Observations on phenotypic variability within populations of elodea Canadensis in County Durham

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Observations on Phenotypic Variability within Populations of *Elodea canadensis* in County Durham

by

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1. INTRODUCTION

1.1 Aims of the work

The obligate submerged macrophyte Elodea canadensis (Canadian Pondweed) has been described by Sculthorpe (1967) as "a habitual experimental subject." Most of the recent work done on this species has however been of a physiological rather than an ecological nature. While the species' ecology was studied after its accidental introduction into the British Isles in the middle of the nineteenth century and at the beginning of this century when it was still flourishing, now it has settled down to a mere denizen, less work is being done on its ecological significance.

This study is concerned with the interaction of the environment on the phenotypic expression of Elodea in County Durham. As the species is dioecious and (with only one exception) only female plants have been observed in the British Isles, vegetation reproduction through budding and fragmentation is the only means by which the reproduction and spread of the species can occur. Mutation is considered ineffective in changing the genotype of the species and therefore all populations in the British Isles can be considered to be members of one clone or major plant unit (Arber, 1920) genetically the same although they may be phenotypically distinct. Elodea is quoted as a good example of the plastic behaviour of hydrophytes (Sculthorpe, 1967).

In an attempt to demonstrate that populations from four sites in County Durham are genetically similar, various culture experiments were carried out. The two aspects of variation that are considered in this context are growth rates and growth morphology. While only one set of culture conditions was used for the investigations on morphology, the conditions were varied for the various growth experiments. This was done in an attempt to assess the effects of

artificially manipulating environmental factors, such as the amount of aeration in the flasks and the level of Fe.EDTA in the culture medium, on the growth rates observed. Growth rates and morphological types were also investigated with respect to Elodea growing in the field in order to assess and quantify the extent of environmental variation.

The final section of the study is concerned with possible causes of phenotypic variation. Differences in water chemistry were determined using spectrophotometric, titimetric and colorimetric techniques and pH measurements of the water are presented and considered in the light of the ability of Elodea itself to have an alkalifying effect on the water. Thirdly, a physical analysis of the bottom mud from each site is documented. These factors are, in all likelihood, some of the main parameters that control the form of the species as found in the selected sites. The phenotypic expression of growth under natural conditions will also largely be affected by biotic factors, namely, the presence or absence of other competing species.

1.2 Description of the species and its history within the British Isles.

Elodea canadensis is a submerged macrophyte belonging to the Hydrocharitaceae. The leaves are generally arranged in whorls of three although basal nodes may only have two leaves. The function of flexibility to the motion of the water is taken over by the axis and the leaves are small and simple (Arber, 1920). It is probably due to the fact that the leaves are only two cells thick and to the extreme delicacy of the plant that the species ows its incapacity to produce a land form (Arber, 1920; Sculthorpe, 1967). Certain anatomical features such as mesophyll and stomata are totally lacking and the vascular system is not highly developed. Frank and Hodgson (1963) report that practically all authors agree that submerged plants represent retrograde species in which a degeneration of the vascular system has occured. A characteristic anatomical feature seen in Elodea is the development of lacunae which arise schizogenously by the enlargement of chinks between the young cells and the subsequent division of cells surrounding the air spaces.

The roots of Elodea, which arise from only certain nodes, form approximately 2.6% of the biomass (Berutskiĭ, 1950). They only develop root hairs when they penetrate the substrate as when they are immersed in water the cutinisation of the epidermis inhibits their development. In the substrate there is insufficient oxygen to bring about the formation of a true cutide (Scullthorpe, 1967).

Early in the twentieth century there developed a controversy over the extent to which the roots of Elodea are important as organs for the absorption of nutrients. The dicthotomy of views arose between the comparable ideas of Pond (1905) and Snell (1908) who considered the roots to be important for absorption and those of Brown (1913) who considered their function to be almost solely for anchorage. Pond was

of the opinion that roots are instrumental in obtaining the necessary nitrate and potassium and Snell, using 10 cm terminal shoots showed that those allowed to root into soil under water grew better than those rooted into sand or whose penetration into soil was inhibited by controlling absorption through the roots stems and leaves using a mechanical arrangement or by removing the roots and cementing the wounds, he claimed to find that the greatest absorption was in fact occuring through the roots. Brown found that if Elodea was cultured in battery jars the pieces in those that had soil in grew whereas those in jars without soil died. However, if carbon dioxide was bubbled through the cultures without soil, the Elodea remained healthy and grew actively. When comparing rooted against non-rooted cultures he found that the rooted specimens did better as dense growths of algae occured on the non-rooted pieces. From these experiments Brown concluded that the roots are important in anchoring the Elodea near to the source of carbon dioxide. He refuted the theory of Pond and Snell as he considered that nutrients absorbed by the roots would diffuse at before reaching the growing spices and leaves. Sculthorpe (1967) notes the free movement of ions from the medium into the leaves and shoots.

Another feature of the physiology of the species which bears relation to this study is documented by Ruttner (1953). He showed that Elodea has the peculiar property of being able to continue photosynthesising when all the free carbon dioxide is exhausted, using half bound carbon dioxide of bicarbonate ions. The bicarbonates are thus decomposed and calcium carbonate is often precipated on the leaves of Elodea as a result. The strongly alkaline reaction that develops in the media is due to the formation of calcium hydroxide from the hydrolysis of the calcium carbonate. A practical case that illustrates this point is quoted by Roddy (1963). He studied a river in Florida and found that whereas the pH ranged from 8.5 to 9.5 in the main channel, on each side

of the channel where the growth of Elodea was very heavy it ranged from 9.0 to 10.0.

The only record of a male plant found in the British Isles is that given by Douglas (1880) who discovered such flowers near Edinburgh which he described and illustrated. All other firm records in the British Isles are of female plants however. Strasburger (1910) reported that while recently imported female plants from America had up to six ovules per ovary, the European counterparts had only two to three and they were sexually weakened. Therefore he concludes that during a long period of vegetative reproduction the sexual tendencies may have diminished. Ernst Schwarzenhach (1945) considers that the European representatives are normally sexual however. Whether he is correct or not it is still evident that vegetative reproduction constitutes the chief method of propagation of the species. Fragmentation is common and any part of the axis is viable and potentially capable of yielding a new plant if it bears a dormant lateral bud from which new growth can occur. In autumn hibernacula are produced which are modified buds, the leaves rather than the axes being the main components. These remain attached through the winter and only cease to be dormant in the spring when the leaves expand, the axis elongates and a new plant is formed (Sculthorpe, 1967).

The history of the spread of Elodea in the British Isles is probably one of the most celebrated examples of the consequences of introducing alien hydrophytes. As such it provides a classic example of the speed by which species can spread by purely vegetative means. How far this vigour is due to the fact that it is polyploid (Gustafsson, 1946) is impossible to say. Elodea canadensis is native in North America where it spans the whole continent but it is adventive in the British Isles, Continental Europe, Western Siberia, Australia and New Zealand. It was first discovered in Ireland in 1836 although how the species was introduced into the British Isles is not known.

Other early records are Berwick on Tweed in 1842 and reservoirs near Foxton in 1847. The Foxton site is a locality of great significance due to its proximity to the strategic centre of the canal system that was then at its heyday. Figure 1 (reproduced from Fritsch and Salsbury, 1938) shows the spread of the species that occured after its appearance at Foxton in 1847. As a result of a portion introduced into the conduit at Cambridge from the Botanic Gardens the species spread into the Cam and thence into the Ouse and the dykes of the East Anglian Fens. Rowing and fishing were severely hampered in the rivers choked with the weed and extra horses were often required for towing canal barges.

In 1909 a questionairre was sent to the various Natural History Societies round the country asking for information about the species. C.E. Robson who was the Honorary Secretary of the Natural History Society of Northumberland and Durham replied:

> "There are no canals in the district and the rivers being swift flowing and not wide, the plant is practically unknown."

Figure 2 (reproduced from a map supplied by the Biological Records Centre) shows the current distribution of the species in the British Isles. Although the greatest concentration occurs in the south and east it is also quite widespread in the Durham area. It is not possible to tell whether Robson's reply in 1909 was based on an incomplete survey or whether the species has increased in the North-east since that time. He makes no mention of ponds, these being the chief habitat in which the species is found in County Durham at the present time.

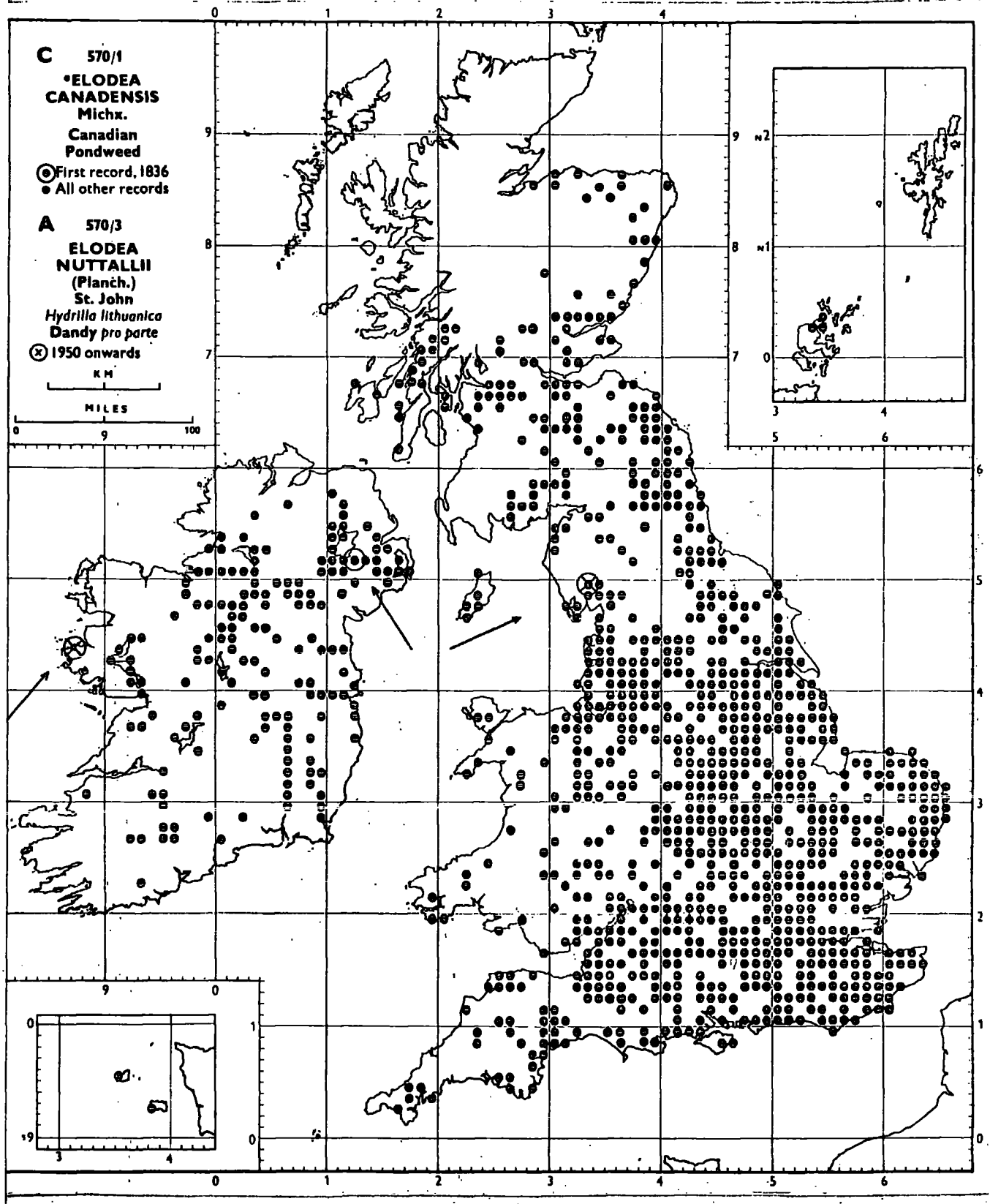
One of the chief findings of the survey was that when Elodea is first introduced into an area it generally goes through a succession of growth phases. Siddall (1885) noticed a phase of active growth and colonisation reaching a climax in five to seven years and then the

vigour of the species was observed to decline. He also noticed that cyclosis became weakened after a period in a certain habitat. It is largely due to this cycle of vigour that by about 1912 the weed ceased to be troublesome in many areas and merely became established on a modest scale as a new member of the vegetation. There are two theories as to the cause of this cycle of abundance. The theory that it was due to the obligate vegetative reproduction and subsequent lack of genetic variability has been largely refuted as there is a repetition of the same rhythm of vigour, abundance and decline in each new habitat colonised. Salisbury (1961) noted that fragments from declining areas grew successfully when taken to new habitats. His theory is that some mineral nutrient or nutrients taken up by the plant become unduly depleted. As the supply of nutrients is diminished the weed may decline until an equilibrium is reached between the population and the amount of nutrient supply made available by leaching or silt deposition. Olsen (1954) concluded that it only grows vigorously where the substrate is anaerobic and that iron may be a critical microelement.

Many authors (Misra, 1938; Butcher, 1933; Brown,1913; Fish and Will, 1966) emphasise the fact that the optimal habitat of Elodea is in eutrophic waters, for example where the rivers receive town sewage or farmland drainage, or where silt gets really organic in a late stage of hydroseral succession. Although the species is capable of withstanding continual smothering with silt or sand it is not however, able to exist in rivers of strong current. This is due to the mechanical strain involved and the coarseness of the river bed which is itself largely controlled by current. Broadly speaking, however, it can be said that Elodea is able to tolerate a wide habitat range and, as such, has the potentiality for displaying a wide spectrum of phenotypic variation.

Figure 1. Map showing the spread of Elodea canadensis after its appearance in England in 1847. (reproduced from Fritsch & Salisbury, 1938).

Figure 2. Map showing the current distribution of Elodea canadensis (reproduced from a map supplied by the Biological Records Centre).



2. MATERIALS AND METHODS

2.1 Study Areas

Four sites in County Durham were chosen for study which were reasonably accessible from Durham and which provided a range of physical habitats. All four sites have a common denominator however, in that they all occur on alluvium in river valleys.

2.11 Hell Kettles ponds (map reference 281109) are situated three miles south of Darlington, less than one mile from the River Tees. They occur on the post glacial river alluvium that overlies the Permian magnesium limestone close to the boundary with the Triassic keuper sandstone. Many legends are attached to the ponds (Nicholson and Nowers, 1926) which are said to have been formed during a terrible upheaval of the ground at Christmas time in 1179. Most probably they are due to the collapse of a cavity in the Magnesium Limestone and as such they are fed by submerged springs. The two ponds which are only separated by a distance of about twenty metres, are sited in a field used for cattle pasture. The two ponds differ markedly in form; whereas the Croft Kettle is circular in shape and very deep (seven metres) the Double Kettle is reniform in shape and considerably less deep (two to five metres). In the Croft Kettle Elodea has only rarely been found growing (Whitton, personal communication) whereas at the present time it is the dominant macrophyte growing round the margins of the Double Kettle. For this reason, attention was confined to the Double Kettle.

2.12 The second site was situated close to the right bank of the River Wear at Bishop Auckland, approximately twenty metres upstream from the main road bridge, (map reference 205303). The geological strata that occur in this area are the carboniferous coal measures but these are overlain by river alluvium. At this site, Elodea only

grows in small patches and there is by no means widespread cover. This is presumably due to the effects of current, for Elodea is only able to grow in slow flowing stretches (Misra, 1938). The patches of Elodea are found along the bank where the current is locally retarded.

2.13 The site at Witton-le-Wear consists of a lake approximately half a mile long which is situated in the Wear valley about four miles above Bishop Auckland (map reference 166312). The lake is the site of former gravel workings, now flooded, which are underlain by the coal measure seams. Fen meadow and fen carr vegetation surrounds the lake which is part of a Naturalist Trust owned nature reserve. Elodea is quite widespread within the lake but it never forms very dense stands.

2.14 Page Bank (map reference 221349) situated about one mile downstream from Willington is similarly the site of former gravel workings adjacent to the River Wear. The pond is somewhat smaller than at Witton-le-Wear and it is also less deep, drying up being more extensive in late summer at this site than at any of the other sites.

Figure 3 is a map illustrating the locality of the four sites in relation to the geological and river systems of the Durham area. Plates 1 and 2 give an impression of each locality with their corresponding types of surrounding vegetation.

Figure 3. Map showing the localities of the four study areas in relation to the geological and river systems of the area.

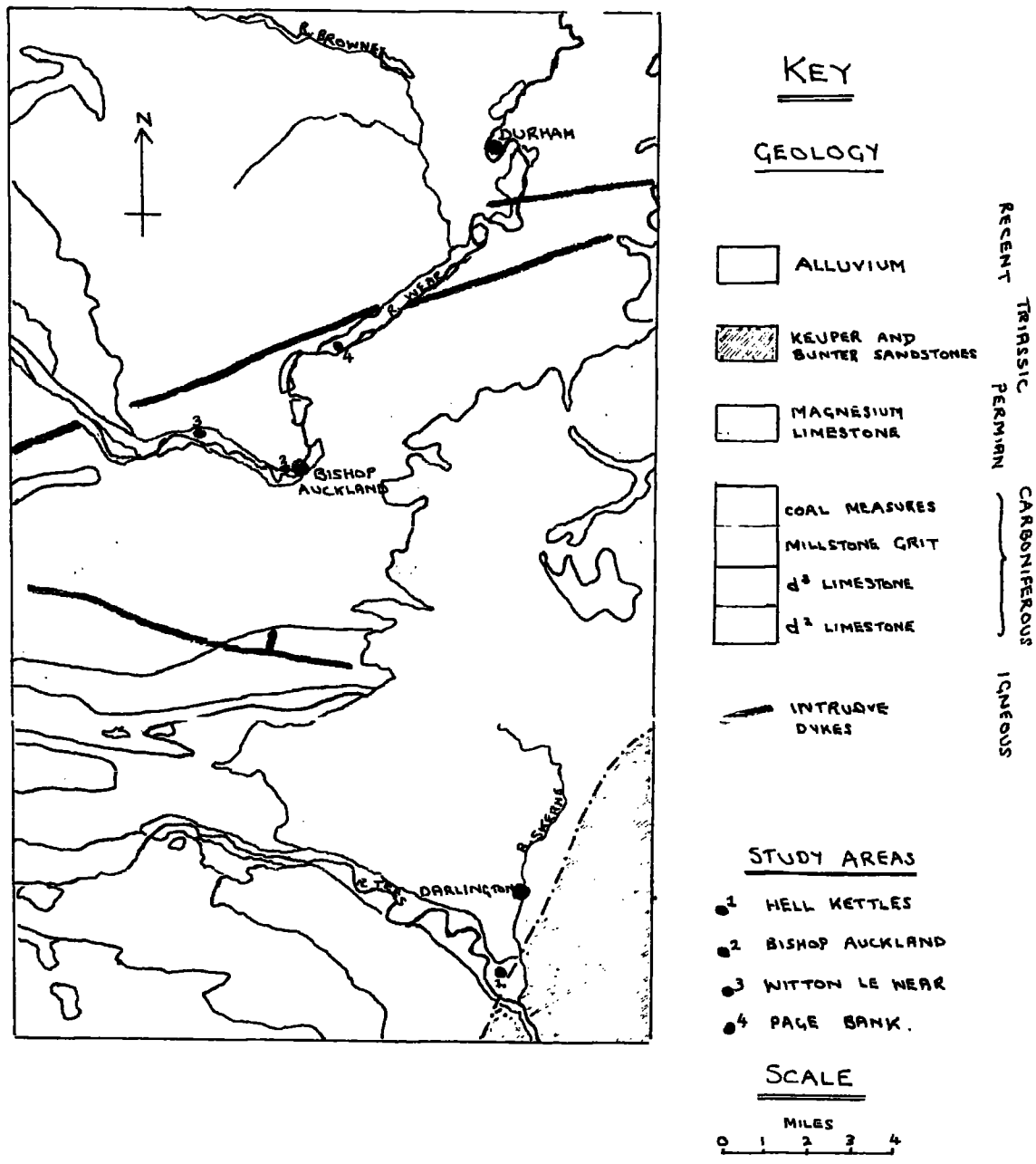
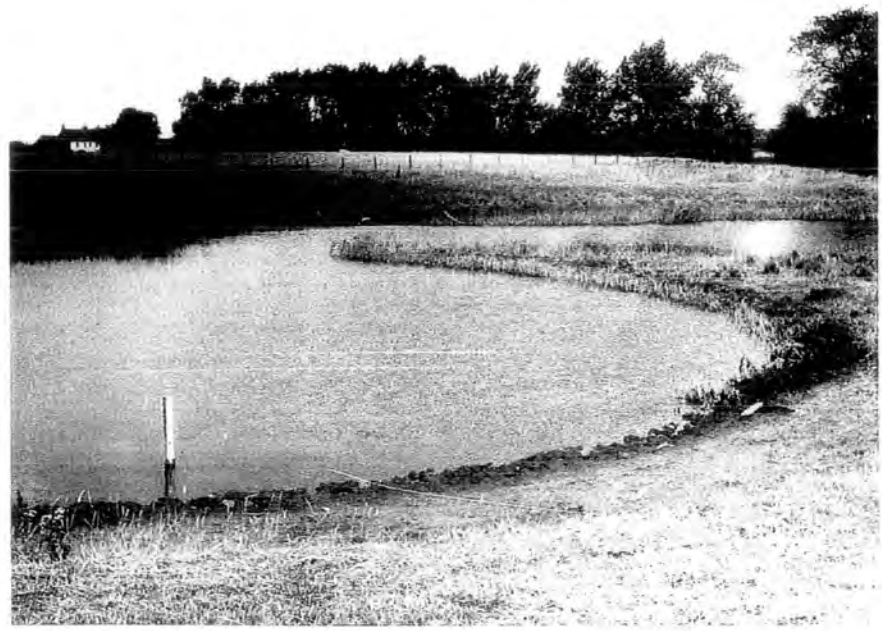


PLATE I.

Study Areas:

1. Hell Kettles



2. Bishop Auckland

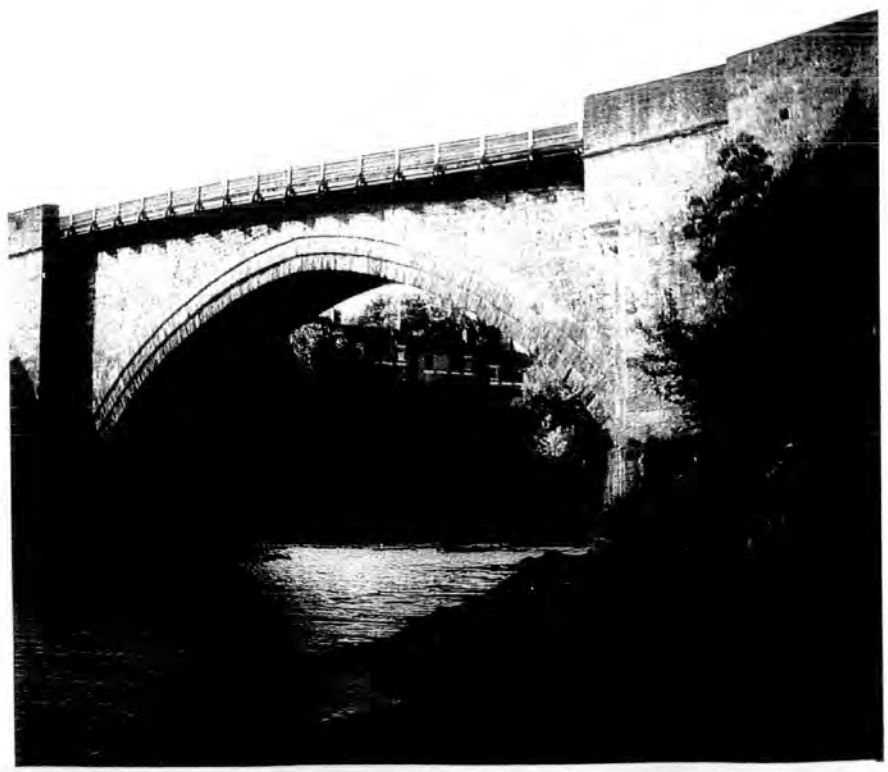


PLATE 2.

Study Areas:

3. Witton - le - Wear

4. Page Bank

2.2 Growth Studies

2.21 Laboratory cultures

In each series of culture experiments in which growth was measured, apical shoots of *Elodea* were cultured individually, the length of the pieces depending upon the experiment concerned. The flasks used for the cultures were 1 L., 500 ml and 250 ml conical flasks and specimen jars. A bung or covering with cotton wool prevented unnecessary contamination by micro-organisms.

The standard culture medium selected for the cultures was a modified form of the No. 10 medium of Chu (1942). This was chosen on the grounds that it is a basically well proven 'all round' medium for the culture of aquatic plant organisms. The medium contained:

25 mg/L KH_2PO_4

25 mg/L $MgSO_4$ $7H_2O$

40 mg/L $Ca(NO_3)_2$

8 mg/L $NaHCO_3$

10 mg/L Na_2SiO_3

0.5 mg/L Fe as Fe (III). EDTA chelate, plus

0.25 ml trace element solution of Kratz and Myers (1955).

The medium was used in this form for all the cultures except one series where the Fe EDTA component was varied in order to investigate the effect on growth of changing the concentration of iron.

The amounts of media used were:-

400 ml in 1 litre flasks

250 ml in 500 ml flasks

150 ml in 250 ml flasks

200 ml in specimen jars.

In each experiment the medium was changed twice weekly. At the same time both the containers and the *Elodea* pieces were thoroughly washed in an attempt to remove as many algal contaminents as possible. A paintbrush was used for the purpose of removing the algae from the *Elodea*.

All the cultures were maintained at a constant temperature of 20°C. Three of the series of cultures were kept in a plant growth room where the illumination was about 2400 lux while the fourth culture experiment was carried out in a tank where the illumination was greater, approximately 5000 lux (from beneath).

Aeration was introduced into two sets of the cultures. A simple electrical air pump with two outlets was fitted up with polythene tubing so that air could be pumped into the surface layers of eight flasks. The cultures contained in the tank were subject to continuous shaking.

Three growth parameters were measured on the Elodea at intervals depending upon the conditions of the experiment. These were:-

1. Length. This was taken as the overall length of the plant and did not include the additional length of the side branches that developed during the course of the experiment.

2. Number of visible internodes. This measurement is approximately proportional to the number of leaves (three leaves per node/internode) but is a more reliable measure as at the growing tip it is difficult to decide how many leaves are actually present.

3. Wet weight. In an attempt to discover the most accurate method for obtaining wet weight measurements, a 50 mm piece of Elodea was weighed ten times by each of four different procedures and the standard deviations were calculated for each method. These were:-

(a) remove Elodea from medium, shake five times, weigh in a beaker of medium of known weight.

(b) remove Elodea from medium, shake five times, weigh on a watch glass of known weight.

(c) remove Elodea from medium, shake five times, blot gently, weigh in a beaker of medium of known weight.

(d) remove Elodea from medium, shake five times, blot gently, weigh on a watch glass of known weight.

The results of the weighings are shown in the Appendix.

The standard deviations for the four methods were:-

(a) .0039 g

(b) .0034 g

(c) .0041 g

(d) .0045 g

As a result method (b) was used as this was shown to give the most accurate weighing despite superficial evaporation from the Elodea while being weighed.

The cultures were maintained for $1\frac{1}{2}$, 2 or 3 weeks depending upon rates of growth and the subsequent limiting effect of the glass containers.

2.2 Field Growth Studies

Tagged individuals

At each of two selected sites, Page Bank and Bishop Auckland, twelve individual *Elodea* plants were tagged with pieces of numbered Dymo tape by tying the tape to the plant with non-rotting nylon thread. The plants which were selected arbitarily were sufficiently far apart that the threads did not become tangled. At weekly intervals for seven weeks at Page Bank and six weeks at Bishop Auckland the lengths of the plants were measured, the measurement taken being the distance between the surface of the mud and the apex of the plant.

Increase in Standing Crop

Page Bank was chosen as the site for standing crop measurements being an accessible site that could be visited weekly.

An apparently homogeneous strip of aquatic vegetation, ten metres long and one metre wide was selected along the southern margin of the pond, far enough out into the water not to dry up as summer proceeded. Metre quadrats were marked out using sticks and stones.

Each metre quadrat was divided into four half metre squares giving forty quadrats in all. Using numbers picked randomly from a hat, eight of these were selected each week and were cropped such that after five weeks the whole strip had been cropped.

A steel frame quadrat was placed over each half metre square in question and the total contents of macrophytes were collected by pulling them up by the roots from the soft mud. They were then brought back to the laboratory in labelled polythene bags and the material from each quadrat was washed and sorted. When all the *Elodea* from each quadrat had been separated from the rest of the vegetation and had been thoroughly washed to remove epiphytes etc., it was whirled for 1 minute in a piece of net and then weighed immediately

to obtain the fresh weight. Following this it was ground up, dried in a hot air oven at 105^{o} for twenty-four hours and cooled in a desiccator. The dry weight was then obtained.

2.3 Studies on Morphology

In order to evaluate the extent of convergence of form with culture using material from the four sites, various morphological descriptions were carried out.

Four 50 mm pieces of Elodea from each site were used in a morphological description of each of the phenotypes collected from the field on 18.V.70 and 16.VII.70. A third description was prepared using a similar quantitiy of material after it had been cultured in the initial growth experiments. From the material that had been cultured, a subculture was carried out under the same temperature and light conditions using ten 40 mm sprigs of Elodea from each site, in order to decrease the effect of ions that were present in the Elodea when it was removed from the field and which would presumably have a carry-over effect. The fourth description was thus made on this material which had been subcultured for two weeks. A second subculture was attempted using four 25 mm sprigs from each site. In this case material from different sites was cultured together rather than individually in order to allow cross contamination of those epiphytic algae that could not be completely eradicated by washing.

The main morphological parameters that were used were internode length, leaf length and leaf width. Anatomical features such as anthocyanin content in the nodal cells, chloroplast form, size of leaf cells and development of larvae were also studied.

2.4 Analysis of Environmental Parameters

2.41 Water chemistry and tissue extraction

Collection and storage of water samples

Each time one of the four sites was visited a water sample was collected. The containers used were screw top polythene bottles which were filled to maximum capacity and brought back to the laboratory where the samples were filtered through a sintered glass funnel. The samples were then stored in a cold store (temperature 9^{o}) until needed for analysis.

In the case of the last sample collected from each site, a slightly different procedure was used in order that phosphate analysis could be carried out. Using the method of Heron (1962) the polythene bottles were first impregnated with iodine by placing a few crystals of solid iodine in the bottle and heating the sealed bottle overnight at a temperature of 60^{o}C. Bottles treated in this way and subsequently well washed minimise bacterial growth. When the water samples were collected they were filtered in the field and on being brought back to the laboratory they were placed immediately in the deep freeze where they were stored until needed for analysis.

Preparation of Digested samples

Samples of Elodea canadensis including roots were collected from each site on 1.VII.70 and brought back to the laboratory where they were thoroughly washed, ground up and mixed. Four subsamples were taken from the material from each site and these were dried at 105^{o}C for twenty-four hours and cooled in a desiccator so that the dry weights could be obtained. Each of the sixteen samples was then treated in the following manner in order to bring the material into solution. The method employed is that outlined by Jefferies and Willis (1964) with the only difference being that double the amount

of hydrochloric acid was used.

Each sample was placed in a labelled 250 ml conical flask to which was added 5 ml conc. perchloric acid, 10 ml conc. hydrochloric acid and 20 ml conc. nitric acid. The flasks were heated on a sand tray so that the vegetative material could be digested. All the excess acid was boiled off so that only a little perchloric remained. When there was no residue left and the liquid was clear, each sample was filtered into a 250 ml volumetric flask, the filter paper of which had previously been soaked in 10% perchloric acid in order to remove trace elements. The process of filtration removes any silica. The flasks were then made up to the mark with distilled water and the contents poured into polythene bottles where they were stored until ready for analysis.

Analysis for sodium and potassium

The analysis of sodium and potassium was carried out spectrophotometrically using the EEL flame photometer (Evans Electroselenium Ltd.). The principle of the apparatus is based on the fact that a solution containing sodium and potassium when introduced as a fine spray into a flame will emit light of characteristic wavelength (K^+ : 769 mμ; Na^+ : 589 mμ). The light emitted by these elements can be separated by passage through suitable filters and measured by means of a photocell and galvanometer. The intensity of light of appropriate wavelength is then related to the concentration of the element emitting it (Mackereth 1963).

Before the anlaysis was carried, stock solutions containing 1 mg/ml Na^+ and 1 mg/ml K^+ were prepared. 2.542 g. of NaCl in a litre of water were used for the former and 1.907 g. of KCl in a litre of water for the latter. From these solutions a series of standards were prepared containing from 0 to 100 mg/l. Dealing first with sodium and then with potassium the scale on the machine was set correcly at 0 (distilled water) and 100 mg/l. and the standards were

run through, the readings obtained being used to form a calibration curve. Readings were then obtained from each of the water samples and the digested samples and the mg/L were read off the 'x' axis of the calibration curve.

Analysis for Magnesium

The determination of magnesium was carried out using the EEL Atomic Absorption Spectrophotometer. The principle of this instrument is that it gives a measure of the light absorption by the element concerned (in this case magnesium) when it is excited by a flame. Light is provided by a hollow cathode lamp containing the relevant element.

A stock solution of magnesium oxide was prepared by dissolving 0.829 g of anhydrous magnesium oxide (AR grade) in 41.5 ml Normal hydrochloric acid. It was then made up to 500 ml with distilled water, this giving a concentration of 1000 mg/L. From this stock solution a series of standards were prepared containing from 0 to 60 mg/L magnesium. The machine was set up as explained in the EEl instruction sheet and the standards were run through in order to produce a calibration curve. The water samples and digested samples were then tested and, using the readings and the calibration curve, the mg/L obtained. For some of the very hard water samples it was necessary to dilute by a factor of two in order to obtain a reading that was on the scale.

Determination of Calcium

The analysis for calcium was carried out volumetrically using the method described in "Standard Methods" (American Public Health Association, 1965).

EDTA (Ethylene diaminetetracetic acid) is used as the titrant as it combines with calcium to form a complex that gives a good colour change when the right indicator is used. As magnesium

also combines with EDTA the pH is maintained at 12-13 in order that the magnesium is largely precipated as the hydroxide.

The following reagents were made up:-

(i) Standard calcium solution. 1 mg/ml calcium carbonate

(ii) EDTA titrant. 3.723 g. of the disodium salt dissolved in 1 L. of water.

(iii) Normal sodium hydroxide

(iv) Eriochrome Blue Black R. indicator

(v) Murexide indicator.

The accuracy of the EDTA was checked by first titrating against the standard calcium solution. The procedure for each titration was as follows:-

50 ml of the sample were pipetted into a conical flask to which was added sufficient sodium hydroxide to bring the pH up to 12 or 13. 0.2 g of the indicator were added and the mixture was then titrated against the EDTA in the burette. For the water samples eriochrome blue black R indicator was used which changes from red through purple to blue (without a trace of pink) at the end point. Murexide which was used as the indicator for the digested samples changed from pink to purple at the end point. Prior to the titrations 0.5 ml hydrochloric acid were added to the four water samples that had been deep frozen in order to bring the calcium carbonate back into solution.

Analysis for Iron

The determination of iron was carried out using the Hilger and Watts AA_2 Atomic Absorption Spectrophotometer. The apparatus was set up as explained in the instruction booklet. A series of standards of 0, 1, 2, 5, 10 and 25 mg/L of iron were set up and were used to form a calibration curve. Analysis was then carried out on the digested samples. Due to only micro amounts of iron being present

in the water samples and the insensitivity of the machine analysis could not be done on the water samples.

Determination of Phosphate

The analysis for phosphate was carried out colorimetrically using the molybdenum blue method described by Murphy and Riley (1962).

The method is based on the fact that an acidified solution of ammonium molybdate containing ascorbic acid and a small amount of antimony will react rapidly with phosphate ion yielding a blue-purple complex containing antimany and phospherous in a 1 : 1 atomic ratio.

A mixed reagent solution was prepared consisting of:-

125 ml N. Hydrochloric acid

37.4 ml ammonium molybdate

75 ml 0.1M ascorbic acid

12.5 ml potassium antimonyl tartrate.

A stock phosphate solution containing 0.1757 g potassium dihydrogen phosphate per litre was prepared. From this solution a series of standards were prepared ranging from 0 to 4 mg/L. Using first the standards and then the samples 40 ml of each were pipetted into a sulphuric acid washed test tube. 8 ml of mixed reagents were then added and the solutions were left to equilibrate for ten minutes. The optical density of each standard and sample was then tested using an EEL Flow-through colorimeter with the wavelength set at 700 mμ.

A calibration curve was drawn up from the readings obtained with the standards and from this the mg/L were obtained for each of the water samples which had been correctly collected and stored for phosphate analysis.

Determination of % dry weight

2 samples of Elodea from each site were used in the determination of the percentage dry weight. The method employed was that described in section 2.22 (Standing Crop Measurements).

Determination of percentage Ash (Inorganic) content

A sample of Elodea of known dry weight from each site was heated in a muffle furnace for 48 hours (i.e. to constant weight) at a temperature of 400°C. Each residue, after it had been cooled in a desiccator, was then weighed.

2.42 pH

During the course of the summer field pH measurements were taken using a portable field pH metre which had been calibrated with a series of buffer solutions.

The ability of Elodea to produce an alkaline reaction was tested in the laboratory. Into four specimen jars containing 250 ml top water were put three 5 cm apical sprigs of Elodea, one jar being used for each site. At staggered time intervals three drops of phenol phthdein were added to each jar and they were placed in the sunlight. After half an hour the alkaline reaction had caused the phenol phthalein indicator to turn bright pink. The depth of colouration produced was tested using an EEL colorimeter with a green filter.

2.43 State of Bottom Mud

Before the tests on the bottom mud from each site could be carried out the "fine earth sample" had first to be obtained. A sample of mud from each site was brought back to the laboratory in a polythene bag where it was spread out on newspaper and left for 48 hours in a warm dry cellar in order to become air dry. The samples were then crushed with a pestle and mortar and passed through a 2mm mesh before being stored in glass jars.

Moisture content

For the analysis of the moisture content of each sample a clean dry crucible was weighed and then weighed again with the

addition of a small amount of air dry mud. The samples were dried in an oven for 24 hours at 105°C, cooled in a desiccator and the weight of the crucible and oven dry mud obtained.

Organic Component (Loss on Ignition)

An estimate of the organic component of each sample was obtained by igniting oven dry samples in a muffle furnace at 500°C for two hours and cooling in a desiccator. Three measurements were again taken, these being the weight of the crucible, the weight of the crucible and oven dry soil and the weight of the soil and inorganic component.

Mechanical Analysis of Mud samples. Bouyoucos Method

50 g. of fine earth sample from each site were weighed out and shaken with 400 ml distilled water and 10 ml sodium hydroxide in a milk bottle overnight. The following morning the samples were transferred to one litre measuring cylinders, and were made up to a litre with distilled water.

At staggered time intervals for each sample the following procedure was carried out:
At time zero the cylinder was shaken for one minute. It was then placed on the bench, a hydrometer was placed in the suspension and a few drops of ethyl alcohol were added to dissipate the froth. Readings on the hydrometer were taken 40 seconds, 4 minutes 48 seconds and two hours after shaking had finished (i.e. from time zero). A temperature reading was taken with the first and third hydrometer reading. The hydrometer measures the density of the suspension.

3. RESULTS

3.1 Growth Studies

3.11 Laboratory cultures

(a) Test Growth Experiment

Length, wet weight and number of internode measurements were obtained at half weekly intervals for fifteen replicates of Elodea from each site, five each of 1 litre, 500 ml and specimen jars being used for each set of material. Possible 'bottle artefacts' are ignored due to the equal distribution of different types of flasks amongst the material from the different sites.

After three weeks culture the pieces of Elodea from Page Bank were becoming too big for the 500 ml flasks and the cultures were therefore terminated.

The two main problems that were met with during the course of the cultures were:-

(i) the problem of death at the cut end. This observation was also made by Brown (1913) in his cultures that neither contained soil nor had carbon dioxide bubbled through. As it was difficult to decide on the exact length of dead/dying material the whole length was included unless the dead material had become detached.

(ii) the problem of persistant epiphytic algae. Despite repeated washing of both the Elodea and the flasks it was found impossible to remove all of the epiphytes, members of the Cyanophyta and Bacillariophyta being particularly persistant. It is probable therefore that the results are somewhat distorted by these algal contaminants.

The table of raw data for the sixty cultures is shown in the appendix. The condensed data is presented in Table 1, with means, standard deviations and standard errors calculated for each parameter measured. The means are plotted graphically in Figures 4, 5 and 6.

All three sets of data show the growth rate to be greatest in the Page Bank Elodea and least in the Witton-le-Wear material with Bishop Auckland and Hell Kettles material having intermediate rates. Figure 4 showing increase in length displays a certain degree of tailing off after one to two weeks culture. This is due to the development of side branches in which much of the growth occurs which are not included in the measurements of overall length. Figure 5 gives a more realistic picture of the steady, almost linear rate of growth. Figure 6 reflects the general state of health of the pieces, Bishop Auckland and Page Bank material producing many new leaves and therefore internodes while in the case of material from Hell Kettles and Witton-le-Wear the rate of growth of new internodes is seen to decrease slightly.

'T' tests were carried out on the data to determine whether there were significant differences in the growth rates of the material from the different sites. The results of these tests (shown in Table 2) show that under the conditions of culture there are significant differences between measurements after three weeks culture in all pairs of sites except Hell Kettles and Witton-le-Wear, and Bishop Auckland and Page Bank which do not show significant differences.

Table 1. Test Growth Experiment. Condensed data

Hell Kettles		WEEKS 0	1	2	3
Length (mms)	Mean	50	73.1	87.6	89.3
	S.D.	-	5.9	8.9	2.2
	S.E.	-	1.5	2.3	5.6
Wet weight (grams)	Mean	0.1734	0.3087	0.4205	0.5559
	S.D.	0.0431	0.0552	0.0789	0.1755
	S.E.	0.0118	0.0142	0.0204	0.0453
Number of internodes	Mean	12.2	24.53	38.4	45.46
	S.D.	1.6	4.62	6.47	9.57
	S.E.	0.41	1.19	1.67	2.47

Bishop Auckland					
Length (mms)	Mean	50	89.3	102.3	109.5
	S.D.	-	8.8	14.9	20.5
	S.E.	-	2.3	3.8	5.4
Wet weight (grams)	Mean	0.2031	0.3602	0.5555	0.7591
	S.D.	0.015	0.0396	0.0531	0.1090
	S.E.	0.004	0.0102	0.0137	0.0281
Number of internodes	Mean	9.6	28.2	43.13	63.20
	S.D.	0.79	3.87	8.82	15.84
	S.E.	0.21	1.00	2.28	4.09

Table 1 (contd.) Test Growth Experiments. Condensed data

		WEEKS			
		0	1	2	3
Witton-le-Wear					
Length (mms)	Mean	50	72.5	79.5	79.6
	S.D.	-	6.2	11.8	14.8
	S.E.	-	1.6	3.1	3.4
Wet weight (grams)	Mean	0.1259	0.2124	0.3565	0.5055
	S.D.	0.0213	0.0381	0.0594	0.0627
	S.E.	0.0055	0.0102	0.0159	0.0167
Number of Internodes	Mean	13.6	27.28	36.85	41.35
	S.D.	1.78	3.58	4.05	8.3
	S.E.	0.46	0.95	1.08	2.22
Page Bank					
Length (mms)	Mean	50	87.5	111.3	121.8
	S.D.	-	10.6	11.1	19.4
	S.E.	-	2.7	3.3	5.0
Wet weight (grams)	Mean	0.1846	0.3624	0.5713	0.7909
	S.D.	0.0206	0.0420	0.0696	0.1256
	S.E.	0.0053	0.0108	0.0179	0.0324
Number of internodes	Mean	12.6	38.6	57.86	68.06
	S.D.	1.3	2.93	7.54	13.82
	S.E.	0.33	0.75	1.9	3.57

Table 2. Results of students 't' tests on condensed data of Test Growth Culture

LENGTH		WEEKS 1	2	3
Hell Kettles / Bishop Auckland	value of 't'	5.89	3.33	2.5
	probability	<.001	<.01	<.02
Hell Kettles / Witton-le-Wear		.2735	2.098	1.4
		n.s.	<.05	n.s.
Hell Kettles / Page Bank		4.6	5.8	4.32
		<.001	<.001	<.001
Bishop Auckland / Witton-le-Wear		5.996	4.53	4.48
		<.001	<.001	<.001
Bishop Auckland / Page Bank		.5074	1.788	1.67
		n.s.	<.1	n.s.
Witton-le-Wear / Page Bank		4.77	7.02	6.65
		<.001	<.001	<.001

Table 2 (Contd.) Results of students 't' tests on condensed data of Test Growth Culture

	WEEKS 0	1	2	3	
WEIGHT					
Hell Kettles / Bishop Auckland	2.38	2.94	5.49	3.8	value of 't'
	<.05	<.01	<.001	<.001	probability
Hell Kettles / Witton-le-Wear	3.648	5.507	2.475	1.0439	
	<.002	<.001	<.02	n.s.	
Hell Kettles / Page Bank	.8658	3.01	5.55	4.219	
	n.s.	<.01	<.001	<.001	
Bishop Auckland / Witton-le-Wear	11.35	10.24	9.48	7.75	
	<.001	<.001	<.001	<.001	
Bishop Auckland / Page Bank	2.786	.1480	.70	.7414	
	<.01	n.s.	n.s.	n.s.	
Witton-le-Wear / Page Bank	7.6	10.09	8.97	7.829	
	<.001	<.001	<.001	<.001	
INTERNODES					
Hell Kettles / Bishop Auckland	5.6	2.36	1.67	3.71	
	<.001	<.05	n.s.	<.001	
Hell Kettles / Witton-le-Wear	6.46	1.8	.779	1.85	
	<.001	n.s.	n.s.	n.s.	
Hell Kettles / Page Bank	.756	10.00	7.69	5.205	
	n.s.	<.001	<.001	<.001	
Bishop Auckland / Witton-le-Wear	7.9	.66	2.48	4.69	
	<.001	n.s.	<.02	<.001	
Bishop Auckland / Page Bank	7.72	8.32	4.96	.895	
	<.001	<.001	<.001	n.s.	
Witton-le-Wear / Page Bank	1.766	9.3	9.6	6.35	
	n.s.	<.001	<.001	<.001	

Figure 4. Test growth experiment. Increase in length of Elodea during three weeks culture.

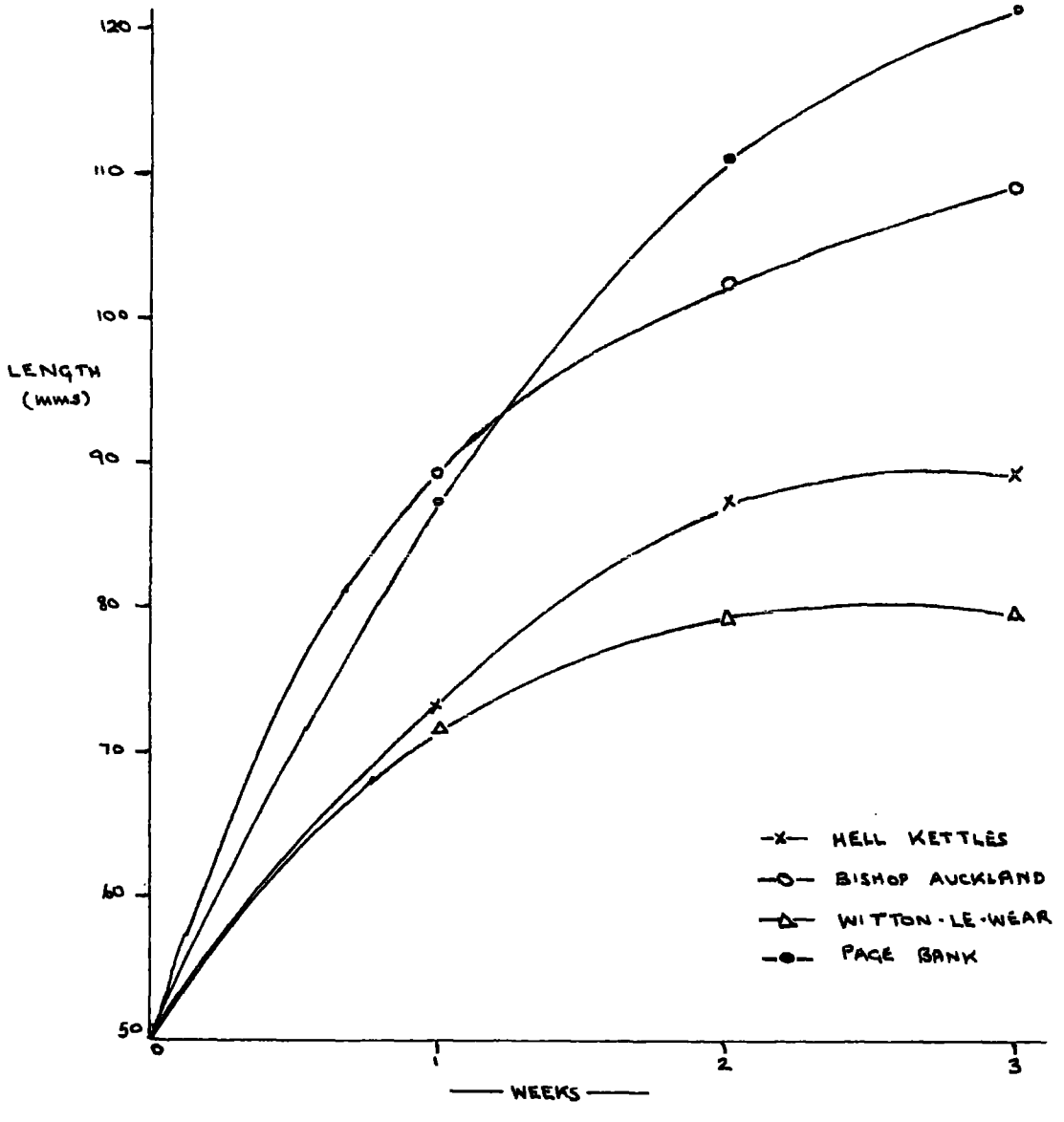


Figure 5. Test growth experiment. Increase in wet weight of Elodea during three weeks culture.

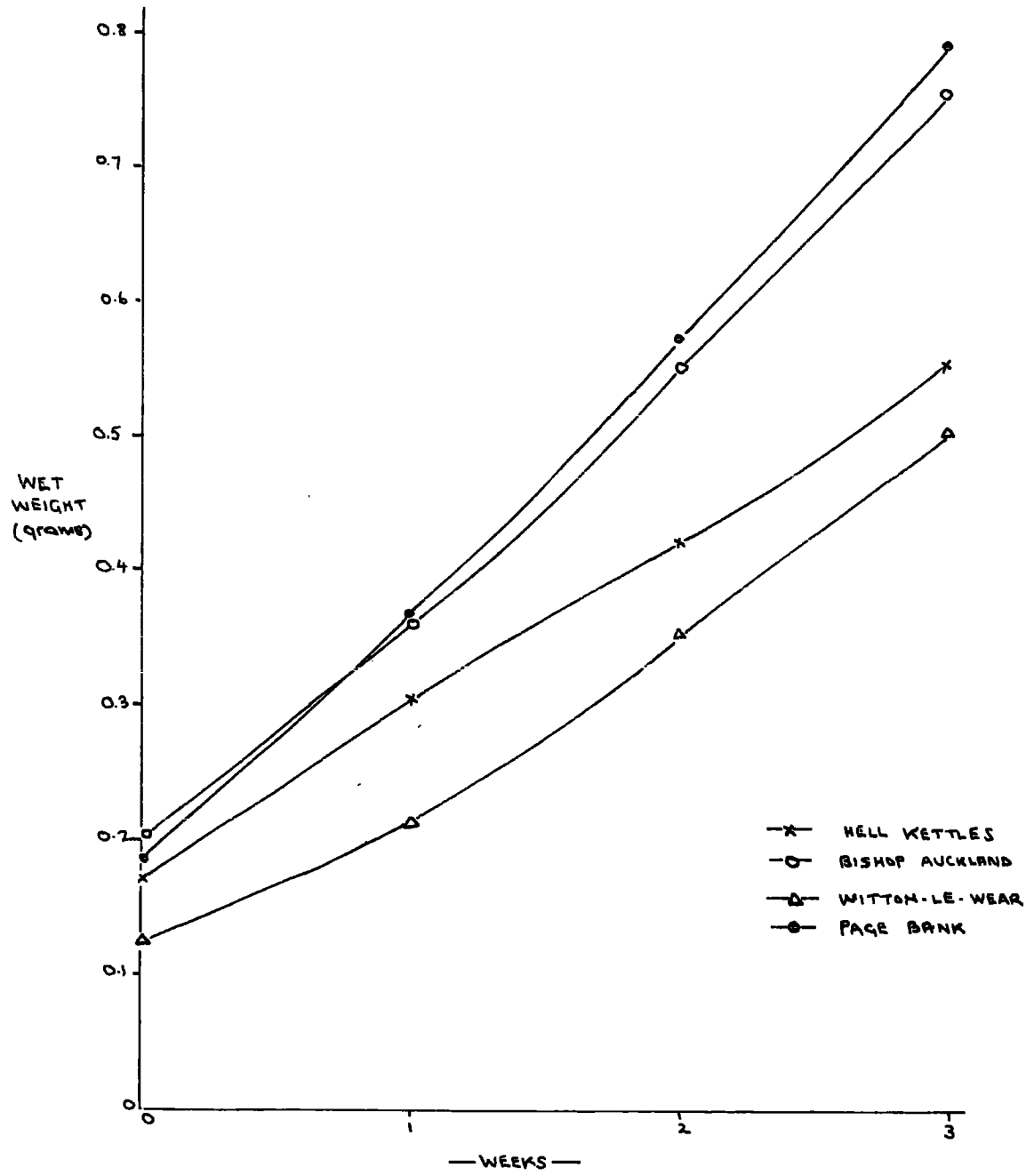
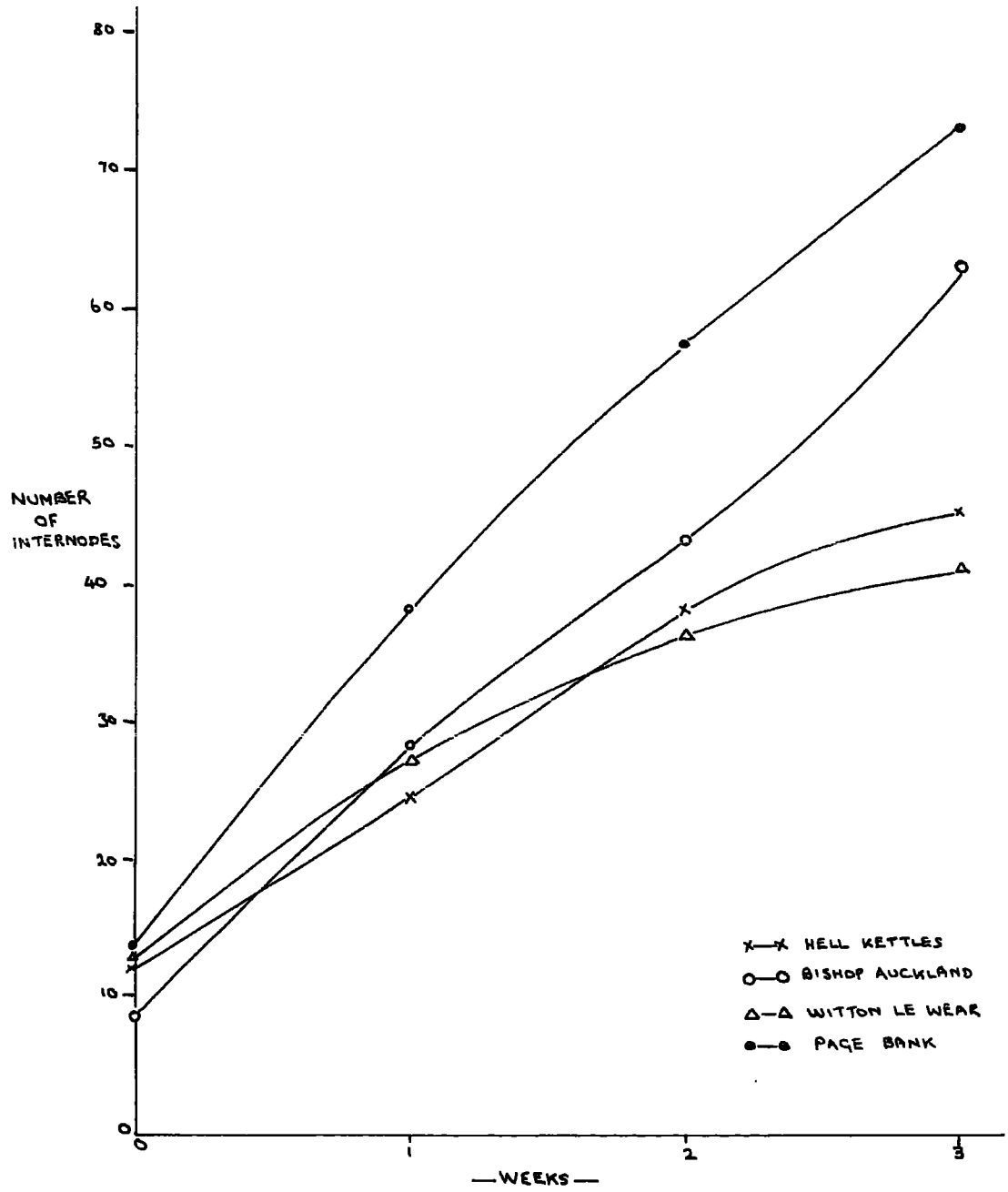


Figure 6. Test growth experiment. Increase in number of internodes during three weeks culture.



(b) Effect of Aerating flasks with pumping

In order to minimise the effect of problem (i) in section (a) above, eight flasks (two replicates from each site) were set up with air pumped into them as explained in Materials and Methods, Section 2.21. The cultures were maintained for one and a half weeks and then terminated, due to the excessive rates of growth that had occured. The results (presented in Table 3) show that comparable increases occured in the aerated flasks in one and a half weeks as occured in the initial cultures in three weeks. In terms of length, Page Bank material again grew fastest with Bishop Auckland, Hell Kettles and Witton-le-Wear material growing at successively slower rates. The graph (Figure 8) showing increase in weight can be explained as follows:- The material from Hell Kettles being study and robust did not loose this character in only one and a half weeks culture and therefore remained comparatively dense throughout the experiment. Page Bank material also weighed heavily at the end of the culture due to a genuinely fast rate of growth. Witton-le-Wear material grew well for the first two weeks but then algal contamination affected one of the pieces very severely and because there were only two replicates this had a marked effect on the mean. Bishop Auckland material is of a less dense growth form and possibly responds least well to the affect of aeration in culture flasks. Figure 9 showing the number of internodes, shows again that the phenotypic character of short internode length in the Page Bank and Hell Kettles material is maintained for the duration of the culture. There is a clear correlation between the number of internodes at the start and at the end of the culture.

Table 3. Effect of Aerating Flasks with Pumping

	Half week periods											
	0 3.VII.70			1 7.VII.70			2 11.VII.70			3 14.VII.70		
	Length mm	Weight g.	Internodes	Length mm	Weight g.	Internodes	Length mm	Weight g.	Internodes	Length mm	Weight g.	Internodes
Hell Kettles 1	50	0.3453	18	69	0.4594	28	93	0.7264	46	115	1.0083	53
Hell Kettles 2	50	0.2154	19	73	0.2884	29	101	0.6014	48	118	0.9594	67
Hell Kettles mean	50	0.2803	18.5	71	0.3740	28.5	97	0.6638	47	116.5	0.9788	60
Bishop Auckland 1	50	0.2513	14	72	0.2755	24	99	0.4889	40	115	0.6899	47
Bishop Auckland 2	50	0.1833	12	78	0.3301	21	105	0.6423	35	120	0.7379	51
Bishop Auckland mean	50	0.2173	13	75	0.3028	22.5	102	0.5655	37.5	117.5	0.7139	49
Witton-le-Wear 1	50	0.2487	13	78	0.4764	23	109	1.0886	49	118	1.0476	60
Witton-le-Wear 2	50	0.1360	11	64	0.2584	20	79	0.4961	27	85	0.6527	37
Witton-le-Wear mean	50	0.2023	12	71	0.3674	21.5	94	0.7923	38	101.5	0.8501	48
Page Bank 1	50	0.1167	29	85	0.2523	40	125	0.6020	57	144	0.8770	63
Page Bank 2	50	0.2157	30	85	0.2682	40	115	0.5874	55	141	0.9450	76
Page Bank mean	50	0.1912	29.5	85	0.2602	40	120	0.5947	56	142.5	0.9110	69.5

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Figure 7. Aerated cultures. Increase in length of Elodea during one and a half weeks culture.

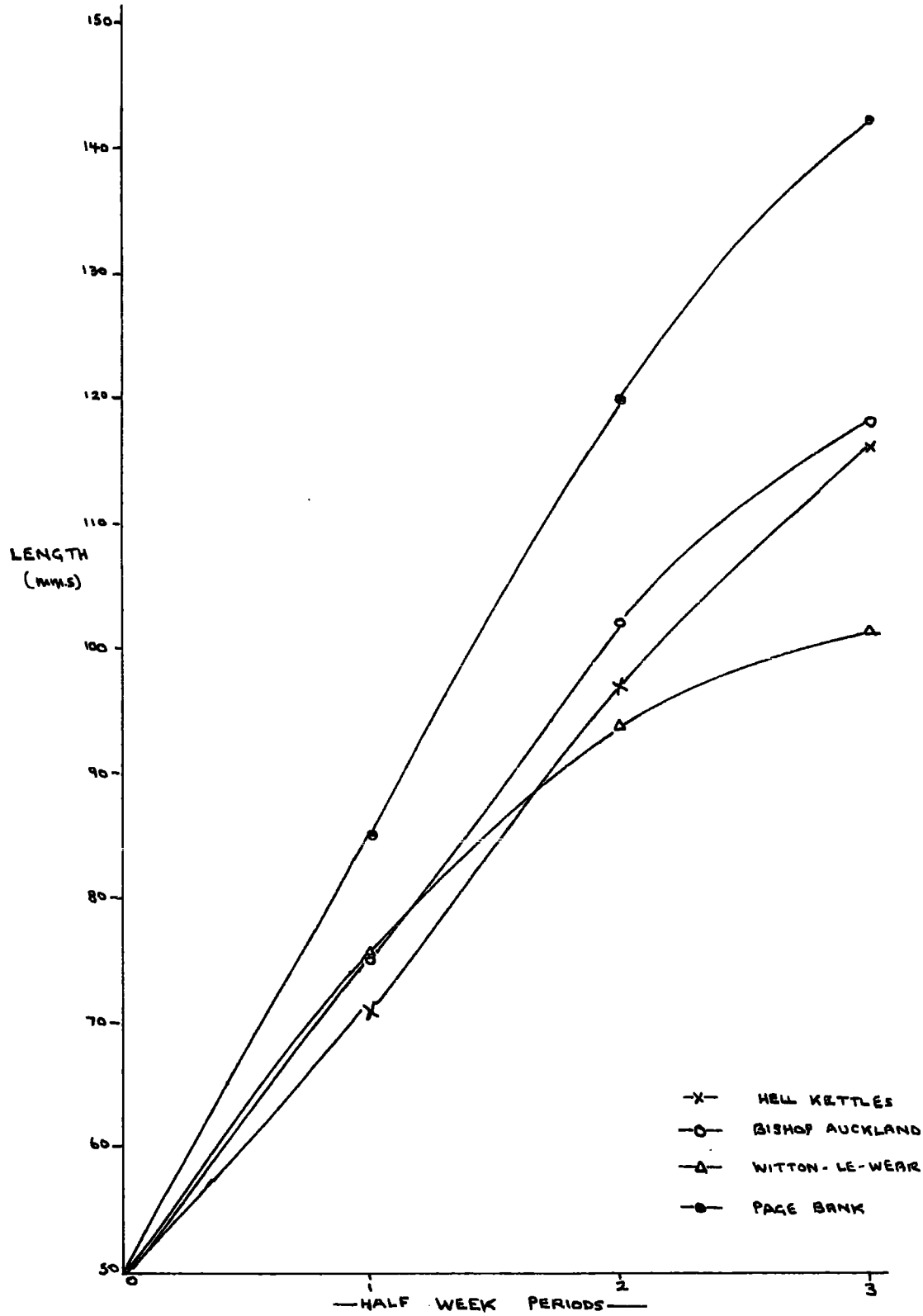


Figure 8. Aerated cultures. Increase in wet weight of Elodea during one and a half weeks culture.

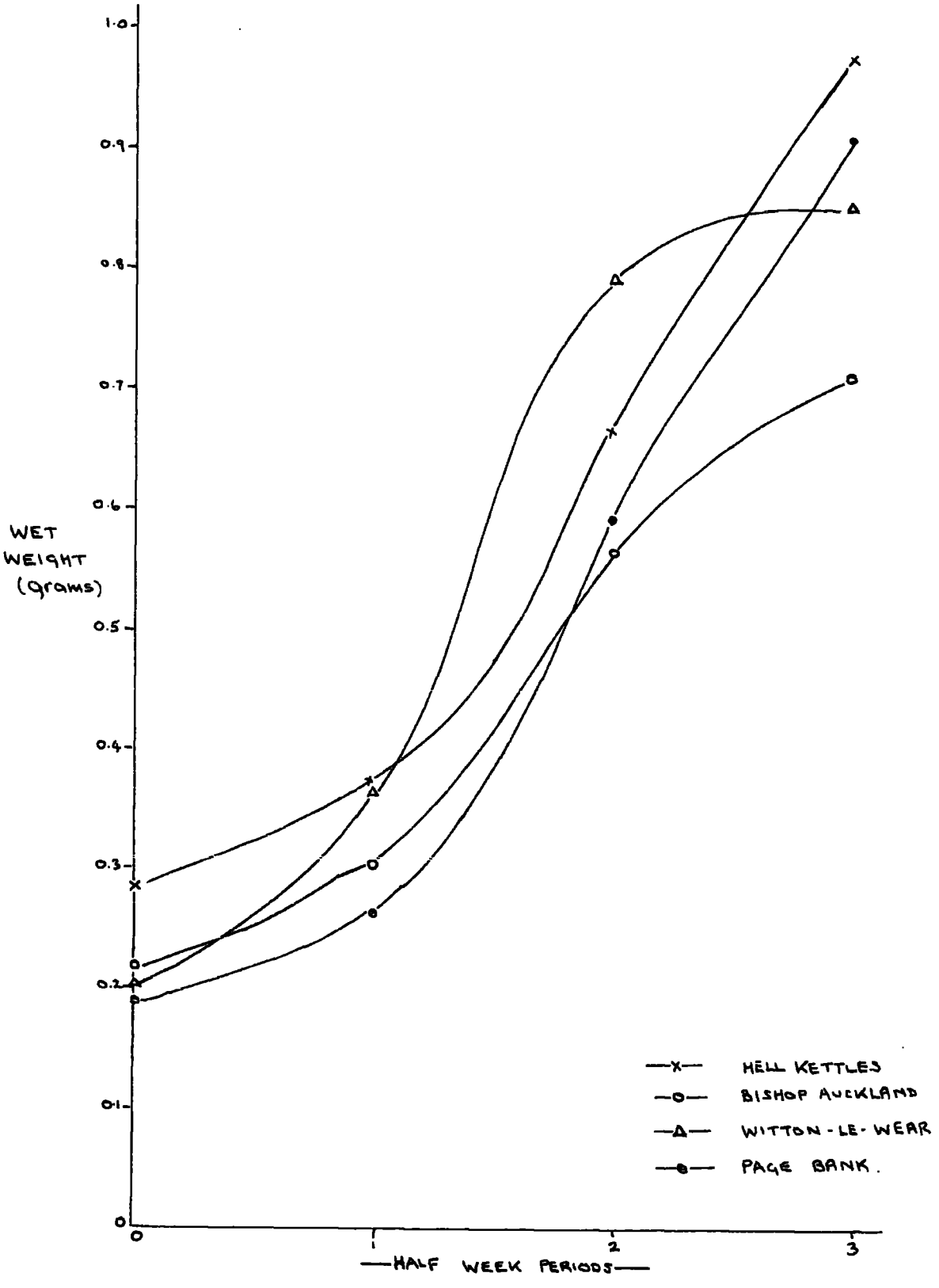
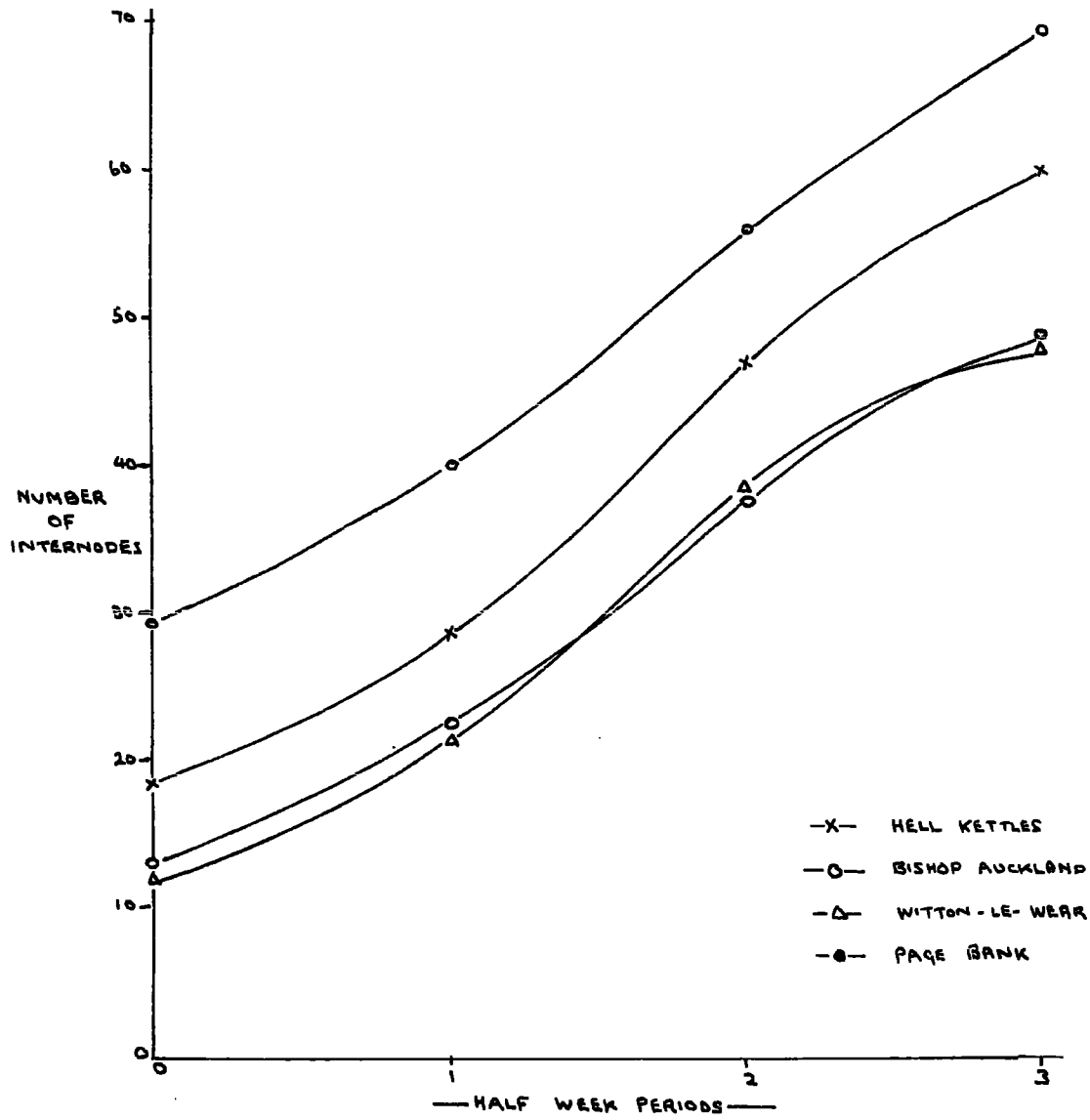


Figure 9. Aerated cultures. Increase in number of internodes during one and a half weeks culture.



(c) Effect of altering the concentration of iron in the culture medium

Eight 40 mm sample pieces of Elodea from each site were grown in Chu 10 medium in which the Fe.EDTA leval was modified to give a range in concentration of iron from 0 to 4 mg/L.

The cultures were grown in specimen jars for a period of two weeks and measurements were taken at the start and finish of the experiment. The data is presented in Table 4. Figures 10, 11 and 12 show the increase in overall length, wet weight and number of internodes in each piece of Elodea over the two weeks, in relation to the Fe.EDTA concentration. The pattern of the graphs shows that maximum growth generally occurs at the lower levels of Fe.EDTA (i.e. 0.3, 0.4 and 1 mg/l.) Growth is slower where no Fe.EDTA is contained in the culture medium and higher levels are also apparently inhibitory. The weight measurements for Witton-le-Wear show clearly the overall trend.

The graphs also show that the Page Bank material is least inhibited by the higher levels of Fe.EDTA. Were the means taken between the different sites, a smoother curve would occur. The variation that is seen in the graphs is due to

(i) A lack of replicates, which, if feasible, would have smoothed out such irregularities.

(ii) A probable lack of uniformity in the innocula.

The experiment does show however, that the iron concentration does affect the rate of growth of Elodea and that the theory of Olsen (1954) that iron is a critical micronutrient for the growth of Elodea in the field has at least some bearing on the growth of the species in culture. Had the cultures been maintained for longer, a more marked 'running down' would probably have been observed in those cultures in which the element was completely lacking from the medium.

Table 4. Effect of iron concentration on growth

Cultures		Start 11.VII.70 Length (mms)	Wet weight (g.)	Internodes	Finish 25.VII.70 Length (mms)	Wet weight (g.)	Internodes
Hell Kettles	1	30	0.1432	11	65	0.2967	32
	2	30	0.1451	12	46	0.3476	37
	3	30	0.0908	16	45	0.2184	23
	4	30	0.1330	14	60	0.2741	30
	5	30	0.1389	11	38	0.2693	20
	6	30	0.1003	10	33	0.2529	20
	7	30	0.1395	11	28	0.1749	13
	8	30	0.1472	11	33	0.2385	8
Bishop Auckland	1	30	0.0725	4	29	0.1348	8
	2	30	0.1355	5	48	0.2710	9
	3	30	0.0870	5	57	0.3133	16
	4	30	0.0833	4	45	0.2431	11
	5	30	0.1240	5	29	0.1537	6
	6	30	0.0976	5	30	0.1376	6
	7	30	0.1045	5	44	0.2029	19
	8	30	0.1284	5	28	0.2044	9
Witton le-Wear	1	30	0.0831	6	39	0.2371	21
	2	30	0.1208	8	72	0.3785	28
	3	30	0.0817	6	78	0.3785	33
	4	30	0.1034	9	65	0.3423	25
	5	30	0.0796	9	69	0.2791	28
	6	30	0.0618	9	70	0.2265	27
	7	30	0.0641	7	49	0.1575	20
	8	30	0.0904	6	42	0.1450	10
Page Bank	1	30	0.0810	10	75	0.2528	30
	2	30	0.0924	11	100	0.3644	35
	3	30	0.0883	13	94	0.2818	30
	4	30	0.0736	11	86	0.2527	32
	5	30	0.0849	11	90	0.3050	37
	6	30	0.0956	12	82	0.2706	34
	7	30	0.0623	10	75	0.1966	25
	8	30	0.1032	16	72	0.2311	36

Figure 10. Increment of growth during two weeks culture in Fe.EDTA levels of 0 to 2 ml per 1. culture solution. Increase in length.

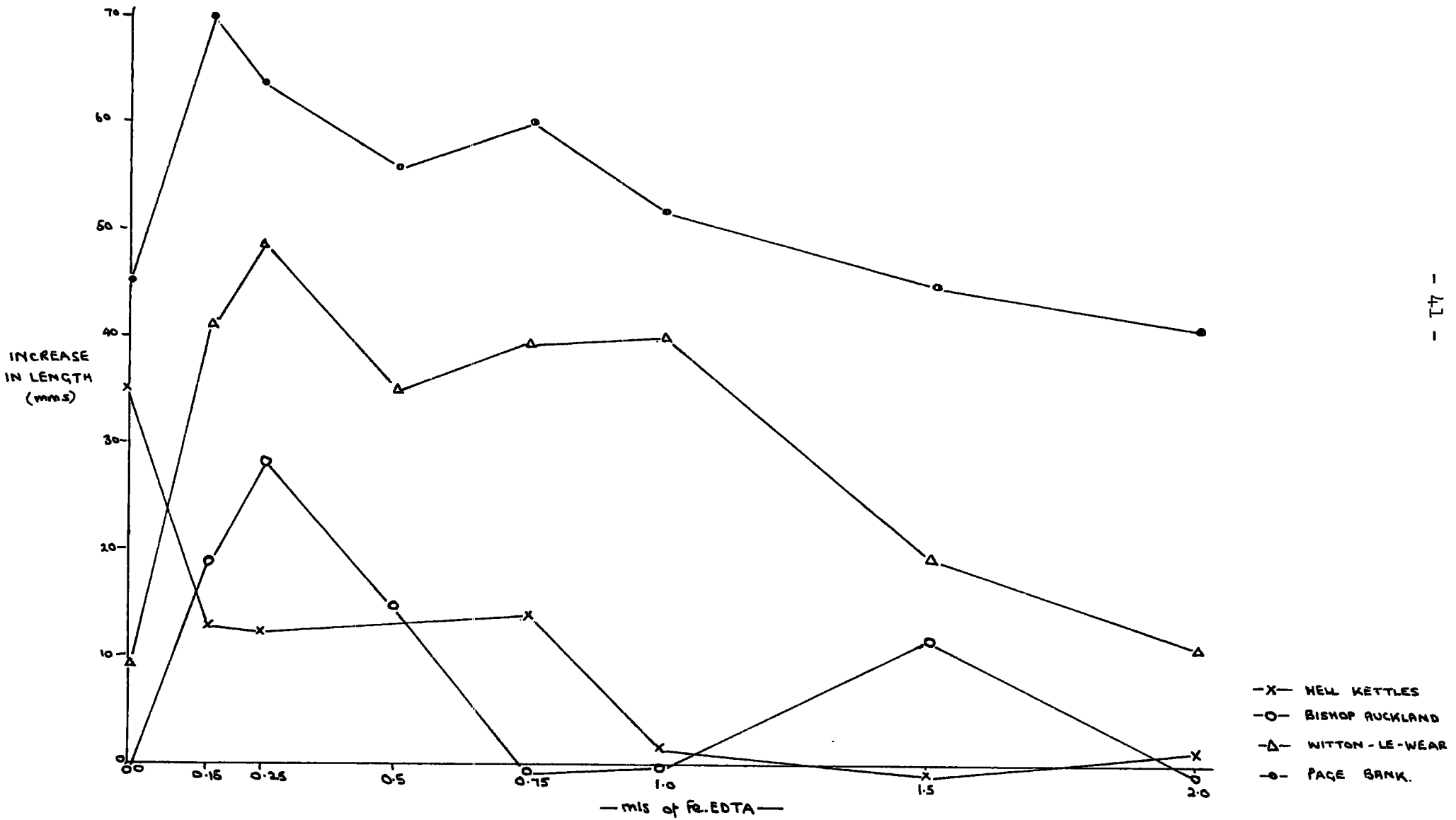


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Figure 11. Increment of growth during two weeks culture in Fe.EDTA levels of 0 to 2 ml per 1. culture solution. Increase in wet weight.

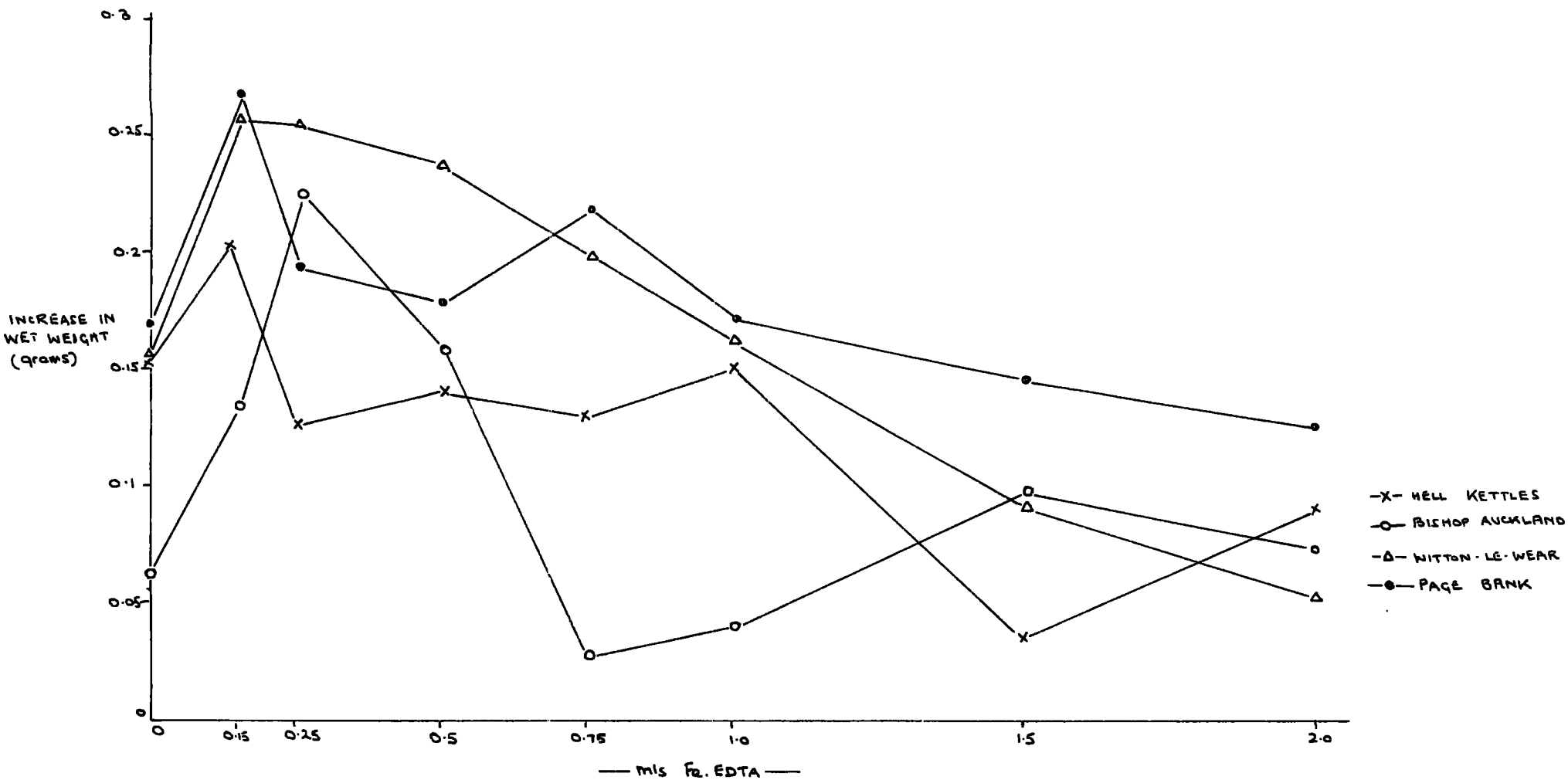

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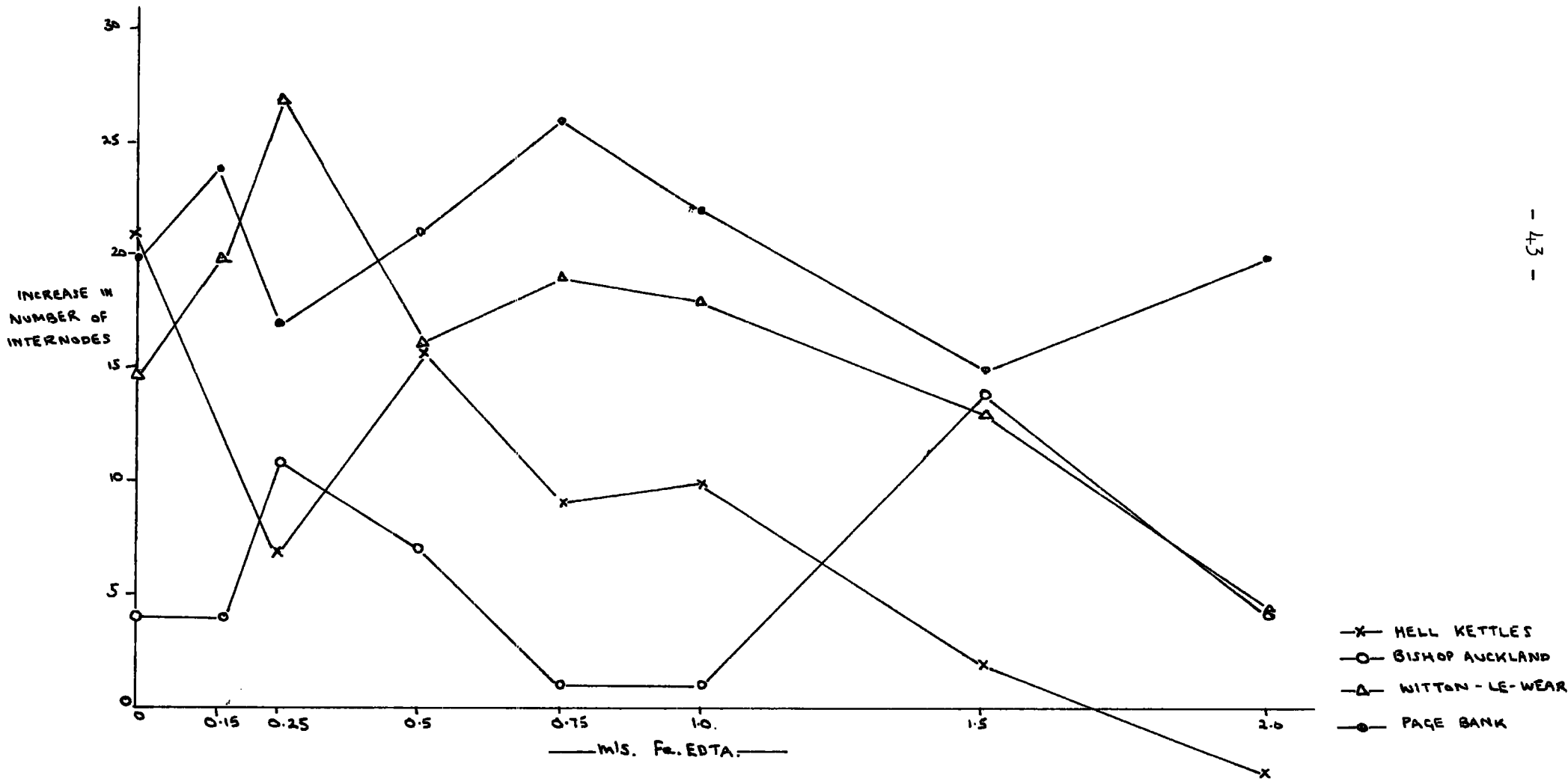
Figure 12. Increment of growth during two weeks culture in Fe.EDTA levels of 0 to 2 ml per l. culture solution. Increase in number of internodes.



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(d) Regeneration of small fragments

A range of Elodea pieces of 5, 10, 15, 20 and 25 mm in length were cultured individually in Chu 10 medium contained in 250 ml flasks which were kept in a tank (see Materials and Methods, Section 2.21) where shaking was continuous. The sprigs used were apical portions from material that had been cultured in the aeration experiment mentioned in (b). The cultures were maintained for one and a half weeks and length and wet weight measurements were taken twice weekly. The results show that

(i) shaking has a marked effect on growth causing an even greater growth rate than occured in the flasks aerated by pumping. As occured in the other aerated cultures however, the Bishop Auckland material was seen to respond least well to this condition.

(ii) even small pieces of Elodea of 5 mm in length are able to regenerate freely under conditions of culture. This finding relates to the situation in the field as it shows that in all likelihood similar pieces that become fragmented under natural conditions are able to form new plants. Although the actual increment of growth in the one and a half weeks is an average smaller than in the larger pieces, with the exception of the Bishop Auckland 5 mm portion, the percentage increase inlength over the original is considerably greater.

(iii) Again with the exception of the Bishop Auckland 5mm anomaly, the lengths regenerated from the smaller pieces have a smaller length over weight ratio than the larger pieces.

The table of raw data is shown in Table 5 and the findings in (ii) and (iii) above are shown in Table 6.

Table 5. Regeneration of small Fragments. Raw data

		Half week periods							
		14.VII.70		17.VII.70		21.VII.70		25.VII.70	
		Length (mms)	wet weight (grams)	Length (mms)	wet weight (grams)	Length (mms)	wet weight (gms)	Length (mms)	wet weight (grams)
Hell Kettles	1	5	0.0324	8	0.0300	23	0.0675	43	0.2171
	2	10	0.0391	14	0.0706	35	0.1658	71	0.3575
	3	15	0.0798	19	0.0964	40	0.1781	49	0.2900
	4	20	0.1043	27	0.1396	60	0.3548	80	0.7037
	5	25	0.2100	35	0.3100	64	0.5686	73	0.7563
Bishop Auckland	1	5	0.0406	9	0.0434	12	0.1037	12	0.1752
	2	10	0.0328	13	0.0562	27	0.1593	45	0.2921
	3	15	0.0685	19	0.0812	26	0.1141	28	0.2327
	4	20	0.0962	25	0.1387	41	0.3120	45	0.5170
	5	25	0.1631	32	0.2875	42	0.3641	52	0.5467
Witton le-Wear	1	5	0.0097	7	0.0174	16	0.0538	33	0.1247
	2	10	0.0533	18	0.0809	39	0.1883	57	0.2891
	3	15	0.0820	22	0.1228	55	0.2567	70	0.3970
	4	20	0.0953	27	0.1081	55	0.3109	67	0.5306
	5	25	0.1442	35	0.2610	67	0.4225	75	0.6461
Page Bank	1	5	0.0176	8	0.0239	15	0.0600	46	0.1839
	2	10	0.0307	15	0.0493	34	0.1249	51	0.2319
	3	15	0.0472	20	0.0678	45	0.1544	69	0.3140
	4	20	0.0969	28	0.1558	61	0.2364	77	0.3656
	5	25	0.1307	33	0.2084	67	0.2963	80	0.4494

Table 6. Regeneration of Small Fragments. Rates of growth.

Cultures		Increase in length in half weeks 0-3. (mm)	% increase in length over original	Length / weight ratio at end of culture
Hell	1	38	760	198.06
Kettles	2	61	610	198.6
	3	54	360	168.96
	4	60	300	113.68
	5	48	192	96.52
Bishop	1	7	140	68.49
Auckland	2	35	350	154.06
	3	13	86	120.32
	4	25	125	87.04
	5	27	108	95.16
Witton-	1	28	560	264.63
le-Wear	2	47	470	197.16
	3	55	366	176.32
	4	47	235	126.27
	5	50	200	116.08
Page	1	41	820	250.13
Bank	2	41	410	219.92
	3	54	360	219.74
	4	57	285	210.61
	5	55	220	178.02

3.12 Field Growth Studies

(a) Growth of Individuals

As a result of the measurement of tagged individuals (as explained in Materials and Methods, Section 2.22) Table 7 was drawn up showing the increase in length of *Elodea* plants growing at Bishop Auckland and Page Bank over, respectively, a five and six week period.

The results show that some of the tags were missing after varying lengths of time. While only one was missing at Page Bank, eventually three were missing at Bishop Auckland. This is presumably a result of both the more brittle nature of the Bishop Auckland phenotype which breaks readily and also the effect of current in the river site.

From the data obtained, the mean percentage increase in length divided by the original length was calculated for each week as the plants were of varying sizes at the start of the measurements.

The results, shown graphically in Figure 13, demonstrate that the growth rate is faster at Bishop Auckland than at Page Bank. In all likelihood this is at least partly due to the biotic factor, the effect of competition by other species having an inhibitory effect on growth and therefore affecting this phenotypic expression. Whereas the *Elodea* at Bishop Auckland grows in pure, though small, stands, at Page Bank, *Elodea* grows amongst *Ceratophyllum demersum* and *Myriophyllum spicatum* which are macrophytes of similar requirements to *Elodea*. Interspecific competition is therefore likely to occur.

For the part of the growing season in which growth was measured (28.V.70 to 8.VII.70) the increments of growth increase approximately linearly.

The weekly increments in growth were also calculated on a percentage increase basis for the cultured *Elodea* from Page Bank and

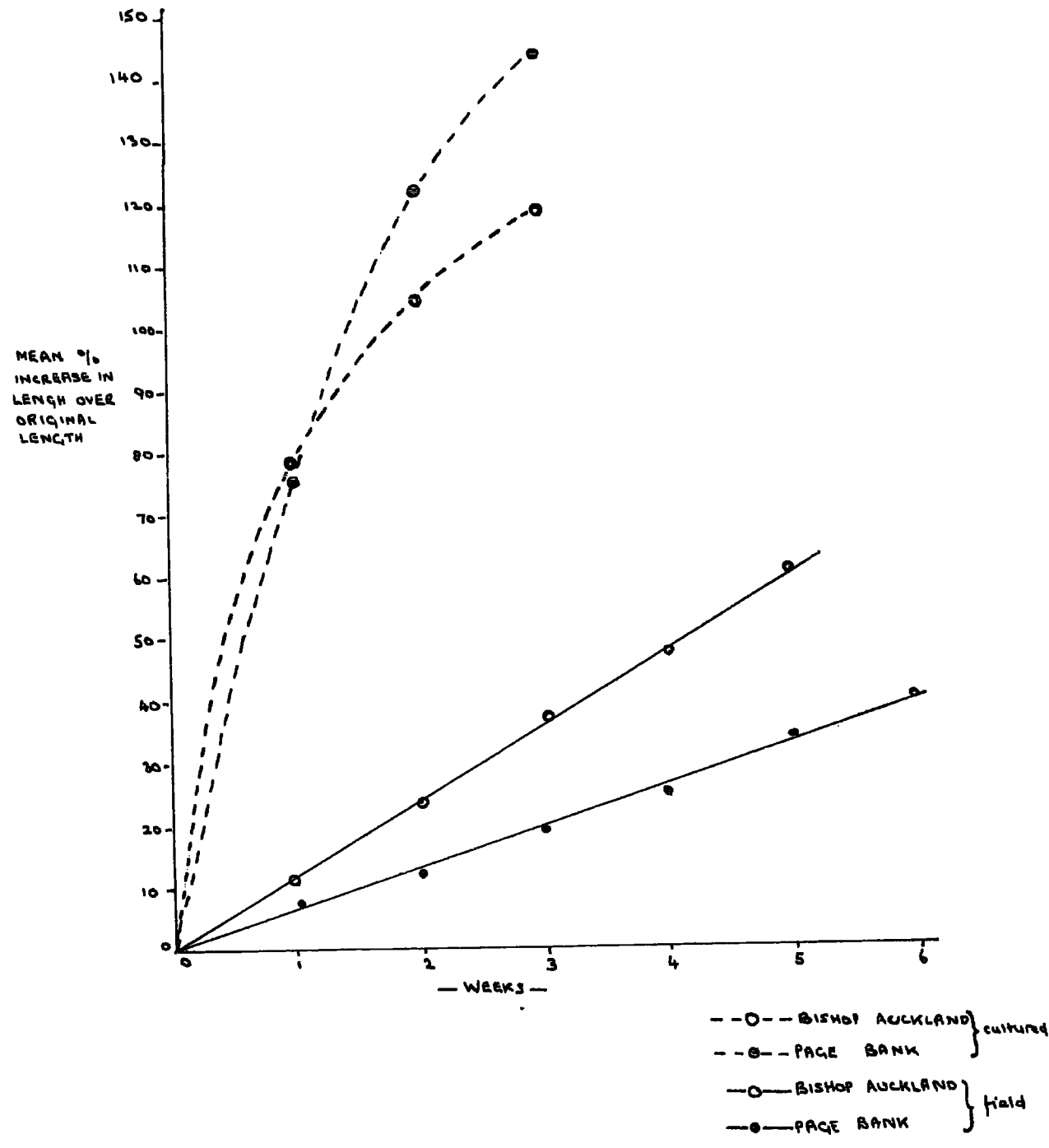
Table 7. Growth of individuals in the Field

(all measurements in mm's)

Elodea plants		May 28	June 4	June 11	June 17	June 24	July 2	July 8
Page	1	120	145	150	170	180	185	200
Bank	2	160	160	170	185	200	220	235
	3	140	155	160	170	190	190	200
	4	125	130	130	145	150	170	180
	5	100	110	115	120	130	140	150
	6	90	100	100	110	130	135	140
	7	150	150	160	175	175	190	200
	8	140	150	155	160	160	170	180
	9	150	165	170	170	180	200	200
	10	155	160	160	170	170	180	180
	11	170	missing					
	12	190	210	21½	22	22	22½	23
Mean % increase over original			7.95	11.77	18.81	24.08	33.57	39.09

Bishop	1		200	230	250	275	290	300
Auckland	2		210	230	260	280	295	320
	3		220	245	270	missing		
	4		220	240	270	290	305	330
	5		220	230	250	260	280	290
	6		130	155	180	210	230	250
	7		160	180	210	235	250	280
	8		150	165	190	220	245	260
	9		245	missing				
	10		170	180	200	220	230	250
	11		160	180	205	230	250	270
	12		260	280	290	310	340	missing
Mean % increase over original				10.66	23.78	37.59	47.02	60.07

Figure 13. Rate of growth of Elodea in the field as against cultured Elodea



Bishop Auckland grown in the Test Growth Experiment. It can be seen from the graph that the rates of growth in these cultured specimens are considerably greater.

(b) Increase in Standing Crop

The standing crop data obtained as explained in Materials and Methods, Section 2.22 is presented in tabular form in Table 8. For each week that samples were collected the mean standing crop (expressed as grams dry weight per half metre squared) was obtained.

By multiplying by a factor of four the grams dry weight per metre squared were obtained. The figures were then:-

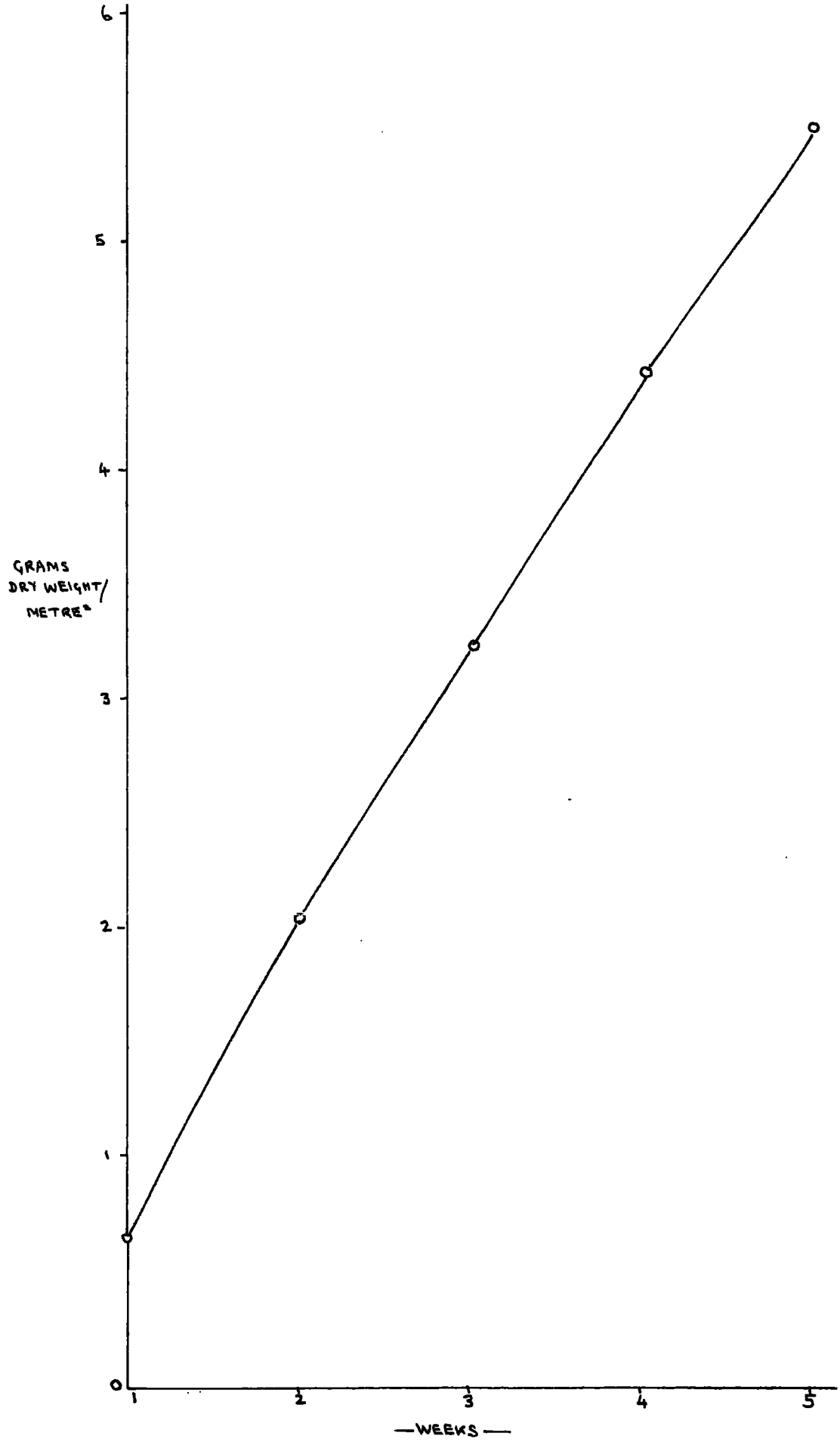
	grams dry weight/metre^2
Week 1	0.65
2	2.02
3	3.66
4	4.46
5	5.51

When plotted on a graph (Figure 14) an almost linear relationship is obtained showing a steady increase in standing crop over that part of the growing season. It seems apparent from the variation that occurs between samples within one given week however, that the graph is at least partly fortuitous and could not therefore be statistically verified. The low levels of standing crop that are observed are a reflection of (i) the high water content of Elodea and (ii) the sparseness of the species which is only subdominant to other macrophytes.

Table 7. Standing crop data expressed as Grams dry weight/half metre^2

Quadrats	1 4.VI.70	2 11.VI.70	3 18.VI.70	4 26.VI.70	5 2.VII.70
1_1			0.1078		
1_2					
1_3			0.1167		2.0497
1_4		0.1129			
2_1				-	
2_2		0.0603			
2_3	0.1705				
2_4					2.7474
3_1		-			
3_2					0.2031
3_3					0.0394
3_4				1.5428	
4_1			0.4390		
4_2	0.0289				
4_3	-				
4_4				0.0357	
5_1		2.5511			
5_2			0.4800		
5_3					2.6035
5_4		0.0478			
6_1					0.8929
6_2					0.1990
6_3	0.0877				
6_4			2.0990		
7_1			1.6586		
7_2		0.1233			
7_3			0.3977		
7_4	-				
8_1	0.0894				
8_2				2.0815	
8_3			1.2226		
8_4					2.2789
9_1				0.4799	
9_2		0.3945			
9_3				1.9376	
9_4		0.7508			
10_1	0.8825				
10_2				2.3858	
10_3	0.0357				
10_4				0.8269	

Figure 14. Increase in standing crop of Elodea at Page Bank during five weeks of the growing season.



3.2 Studies on Morphology

Recordings were made for various morphological characters using phenotypes collected on two different dates and sets of Elodea that had been cultured and subcultured as explained in Section 2.3 of Materials and Methods. An analysis of the material that had been twice subcultured was not possible as the material had largely died. Plate 3 shows the form of the earlier of the two phenotypes collected from each site and the form of the corresponding material that had subsequently been cultured and twice subcultured. Although the second set of material which had been in culture for about seven weeks is clearly very unhealthy there is nevertheless a similarity in form that is not apparent in the phenotypes displayed on the left hand side of the plate.

Briefly the phenotypes can be descriptively characterised as follows:

Hell Kettles Elodea, when growing under natural conditions, is olive green in colour. At the beginning of the season the internodes were relatively long but these become shorter as the season progressed. The leaves are wide and give the material a sturdy and robust appearance.

Elodea growing under natural conditions at Bishop Auckland, is leaf green in colour. The leaves are longer but less wide than at Hell Kettles. A feature of the material from this site is the length of the internodes which are appreciably longer. They are brittle and therefore the plant becomes fragmented relatively easily.

Witton-le-Wear material is dark green in colour; in form it has somewhat intermediate characteristics. The phenotype changed markedly between the two times of collection, the leaves being considerably larger in the case of the second phenotype described.

Page Bank material also changed through the season, the leaves becoming longer and narrower and aquiring a less lanceolate and

more strap-shaped or oblong form. Anthocyanin pigmentation of the nodes was very marked in the second phenotype examined and could be clearly seen when viewed macroscopically.

Within the limits of the experimental conditions under which the Elodea was cultured, most of the phenotypic identifities were lost. Internodes become shorter (this is presumably due to the high light intensity) the leaves became narrower and colours became more uniform. Measurements for anatomical features are not included in the table of results as they proved difficult to quantify and did not show the same degree of environmental plasticity. The figures quoted in the table for leaf length and width indicate the range that was observed in the material considered (four 50 mm pieces in each description). The length/width ratio was calculated using the means of the two outer limits for these characters. Figure 15 shows a series of polygonal graphs for each site, the values for leaf length, leaf width, leaf length/width ratio and length of internodes being plotted on different axes radiating from the centre points. The focalisation of form that occurs with culture is clearly apparent from the shape of the polygons.

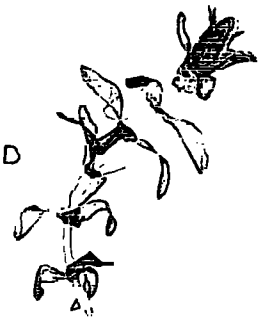
PLATE 3: MORPHOLOGICAL COMPARISONS

PHENOTYPE 2.

POST SUBCULTURE

HELL KETTLES

BISHOP AUCKLAND



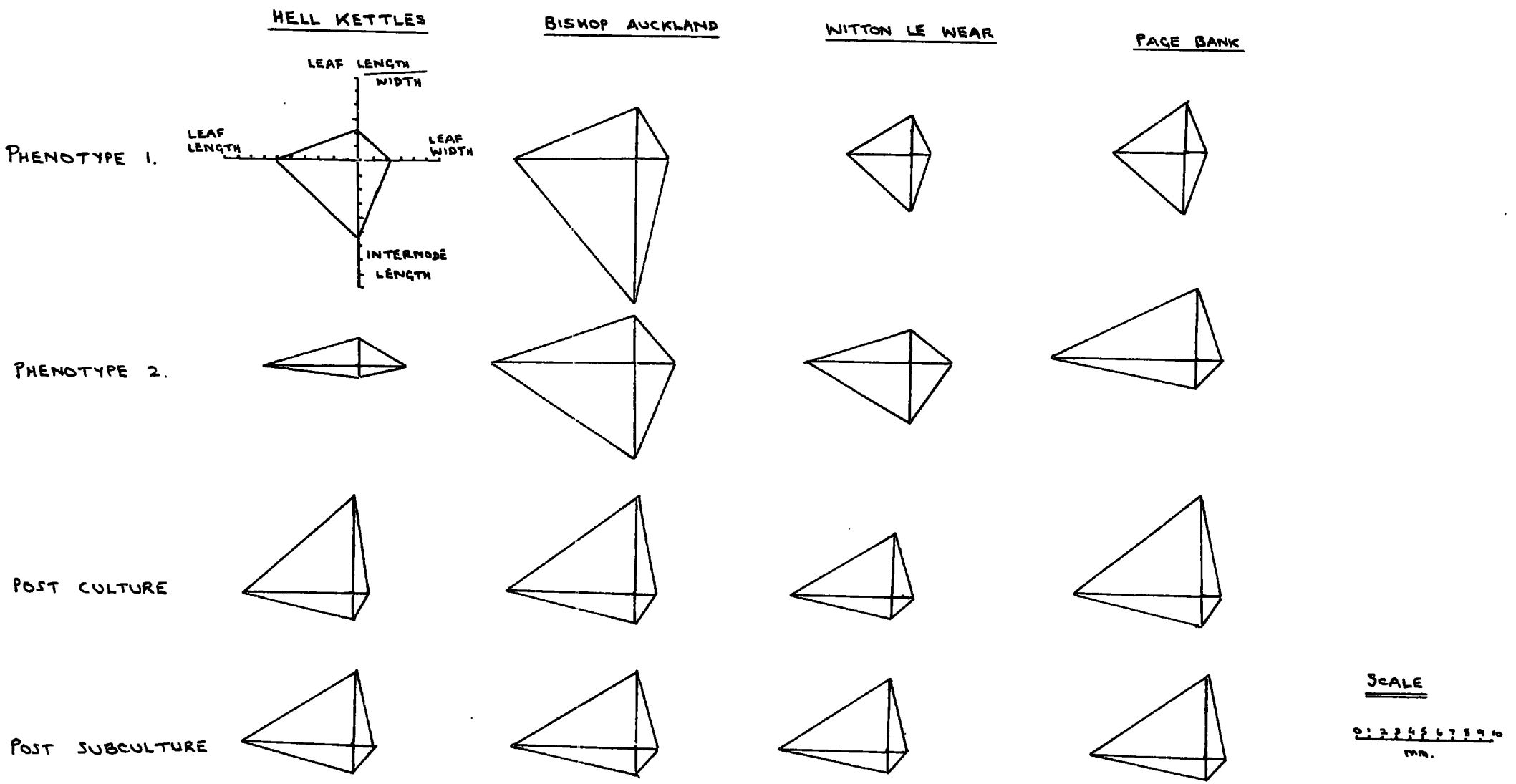
WITTON LE WEAR

PAGE BANK

Table 9. Morphological comparisons of quantifiable Characters

	Leaf length (mm)	Leaf width (mms)	Leaf length/ width	Internode Length (mms)
Hell Kettles				
Phenotype (1)	5-7	2-3	2.4	3-8
Phenotype (2)	6-8	3-4	2	0.5-0.75
Post culture	6-10	0.75-1.5	7.09	1-3
Post subculture	7-9	1-2	5.3	1-3
Bishop Auckland				
Phenotype (1)	8-10	2-3	3.6	5-16
Phenotype (2)	10-11	3	3.6	3-11
Post culture	8-11	1.25-1.75	7.0	1-3
Post subculture	8-10	1-2	6.0	1.5-2
Witton-le-Wear				
Phenotype (1)	4-5	1-2	3.0	2-6
Phenotype (2)	7-8	3	2.4	3-6
Post culture	7-8	1.25-1.75	5	1-2
Post subculture	7-9	1-2	5.3	1.5-2
Page Bank				
Phenotype (1)	5-6	1-2	3.75	2-7
Phenotype (2)	10-11	2	5.25	1-3
Post culture	8-11	1-1.5	7.6	1.5-2.5
Post subculture	8-10	1-2	6.00	1.5-2

Figure 15. Polygonal graphs showing morphological differences between Elodea phenotypes and cultured Elodea



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3.3 Analysis of Environmental Parameters

3.31 Water Chemistry

For each of the ions sodium, potassium, magnesium and phosphate readings were obtained for each water sample respectively, off the machines being used. From the calibration curves that were prepared the mg/l were read off the 'x' axis of each curve. Figure 16, serving as an example, shows th· · calibration curve that was prepared for sodium, its typically concave form being readily apparent.

In the case of calcium the following calculation was required for each sample:

Standard EDTA titrant (0.01M) is equivalent to0.4008 Mg.Ca per 1.0 ml

$$\text{Mg/l Ca} = \frac{\text{ml EDTA x to 1 ml EDTA (=1)} \quad \text{mg } CaCO_3 \text{ equivalent} \times 400.8}{\text{ml sample (=50)}}$$

The results of the analysis of the water samples are shown in Table 10. These show that Hell Kettles has a higher concentration of the four major cations, sodium, potassium, calcium and magnesium although Bishop Auckland, rather surprisingly, is the only site that shows appreciable amounts of phosphate (the only anion tested).

Hell Kettles has the highest concentration of sodium while the River Wear at Bishop Auckland has the lowest concentration. Witton-le-Wear and Page Bank, which contain intermediate levels, both display an increase in the amount of the ion as summer progresses.

Concentrations of potassium are about four times as low as those of sodium. Levels proportional to the sodium content are found at each site although Witton-le-Wear and Page Bank do not show the same increase with time as occured in the case of sodium.

The water at Hell Kettles which occurs in alluvium overlying the magnesium limestone is considerably harder than at the other sites having a total hardness of around 350 mg/l. The other three sites show calcium levels which compare with each other although Page Bank has considerably more magnesium than the other two sites. At Bishop

Figure 16. Calibration curve for sodium.

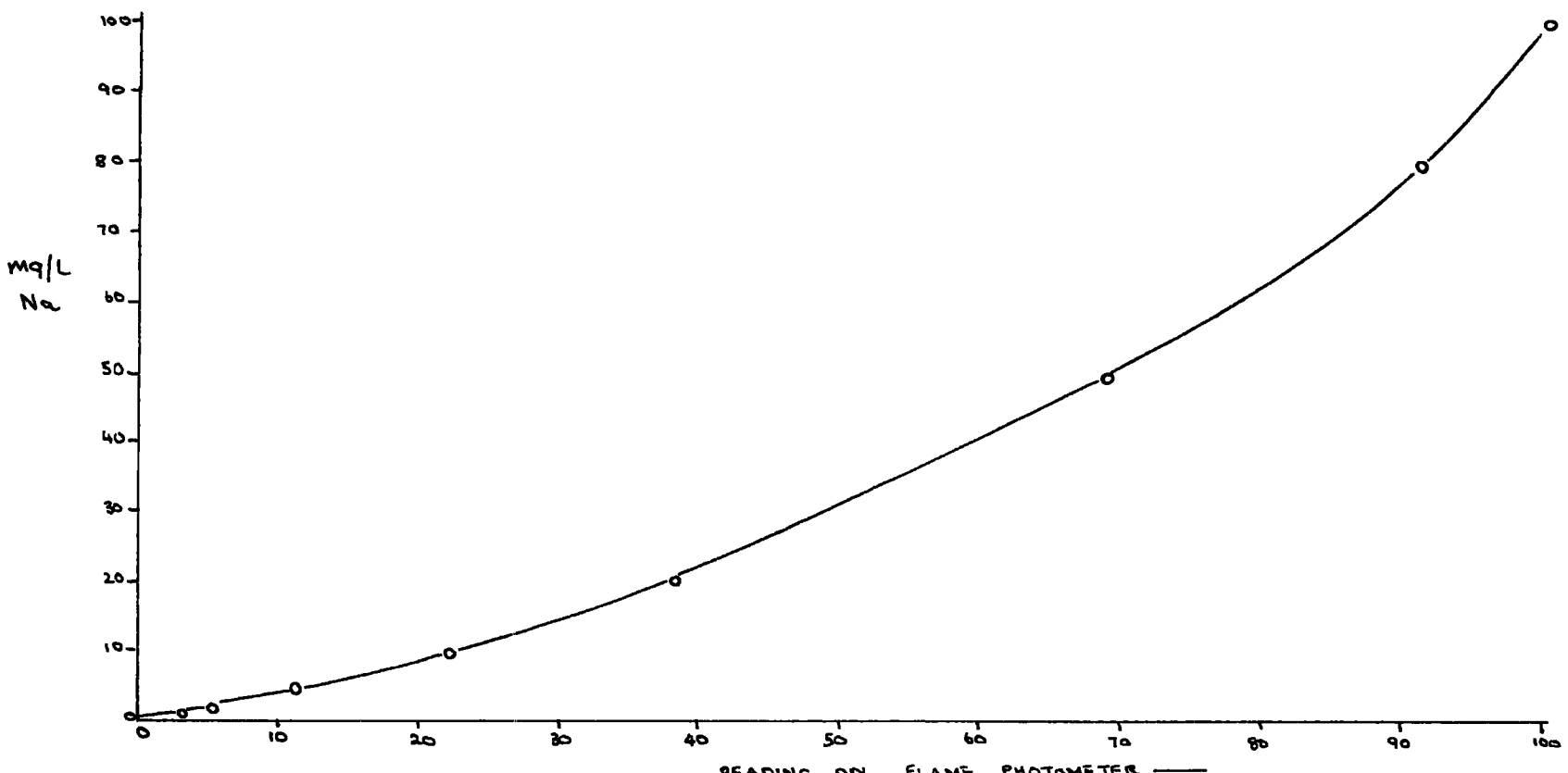

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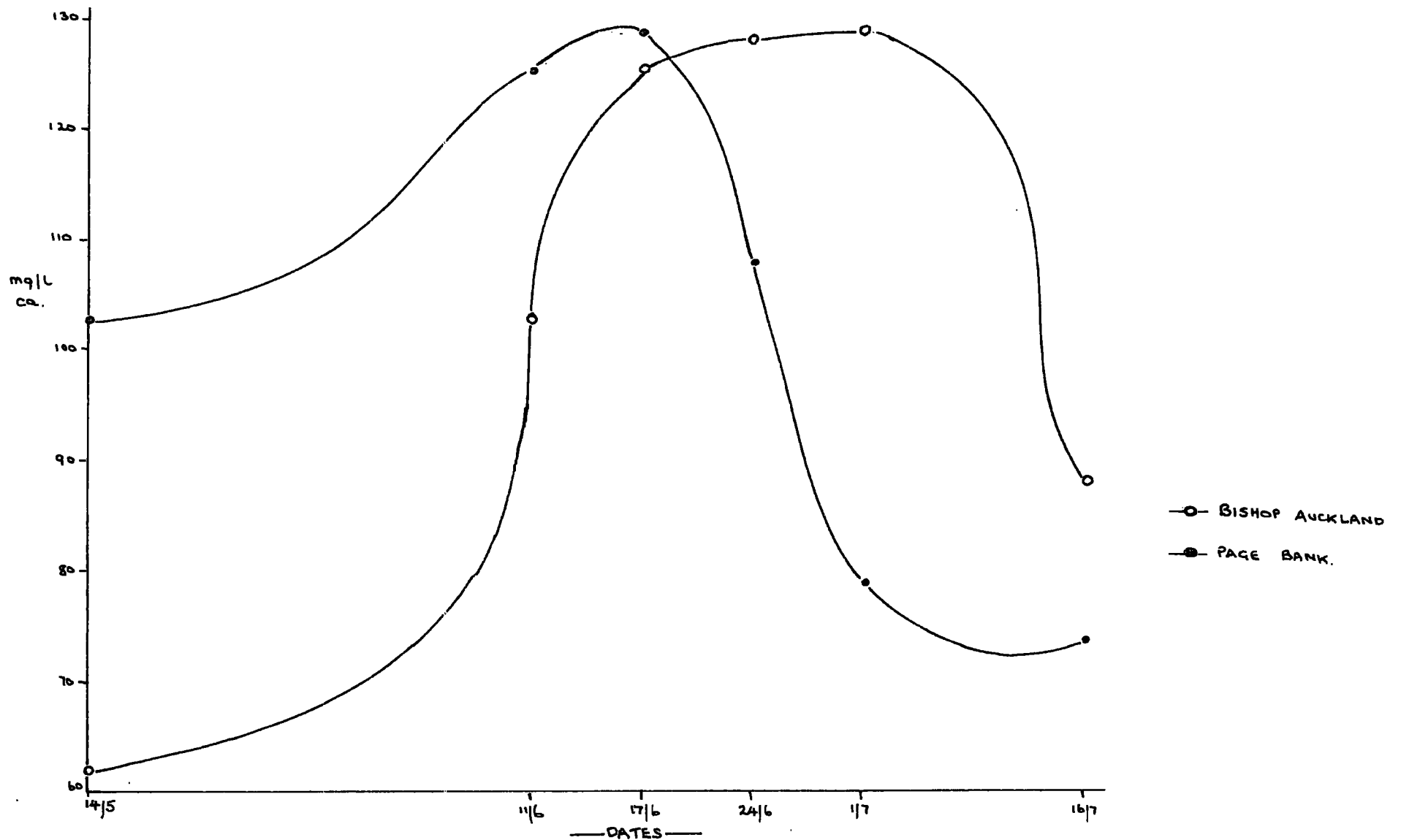
TABLE 10

Water Analysis mg/l

H.K. Double Kettle	Na^+	K^+	Ca^{++}	Mg^{++}	Total hardness (Ca^{++} + Mg^{++}) [mg/l]	PO_4
6. V.70	32	9	307	52	359	
4. VI.70	32	9.5	318	49	367	
1.VII.70	33	9.5	301	52	353	
15.VII.70	30	8	302	46.5	348.5	0.025
Bishop Auckland						
14. V.70	8	2	62	8.5	68.5	
11. VI.70	10.5	2.5	103	12	125	
17. VI.70	11	2.5	126	13	139	
24. VI.70	11	2.5	128	12	140	
1.VII.70	10	2.5	129	11	140	
15.VII.70	10	2.5	88	12	100	0.055
Witton-le-Wear						
14. V.70	14	3.5	103.4	19	122.4	
2. VI.70	14.5	2.5	73.7	19	92.7	
15.VII.70	17.5	2.5	76.9	22	98.9	0.015
Page Bank						
14. V.70	12	3.5	103	33	136	
11. VI.70	14	3.5	126	32	158	
17. VI.70	16	4	129	35.5	164.5	
24. VI.70	18	3.5	108	35.5	143.5	
2.VII.70	17.5	3.5	79	33	122	
16.VII.70	18	2	74	31	115	0.01

Figure 17. Changes in the levels of calcium in the water at Bishop Auckland and Page Bank between May and July.



Auckland and Page Bank, at which six samples were collected through the summer, there is a general concentration of dissolved solids during June. Figure 17 shows graphically the rise and fall in calcium concentration that occurs at the two sites.

Tissue Analysis

The mg/l of the four major cations and iron were obtained for each 250 ml subsample of digested material in the same manner as they were obtained for the water samples. To determine the percentage dry weight in each sample the following calculation was carried out:-

In 1 l. there are $\frac{mg/l}{1000}$ grams of ion

In 1 l. there are 4 x dry weight of sample (grams)

$$\text{proportion of ion} = \frac{\frac{mg/l}{1000}}{4 \times \text{dry weight}} = \frac{0.001 \text{ mg/l}}{4 \times \text{dry weight}}$$

$$\% \text{ dry weight of ion} = \frac{mg/l}{40 \times \text{dry weight}}$$

For the four subsamples from each site the mean, standard deviation, and standard error were calculated for each ion (see Table 11). To these statistics were applied 't' tests to discover whether there were significant differences between the amounts of the various elements in the digested Elodea from the four sites.

Calcium levels were particularly high in the material from Hell Kettles reflecting the hard water in which it grows. Levels were second highest at Bishop Auckland, with Witton-le-Wear and Page Bank displaying lower levels. Significant differences occured between the concentrations in all six combinations of sites.

In terms of magnesium, the Page Bank Elodea had a strikingly high content (mean = 0.88% dry weight). This was significantly different from the levels in the material from the other three sites ($p = <.001$) but there were no significant differences within the material from

those other sites.

With regard to the levels of iron in the Elodea there was no significant difference between the higher levels in the material from Witton-le-Wear and Page Bank and between the lower levels from Hell Kettles and Bishop Auckland. There were differences ($p = <.1$) however, between the other four combinations of sites.

TABLE 11

Analysis of Elodea collected 1-2.VII.70

Expressed as percentage dry weight

H.K. Double Kettle Sub samples	Na^+	K^+	Ca^{++}	Mg^{++}	Fe
1	0.51	2.02	5.67	0.45	0.51
2	0.51	2.52	5.90	0.57	0.14
3	0.37	2.25	5.24	0.41	0.18
4	0.47	2.39	5.79	0.47	0.16
Mean	0.47	2.3	5.65	0.47	0.25
S.D.	0.06	0.19	0.25	0.06	0.15
S.E.	0.03	0.09	0.12	0.03	0.07
Bishop Auckland					
1	0.24	2.62	4.6	0.44	0.14
2	0.36	3.17	5.2	0.43	0.14
3	0.14	2.44	4.47	0.42	0.14
4	0.33	1.49	4.34	0.33	0.11
Mean	0.27	2.43	4.65	0.41	0.13
S.D.	0.08	0.61	0.33	0.04	0.01
S.E.	0.04	0.3	0.16	0.02	0.006
Witton-le-Wear					
1	0.32	3.17	3.05	0.47	1.59
2	0.25	2.14	3.23	0.38	0.51
3	0.28	2.65	2.43	0.47	0.66
4	0.33	2.42	2.67	0.42	0.33
Mean	0.29	2.6	2.84	0.44	0.77
S.D.	0.03	0.38	0.31	0.04	0.48
S.E.	0.01	0.19	0.15	0.02	0.24
Page Bank					
1	0.28	2.68	2.67	0.92	0.92
2	0.31	2.57	1.96	0.8	0.61
3	0.22	1.77	2.01	0.89	1.47
4	0.23	2.59	2.38	0.89	0.74
Mean	0.26	2.4	2.25	0.88	0.93
S.D.	0.04	0.37	0.29	0.05	0.33
S.E.	0.02	0.18	0.14	0.02	0.16

Percentage dry weight and percentage ash weight

The percentage dry weight and percentage ash weight (proportion of inorganic matter in total dry matter) of the Elodea from each site are shown in Table 12 below.

	Hell Kettles	Bishop Auckland	Witton-le-Wear	Page Bank
Mean % dry weight	10.8	8.73	10.62	9.37
Ash weight (dry basis)	24.35	27.15	24.24	22.17

The results show that the variation in the percentage dry weight is not as great as in the percentage ash weight. In terms of dry weight the Hell Kettles material has the largest proportion of dry matter whereas that from Bishop Auckland has the lowest proportion. The low proportion of inorganic material in the Page Bank Elodea, shown by the low percentage ash weight, is possibly a reflection of the habitat which appears to be very rich in organic matter (see section 3.33).

3.32 pH

The pH measurements taken at the various sites are shown below in Table 13.

Table 13. pH Measurements

	Hell Kettles	Bishop Auckland	Witton-le-Wear	Page Bank
4. VI.70	7.5 (7.8)			
17. VI.70		7.4 (7.8)		8.5 (8.8)
24. VI.70		7.6 (8.0)		9.6 (9.8)
1.VII.70	7.5 (7.9)		8.2 (8.6)	
15.VII.70	7.6 (8.0)	8.4 (9.0)	8.4 (8.8)	
16.VII.70				9.6 (10.2)

In each case the first figure shown in the above table is the pH measurement taken in the water at large while the figure in brackets is the pH taken when the cathode was placed in the water actually in amongst the Elodea plants.

It is at once apparent that the pH measurement are extremely high. This is in all probability largely due to the effect of the presence of Elodea itself as it is noted for its alkalifying effect (see Introduction, Section 1.2). The results also show that there is an increase in pH during the growing season which can be correlated with increased photosynthetic activity as the summer progresses. Further more it is evident that of the four sites Page Bank has the most alkaline water.

The depth of colouration produced by the Elodea in pairs to which phenolphthalein had been added (as explained in Materials and Methods, section 2.42) was tested with an EEL colorimeter with a green filter. The results were as follows:-

Distilled water (set to)	0
Hell Kettles	2.58
Bishop Auckland	3.03
Witton-le-Wear	3.08
Page Bank	3.13

It is thus evident that in the given material, Page Bank displays to the greatest extent, this property of alkalifying the water.

3.33 State of Bottom mud

Moisture and organic content

The results of the analysis of the bottom muds are shown in Table 14 below.

Table 14.

	Hell Kettles	Bishop Auckland	Witton-le-Wear	Page Bank
% moisture content by weight in air dry soil	0.76%	0.78%	0.47%	2.06%
% loss on ignition of air dry soil	4.91%	4.15%	4.02%	12.36%

Page Bank has clearly the highest moisture and organic content. Whereas the organic content of the mud from the other three sites is comparable (4-5%) the Witton-le-Wear mud has a significantly lower moisture content than that of Hell Kettles or Bishop Auckland.

Mechanical Analysis

Calculations were necessary to obtain an estimate of the different textural fractions of the mud samples from each site.

Firstly, in order to correct for temperature a value of 0.4 was deducted from each of the hydrometer readings.

The % sand based on the United States Department of Agriculture limits was calculated as follows.

$$\% \text{ sand} = 100 - \frac{(\text{corrected 1st reading} \times 100)}{50 - \frac{1}{2} \text{ moisture}}$$

Using the international limits the percentage sandis equal to

$$100 - \frac{(\text{corrected second reading} \times 100)}{50 - \frac{1}{2} \text{ moisture}}$$

If the sand content was lessthan 70% (as in the case of the Page Bank substrate) half the loss on ignition value had to be deducted from the sand.

The percentage clay is calculated as

$$\frac{\text{3rd corrected reading}}{50-\frac{1}{2}\text{ moisture}} \times 100$$

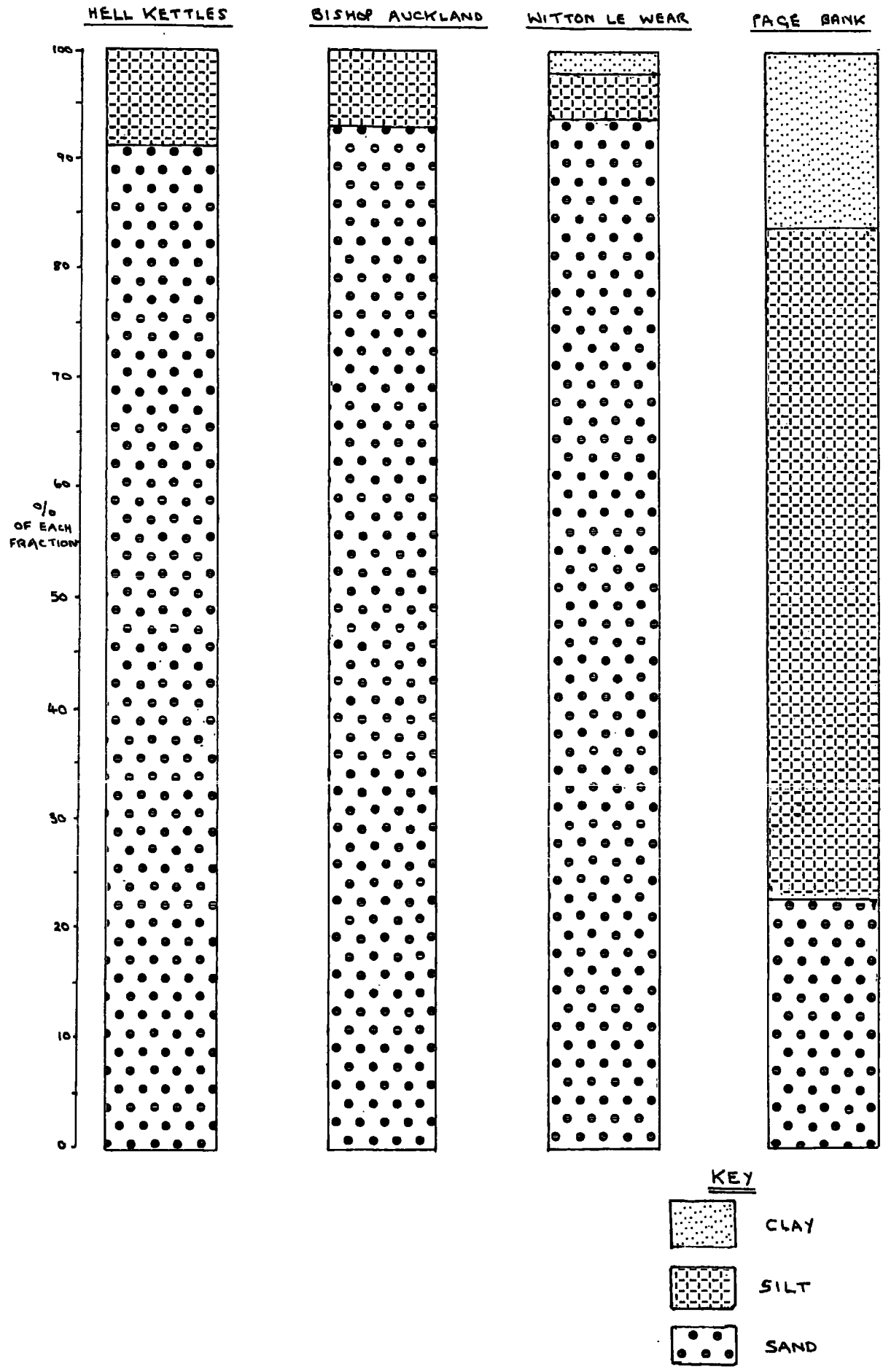
The percentage silt is calculated by subtraction.

The results are tabulated in Table 15 and the mechanical analysis is also shown histogramatically in Figure 18 using the U.S.D.A. limits. The U.S.D.A. scale appears to be the more satisfactory of the two scales for it would be hard to call the Page Bank substrate a "sandy" soil. The other substrates are largely of sand grade; this can be felt by the gritty nature of the material. It is thus apparent that in all three properties it is the Page Bank substrate that differs markedly from the others.

Table 15. Percentage of each of the different fractions in the bottom muds

U.S.D.A. limits	Size limits	Hell Kettles	Bishop Auckland	Witton le-Wear	Page Bank
sand	2mm - 0.05 mm	90.73	92.76	94.775	23.16
silt	0.05 - 0.0002	9.27	7.26	4.019	61.32
clay	0.002 mm	0	0	1.206	15.52
International limits					
sand	2mm -.02 mm	94.76	98.79	96.78	57.68
silt	0.02 - 0.002	5.24	1.21	2.009	26.80
clay	0.002 mm	0	0	1.206	15.52

Figure 18. Mechanical Analysis of the bottom mud from each study area. (U.S.D.A. limits).



4. DISCUSSION

In order to discuss phenotypic plasticity in Elodea canadensis the first problem to consider is whether the different populations are in fact genetically the same. There is clearly no doubt that the populations all belong to the same species and theoretically intraspecific variation can be ruled out as Elodea reproduces apomictically in the British Isles and is not therefore subject to the recombination of genes at meiosis. Most authors agree that mutation along would be unlikely to alter the genotype and the bulk of the literature does in fact indicate that, in England, if not the whole of the British Isles, the different populations are all members of one clone. Arber (1970) states "Possibly the whole Elodea population of England may be regarded, in one sense, as a single individual, with an enormous vegetative output, mechanically sub-divided into vast numbers of apparently distinct plants; in other words, it is not improbable that it may represent the soma developed from a single fertilised ovum".

The results of this work suggest that, due to the morphological convergence that occurs with culture, the potential genetic control of grass morphology may be the same from site to site. However cases are cited where taxa that vary genetically have closely resembled each other after being cultured and therefore the evidence is not conclusive.

The results of the growth studies (Section 3.1), showed, however, that in culture Hell Kettles and Witton-le-Wear material grew at comparable rates and that Bishop Auckland and Page Bank material had a similar growth rate. The distinction between the two pairs of sites is not however sufficient evidence that they are genetically different; more likely, it is an artefact caused by experimental difficuties, these being:

(1) A carry over effect from the environment that was maintained during the three weeks culture. Although the hereditary material is presumably

the same, the protoplasm of the different specimens will differ according to the environment, even within material from one site, thus giving rise to a lack of uniformity in the innocula. During further culturing much of the material died and therefore it was not possible to obtain accurate measurements after sufficient time had elapsed to eliminate this carry over or lag effect.

(2) Algal contamination could not be sufficiently suppressed. Despite repeated washings at the end of each half week period, growths of Cyanophyta and Bacillariophyta remained troublesome. These would be competing with the Elodea for the nutrients in the medium and for the carbon dioxide absorbed by the atmosphere which has been shown by the aeration experiments to be an important factor. Therefore it seemed reasonable to maintain the basic premise that the populations are, in all probability, genetically similar.

Heslop-Harrison (1953) states that among higher plants aquatic and amphibious plants are probably the most plastic of all and, as noted in the introduction, Sculthorpe (1967) quotes Elodea canadensis as a good example of the plastic behaviour of hydrophytes. Being a species of wide tolerance limits that inhabits a broad spectrum of habitats it provides the potential for a high degree of plasticity. The broad range of habitats is seen in the analysis of the environmental parameters at the sites investigated.

The analysis for magnesium and calcium showed that the water at Hell Kettles was the hardest of the four sites. Nicholson and Nowers (1926) obtained measurements of 129.3 mg/l Ca (as $CaSO_4$ and $CaCO_3$) and 30.3 mg/l Mg (as $MgCO_3$). These results show that in nearly fifty years the concentrations of these elements have risen quite dramatically. This finding is consistent with the observation of Fell (personal communication) that in recent years the level of water in the Double Kettle has fallen several feet. The only other site at which measurements have been obtained by other workers is Bishop Auckland. Below the confluence of the River

Gaunless (downstream of the study site). Snow and Whitton (in press) obtained mean values of 23 mg/l Na, 3.5 mg/l K, 65 mg/l Ca, 19 mg/l Mg and 0.05 mg/l PO_4-P. Whereas the data in the present work shows a similar level for phosphate, levels are generally rather higher for calcium and lower for the three other cations.

The measurements obtained for percentage dry weight, percentage ash content and the analysis of the material for sodium, potassium, calcium, magnesium and iron are all within the range found by other workers, for example Harper and Daniel (1934), Nelson and Palmer (1938), Misra (1938) and Fish and Will (1966). Considerable variation in the values is observed in the literature. In all probability this is partly due to the methods employed (for example, it seems unlikely that all workers will have a uniform method for obtaining percentage dry weight), and also due to genuine differences within the phenotypic make-up of the plants concerned. These differences will depend on season, habitat and geographical locations. Fish and Will (1966) state that Elodea reflects the composition of the water in which it grows. In this study this is clearly seen in the case of calcium and sodium. Calcium levels are highest in the Elodea from Hell Kettles, the site with by far the hardest water. The level of sodium in the Hell Kettles material is also significanly higher, mirroring the high levels in the water. Magnesium and potassium do not show such a high degree of correlation however.

The levels of iron found, 0.13 - 0.77% dry weight compare with the results of other workers (mentioned by name in the preceeding paragraph). It is pertinent to note here that Elodea may have potential as use for a fodder crop. It has a higher concentration of iron than is found in either spinach or yeast and a higher concentration of nitrate than in weeds or grasses (Nelson and Palmer, 1938; Fish and Will, 1966). Although it is evidently "relished by cattle" the high water content (approximately 92% as opposed to 82% in Lolium perenne) may diminish its value as food for stock however. Nevertheless, if the weed is to be cleared in certain parts of the world the financial burden of such a task will be eased if

the weed can be used to manufacture a product of commercial value. If Elodea can be exploited commercially it will be important to choose the sites where phenotypes containing high nutrient levels are found.

It would seem likely that the morphology of Elodea is largely affected by physical factors of the environment rather than by the biotic. At this stage a correlation between environmental differences and phenotypic form would seem desirable but is not possible. The principles of ecology are based on the fact that the environment is composed of large numbers of interacting variables and therefore it is impossible to analyse environments and their effects completely. Other highly variable factors such as light intensity, temperature, oxygen levels and turbidity were not examined in this study and these must play some part in the complex mesh of environmental influences that together mould the various phenotypes.

In the field the growth rates are affected by biotic influences as well as the purely physical. It is suggested that these largely take the form of competition by other macrophytes. Grazing pressures on macrophytes are often slight (Vollenweider, 1969) whereas the populations of phytoplankton may be controlled by herbivorous animals to a rather greater extent. Although no quantitative measurements were obtained for growth at Hell Kettles, subjective estimates at successive visits were adequate to show that the growth rate was faster at this site than at any of the other three. The results (section 3.12b) show that at Bishop Auckland the growth rate is faster than at Page Bank. Wherease at Hell Kettles and Bishop Auckland Elodea is the dominant macrophyte, at Page Bank it is subject to interspecific competition with Certophyllum demersum and Myriophyllum spicatum. A physical factor that may encourage growth at Hell Kettles and Bishop Auckland is the higher phosphate level of the water. Mulligan and Baranowski (1969) state that Elodea grows best in phosphate levels of 0.02 - 0.065 mg/l. Both Witton-le-Wear and Page Bank waters fall short of these levels. The results of the culture experiments have shown that, as Olsen (1954) postulated, iron is important for the

growth of Elodea. Although only non-detectable trace amounts were present in the water samples there is, in all probability, a range of concentrations as shown by the variations in the amounts contained in the Elodea.

In concluding this antecological study, it is suggested that sufficient variation in habitat conditions has been observed to account for the range of phenotypic plasticity observed in Elodea canadensis. The weight of evidence indicates that at least the genetic potential of the morphology of the species is the same throughout the experimental material although conclusive evidence was not obtained to show that thephysiological control over growth in culture was the same between all four sites.

5. SUMMARY

1. Four sites in County Durham were selected at which to examine phenotypic variation in Elodea canadensis. The aspects studied were growth rate and gross morphology.

2. The growth rates of the different phenotypes were observed to differ in the field. Under culture conditions Elodea from two of the sites grew at a significantly faster rate than that from the other two sites. Due to algal contamination and an environmental carry-over effect caused by a conditioning to the previous habitat, this is not sufficient evidence that the two pairs of phenotypes are genetically distinct. Aeration had a promotory effect on growth whereas high and low levels of Fe.EDTA had an inhibitory effect.

3. Morphological form as seen in leaf dimensions and internode length varies considerably between sites. These characters converge with culture suggesting that thepotential genetic control of morphology may be the same from site to site.

4. The study of certain environmental parameters was carried out. Differences in water chemistry were determined and in some cases these could be correlated with differences in the chemical constituents of the Elodea itself. pH measurements were obtained and an analysis of the bottom sediment from each site was carried out.

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7. APPENDIX

Table A. Comparison of Accuracy obtained from different methods of measuring fresh weight

Measurements (grams)	Methods a	b	c	d
1	0.099	0.094	0.085	0.075
2	0.098	0.087	0.080	0.075
3	0.093	0.082	0.090	0.069
4	0.105	0.085	0.079	0.079
5	0.093	0.083	0.081	0.082
6	0.095	0.089	0.078	0.072
7	0.100	0.081	0.087	0.070
8	0.094	0.083	0.080	0.068
9	0.103	0.086	0.083	0.068
10	0.906	0.081	0.091	0.069
Mean	0.098	0.085	0.084	0.072
Standard deviation	0.0039	0.0034	0.0041	0.0045

TABLE B

TEST GROWTH EXPERIMENT, RAW DATA

HELL KETTLES

LENGTH (MM'S)
WET WEIGHT (GRAMS)
NO. OF VISIBLE INTERNODES.

FLASK		WEEK 0			WEEK 1			WEEK 2			WEEK 3		
		LENGTH	WET WEIGHT	INTER-NODES	LENGTH	WET WEIGHT	INTER-NODES	LENGTH	WET WEIGHT	INTER-NODES	LENGTH	WET WEIGHT	INTER-NODES
1 L. FLASKS	1	50	0.2350	12	75	0.4262	27	105	0.4100	39	90	0.3584	44
	2	50	0.1584	11	72	0.2485	22	82	0.5790	34	79	0.4055	36
	3	50	0.1692	11	60	0.3031	17	60	0.3553	29	59	0.4186	31
	4	50	0.2787	12	75	0.3553	27	55	0.3127	28	44	0.3342	23
	5	50	0.2153	11	85	0.3554	27	102	0.5536	52	85	0.6353	48
500 ML. FLASKS	1	50	0.1348	14	90	0.4086	34	102	0.5021	47	98	0.5476	53
	2	50	0.0967	12	50	0.2463	23	52	0.2783	28	50	0.3687	25
	3	50	0.1547	11	80	0.2754	22	95	0.3822	38	69	0.4106	35
	4	50	0.1692	14	60	0.2299	21	75	0.3031	36	72	0.3715	40
	5	50	0.2133	13	55	0.3213	14	65	0.4041	34	79	0.5843	50
SPECIMEN JARS	1	50	0.1811	10	85	0.2923	25	115	0,4298	42	140	0.7493	53
	2	50	0.1711	13	80	0.3860	31	102	0.5607	58	116	1.0189	80
	3	50	0.1303	16	55	0.1882	21	75	0.3359	32	92	0.6226	49
	4	50	0.1187	13	85	0.2647	28	112	0.4759	41	132	0.8094	62
	5	50	0.1750	10	90	0.3306	29	118	0.4247	38	135	0.7044	53

TABLE B

TEST GROWTH EXPERIMENT, RAW DATA

BISHOP AUCKLAND

LENGTH (MM'S)
WET WEIGHT (GRAMS)
No. OF VISIBLE INTERNODES

FLASK		WEEK 0			WEEK 1			WEEK 2			WEEK 3		
		LENGTH	WET WEIGHT	INTER-NODES	LENGTH	WET WEIGHT	INTER-NODES	LENGTH	WET WEIGHT	INTER-NODES	LENGTH	WET WEIGHT	INTER-NODES
1 L. FLASKS	1	50	0.1956	10	95	0.3998	34	115	0.5902	60	115	0.5902	77
	2	50	0.2231	10	82	0.3940	30	80	0.6194	51	80	0.7735	64
	3	50	0.1774	10	84	0.3022	24	90	0.5283	35	99	0.6929	48
	4	50	0.1824	8	87	0.3352	26	95	0.4777	40	93	0.6753	54
	5	50	0.1989	10	87	0.1909	28	90	0.4765	35	92	0.4907	35
500 ML. FLASKS	1	50	0.2397	10	97	0.4131	27	112	0.5095	41	120	0.7245	58
	2	50	0.2183	8	83	0.3235	26	85	0.5412	37	75	0.7490	41
	3	50	0.2056	10	93	0.3755	32	114	0.5554	61	120	0.8040	70
	4	50	0.2008	10	65	0.2844	22	79	0.4996	33	90	0.6280	52
	5	50	0.2064	9	87	0.3586	26	95	0.5105	34	95	0.6919	49
SPECIMEN JARS	1	50	0.2028	10	99	0.3704	26	123	0.6027	45	150	0.7934	82
	2	50	0.21	10	90	0.4060	32	120	0.6474	50	110	0.8547	89
	3	50	0-2147	10	99	0.3728	28	102	0.6067	35	131	0.2959	73
	4	50	0.1846	9	100	0.3867	26	120	0.6127	43	122	0.9327	75
	5	50	0.1871	11	95	0.3792	27	114	0.5557	47	139	0.8546	81

TABLE B

TEST GROWTH EXPERIMENT, RAW DATA

WITTON-LE-WEAR

LENGTH (MM'S)
WET WEIGHT (GRAMS)
No. OF VISIBLE INTERNODES

FLASK		WEEK 0			WEEK 1			WEEK 2			WEEK 3		
		LENGTH	WET WEIGHT	INTER-NODES	LENGTH	WET WEIGHT	INTER-NODES	LENGTH	WET WEIGHT	INTER-NODES	LENGTH	WET WEIGHT	INTER-NODES
1 L. FLASKS	1	50	0.2170	15	85	0.2748	30	99	0.4252	38	110	0.6329	44
	2	50	0.1073	13	75	0.1281	26	77	0.3778	40	70	0.5510	46
	3	50	0.0822	13	70	0.1533	21	74	0.2664	30	65	0.2489	29
	4	50	0.1547	14	80	0.2357	29	68	0.2541	38	85	0.4134	50
	5	50	0.1152	12	75	0.2092	29	80	0.2541	47	74	0.5697	51
500 ML. FLASKS	1	50	0.1341	15	78	0.2247	27	84	0.4240	34	75	0.4726	34
	2	50	0.1389	12	76	0.2558	36	85	0.4517	35	85	0.5472	37
	3	50	0.1165	11	70	0.1990	23	72	0.3101	34	80	0.4433	44
	4	50	0.1236	16	ABANDONED AS COMPLETELY DEAD								
	5	50	0.1114	14	55	0.2826	22	55	0.3964	28	45	0.2272	26
SPECIMEN JARS	1	50	0.0881	18	65	0.2084	30	77	0.3465	38	88	0.9711	40
	2	50	0.1594	12	80	0.2487	27	103	0.4346	38	110	0.6870	52
	3	50	0.1379	13	72	0.2074	28	85	0.3430	46	76	0.5005	52
	4	50	0.1057	14	75	0.1922	28	84	0.3913	34	60	0.3486	29
	5	50	0.0975	12	60	0.1540	26	70	0-3166	36	92	0.4639	45

TABLE B

TEST GROWTH EXPERIMENT, RAW DATA

PAGE BANK

LENGTH (MM'S)
WET WEIGHT (GRAMS)
NO. OF VISIBLE INTERNODES

FLASK		WEEK 0			WEEK 1			WEEK 2			WEEK 3		
		LENGTH	WET WEIGHT	INTER-NODES	LENGTH	WET WEIGHT	INTER-NODES	LENGTH	WET WEIGHT	INTER-NODES	LENGTH	WET WEIGHT	INTER-NODES
1 L. FLASK	1	50	0.2112	13	85	0.3387	38	105	0.5018	49	115	0.5972	51
	2	50	0.2080	12	100	0.4390	43	125	0.6987	74	130	0.8633	71
	3	50	0.1953	12	88	0.4271	43	100	0.6502	57+15(2)	95	0.8365	66
	4	50	0-1850	13	102	0.3952	41	129	0.5480	60	130	0.8394	59
	5	50	0.2073	12	88	0.2728	40	101	0.4419	64	104	0.5624	61
500 ML. FLASK	1	50	0.1821	15	85	0.3374	41	105	0.4711	60	115	0.5810	72
	2	50	0.2029	12	95	0.3293	40	115	0.6651	60	116	0.8372	57
	3	50	0.1953	13	90	0.3769	40	112	0.6194	58	118	0.8436	53
	4	50	0.1895	12	84	0.3376	33	100	0.5527	46	94	0.6780	45
	5	50	0.1577	16	65	0.3951	36	80	0.5467	52	85	0.8942	64
Specimen Jars	1	50	0.1890	13	95	0.3860	40	124	0.6871	52	156	0.9172	84
	2	50	0.1370	11	105	0.3374	34	130	0.5428	59(2)	160	0.7700	81
	3	50	0.2035	11	90	0.3356	37	111	0.5353	59	140	0.9300	82
	4	50	0.1533	13	68	0.3389	36	110	0.4865	50	140	0.7691	79
	5	50	0.1516	12	73	0.3901	37	123	0.6236	53	130	0.9537	96