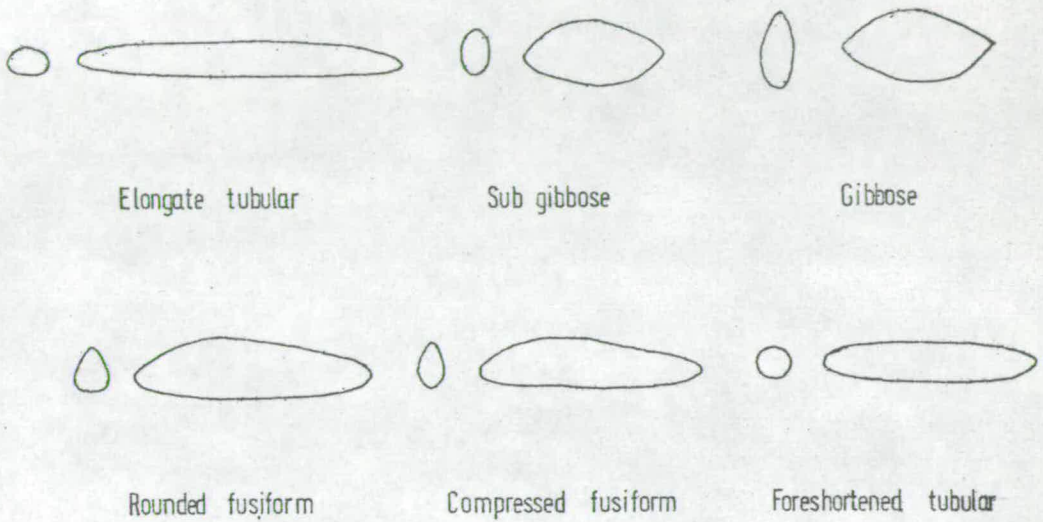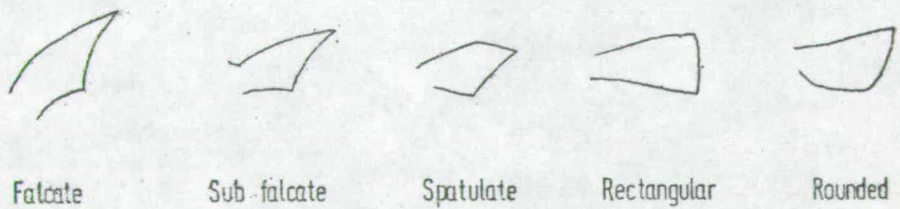
RESULTS

A summary of body, mouth and fin forms is presented in Table 26. The bodies of both the grayling and the trout are fusiform, but the grayling body is slightly more rounded and the trout's more compressed(Plts.6ab). The mouth position of the grayling is termed 'semi-ventral' for better precision and this is pictured in Plt.7a which indicates that the mouth lies nearly under the line of the body axis; the mouth of the trout is clearly terminal and this is shown in Plt.7b. The mouth forms of both species are grasping by definition (Nikolskii 1963). The pectoral fins of the grayling and trout are falcate and their caudal fins are homo-cercal.

Table 27 presents the figures and ratios for body form for each specimen. The grayling width to length ratio is low for the two immature fish but the ratios for both immature and mature grayling were in every instance higher than those for trout of corresponding maturity (size). Similarly, grayling height to length ratio was lwer for immature fish and this was the same ratio for mature trout (1:5). The body height ratio for both immature and mature grayling and trout was always 2:1. The pectoral to pelvis distance of the young and mature fish of both species were similar, with combined means of 31.02 ± 1.2 and 32.66 ± 0.3, respecitively (p= 90%). There was no significant difference between the mean caudal peduncle distance for immature and mature grayling and trout (91%).

The final table presents figures relating the sizes and forms of the mouths to the lengths of the fishes. There is no significant difference between the mean widths of the grayling and trout mouths ($p = 10\%$); how-ever,there is a significant difference between the lower jaw extensibility of the grayling and of the trout - the mean grayling percentage is 4.62 ± 0.2 and the mean for trout is 8.6 ± 1.7 (p= 99.9%). The measurement

Table 26: <u>Summary of Body and Fin Form of Grayling and Trout.</u>

	Grayling	Trout
Body Type	fusiform	compressed fusiform
Fin Form[a]	falcate	falcate
Mouth Position	semi-ventral	terminal

a. It should be noted that although the pectoral, pelvis, anal, caudal, and adipose fins of the grayling and trout are similar, the dorsal fin of the grayling is proportionately larger and is in fact an outstanding species' characteristic.

A COMPARISON OF THE BODY TYPES OF GRAYLING AND TROUT
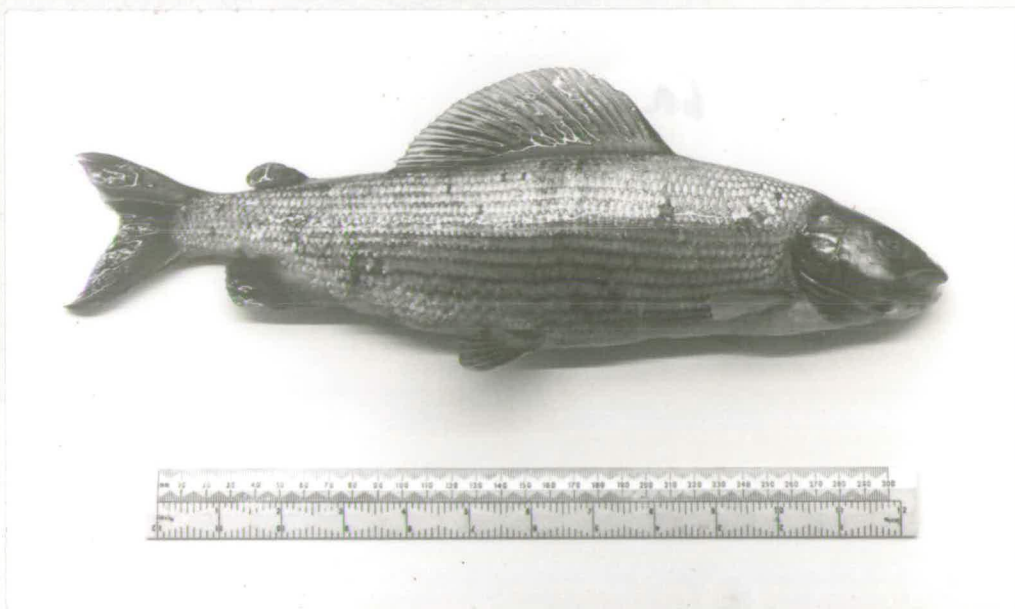
Plate 6a. Grayling (33.0 cm)
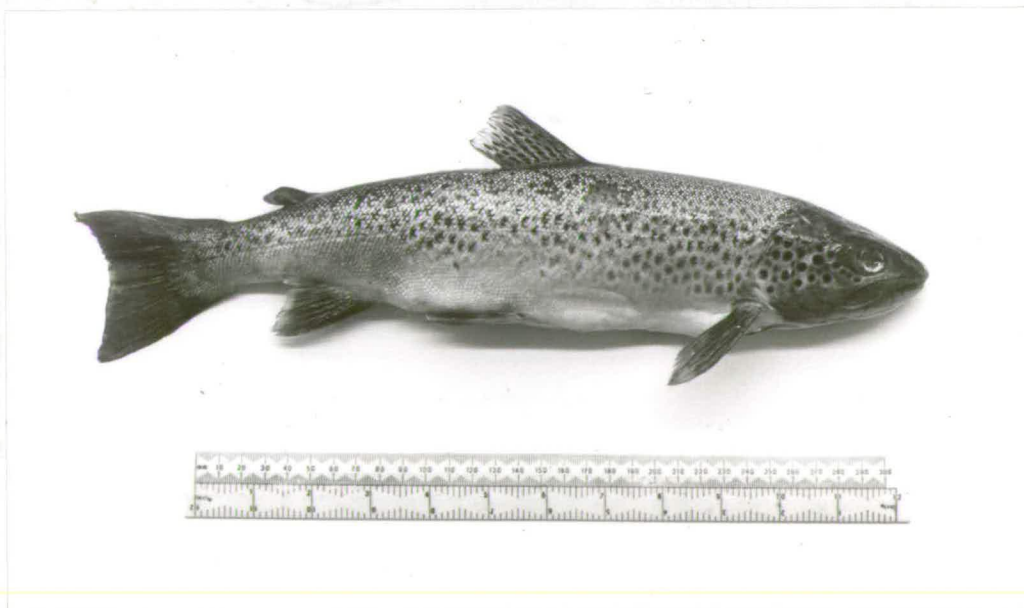
Plate 6b. Trout (34.2 cm)

EXTERNAL SIDE-VIEW OF GRAYLING AND TROUT MOUTHS

Plate 7a. Trout

Plate 7b. Grayling

Table 27: A Summary of Ratios Describing Body Form of Grayling and Trout

Species	Body Width: Body Length	Body Height: Body Length	Body Height: Body Width	Pect. to Pelvis	Caudal p. to anus
Grayling	1:8	1:5	2:1	28.5	29.6
Grayling	1:8	1:5	2:1	28.6	30.0
Grayling	1:8	1:4	2:1	31.2	29.3
Grayling	1:7	1:4	2:1	35.1	32.0
Grayling	1:7	1:4	2:1	31.7	27.2
mean ± S.E.				31.0 ±1.2	29.6± 0.8
Trout	1:9	1:5	2:1	32.6	32.9
Trout	1:9	1:5	2:1	31.7	32.2
Trout	1:9	1:5	2:1	32.0	28.9
Trout	1:9	1:5	2:1	32.8	30.8
Trout	1:9	1:5	2:1	31.2	33.0
mean ± S.E.				32.1±0.3	31.6±0.8

Table 28.

A Summary of Measurements for Size and Mouth Form of Grayling and Trout

Species	St. Len. (mm)	W. mouth (mm)	" as % St. Len.	L. jaw extens. (mm)	"as % St. Len.	T - B mouth (mm)	" as % St. Len.
Grayling	134	11.3	8.4	6.6	4.9	16.1	12.0
Grayling	138	11.8	8.6	6.6	4.8	17.0	12.3
Grayling	259	21.3	8.2	12.7	4.9	26.7	10.3
Grayling	310	20.5	6.6	13.6	4.4	29.0	9.4
Grayling	330	24.4	7.4	13.5	4.1	32.5	9.8
mean± S.E.			7.8+0.4		4.6+0.2		10.8+0.6
Trout	139	12.3	8.8	12.4	8.9	33.8	24.3
Trout	135	11.5	8.5	11.7	8.7	32.5	24.1
Trout	250	20.0	8.0	21.5	8.6	45.2	16.0
Trout	284	20.5	7.2	22.4	7.9	45.5	16.0
Trout	342	22.3	6.5	29.8	8.7	50.4	14.7
mean ± S.E.			7.8+0.4		8.6+0.2		20.3+2.1

(St. Len.= Standard length; W. mouth = Width of mouth; " = ditto; L. jaw extens = Lower jaw extensibility; T-B = Top to bottom.)

for the vertical span of the open mouth also indicates a significant difference in the degree of articulation of the species- the mean grayling span was 10.7 ± 0.6 and the mean for trout was 20.3 ± 2.1 (p= 99%). The immature fish of both species exhibit a wider span per body length than the larger mature fish.

Photographs of the dentition of a 25.9 cm grayling and a 25.0 cm trout are presented in Plt. 8a and 8b. The dentition of the grayling consists of tiny teeth near the head of the vomer and the anterior side of the palatine and along the jaw line. The trout mouth exhibits large, sharply pointed teeth which curve inwards towards the pharynx and these teeth are found along the jaw and on the tongue and vomerine and palatine bones as well.

DISCUSSION

The form of the body to a great extent limits the way of life of a fish: The type of habitat (i.e. lotic or lentic) will be indirectly related to the swimming demands required for routine activities. Natural selection must tend to favour shapes which give the fish the least possible drag at any given swimming speed and the ideal body proportions for a stream-lined form are 4.5 times as long as the maximum diameter (Alexander 1970). The maximum diameter of grayling and trout is body height and the ratio of this diameter to body length is generally 1:5. It is therefore considered that the fusiform body type of both grayling and trout are reasonably stream-lined and well-adapted to lotic conditions.

There is a slight difference in the shapes of the grayling and trout (Plts. 6a,b) as is indicated by their classifications as rounded and compressed fusiform, respectively. According to Alexander (1970) a more rounded form has less surface area and presumably less drag and it therefore seems likely that the grayling is actually marginally

CLOSE-UP VIEWS INSIDE GRAYLING AND TROUT MOUTHS

Plate 8a. Grayling mouth. (note horny tubercles on jaw)

Plate 8b. Trout mouth. (note sharply pointed teeth visible
on the jaw and on the tongue)

COMPARISON OF GRAYLING AND TROUT TEETH
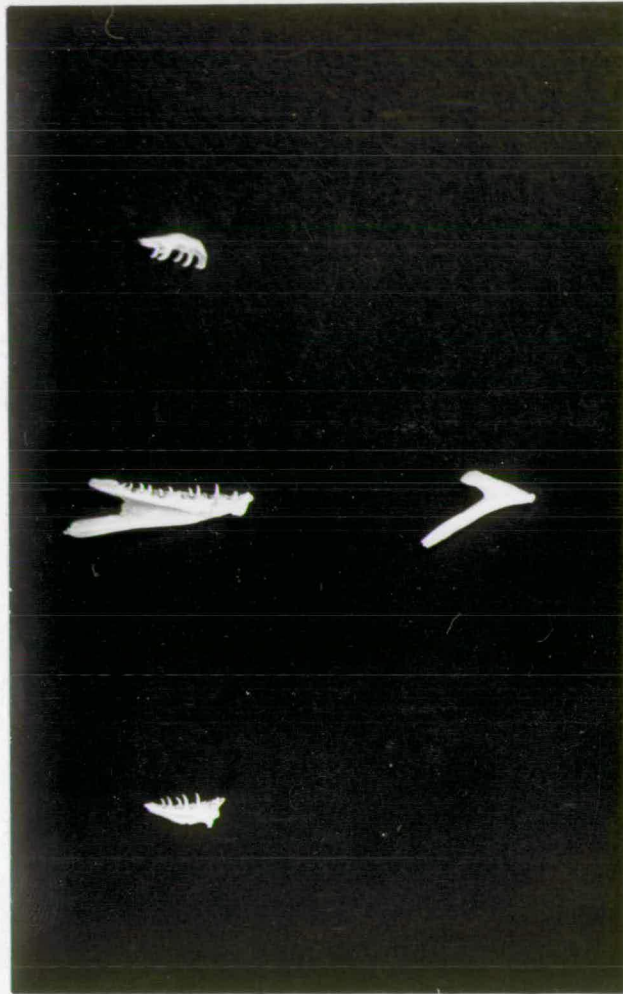
Trout   Grayling

Plate 9.

more stream-lined for swimming than the trout.

Paired fins are generally used for stabilising and manuevering and the basic function of the pectoral fin is for rising, turning, diving, and stopping; The fish can only stay the right way up by using its fins to correct for any accidental rolling movements and these fins are also required to counter-balance the force produced by the respiratory current (Alexander 1970); the pectoral fins are particularly important to the fish for braking because they can be spread at more or less right angles to the direction of motion. In salmonids, these fins are placed low on the body and can be flattened against it when swimming in order to reduce drag. The shape of the grayling and trout pectoral fins is considered todiminishdrag and further suggests that they have developed a fin design which complements their stream-lined body shape.

The length of the caudal peduncle has been related to both the standard of swimming and to the type of habitat of the fish (Nikolskii 1963). The caudal peduncle of a goodswimmer in flowing water is short and high like the trout, whereas in still water it is longer and lower like the mackeral (Scomber scombrus). A comparison of the grayling caudal peduncle with that of the trout indicates that they are similar and these results again confirm that both species exhibit an efficient over-all swimming design for a lotic environment.

Against the great and consistent similarity in the body form of the grayling and the trout are the obvious differences in their mouths. The mouth of a fish is an extremely important and specialised structure because it not only obtains the food but also restricts the size and type of prey which can be eaten. The position of the mouth of the grayling is located in a plane lower than the line of the body axis which immediately suggests a ventral feeding focus; conversely, the mouth of the trout which is in line with the body axis is in a position

to feed in most directions - above, below, or directly in front of the fish. Therefore, the slight differences seen in the mouth positions of these sympatric salmonids presumably indicate that they exhibit physical propensities to feed in different strata of the invertebrate fauna: The design of the grayling mouth directs it to feed in the benthos and that of the trout, in the drift, surface, and benthos, and these specific structural inclinations confirm my earlier results for stomach analyses (see Chapter II) and those of other researchers (Muller 1954b, Dahl 1962, Jankovic 1964, Petersen 1968, and Hellawell 1971).

The aperture of the grayling mouth appears to be small in relation to the size of the fish and somewhat rounded (Pennel 1863), whereas the trout mouth is comparatively larger for the same size of fish and forms something like a grin (Alexander 1970). The round mouth is thought by Alexander to represent an adaptation for sucking rather than grasping prey (Platts 1935) because the effect of the sucking is intensified by the narrowness of the aperture; the round mouth (unlike the true grasping mouth) is incapable of seizing prey from any angle. Evidence that grayling do in fact suck their prey is given by the presence of stones in their stomachs (see Chapter II, and Siddiqui 1967, Hellawell 1971); grayling have also been seen to inhale and expel stones in captivity (Mitchell 1967).

A closer examination of the actual gapes of the grayling and the trout reveals further critical differences in the structure of their mouths which reinforces the suggestion that slight variations in the feeding structure of co-habiting euryphagic species tend to promotes some degree of specialisation in feeding. The gape of the fish mouth is directly related to the extensibility of the lower jaw: In every instance, the lower jaw extensibility of the trout was (approximately)

twice that of the corresponding size of grayling and this clearly demonstrates quantitatively that the gape of the trout is significantly greater than that of the grayling (p=99%). This indicates that in terms of hunting, not only can the trout take prey from any angle and from virtually any stratum of the fauna, but also that it can take larger prey than a grayling of a similar body size. The larger gape of the trout probably allows it to feed from a wider range of prey, i.e. to exploit the fauna more fully, and this may be an extremely useful characteristic in times of scarcity (Magnuson 1962) and may also act to increasingly separate these species as regards feeding.

This introduces the important notion of the advantages of fish size (and of mouth size in particular) in relation to food size and its influence in the feeding and growth relations of a fish community. In an inter-specific context, a larger gape would tend to afford a species an advantage both in the range of prey and in the calorific reward for searching. (It is assumed that a fish achieves greater metabolic efficiency because of the reduced energy expenditures for searching.) In an intra-specific context, immature fish typically exhibit a larger gape to body size than a mature fish of the same species and again this tends to reduce the energy costs associated with hunting and thereby to promote the rapid growth which is typical of the first years of life.

The teeth on the jaws of bony fish are usually conical and they generally function to hold prey, however the dentition of the grayling and the trout are dramatically different. The trout has a true grasping mouth which necessitates a wide opening to seize prey; it has very sharp teeth which are inclined inwards (posteriorly) and these are instrumental for piercing prey as well as for preventing their escape. The grayling mouth is designed more for sucking than grasping prey and therefore it possesses only tiny teeth (horny tubercles) which it may

use as friction grips to restrain prey rather than to actually pierce them (Alexander 1970). Henderson (1951) discusses some of the details of the grayling mouth: "The mouth is carp-like without incurved teeth on the tongue or in the mouth as in other salmonids. It has small, stunted teeth near the head of the vomer and on the anterior portion of the palatine bones..." (p. 220).

Other authors have commented on certain morphological characteristics of the grayling. The grayling has a proportionately larger dorsal fin to body length than the trout and this may indicate that the former has greater manueverability because of the rudder-like function ascribed to the dorsal fin (Nikolskii 1963, Alexander 1970). The grayling's manueverability may also be enhanced by its relatively larger air bladder: "It has the power of raising itself rapidly to the surface and of descending with a stone-like velocity, a facility which has been ascribed to the large dorsal fin striking either upwards or downwards against the current, but which is more probably attributable to the unusual size of the swimming or air bladder" (Pennel 1863, p.204). Furthermore, the manueverability of the grayling appears to be matched by its visual acuity. The eye of the grayling is distinctively shaped - rather pear-like - and is thought to provide better vision than the round eye of the trout (Sawyer 1946).

SUMMARY AND CONCLUSIONS

1. The body form of the grayling and trout were assessed quantitatively and qualitatively and were shown to be a similar shape and form and were assumed to be equally well designed for rapid swimming.

2. A critical examination of the mouths of grayling and trout of similar sizes consistently demonstrated significant differences in the type, position, gape, and dentition of the species.

   i. The grayling mouth is small and rounded which enhances its sucking mode of feeding. The mouth of the trout is large and yawning enabling it to grasp prey from any angle.

   ii. The grayling mouth is positioned below the mainline of the body axis, inclining the fish to feed from the benthos. The trout mouth is placed in line with the body axis permitting it to feed from any part of the fauna.

   iii. The gape of the trout was nearly twice that of the grayling which suggests that the trout can take larger prey than a grayling of a similar size.

   iv. The dentition of the species was strikingly different: The grayling mouth contained small tubercles along the jaw bone and also to a limited extent on the vomer and palatine bones; the trout mouth exhibited sharply pointed conical teeth along the jaw bone, on the tongue, and on the vomerine and palatine bones as well.

In conclusion, the present study is considered to show that the feeding related structures of grayling and trout do to some extent direct and or delimit the mode of feeding of these species by providing them with some structural specialisation which probably confers a degree of feeding expertise and in some situations has the effect of reducing competition between them.

CHAPTER   VI

FOOD CONSUMPTION AND GROWTH STUDIES

INTRODUCTION

One of the most important differences between grayling and trout may
be in their metabolisms and this may largely influence the seasonal
periodocity seen in their feeding (see Part I). Metabolism is defined
as the general chemical process occurring within the organism or within
part of one: It involves both 'catabolism', the breaking down of organic
compounds from complex to simple form to liberate energy, and 'anabolism',
the synthesising of organic compounds from simple to complex form,
utilising the energy released in catabolism, and these processes pro-
ceed continuously within the organism although at various rates
(Abercrombie, Hickman, and Johnson 1970).

   The rates of chemical reactions are largely regulated by temperature
and similarly, the bio-chemical reactions effecting digestion are greatly
influenced by the relationship between temperature and enzyme kinetics:
Axiomatic to enzyme kinetics is that every enzyme has a temperature
optimum (for efficiency). In the pokiliotherm, such as a fish, where
the metabolism of the organism is directly affected by the environment
(and by temperature in particular), the enzyme systems which regulate
digestion process will be governed by the 'controlling'[4] factors of the
the environment.

   Various methods have been developed for the study of metabolism
in fish. The basal, routine, and active levels of metabolism (section
2 of Methods for definitions of these terms) may be derived from

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4. Controlling factors govern the rate of metabolism by their influence
on the state of molecular activation of the components of the metabolic
chain. Temperature is the most outstanding of the controlling factors.
(Fry 1971).

oxygen consumption as related to activity over a short period of time, generally 24 to 36 hours (Krogh 1916, Fry 1947, Beamish 1964, Brett 1962, 1964, 1965). In such experiments, termed 'respirometry studies', the specimen is placed in a chamber where water temperature and flow are controlled and the amount of oxygen consumed over unit of time is recorded along with the level of random or forced activity. As Winberg (1956) pointed out, there is great potential for error in respirometry work because of the difficulties in maintaining constant conditions throughout the experiment. Water quality requires extremely fine control as a change in its component concentrations could have the effect of stimulating oxygen consumption: The constancy of water flow is crucial to the determination of the level of activity, as well as to the complete circulation of the water which will affect mixing and ultimately the accuracy of the measurements of changes in oxygen level; the efficiency of the flow pattern is in turn related to the chamber design and size and this flow prevents the accumulation of stagnant water and subsequent fallacious results.

Species differences will also affect respirometry results. Fish exhibit individual levels of excitability and may react differently to the experiment when placed in the chamber singly or in a group (Brown 1946 a,b, Winberg 1956). Pre-experimental treatment is extremely important in that the pre-acclimated state (or lack of), particularly as regards temperature and feeding, will greatly influence the rate of metabolism of the fish in the apparatus (Winberg 1956): A sudden change in temperature causes severe metabolic upset (Fry 1947); Whether or not the fish is fed or starved in the days preceding the experiment will alter their level of metabolism upwards or downwards, respectively (Winberg 1956, Brett 1962, Brett and Glass 1973, Beamish 1964 d).

The food consumption and growth experiments provide a means for the indirect measurement of metabolism. This type of experiment takes into

account the total energy system in the fish over the long term, rather than a fraction of it over a short period, as in respirometry studies. Metabolism is deduced from daily records of food intake and weekly measurements of growth and therefore provides a means of quantifying the long-term energy demands of the fish under well-monitored controlled conditions (Paloheimo and Dickie 1966a).

Both the repirometry experiments and the food consumption experiments have shown that fish metabolism varies with the environment and with temperature in particular. Constant light has been shown to increase the rate of oxygen consumption (Winberg and Khartova 1953 in Winberg 1956). The seasonal influence of the photoperiod on metabolism has also been demonstrated (Bullock 1955). Roberts (1964) observed an increase in the oxygen consumption of carp (Cyprinus carpio) with an artificial photoperiod.

An increase in water flow will cause a corresponding increase in the amount of energy required for the fish to maintain itself in that environment. Washbourn (1936) found that trout fry reared in two tanks at the same temperature consumed more oxygen in swift water than in slower water; this experiment, however, provides only superficial view of the relationship between fish metabolism and the environmental demand of flow.

Standard oxygen consumption for routine metabolism remains constant over a wide range of partial oxygen concentrations whereas the energy demands for active metabolism increase curvilinearly as oxygen is reduced (Graham 1949, Fry and Hart 1948). Fry (1957) attributed the dependent phase of active oxygen consumption to the restrictions of the volume of water presented to the gills, which is supported by Saunder's research (1962) on the irrigation of gills in which he demonstrated that bullheads could remove 19% of the available oxygen against the carp which removed

up to 43%. Carbon dioxide can depress oxygen consumption at high levels of activity (Black, Fry, and Black 1954). Beamish (1964c) showed that increased carbon dioxide concentration did not affect standard metabolism. The presence of certain chemicals in the water may stimulate an increase in the oxygen consumption of fish (Burrows 1964). The effect of ammonia (unionised) on the gills and respiration of fish has been the subject of many studies for fish culture. Ammonia is a primary excretory product which has been shown to limit fish growth when allowed to accumulate (Merkens and Downing 1957, Burrows 1964, FAO EIFAC/T11 1970).

Beamish (1964e) found a seasonal variation in the standard rate of oxygen consumption for brook and brown trout acclimated to $10^{\circ}C$ and exposed to natural daylight; oxygen consumption was lowest in March and at a maximum during the late fall and these times correspond with 'over-wintering' and spawning, respectively. Over-wintering typically involves a reduction of food intake and experiments have shown that metabolism decreases in compensatory response to starvation (Lindstedt 1914, Smith 1935): Beamish (1964d) found that there was a reduction in the standard metabolism requirements of the brook trout for the first three days of starvation, but that metabolism leveled off and remained constant for the duration of the experiment (10 days). Beamish (1964e) also demonstrated that there is an increase in metabolism related to spawning.

The relationship between temperature and enzyme kinetics has been noted and this has been quantified for fish as long ago as 1914 by Ege and Krogh: The Krogh normal curve relates (total) metabolism with temperature and was determined empirically from data on the oxygen consumption of a motionless goldfish. Winberg (1956) queried the validity of this relationship and examined the results of much research representing fish of the most diverse systemic positions, sizes, ages and habitats (fresh, salt, and brackish waters) and found that fish

metabolism did in fact follow the Krogh temperature curve reasonably well (Gardner 1926, Scholander et. al. 1953). The Krogh normal curve is described approximately by the following values for the temperature coefficient (Q_{10}):

	0-5°C	5-10°C	10-15°C	15-20°C	20-25°C	25-30°C
Q_{10}	10.9	3.5	2.9	2.5	2.3	2.2

(after Winberg 1956)

Fish are frequently classified into three groups according to their upper lethal limit to temperature:

1. Fish with upper lethal limits below 28°C, such as brown trout and grayling are termed steno therms that is they can only live in water with a narrow range of temperatures.

2. Fish with upper limits between 28°C and 34°C, such as pike and perch,

3. Fish with upper limits of temperature above 34°C, such as carp, tench, and bream are termed eurytherms, that is they can tolerate a wide range of temperatures. (after Varley 1967).

The relationships of the stenotherm and eurytherm metabolisms with temperature have been examined for arctic and tropical fish in the work of Scholander et. al. (1953); the metabolic rate of tropical fish measured at a temperature close to that of the water in their natural habitat was only 3 to 4 times greater than that of the arctic fish measured at a corresponding (natural) temperature of 0°C and therefore the authors concluded that the arctic fish display a high level of basal metabolism. Stenotherms are therefore expected to exhibit a relatively high level of metabolism which enables them to remain active at low temperatures, whereas eurytherms, though active over a wider range of temperatures, are typically inactive in cold water (Varley 1967).

Grayling and trout are both classified as stenotherms and hence may be expected to display a high metabolic level at low temperatures

(0° to 5°C) and to show the greatest activity at cooler times of the year. However, as some seasonal differences have been demonstrated in the feeding activity of the species (see stomach fullness and condition factors in Part I) and given the universality of metabolism and temperature relations (Winberg 1956, Paloheimo and Dickie 1965), it is therefore reasonable to suspect that there is some physiological difference between grayling and trout in the adjustment of their basal metabolism to temperature.

A series of food consumption and growth experiments were designed to detect the occurrence of any seasonal (temperature) change in the routine metabolism of grayling and trout and its referred effect on their feeding activity (appetite). These investigations permit the direct calculation of a species' appetite as well as an indirect assessment of a species' mean daily (routine) metabolic demands at various temperatures within its normal bio-kinetic range.

METHODS

1. Study Areas.

There were two criteria which restricted the sites selected for collecting wild grayling and trout. The first problem was to locate a site which held a sufficient density of fish to provide for experimental needs which amounted to a minimum of 16 fish every 10 to 12 weeks. It was found that in the instance of the grayling that there were difficulties in obtaining fish from the Eden in the required size and numbers (see section 5, iii to follow); the scarcity of grayling fingerlings was probably related to the accepted phenomenon that grayling migrate downstream (into the main river) a few months after hatching (Fabricius and Gustafson 1955, Dahl 1962). An alternative site was found at Norham Boat House on the Tweed River (see Chapter I, Study Areas,

section 2) and this proved to be a fairly reliable source of under-
yearling grayling; typically a 'shoal' of young grayling (up to 30 fish)
were seined in one successful shot.

There are many sites which would have provided sufficient numbers
of brown trout for experimental purposes. However, it was considered
necessary to locate a stream which did not contain sea trout because
brown trout and sea trout are likely to exhibit different growth rates.
Glentress Burn was selected as a sampling site because it contains
brown trout exclusively(see Chapter I, section 2).

2. Terminology Related to Food Consumption, Growth, and Metabolism
   Experiments.

i. Basal Metabolism. This is the minimum level of metabolism of an
animal when resting under unconfined conditions (Brett 1964).

ii. Routine Metabolism. This level of metabolism provides a measure
of the amount of energy required by an animal exhibiting spontaneous
activity in semi-restricted conditions (Brett 1964). The significance
of routine metabolism is that it reflects the animal's response to the
directive[5.] factors of the environment.

iii. Active Metabolism. The active level of metabolism is a measure-
ment of the energy demands of the animal in a maximum steady state
(Brett 1969). This level of metabolism is thought to reflect the exist-
ence of any specific metabolic adaptations to brief or prolonged periods
of movement and to constant or changing swimming speeds.

iv. Total Metabolism (T). This represents the sum total of the var-
ious energy expenditures of an organism in ml O_2/fish/hour.

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5. Directive factors "allow or require a response on the part of the
organism directed in some relation to a gradient of the factor in space
or time....the directive factors also trigger physiological responses
without the mediation of the senses, as in the effect of photoperiod
on the pituitary"(p.18 Fry 1971).

v. Metabolic Coefficient (α). The metabolic coefficient, alpha, represents the level of metabolic adaptation to living conditions, and to temperature, activity, food supply, and habitat in particular (Paloheimo and Dickie 1966a). Winberg (1956) analysed data on salmonid routine metabolism and computed a metabolic coefficient for salmonid metabolism of 0.498 at 20° C. Brett (1973) <ins>and Glass</ins> calculated a metabolic coefficient of 0.824 for active metabolism at 20°C in the sockeye salmon.

vi. Weight Exponent (γ). The weight exponent, gamma, is generally accepted to express the relationship between metabolism and body weight. It has been demonstrated repeatedly (from various experimental methods) that the log of oxygen consumption is linearly related to the log of body weight and a parabolic equation to describe this relationship is included in the following section (3). The conservatism of this relationship between metabolism and body weight is considered to represent the animal's capacity to adapt through an alteration in feeding and general activity or through changes at a cellular level, which permit the establishment of a state of equilibrium (Paloheimo and Dickie 1966b).

vii. Oxy-calorific Coefficient. This coefficient represents the amount of energy (calories) released by the animal combusting oxygen. A working value of 1 ml of oxygen = 4.8 calories is generally accepted (Winberg 1956, Paloheimo and Dickie 1965, Elliott 1975).

viii. Ration.(R) The ration is the amount of food given to the animal. The ration may be presented continuously or intermittently at fixed intervals, in wet or dry form. Rations are termed 'restricted' when the amount of food given to the animal is less than satiation, and 'ad libitum' when fish are fed to excess (Brown 1946b). Both the levels and patterns of feeding have been shown to affect fish metabolism (Brown 1946 b, Kerr 1971).

ix. Assimilated Ration (pR). The pR of a ration is that portion of the food ingested which is physiologically useful (Winberg 1956). When food is digested, some energy is excreted and some is lost through'specific dynamic action', i.e. energy utilised for the processes of digestion and freed through deamination, and the remaining energy which is assimilated is termed the pR of the ration. A working value of 0.8 has been demonstrated (and accepted) as a reasonable approximation of assimilation (Winberg 1956, Paloheimo and Dickie 1966a,b). It should be noted that the ratio of the physiologically useful energy to the total energy content of the ration can vary depending on the kind of food, species of fish. and its state of development, and other environmental conditions (Winberg 1956).

x. Appetite. The appetite of an organism is its voluntary intake of food (Elliott 1975). Appetite will vary in accordance with the metabolic demands of the organism and therefore in a pokilotherm it will reflect environmental influences such as temperature.

3. Equations.

i. Relationship Between Metabolism and Weight (Winberg 1956).

$T = \alpha W^{\gamma}$ or in logarithmic form $\log T = \log \alpha + \gamma \log W$

T= total metabolism;   = metabolic coefficient; W = weight;
 = weight exponent.

The level of metabolism which is described by this equation was termed routine by Winberg (1956) to emphasise the fact that data on fish metabolism and weight (which he re-analyses to derive the above equation) were obtained by diverse methods and were not strictly comparable. However, in practice, the log-linear equation fairly accurately describes the total expenditure of a fish of a given weight at a level between basal and routine (Paloheimo and Dickie 1966a, Elliott 1975). Winberg also suggested that by doubling this figure (termed T_{W_1}, T_{W_2}), one can

estimate routine metabolism in natural conditions (T_{W_2}).

ii. Metabolism as Related to Food and Growth. (Winberg 1956)

$$T = pR - W/t$$

T = total metabolism; pR = physiologically useful (assimilated) portion of the ration; W = weight; t = time.

Winberg (1956) analysed the results of many feeding and metabolism studies (Pentelow 1939, Ivlev 1961, and others) and derived the above formula which specifies the quantitative relationship between the main components of the energy system: Given a certain energy supply, the proportion of the total energy which is used for growth is the reciprocal of that which is dissipated in metabolism. "Thanks to the fact the growth rate, food rations, and metabolic rates are strictly and definitely inter-related, it is possible if one knows two of these values to compute the third" (Winberg 1956, p. 155).

4. Pre-experimental Procedures.

i. Collection of Fish. Grayling and trout were collected from the two study areas within a 3 day period for each experiment: Grayling were seined from the Tweed off Norham Boat House and the brown trout were electro-fished from Glentress Burn, and in both instances the fish were transported back to the department in a large plastic tub which contained river or burn water. It has already been stated that different methods of fishing stress the fish in varying degrees, however it has been assumed for this research that any stressful side-effects associated with the collection methods would disappear during the three week acclimation period prior to the experiments.

ii. Pre-experimental Holding Conditions in the Laboratory. Fish were placed in groups of 8 in each 62x32x32 cm aquarium and the water was filtered and aerated by a West German Turbo-filter (a glass-wool and charcoal chip system). Light and temperature and disturbance

were assumed to be similar for all the aquaria as they were located in close proximity to each other in the animal room.

Fish were starved for the first three days because in all the previous attempts to keep fish in the laboratory it was found that fish initially showed no interest in food (live gammarus) which may have been related to the stress of captivity. Thereafter, fish were fed twice daily on a restricted ration of live food. All fish were judged to take a 'fair share' of the food because their condition did not change during the three weeks in these holding conditions.

iii. Acclimation. Acclimation involves bringing the animal's metabolism into a steady state biochemically by setting one or more of the conditions to which it will be subjected for an appropriate period of time before the experiment (Fry 1947). For food consumption and growth studies, fish were acclimated to both temperature and ration. In experiment I and experiment III, the mean temperatures in the aquaria were sufficiently near the projected experimental temperatures, i.e. within 2°C, that a controlled temperature acclimation upwards for 1 or 2 degrees was unnecessary; Fry (1947) reported that fish acclimate to higher temperatures more easily than to lower ones.

Temperature acclimation procedures were carried out for experiment II because the water temperature in the aquaria was found to be 4.5°C higher than the projected experimental temperature of 5°C (winter), which was probably related to the internal heating system of the building. In order to lower the temperature in these aquaria, a polythene bag filled with crushed ice was placed in each aquarium during the 4 days preceding the experiment. Although this procedure was rather primitive, the temperature was successfully lowered from 9.5°C to 5 - 6°C.

Grayling and trout received the same ration (both type and quantity of prey) during the acclimation period as they were fed in the actual

experiment (See Section 5, iii).

5. Experimental Variables.

All experimental variables were chosen to represent as nearly as possible those conditions which the fish experiences in nature. It would have been preferable to have designed a completely simulated experiment whereby the fish were maintained in individual sections of a streambank aquarium and where temperature, light, and food followed the natural cycles of increase and decrease appropriate to that time of year. However, this sort of experiment was unfortunately not practicable in terms of facilities, finance, and time, and therefore a more clinical approach to the experiment was taken: The experimental design still permitted monitoring of the food and growth of individual fish, however the environmental variables of temperature and light and food were strictly regulated to provide constant conditions in a 'controlled environment' room in the department.

i. Temperature. The temperatures which were chosen for these experiments were sufficiently distinct from one another to accentuate any differences between grayling and trout in their appetites and metabolisms at different seasons of the year: Experiment I occurred over July and August and therefore a summer temperature of 15°C was chosen; the second experiment took place in the autumn, over September and October, and the experimental temperature was set at 10°C; experiment III was run during January and February and a temperature of 5°C was taken as typical of winter.

The air temperature in the controlled environment room was nearly always constant ($\pm$ 0.5°C) except on the one occasion during experiment III when the compressor stopped. It is likely that the compressor had siezed up from the cold and moisture and it was therefore necessary to turn off the machinery for the weekend to allow it to thaw.

It was extremely fortunate that the air temperature outside the building was very cold (below freezing each night) because it was possible to maintain the fish outside over the weekend at near experimental temperature. (The experimental apparatus was portable, i.e. it was supported by a trolley, which enabled easy transport of the experiment to the bird-house outside the department.) Following this incident, it was necessary to maintain a slightly higher temperature in the controlled environment room - $6^{\circ}C$ - to avoid freezing the machinery again.

The air temperature in the controlled environment room was measured by a min-max thermometer and the water temperature was continuously recorded by a Grant's temperature recorder. Both the air and water temperatures exhibited a small amount of variation over the 28 days of the experiment ($\pm$ 0.8° and $0.5^{\circ}C$, respectively).

ii. <u>Light</u>. Similarly to temperature, the light periods which were adopted for these experiments were those which closely paralleled those of natural daylight and the following photo-periods were chosen: Experiment I was an 18 hour day; experiment II was a 12 hour day; and experiment III was an 8 hour day. The change from light to darkness was sudden (rather than a gradual natural transition) and was governed by a time-clock. Light in the controlled environment room was provided by one 60 watt bulb which diffused by aiming the angle-poise lamp towards the ceiling. The intensity of the light was further diffused by the experimental apparatus - opaque plastic bottles.

iii. <u>Food</u>. Grayling and trout were of the smallest size in the first experiment and therefore they were fed a small prey species which was readily accepted by both salmonids - <u>Daphnia</u>. These crustaceans were cultured in a small pond dug behind the department and the pond was routinely enriched with a combination of soya bean meal and horse manure (Needham 1937). However, the <u>Daphnia</u> proved to be unsuitable (see the

discussion of this chapter) and therefore the food species was changed to another crustacean for the second and third experiments – Gammarus. These crustaceans were collected twice a week from the Braid Burn which flows behind the department and they were maintained over the interim days in two aquaria, being fed on rotten lettuce,etc. (Needham 1937).

The amount of food per fish was calculated as a percentage of body weight (wet). An indication of the sort of levels of feeding to be expected are given by Brett *et al.* (1969) who experimented with the food requirements of sockeye salmon at 6 different temperatures; the ration level which Brett described as optimum (per temperature) was applied to grayling and trout in both pre-experimental and experimental treatments. It was obviously advantageous to determine the ration level at which most fish consumed all of their food because this precluded any problems relating to food de-composition in the experimental apparatus. Ration levels of 2% body weight were adopted in experiments II and III and a slightly higher percentage – 3% – for experiment I.

This method for determining the size of ration assumes that the calorific value of the food is the same as that of the fish (Winberg 1956). The calorific value of Daphnia has a mean value of 910 calories per gram wet weight (Slobodkin 1959). Mann (1965) and Solomon and Braefield (1972) carefully determined the calorific value of a gram of wet Gammarus and reported means of 854.4 and 847.0 calories, respectively. If it is remembered that 1 gram of wet grayling or trout constitutes approximately 0.2 gram when dehydrated (see Chapter IV, section 2), and that the value of 1 gram of dried grayling or trout is 4900 calories, then the value of one wet gram of fish is equal to approximately 980 calories. Although some differences have been shown to exist between these various calorific values, the discrepancies were considered to be small and were assumed to be similar for all experiments.

6. Experimental Apparatus.

The apparatus used in food consumption and growth studies was designed in such a way as to allow the monitoring of both growth and appetite of each(individual) fish. Following careful studies of the apparati used by other researchers (Pentelow 1939, Brown 1946b, Warren and Davis 1965, Brett, Shelbourn, and Shoop 1969), a comparatively inexpensive scheme was devised and developed with the assistance of the (ingenious) technical staff in this department (see Plt. 10 and Fig.19).

Four independent open re-circulating systems were constructed, each of which was comprised of 8 half-litre polythene bottles to hold 8 fish of the same species. Altogether there were 32 such bottles which were modified for this research by aralditing a 5 mm (I.D.) polythene tube into the middle of the bottle top to provide an inflow and a 5 mm (I.D.) polythene tube into the bottle bottom near theedge for the out flow; a small rectangular window (7 cm x 3 cm) was cut out of the dorsal surface of each bottle to facilitate oxygen exchange and to permit feeding the fish. The bottles were placed in a series of 8(on their ventral surface on a wooden rack which supported each bottle individually.

Water circulation for each of the four systems was achieved by a series of polythene tubing and Y-connectors - the tubes decreased from 12 mm at the main artery off the filter to 9 mm intermediately (4 tubes) down to 5 mm at the inflow of each bottle. The outflow tubes were loosely wired together to direct the effluents into a common reservoir (plastic pail), where the water was allowed to collect before being pumped back up through the filter system. (The filter system was the same type which was used in pre-experimental holding conditions.)

Maintaining identical constant conditions in each re-circulating system, as regards water quality and flow rate was problematic. If a system was clogged due to air bubbles or if one of the outflow tubes

APPARATUS USED FOR FOOD CONSUMPTION AND GROWTH STUDIES

Plate 10. Experimental re-circulating apparatus situated
on a trolley in the controlled environment room

Fig. 19   Diagram of open recirculating system used in food consumption and growth experiments

collection of uneaten
rations and faeces

polythene bottle (series of eight)

distribution
chamber

to filter

reservoir
with flowerpot filter

charcoal chips and glasswool filter
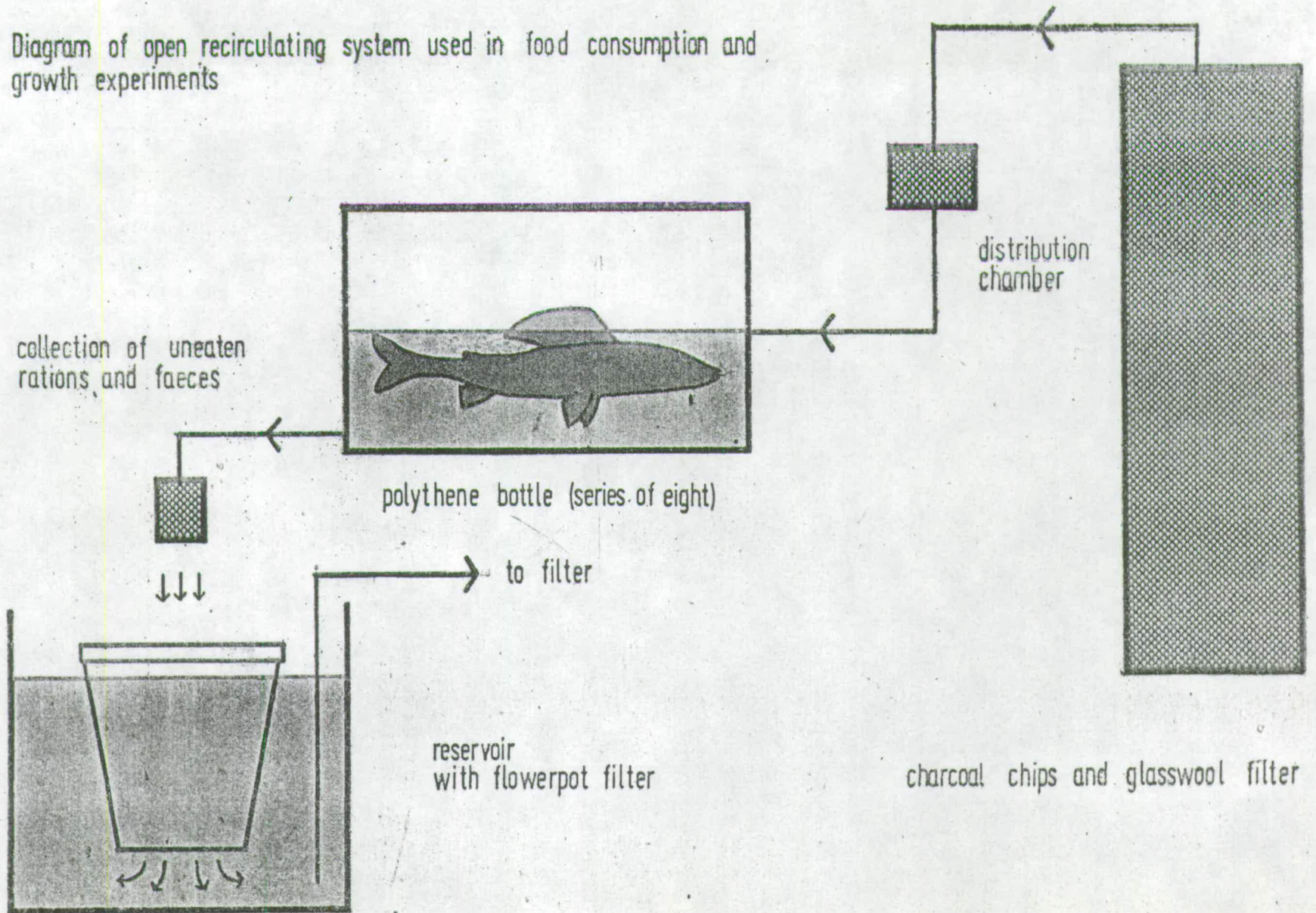
moved slightly and sprayed water outside the reservoir,the amount of water in the system dropped and it was impossible to replace this water with 'matured' make-up water; similarly, a constant flow rate (which was obligatory to this experiment where metabolic rate is expected to respond to a change in activity demands) was difficult to maintain because occasionaly organic matter accumulated in the filter which tended to impede the 'ideal flow rate' of 3.6 litres per minute (clean water and no fish in the system). An assessment of these factors was considered to be beyond the scope of these preliminary investigations and therefore it was assumed that all systems would be affected to a similar extent.

One aspect of water quality which did warrant careful attention was the concentration of un-ionised ammonia (NH_3). In any re-circulating system, ammonia is the chief factor which limits the growth of fish. "The harmful effects of ammonia on fish are related to the pH value and the temperature of the water due to the fact that only the un-ionised fraction of ammonia is poisonous. The un-ionised fraction increases with rising pH value and with rising temperature." (p. iv FAO 1970). The concentration of ammonia in each re-circulating system was measured at the end of a experiment with an ammonia probe. The adverse effects of NH_3(un-ionised) are only absent at concentrations lower than 0.025 mg/l.

The amount of oxygen in the water will also limit fish growth and therefore aeration was carefully monitored in each system. Aeration was partially achieved by natural saturation of the water which was facilitated by the large surface area of water exposed to the atmosphere. A good oxygen supply was assured by supplementary aeration in the form of air stones which were placed in the reservoirs.

7. Experimental Procedures.

i. Weighing and Dispensing the Ration. Both Daphnia and Gammarus

were weighed volumetrically to facilitate the feeding procedure. The accuracy of weighing rations was of considerable importance to this kind of experiment and therefore it was considered necessary to validate volumetric measuring by assessing the magnitude of experimental error. Weighing accuracy was attested by twenty replicates of a volumetric measure of ration which were then weighed on a Vernier balance to the nearest 0.1 mg. As both Daphnia and Gammarus are aquatic species, care was taken to remove surface water before measuring out a ration: Solomon and Braefield (1972) allowed Gammarus to hop on a kim wipe for 5 seconds before weighing them and this method was adopted in this research. The standard error for weighing a ration of Daphnia was ± 0.002 mg and for Gammarus was ± 0.007 mg.

Because the calorific value of Gammarus has been shown to vary with the size of the individual (Solomon and Braefield 1972), it was necessary to screen these invertebrates through two sieves (3 mm and 6 mm mesh) to provide a reasonably uniform size of prey. Again 20 replicates were made of this seiving process and the uniformity of prey was demonstrated - 4 to 5 mm.

The time of feeding was fixed at 11:00 and 17:00 hours. To avoid disturbing the fish, the rations were dispensed at a distance of 40 cm by means of a dowel rod with the pre-determined volumetric measure attached to the end of the dowel. This 'Heath-Robinson' device enabled one to slip food through the rectangular opening(in the dorsal surface of the bottle) into the water where it was rotated until all the prey had been liberated in the water.

The flow of the re-circulating system tended to carry the prey out of the system (via the outflow tubes) and therefore it was necessary to divert the flow during the feeding period. 15 minutes was considered to allow sufficient time for the fish to consume their ration.

There was a possibility that some of the fish would not consume all of their rations (Elliott 1975) and therefore a means of collecting any uneaten food was devised: Individual nylon bags (10cm x 2.5cm) were attached to each effluent tube and these collected any uneaten ration once the flow of water was restored to the system. Because the food was 'live', there was no measurable loss of food value through decomposition which would affect the records of food consumption.

ii. Growth Measurements. Fish were weighed live at the beginning and end of each experiment. The method which was adopted for weighing fish was the one which caused the least amount of stress (Wedemeyer 1972) - metabolic excitability and damage to scales and mucous film. Live weight is best taken in water by weighing the container with sufficient water to hold a fish and then by adding the fish and taking the difference between the two weights (Lagler 1968). The S.E. for weighing was $\pm$ 0.02g.

It should be noted that because fish were weighed only once, at the beginning of the experiment and once at the end, that results will not indicate any interim changes in growth during the experiment.

iii. Fish Condition. It was considered important to assess the condition of fish at the end of the experiment and this was done in two ways: (1) fish were superificially described as either full-bodied or emaciated; (2) fish gills were examined histologically to provide an index of fish health. The occurrence of hyper-plasia of the gill tissue is a general indication of some stress to the fish, be it ammonia, or bacterial or protozoan infection of the gills.

The histological procedures for preserving, dehydrating, embedding, sectioning, and staining gills were the same as those used in histological investigations of the omenta around the pancreas (see Chapter IV, methods, section 3).

RESULTS.

1. Experiment I: 15° C, an 18 hour day with 3.0% of body weight for ration.

The changes in weight for 16 grayling and 16 trout are recorded in
Table 29 . All the fish lost weight during the experiment, grayling lost
between 2.4% and 19.5% of body weight - a mean loss of 11.8% ± 1.21 - and
the trout lost 0.3% to 28.5% of body weight - a mean drop of 12.4% ± 2.01.
Fish appetites were consistent throughout the experiment except in those
instances where fish stopped feeding which occurred a few days before
death; mortalities occurred in grayling only - 3 fish died during the
first week of the experiment and 2 died in the third week. The fish
which did survive until the finish of the experiment, however, were not
in good condition: Both grayling and trout were emaciated and their gills
exhibited hyper-plasia (see Plt.11a). The results of water analyses
indicate that there was a definite accumulation of calcium, magnesium,
potassium, sodium, and of pH and ammonia during the course of the
experiment. Finally, it was considered that any deductions for routine
metabolism from this food and growth study would be erroneous because
of the obviously stressed condition of the fish and therefore no calcu-
lations were made for daily metabolic demands of grayling and trout
at 15° C.

2. Experiment II: 10° C, a 12 hour day with 2% body weight ration.

Figures for the change in weight, appetite and routine metabolism for
8 grayling and 16 trout are presented in Tables 30 and 31, respectively.
One grayling gained weight and one grayling lost weight and the weights
of the remaining fish were unchanged at the end of the experiment. 11
of the trout maintained weight and 5 lost weight during the experiment
(weight losses ranged from 1.2% to 10.9% body weight). All fish fed
consistently during the experiment and the mean grayling appetite was

Table 29. Summary of the changes in weight for grayling and trout
in Experiment I ($15°$ C, 18 hour day).

(W = weight in grams)

Grayling			Trout		
W at t_1	W at t_2	t_1-t_2	W at t_1	W at t_2	t_1-t_2
6.69	6.53	-0.16	3.02	2.72	-0.30
7.53	6.06	-1.47	4.18	2.99	-1.19
6.16	5.55	-0.61	5.09	4.48	-0.61
7.68	6.89	-0.79	3.14	2.82	-0.32
6.41	5.64	-0.77	4.52	4.07	-0.45
6.48	5.77	-0.71	2.63	2.55	-0.08
8.19	7.21	-0.98	3.37	3.01	-0.36
7.45	6.41	-1.04	6.28	5.75	-0.53
6.75	6.01	-0.74	4.21	4.05	-0.16
7.93	6.90	-1.03	3.11	2.71	-0.40
8.89	7.91	-0.98	3.39	3.12	-0.27
6.31	5.62	-0.69	3.57	3.18	-0.39
8.21	7.22	-0.99	3.68	3.24	-0.44
6.84	6.02	-0.82	4.05	3.57	-0.48
8.26	7.52	-0.74	4.34	4.05	-0.29
7.71	6.99	-0.72	4.73	4.31	-0.42
Means 7.34$\pm$0.21		-0.83$\pm$0.07	3.39$\pm$0.64		-0.42$\pm$0.06

HISTOLOGICAL EXAMINATIONS OF CHANGES IN THE LAMELLAE STRUCTURE OF GILLS
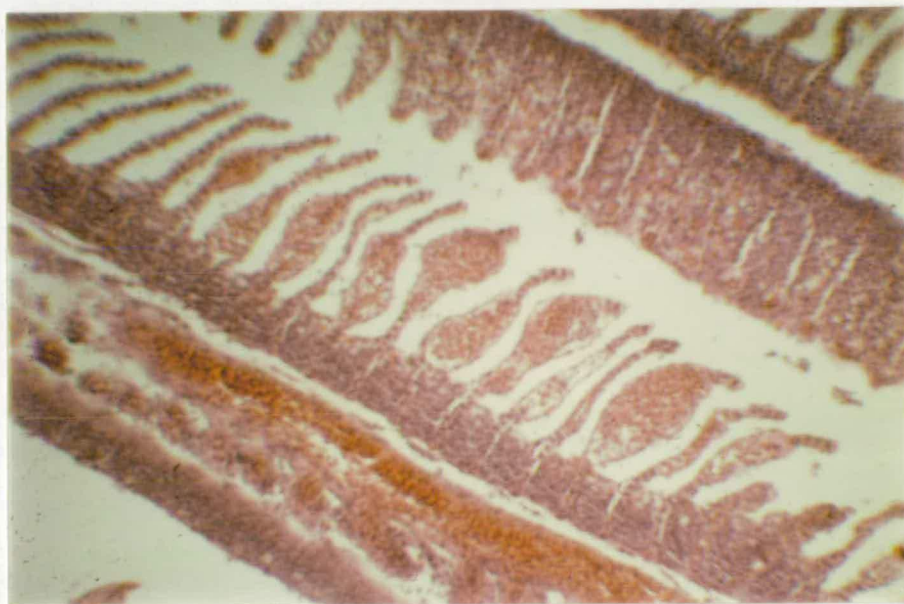
Plate 10a. Hyperplasic gill taken from a grayling in
Experiment I (x 100)
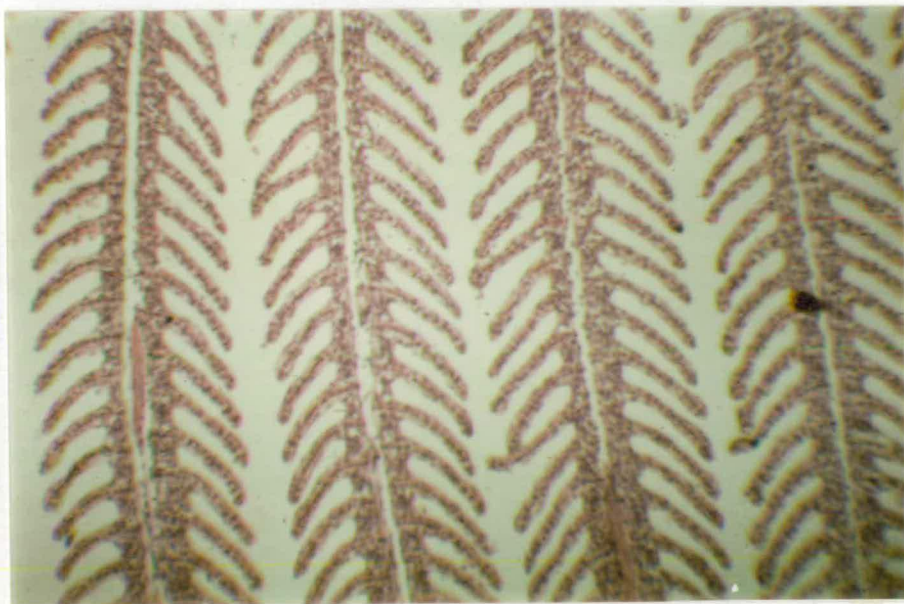
Plate 10b. Gill taken from a grayling in Experiment II (x 100)

Table 30 : Summary of food consumption, changes in weight, and calculations for routine metabolism in grayling in Experiment II (10 C, 12 hour day 2% ration).

Weight at t_1 (g)[1]	Weight at t_2 (g)[2]	Growth $\Delta W/t_1-t_2$ ($\frac{1}{g}$)[2]	Daily food consumption (g) cal.	Total food consumption (g) cal.	Appetite $\frac{daily\ ration}{fish\ weight}$ (g)	Deduced metabolism $T = pR - G$ (g) cal/fish/h	cal/g fish/h	Daily routine metabolism deduced cal/fish/d	estimated (T_W) cal/fish/d [1]
28.0	28.0	0.0	(0.45)380.3	(11.69)9869.6	1.61	12.67	0.45	304.1	265.4
26.0	26.2	+0.2	(0.47)397.2	(12.22)10325.9	1.81	12.85	0.49	308.4	251.5
21.0	21.0	0.0	(0.39)329.6	(10.14)8568.3	1.86	10.99	0.52	263.7	213.8
23.9	23.9	0.0	(0.43)363.4	(11.18)9447.1	1.80	12.11	0.51	290.6	235.6
24.7	24.6	-0.1	(0.39)329.6	(10.14)8568.3	1.58	10.79	0.44	259.0	241.9
26.8	26.8	0.0	(0.46)388.7	(11.96)10106.2	1.72	12.96	0.48	311.1	257.3
17.5	17.5	0.0	(0.34)287.3	(8.84)7469.8	1.94	9.58	0.55	229.9	186.2
27.0	27.0	0.0	(0.49)414.1	(12.74)10766.3	1.81	13.80	0.51	331.2	259.0
Means 24.4±0.8			(0.43±0.02) 361.2±15.1	(11.11±0.73) 9387.7±606	1.77±0.4	11.97±0.49	0.49±0.01	287.2±11.9	213.8±8.4

Table 31 : Summary of food consumption, changes in weight, and calculations for routine metabolism in trout in
Experiment II (10 C, 12 hour day, and 2% ration).

Weight at t_1 (g)[1]	Weight at t_2 (g)[2]	Growth $\Delta W/t_1-t_2$ (g)[2]	Daily food consumption (g) cal.	Total food consumption (g) cal.	Appetite daily ration (g) / fish weight (g)	Deduced metabolism $T = pR - G$ cal/fish/h	cal/g fish/h	Daily routine metabolism deduced cal/fish/d	estimated (T_W) cal/fish/d 1
20.5	20.5	0.0	(0.34)287.3	(8.84)7469.8	1.66	9.58	0.47	229.9	221.7
28.0	28.0	0.0	(0.42)354.9	(10.92)9227.4	1.50	11.83	0.42	283.9	266.2
13.8	12.3	-1.5	(0.20)169.0	(5.20)4394.0	1.45	8.64	0.63	207.4	155.3
23.5	23.5	0.0	(0.33)278.9	(8.67)7326.2	1.42	9.39	0.40	225.4	233.0
22.4	22.4	0.0	(0.37)312.7	(9.55)8069.8	1.65	10.42	0.47	250.1	224.5
16.4	15.5	0.0	(0.22)185.9	(5.72)4838.4	1.34	7.99	0.49	191.8	177.1
16.9	16.7	-0.2	(0.27)228.2	(7.02)5931.9	1.60	8.01	0.47	192.2	181.2
26.5	26.5	0.0	(0.39)329.5	(10.14)8568.3	1.47	10.98	0.41	263.5	255.1
21.0	21.0	0.0	(0.30)253.5	(7.69)6498.1	1.42	8.45	0.40	202.8	215.5
30.0	30.0	0.0	(0.47)397.2	(12.22)10309.0	1.57	13.24	0.44	317.8	280.3
9.3	8.1	-1.2	(0.19)160.5	(4.94)4174.3	0.62	7.76	0.17	186.2	115.2
25.3	25.3	0.0	(0.35)295.8	(9.10)7689.5	1.38	9.86	0.39	236.6	246.3
35.5	35.5	0.0	(0.48)405.6	(12.48)10545.6	1.35	13.52	0.38	324.5	319.2
16.4	16.0	-0.4	(0.25)211.3	(6.50)5492.5	1.52	7.84	0.48	188.2	177.4
21.4	21.4	0.0	(0.31)262.0	(8.06)6810.7	1.45	8.73	0.41	209.5	218.9
22.7	22.5	-0.2	(0.29)245.1	(7.54)6371.3	1.28	8.17	0.36	196.1	226.8
Means 21.9±1.6			(0.32±0.02) 270.4±15.3	(8.41±0.57) 7107.9±507	1.42±0.06	9.65±0.37	0.48±0.04	231.6±11.2	219.6±12.6

1.77 $\pm$ 0.04 which was significantly higher than the mean trout appetite of 1.42 $\pm$ 0.06 (p = 99%). Data for the deduced daily routine metabolism of grayling and trout is presented both in terms of calories per gram of fish and calories per whole fish because the samples are not identical (size and number). Grayling metabolism (T_g) was significantly higher than that of trout (T_t) per gram of fish (p = 98%). The relationship between daily deduced metabolisma and various weights of fish (in terms of whole fish) is presented in Fig. 20 for both grayling and trout, along with Winberg's routine metabolism for salmonids (T_{W_1}) which was estimated from a similar range of fish weights and modified with the appropriate temperature correction (Q_{10}). Table 32 shows no significant differences between the regression coefficients for T_g, T_t, and T_{W_1}. Finally, there were no mortalities in this experiment and the water quality did not indicate any dramatic changes in the concentrations of calcium, magnesium, potassium, sodium, ammonia or pH (see Table 33). Gills did not exhbit hyperplasia (see Plate 11b).

3. Experiment III: 6°C, an 8 hour day with 2% body ration.

Figures for the changes in weights, appetites, and routine metabolisms of 15 grayling and 8 trout are presented in Tables 34 and 35 . Ten grayling gained weight (ranging from 0.9% to 6.8% body weight), 1 lost weight, and 4 showed no change in weight at the end of the experiment. 3 trout lost weight (ranging from 1.1% to 3.9% body weight) and 5 were the same weight at the finish of the experiment. All fish took food regularly and the mean daily ration consumed by the grayling - 1.01 $\pm$ 0.04 - was significantly higher than that of the trout - 0.79 $\pm$ 0.03 (p = 99%). Grayling metabolism (T_g) was significantly higher than that of trout (T_t) in terms of calories per gram of fish (p = 94%). The relationship between daily deduced metabolism and various weights of fish in terms of the whole fish is presented in Fig. 21 along with Winberg's estimate

at 10°C
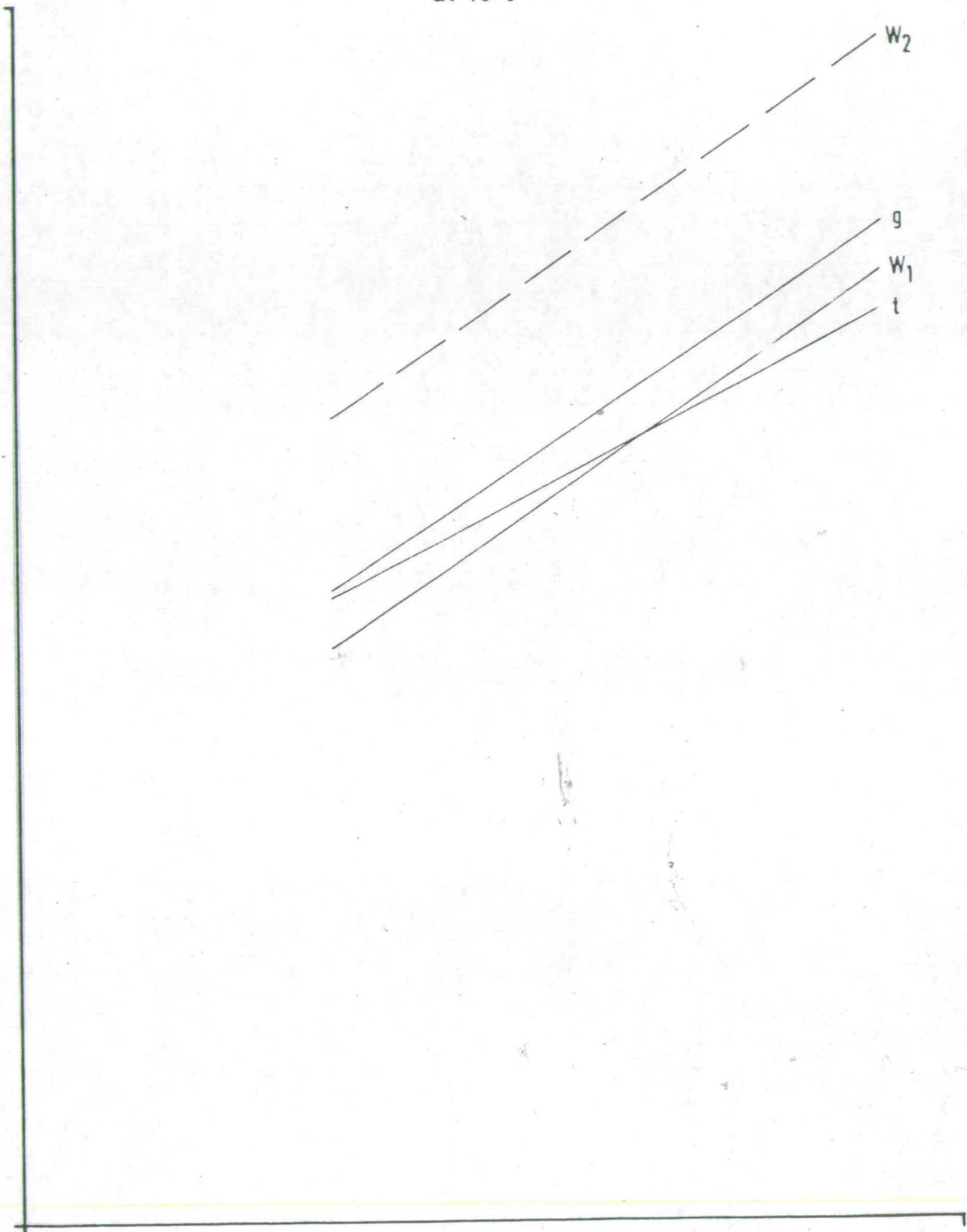
Fig. 21 Comparison of the relationship between deduced routine metabolism and body weight of grayling (T_g) and trout (T_t) with estimated routine metabolism for fish of a similar range of weights (T_{W_1}; T_{W_2}) at $6°C$
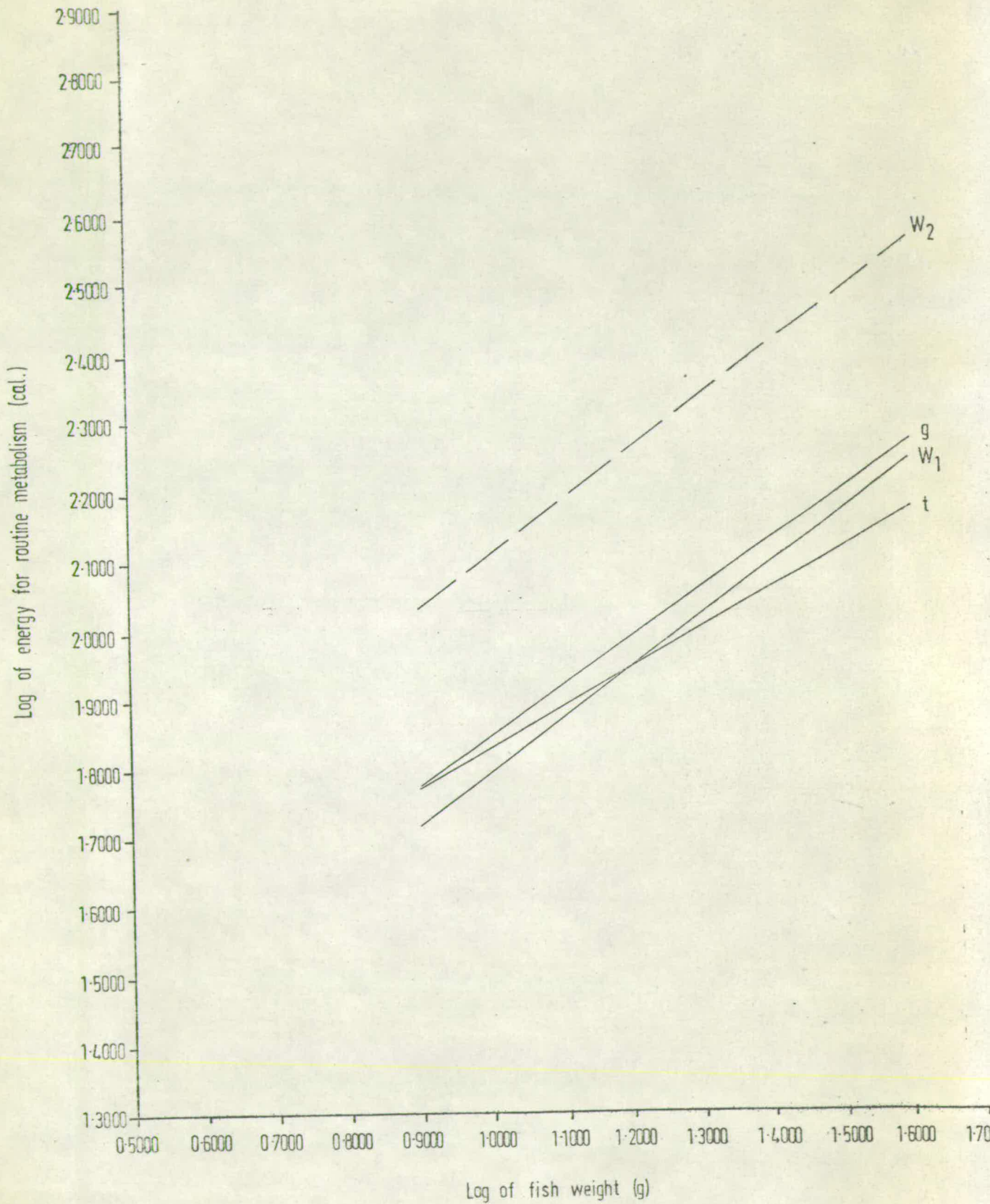
Table 32.

Comparison of the Regression Coefficients for Deduced and Estimated

Routine Metabolism for Grayling and Trout.

(T_g = deduced grayling metabolism; T_t = deduced trout metabolism; T_{w_1} = estimated routine metabolism after Winberg 1956.) Student's-t.

Samples	number	deg. free.	r_1-r_2	t	p
$T_g - T_t$	15, 8	19	0.1261	1.032	69%
$T_g - T_{w_1}$	15, 8	19	0.0555	0.645	48%
$T_t - T_{w_1}$	8, 8	12	0.1816	2.067	93%

(experimental temperature $6°C$)

$T_g - T_t$	18, 16	20	0.2268	1.36	81%
$T_g - T_{w_1}$	8, 16	20	0.0001	-	-
$T_t - T_{w_1}$	8, 8	12	0.2249	1.36	80%

(experimental temperature $10°C$)

Table 33. Summary of the water analyses for food consumption studies.

Sample	pH	Ca	Mg	Na	K	NH_3
Experiment I	8.0	25.0	5.0	6.2	10.0	9.8
Mains Supply	7.1	-	-	-	-	trace
Experiment II	7.4	14.0	3.0	5.0	1.5	1.6
Mains Supply	7.0	-	-	-	-	trace
Experiment III	7.4	11.0	3.0	4.0	0.5	0.7
Mains Supply	7.0	-	-	-	-	trace

Table 34 : Summary of food consumption, changes in weight, and calculations for routine metabolism for grayling in Experiment III (5-6 C, 8 hour day, and 2% ration).

Weight at t_1 (g)	Weight at t_2 (g)	Growth $\Delta W/t_1-t_2$ (g)	Daily food consumption (g) cal.	Total food consumption (g) cal.	Appetite daily ration/fish weight	Deduced metabolism $T = pR - G$ cal/fish/h	cal/g fish/h	Daily routine metabolism deduced cal/fish	estimated (T_{W_1}) cal/fish
26.0	27.5	+1.5	(0.38)321.1	(9.88)8438.6	1.46	7.21	0.28	173.0	147.6
27.5	28.7	+1.2	(0.33)278.9	(8.58)7250.1	1.20	6.44	0.23	154.6	154.0
39.0	40.2	+1.2	(0.44)371.8	(11.44)9666.8	1.13	9.32	0.24	223.7	200.9
33.5	33.5	0.0	(0.29)245.1	(7.54)6371.3	0.87	7.58	0.23	181.9	178.9
28.0	28.4	+0.4	(0.28)236.6	(7.28)6151.6	1.00	6.59	0.24	158.2	156.2
27.0	26.5	-0.5	(0.24)202.8	(3.84)3244.8	0.89	7.04	0.26	169.0	151.9
25.5	25.9	+0.4	(0.26)219.7	(6.76)5712.2	1.02	6.07	0.24	145.7	145.2
33.5	33.5	0.0	(0.29)245.1	(7.54)6371.3	0.87	7.58	0.23	181.9	178.6
32.3	32.3	0.0	(0.28)236.6	(7.28)6151.6	0.87	7.32	0.23	175.7	174.0
27.2	27.6	+0.4	(0.28)236.6	(7.28)6151.6	1.03	6.59	0.24	158.2	151.5
28.0	28.8	+0.8	(0.30)253.5	(7.80)6591.0	1.07	6.52	0.23	156.5	153.1
29.1	29.4	+0.3	(0.28)236.6	(7.28)6151.6	0.96	6.78	0.23	162.7	155.8
21.5	21.7	+0.2	(0.23)194.4	(5.98)5053.1	1.07	5.65	0.26	135.6	132.6
38.1	38.1	0.0	(0.31)262.0	(8.06)6810.7	0.81	8.11	0.21	194.6	185.4
31.5	31.8	+0.3	(0.30)253.5	(7.80)6591.0	0.95	7.29	0.23	175.0	168.4
Means 29.8 ± 1.23 ± 1.23			(0.30 ± 0.01) 253.5 ± 10.9	(7.62 ± 1.00) 6438.9 ± 845.0	1.01 ± 0.04	7.11 ± 0.23	0.24 ± 0.004	169.8 ± 5.5	162.3 ± 4.67

Table 35 : Summary of food consumption, changes in weight, and calculations for routine metabolism for trout in Experiment III (5-6 C, 8 hour day, and 2% ration).

Weight at t_1 (g)[1]	Weight at t_2 (g)[2]	Growth $\Delta W/t_1-t_2$	Daily food consumption (g) cal.	Total food consumption (g) cal.	Appetite daily ration/fish weight (g)	Deduced metabolism $T = pR - G$ cal/fish/hr	cal/g of fish	Daily routine metabolism deduced cal/fish	estimated (T_W)[1] cal/fish
22.00	22.00	0.00	(0.19)160.6	(4.94)4174.3	0.86	4.97	0.23	119.3	130.6
25.00	25.00	0.00	(0.21)177.5	(5.46)4613.7	0.84	5.49	0.22	131.8	142.8
18.30	18.10	-0.20	(0.16)135.2	(4.16)3515.2	0.87	4.56	0.25	109.4	113.0
26.70	26.70	0.00	(0.20)169.0	(5.20)4394.0	0.75	5.23	0.20	125.5	150.5
20.50	20.50	0.00	(0.18)152.1	(4.68)3954.6	0.88	4.71	0.23	113.0	123.6
29.60	29.60	0.00	(0.21)177.5	(5.46)4613.7	0.71	5.49	0.19	131.8	162.5
23.40	23.10	-0.30	(0.18)152.1	(4.68)3954.6	0.77	5.26	0.23	126.2	136.3
15.50	14.90	-0.60	(0.10) 84.5	(2.60)2197.0	0.65	3.73	0.25	89.5	99.6
Means 22.62±1.61			(0.18±0.01) 151.0±8.45	(4.64±1.04) 3920.8±878.9	0.79±0.03	4.93±0.21	0.22±0.007	118.3±5.0	132.4±7.18

of metabolism for fish of a similar weight range. Table 32 shows significant differences between the regression coefficients for T_g and T_t and T_{W_1}. Finally, there were no mortalities in the experiment and the water quality did not indicate any dramatic changes in the concentrations of calcium, magnesium, potassium, sodium, ammonia or pH (see Table 33).

DISCUSSION

1. An Assessment of the Experiment and the Validity of the Results.

   i. Experiment I. There were two practical problems which were probably largely responsible for the mortalities and the generally poor condition of surviving fish in this experiment - water and food quality. As indicated in the water analyses, there were substantial changes in the concentrations of certain ions in the re-circulating water during the twenty-eight days of the experiment, namely in calcium, sodium, potassium, and ammonia. The regular ventilation of the gills in such adverse conditions has been shown to result in concentrations of these ions in the gill tissue which may lead to hyperplasia (Hughes and Morgan 1973); the occurrence of hyperplasia in the gills of experimental grayling and trout is illustrated in Plate 11a , exhibiting the characteristic thickening of the epithelium and the fusion of adjacent lamellae. Potassium and sodium in partiular play a major role in the ion-osmoregulation of freshwater fish and the alterations in the concentrations of these ions suggest that there was considerable stress to the ion-osmoregulation system of experimental fish. NH_3 constituted an additional stress.

   Most fish were in an emaciated condition at the end of the experiment which probably related to the type of diet. Fish were fed a ration size which calorifically was in excess of estimated routine requirements; however, Daphnia are extremely small crustaceans (0.7 to 1.88 mm), and a great number of them are necessary to make up the total daily calorific

requirements of even small fish with the consequence that the energy cost per unit prey is higher than for other larger prey species. It therefore seems likely that the potential energy of the ration of Daphnia was not sufficient to meet the daily metabolic demands of the fish nor to allow for growth. Kazarkin (in Winberg 1956) noted an increase in metabolic costs associated with increased searching costs for pike fry feeding on daphnids and although rations were relatively high, growth rate was slow, presumably because of the high expenditure of energy for searching.

In conclusion, it appears that fish were not only stressed by large changes in the water chemistry, which damaged their gills, but also that fish were unable to reap sufficient calorific reward from their ration . Both these factors are thought to have stressed fish metabolically and physiologically which led to poor general condition and in some instances, to mortalities. The results of experiment I were therefore considered invalid for calculations of appetite and for deductions of routine metabolism.

As a consequence of the problems experienced in this experiment, two alterations were introduced. An attempt was made to improve the water quality by the following additions to the experimental apparatus: Additional filtering material was provided (layers of charcoal chips and glass wool) inside a flower pot which was placed in each pail underneath the out-flow tubes; two air stones were also placed in each pail. The second alteration concerned the type of ration; it was decided to feed fish on Gammarus because the fish in the following experiments would be big enough to take this food species and because it is a food type which has been used successfully in food consumption experiments conducted by others. (Solomon and Braefield 1972).

ii. _Experiment II_. The problems experienced with water quality and
food in the first experiment did not appear in this experiment. The
changes in ion concentrations were small and were not shown to affect
the fish adversely as regards their gills. As the food was readily
accepted by the fish and fish appeared to be in good condition at the
end of the experiment, _Gammarus_ was considered to provide a satisfact-
ory diet for the fish. Because fish were not seen to be stressed as
in the previous experiment, the calculations made for appetite and
routine metabolism based on the food and weight change data were
assumed to be valid. Furthermore, because of the high degree of
accuracy shown for measurements of both fish and food species, even
the small changes in weight - on the order of 0.1 of a gram - were
assumed to be valid.

iii. _Experiment III_. The assessment is as above (ii.).

2. Appetite.

"From comparisons with other workers, it was concluded that the maxi-
mum amount of food consumed in a meal provides sufficient calories for
both the daily metabolic demands and maintenance requirements of
(brown) trout at temperatures between 3.8° C and 18.4° C, but not at
temperatures above 18.4° C"(Elliott 1975, p. 51). Appetite as a measure
of the voluntary intake of food reflects an organism's routine (or
active) metabolic demands; it is a function of the inter-relationship
of the size and frequency of meals and the size and composition of the
prey and the rate of evacuation which is temperature dependent. Larger
meal size has been shown by Hunt (1960) to increase the rate of evacu-
ation, probably through increased specific dynamic action, with the con-
comitant rise in metabolic demands and to peristalsis due to the greater
mechanical stimulation of the stomach walls. Windell (1969) did not
find an increase in the evacuation rate related to meal size or frequency,

whereas Brown (1951) demonstrated that the rate of evacuation in the brown trout was influenced both by the amount and the frequency of feeding.

The effect of the meal type and frequency and prey size on the rate of evacuation and ultimately on appetite can be clearly and consistently seen in Elliott's research (1972) on the brown trout: Evacuation rates were similar for food organisms of different size groups of the same taxon or for mixed and multiple meals; however, these rates did vary with temperature, increasing exponentially with rising water temperatures between $3.8°C$ and $19.1°C$. Similarly, Molnar and Tolg (1962) found in a field study on gastric evacuation in pike in winter ($5°C$) that evacuation was 5 to 6 times slower than in the summer ($25°C$).

As the feeding regime was the same for all fish in each experiment, it is possible to evaluate the influence of temperature on fish appetite and indirectly on metabolism. Intra-specifically, the appetites of grayling and trout were higher at $10°C$ than at $6°C$ (p = 99%); These results display the expected increase in appetite with temperature — Elliott (1975) found that brown trout appetite increased with temperatures up to $18.4°C$. Inter-specifically, grayling appetite was higher than trout at both temperatures (p = 99% and 99%, respectively). These differences in appetites suggest certain differences in the evacuation and metabolic rates of grayling and trout: A grayling of a given weight will consume more food than a trout of the same weight which indicates that the grayling is turning over food more rapidly than the trout which is presumably related to its higher metabolic demands.

It was mentioned earlier that the digestion process is dependent on enzymes and their rates of activity which are themselves regulated by temperature. It is likely that the inferred differences in the

rates of digestion of grayling and trout may reflect specific differ-
ences in their respective enzyme kinetics as regards temperature, which
in turn will delimit those periods of greater appetite. Because
there is little (published) work on digestion and enzyme activity in
grayling, for the purpose of discussion, the results of work carried
out on the relationship between digestive enzyme activity and tempera-
ture for distantly related species will be included.

Smit (1967) studied the relationship between gastric juice secre-
tion and body temperature in brown bullheads (Ictalurus nebulosus)
which were acclimated to $10°C$, $15°C$, $20°C$, $25°C$, and $30°C$, and found
that the composition of the gastric juice depended on the rate of
secretion, the latter being temperature dependent. Ananichev (1959)
assessed the activities of certain digestive enzymes - pepsin, trypsin,
amylase, and lipase - in the burbot (Lota lota), a winter feeder, and
the pike perch (Lucioperca lucioperca), a summer feeder. (In view of
the seasonal periodocity in feeding exhibited by these species, this
research provides an extremely valuable and provocative biochemical
examination of the mechanisms which regulate feeding activity in fish
and in grayling and trout in particular). His results demonstrated
that the activities of these enzymes vary at different times of the
year : Pepsin, which is the enzyme largely responsible for the initial
break-down of food material, showed a marked change in its activity
between summer and winter which corresponded with known changes in
their feeding intensity; the maximum activities of trypsin and lipase
also coincided with known feeding periods of these species which sug-
gested to Ananichev that the production of all three enzymes was related
to the level of food intake. The results of this work not only indicate
the influence of temperature on the activities of digestive enzymes, but
also provide further confirmation of the inter-relationships between
appetite, metabolism and temperature at a molecular level.

3. Routine Metabolism.

Paloheimo and Dickie (1966a) concluded that the food and growth method for measuring metabolism may be used to obtain estimates of metabolic levels ranging from low routine to active; they also concluded that when fish were allowed to feed voluntarily, but for a restricted period of time so that food consumption and growth were at less than maximum, the value of the metabolism was between standard and active. In food consumption and growth studies conducted on grayling and trout, fish were maintained in what was considered to represent routine conditions (as per flow rate) and were fed on a restricted ration, and therefore it is assumed that the deductions for metabolism based on these experimental results will provide estimates of their routine metabolic demands (see Fig. 22 for comparisons with Winberg and Elliott).

Deductions for the routine metabolic demands for grayling (T_g) and trout (T_t) in terms of gram per fish indicated that there are significant differences in their metabolismss at both 10 C$^\circ$ and 6°C: At both temperatures, the grayling exhibit a higher level of metabolism. The relationship between the log of total body weight and the log of metabolism as presented in Figs 20 and 21, also suggest that there certain metabolic differences between these species at both experimental temperatures, although these differences were not significant statistically. If these suggested differences were valid, they might suggest that the grayling has an intrinsically higher metabolic level than the trout. High metabolic levels, however, may be attributable to stress: Other workers have recorded an increase in fish metabolism, (which in some instances approached the active level) because of the amount of stress in the fish's environment (Beamish 1964). The high metabolic levels recorded in these experiments, however, were not considered to result from stress related to the experimental environment.

Fig. 22: Comparison of the calorific value of the daily ration for
routine metabolism in 50 g grayling and trout.
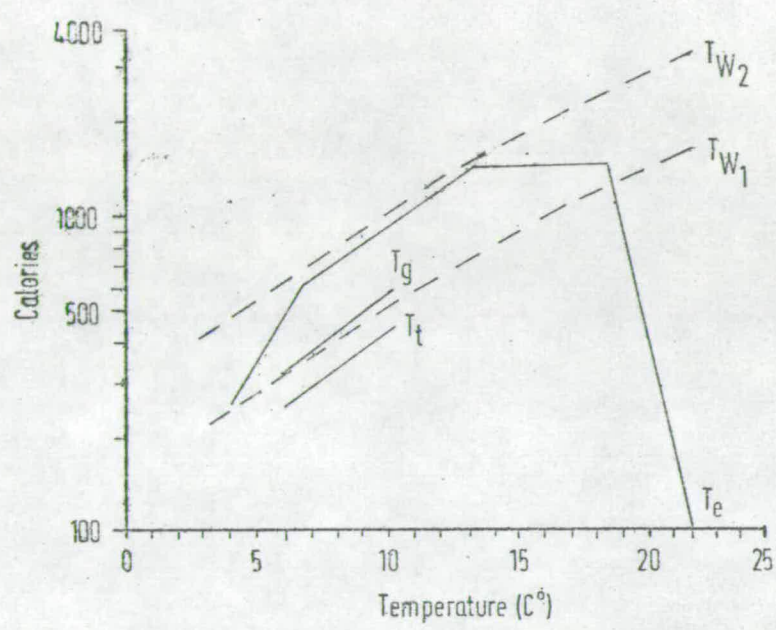
T_E = calorific value of the daily meal of a brown trout to meet the
energy demands of routine metabolism, from 3.8 C to 21.6 C (Elliott 1975

T_g = calorific value of the daily meal of a grayling to meet the
energy demands of routine metabolism, at 6 C and 10 C.

T_t = calorific value of the daily meal of a brown trout to meet the
energy demands of routine metabolism.

T_{W_1} = calorific value of the daily meal of a salmonid to meet the
energy demands of routine metabolism, from 4 C to 22 C (Winberg 1956)

T_{W_2} = calorific value of the daily meal of a salmonid to meet the
energy demands of routine metabolism in nature, from 4 C to 22 C (Winber

In view of the previously mentioned direct relationship between appetite and metabolic demands (Elliott 1975), the results of food consumption and growth studies may be interpreted to mean that the grayling consumes a larger ration (relative to its size) than the trout because of its intrinsically higher level of metabolism, or conversely, that the trout has a smaller appetite (relative to body size) than the grayling and a corresponding lower level of metabolism. Furthermore, these results (and those from Experiment III, $6\overset{\circ}{C}$, in particular) indicate that the grayling is more efficient than the trout in assimilating its ration which is probably related to differences in their digestive enzyme optima. The grayling is reputed to be in prime condition in late winter (Pennel 1863) which presupposes that its metabolism is not only active but that it is functioning efficiently. The trout, however, has been shown to exhibit a decrease in metabolic efficiency at low temperatures: Leonard (1941) placed trout in a holding basket for three hours prior to killing them, and despite the delay in time(as well as an expected increase in metabolic activity related to stress and its effect on digestion), the invertebrates in the trout stomachs were sufficiently vigourous at the time of examination to walk out of the stomachs; Elliott (1975) found that at temperatures below $6^{\circ}C$ trout were inactive and reluctant to feed.

CHAPTER VI

SUMMARY AND CONCLUSIONS

A series of food consumption and growth studies were conducted to investigate any fundamental differences between grayling and trout metabolisms which could largely regulate their seasonal patterns of feeding and would thereby contribute to their interactive segregation.

1. Experiment I ($15°C$, 18 hour day). Most fish lost weight and some fish died. The poor condition of surviving fish was attributed to the type of diet - Daphnia - which provided a low calorific reward per unit of food and to deleterious changes in the water chemistry, i.e. the increases in the concentrations of calcium, magnesium, sodium, potassium, and ammonia. Alterations were applied to the experimental apparatus as well as to the food type, but no conclusions were made about fish appetite or metabolism based on the results of this experiment.

2. Experiment II ($10°C$, 12 hour day). Most fish maintained weight and no fish died during this experiment which was thought to be related to a satisfactory change in the type of diet - Gammarus - and to the improvements in the water chemistry. The direct assessment of appetite indicated that grayling had a significantly higher appetite than trout. Indirect calculations for routine metabolism suggested that grayling metabolism was higher than trout metabolism.

3. Experiment III ($6°C$, 8 hour day). Most grayling gained weight and most trout maintained weight. The appetite of grayling was significantly higher than that of the trout. Deductions for routine metabolism suggested that grayling metabolism was higher than that of the trout.

This series of experiments are considered to provide some preliminary evidence towards the demonstration of certain metabolic differences

between grayling and brown trout. Because of the direct relationship between appetite and routine energy demands (Elliott 1975), the calculations for appetite and deductions for routine metabolism can be interpreted together to suggest that the grayling has an intrinsically higher metabolism than the trout at 10°C and 6°C. If these results are valid, they indicate the occurrence of some degree of metabolic compensation which permits the grayling to not only feed, but also to assimilate efficiently at a winter temperature, whereas the trout exhibits a reduction in appetite and metabolism which is typical of over-wintering.

PART II

FINAL DISCUSSION

Interactive segregation presupposes considerable plasticity in the habits of a species because its optima or preferences will by definition be modified by inter-actions with other species. As regards flexibility in feeding habits, fish of northern temperate areas typically do exhibit euryphagy (Nikolskii 1963), although some degree of feeding specialisation does take place which is thought to confer a survival or nutritional advantage in some situations (Keast 1970). In the instance of interactive segregation in the Eden, where some degree of feeding specialisation was shown to occur in both the type of prey consumed and the seasonal periodocity in feeding, morphological and physiological studies which were conducted on grayling and trout revealed some of the important mechanisms which influence fish behaviour and ultimately effect dietary specialisation in this freshwater community.

The results of morphological examinations of feeding-related structures of grayling and trout indicate that the mouth form and structure exert a fundemental influence on the species' feeding habits, channeling them into particular ways of life and feeding niches. The position of the grayling mouth as well as its shape and gape taper its hunting towards the benthos, whereas the design, orientation, and size of the trout mouth equip it to feed out of the drift as well as from the benthos.

These morphological indexes provide some explanations for the dietary specialisation recorded in the results of Chapter II. The coefficients for overlap in diet demonstrated that on the whole the grayling and trout diets did not overlap significantly, although a significant overlap was found in the diets of immature fish in the summer. This age-specific

dietary overlap most probably is related to the fact that grayling and trout grow at different rates (see Chapter III); because of the extremely rapid growth of the under-yearling grayling, both species are likely to have mouths of similar capacities which could enable them to take the same size of prey during the first year.

Morphological differences in the mouths of grayling and trout also supply tangible evidence for their specialisation in feeding which was seen in the trophic diversity estimates for the summer (Chapter II). Each species was shown to take a major portion of its diet from a particular prey category for which it had been demonstrated to be especially well designed to hunt - the grayling took more than half of its food from the benthos and the trout fed nearly exclusively from the surface.

Food consumption experiments which assessed growth, appetite, and metabolism at different seasonal temperatures suggested that the grayling and trout are specialised as to the time of year which they can consume and assimilate food efficiently for growth. These results permit a better understanding of earlier results which indicated a seasonal periodocity in stomach fullness, condition, and growth: Good condition and growth are related to high food consumption and to efficient assim-ilation, and grayling displayed their peak condition at the end of the winter when stomach fullness indexes were also high, whereas the trout did not attain top condtion until late summer when stomach fullness indexes were at an intermediate level.

The results of food consumption studies also offer further insight into some of the mechanisms which are probably responsible for seasonal periodocity in the feeding and growth of grayling and trout. Indirect deductions suggest that there are certain bio-chemical differences between these species in the ways their metabolisms respond to temper-ature which influence both the rate of digestion and the rate of

food consumption. It appears that grayling and trout are adapted
to different temperature optima: The grayling seems to be more
cold-adapted than the trout and this is to be expected when it is
recalled that the grayling is considered to be an arctic species(Platts 1936,
Henderseon 1951). "Arctic fish feed all year round and may even
increase activity in the winter" (Varley 1967, p. 129). Similarly,
the cold-adapted fish will exhibit a more efficient metabolism at
lower temperatures (less than $6^{\circ}C$) which relies on a compensatory
higher basal metabolism that enables them to feed and to remain
active at cold temperatures (Scholander et al. 1964), and the occur-
rence of some degree of metabolic compensation was inferred from
deductions for grayling metabolism in Chapter VI.

However, morphological and physiological characteristics alone
do not determine the specific feeding patterns and behaviour of fish —
instinctive, individual, and conditioned behavioural patterns incorpor-
ate specific morphological and physiological constraints and together
these will delimit the feeding habits of a species. Instinct will
dictate such fundemental behaviour as whether or not a species is soli-
tary and territorial or gregarious and shoaling which in turn will
greatly influence the species' mode of feeding. The territorial fish
defends an area which for salmonids is commonly related to spawning
and to a varying extent to feeding; the territorial system of the trout
is characterised by the possession of a strictly localised 'station'
within its territory where the fish spends most of its time and from
which it defends its territory and begins its feeding excursions(Noble
1939). The shoaling fish by definition is more sociable in that it
forms groups for a prolonged period of time, usually with fish of a
similar age and biological condition, which are united by similar
behaviour (Nikolskii 1963); the grayling is generally accepted as a

shoaling species (Day 1887, Fabricius and Gustafson 1955, Dahl 1962)
- by the end of the first year, most grayling drop down from the trib-
utaries to the main stream or river where they are often found in
shoals. A shoaling species is considered to be efficient in feeding
because unlike the solitary feeder which is limited to a comparatively
more fixed (restricted) food supply within its territory, the shoaling
fish is able to crop a food supply from a large area and therefore
to more fully exploit any temporary abundance of food.

Certain individual responses of fish may also affect feeding
behaviour. Bryan and Larkin (1972) demonstrated that there are indi-
vidual differences in the foods selected by individual fish which may
be attributable to both taste and learning. Food preference experiments
conducted on brook trout (Salvelinus fontinalis), cut-throat (Salmo clarkii),
and rainbow trout (Salmo gairdneri) in streams and ponds indicated that
different prey types were eaten in proportions which were characteristic
for an individual: "... in field situations, individual trout demonstrate
differences in the food they eat and that these differences persist to
result in specialisation.... the cause of food specialisation in natural
populations of trout probably results from individual differences in
detecting a particular type of prey" (Bryan and Larkin 1972, pp. 1623-4).

Learning is therefore considered to be a major influence in the
choice of food. "There is a tendency for salmonids to become adapted to
a certain food object when that object reaches an abundance that makes
it more available to the fish species than other food in the fauna"
Nilsson 1957, p.   ), which is similar to Tinbergen's concept of the
'searching image'(Tinbergen 1960). This tendency was noted for grayling
in particular by Muller (1954b), who found a marked correlation between the
species in the invertebrate fauna and those species found in grayling
stomachs and concluded that the short productive season regulated the
sort of prey which were taken by the grayling. This facility to adapt

to abundant food species was demonstrated experimentally in the work carried out by Ware (1971) on rainbow trout: Naive fish which were presented with an unfamiliar food changed their feeding habits and developed searching images for these prey species after an average exposure period of 4 days.

This research was undertaken to assess the inter-relationship of grayling and trout in the river Eden with a particular view to competition over food. "Inter-specific competition among fish may be continually shifting in intensity and emphasis. The physical and biological environment and distribution and behaviour of competitors may be in states of continual flux in which case, the niches of competitors cannot be considered constant"(Johannes and Larkin 1961, p. 219). Given the transient nature of competition, intense inter-specific competition in sympatric populations of closely related species may force each species to specialise (temporarily) in a 'realised niche' and therefore to compete at its ecological optimum, rather than to limit or eliminate a species. It is this tendency of sympatric species to utilise their ecological optima which results in the interactive segregation of species (Hartman 1965) - the amount of specialisation as well as the degree of similarity of species will greatly determine the effects of competition.

Results of this research indicated that cohabitating grayling and trout do exhibit some specialisation in feeding which is related to behavioural, morphological and physiological characteristics which effect different feeding optima in these species. Specialisation in feeding is therefore considered as instrumental to the process of interactive segregation and to the avoidance of competition between the grayling and trout populations in the Eden.

APPLICATIONS OF THIS RESEARCH AND SUGGESTIONS FOR FUTURE WORK

This research was undertaken to assess the inter-relationship
of grayling and trout living in the Eden to provide some insight into
the problem of freshwater community relations and of competition in
particular. The results of these investigations which repeatedly
indicated that grayling and trout do not compete to any measurably
limiting extent are of direct value for both public and private fish-
eries management. In Britain, and in Scotland especially, where the
grayling has always been regarded by the angler as a coarse fish
and as a competitor of the brown trout, it is hoped that these results
will contribute to correcting and improving both the image and the
reputation of this species and therefore ultimately to generate fuller
use of British freshwater fisheries.

Apart from demonstrating the ecological innocence of the grayling
in 'trout waters', this research is immediately applicable to the
topical enterprise of fish farming. Results for growth analyses toge-
ther with those for food consumption experiments indicate that the
grayling has a dramatically great potential for growth at low temper-
atures. In a country such as Scotland, where 6 months out of the year
the mean temperature is $3°C$, it would obviously be of tremendous value
to cultivate a salmonid species which can physiologically continue to
feed and assimilate throughout the year. On the continent, the gray-
ling is not only highly regarded as a game fish, but it is also greatly
appreciated for the human palate and consequently, it is not surprising
that the artificial propagation and cultivation of the species has
been practiced for more than a century (Day 1887, Vivier 1958). "In
ancient times, the flesh of the grayling was highly prized as the
numerous laws in the Codex Austriacus give testimony to the care which
its delicate life was protected by the early rulers of Austria"(Seeley 1886).

There are a number of questions which demand investigation before the grayling could be cultivated on a commercial basis - what is the public market for grayling, what are the optimum growing conditions for grayling, and what are the genetic possibilities as regards hybrid grayling? Experiments in the optimum growth conditions for grayling could entail investigations into the actual enzyme systems which enable the grayling metabolism to assimilate food at low temperatures. Hochachka and Somero (1968) and Baldwin (1971) suggest that the crucial process in such cold acclimation is not the bio-synthesis of more of the same kinds of enzymes which are present in the warm-acclimated state, but rather the synthesis of new enzymes which are better adapted to catalyse at cooler temperatures - the isozyme. The isozyme provides the pokiliotherm with the means of achieving greater enzyme efficiency over a wide range of temperatures. Bio-chemical studies on the enzymes of the grayling and their isozyme variants at different seasons of the year may provide valuable insight into the dynamic process of fish metabolism.

There is certainly the possibility of developing(genetic)crosses between the grayling and other fast-growing or disease-tolerant salmonids. Hybridisation experiments involving the grayling and the sea trout were undertaken as long ago as 1873 in France, at the School of Fish Culture (Day 1887); there were certain problems related to the actual fertilisation of grayling eggs because of the temporal separation in spawning seasons, however experiments were still partially successful and results did indicate an increase in the growth rate of the hybrid grayling.

APPENDIX I

ARTIFICIAL PROPAGATION OF GRAYLING

INTRODUCTION

It was attempted to raise grayling from eggs in the laboratory to provide a supply of fish for food consumption studies as well as information on scales and length at hatching for age and growth determinations.

METHODS

1. Stripping. Ova were removed from ripe females taken from the Eden and were placed in a plastic pail with a small amount of water in the bottom; the eggs were carefully fertilised with milt taken in the conventional way from male Eden grayling (Braum 1968). The eggs were then washed until the water was clean and were allowed to harden before transporting them back to the department.

2. Incubation. Eggs were incubated in a set of 4 Menzies Curtis vertical trays. The trays were continuously supplied with mains water which was collected in a header tank to allow most of the chlorine to settle off before flowing through the trays. The set of hatching trays were located in a recess, approximately 8 metres from a wall with high windows (which faced east)and therefore the trays of eggs were exposed to indirect natural light which was not thought to be harmful.

   i. Care of eggs during incubation and hatching. It was imperative to remove any dead eggs immediately to discourage the growth of Saprolegnia . (In fact, from the time of stripping on 31st March,1972, to the time of hatching on the afternoon of the 19th of April, egg losses were minimal- 33.) Dead eggs were removed with forceps and

these were also employed to remove all remnants of egg shells and any dead fry after hatching.

RESULTS

The time required for hatching grayling eggs was 187 degree days, i.e. the number of days for hatching times the mean temperature for those days. The mean length at hatching was 0.95 cm.

Grayling propagation in the laboratory proceeded smoothly from the commencement of incubation to the time of yolk sac absorption, which for the majority of fish occurred on the 28th April, and fish were seen to take dry food readily. On the 9th May, however, there was a tremendous mortality of fry which continued to increase in the following days. On the 13th May, 523 dead fry were removed and at this point the project was abandoned.

DISCUSSION

There is little data available in published literature on the embryonic stages of grayling development (Jankovic 1964). According to Dyk (1956) and to Muller (1961), the process of egg development requires 20 - 25 days in warm water. Peterson (1968) reported that grayling eggs require three weeks to hatch when the water temperature is rising from $5°C$ to $12°C$. Laboratory results therefore concur with these findings, with eggs hatching after 19 days at a mean temperature of $9.8°C$.

There were two aspects of this project which warrant discussion. The first is the question of grayling fry taking an artificial food. Grayling fry appeared to take a powdered food directly following the absorbtion of the yolk sac; This has not been the experience of other researchers: Svetina (pers. comm. 1974) expressed that the grayling had difficulties is digesting dry fish food, and therefore fry were fed on live plankton.

The second problem related to this project was the question of the sudden and dramatic die-off of grayling fry, three weeks after hatching. The common causes of the death of fry are starvation and disease. As regards starvation, it has already been mentioned that grayling fry accepted dry food, however it is possible that they did experience certain problems in digesting and assimilating this food which could have resulted in starvation. Fish were seen to expel gas bubbles and it is therefore likely that some fish died as a result of gas bubble disease. A final factor which may have directly or indirectly caused mortality in these young fish was the presence of chlorine in the water. Dandy (1972) found that brook trout fingerlings (11 to 15 cm) lost equilibrium and died when subjected to 0.04 ppm chlorine in the water. This description closely resembles the final throes of dying grayling fry, who were seen to swim in vertical and diagonal postures and to die with their mouths open. The local water board, the Lothian's Purification Board, was consulted and it was discovered that the additions of chlorine into the mains supply were on an _ad hoc_ basis, dependent on current weather conditions and water levels in the reservoirs, and that these doses were administered once a week in close proximity to the department. It is therefore quite likely that this weekly unmeasured dose of chlorine was of sufficient concentration when it reached the department to interrupt the delicate metabolisms of 17 mm grayling fry.

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